Puneet Kumar
Pran Kishore Deb *Editors*

Frontiers in Pharmacology of Neurotransmitters

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“This book is dedicated to our mentors, students, and researchers.”

Preface

It is our pleasure and privilege to introduce the book *Frontiers in Pharmacology of Neurotransmitters* covering a wide range of information about neurotransmitters and their receptors involved in the pathophysiology of various neurological disorders. In the last decade, substantial progress has been made in understanding the pathophysiological basis of various neurological disorders; however, the discovery and development of drugs for the treatment of such disorders is still an uphill task to the world of neuroscientists. Moreover, the prevalence of neurological disorders is increasing day by day, thereby imposing a great challenge to the researchers to fulfill the unmet demand and develop new therapeutic strategies. This is possible only with the complete understanding of the pathophysiological role neurotransmitters play in the development of such diseases.

This book provides an updated insight into the pathophysiology and pharmacology of neurotransmitters in order to upgrade the knowledge and understanding of researchers about the involvement of these neurotransmitters in the progression of the disease as well as provide innovative ideas to develop new therapeutic interventions targeting various neurological diseases. A total of 20 chapters have contextualized in this book, covering the history, basic science, classification, and pathophysiological mechanisms involved at molecular and ionic levels along with the pharmacology of neurotransmitters. Various research findings in preclinical and clinical settings, highlighting the current status and potentials of various novel molecules targeting these neurotransmitters as promising therapeutic agents, are also discussed. The authors have provided a wide range of literature reviews to make this book an excellent source of information including various diagrammatic pathways and tables in order to facilitate the reader not only to get deeper information in minimum time but also to contribute towards better research in this field. Readers will get to know the various types of pathological changes that occur in a neurological disorder and which types of neurotransmitter is actually involved to elicit specific signs and symptoms of a particular disease, and how it affects the normal life of a person.

This book will be helpful to the undergraduate and postgraduate students and researchers from academia and pharmaceutical industries to gain insight into the different neurological pathways involving the neurotransmitters and their receptors in the initiation and progression of various diseases. This book will attract and

motivate the young scientists and researchers from the multidisciplinary international research community to design innovative drug discovery strategies for the development of novel therapeutic interventions to cure various neurological disorders and improve the human health in future.

Bathinda, Punjab — Puneet Kumar
Amman, Jordan — Pran Kishore Deb

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Neurotransmitters and Their Receptors—State of the Art

1

Puneet Kumar, Sara Nidal Abed, Yazan A. Bataineh, and Mutaz Sheikh Salem

Abstract

Neurotransmitters are endogenous chemical messengers that are responsible for neuronal communication throughout the body. These compounds serve to facilitate various functions controlled by the central nervous system via a process known as chemical synaptic transmission. The discovery of various types of neurotransmitters has taken place over the past years where the neurotransmitters have been classified based on their chemical, functional, and molecular properties along with their location in the body. This chapter highlights all the important neurotransmitters including GABA and glycine, glutamate, melatonin, histamine, serotonin, acetylcholine along with other neurotransmitters, taking into account their synthesis, release, mechanism of action, and metabolism. This chapter also briefly discusses the physiological roles of these neurotransmitters and their contribution in different pathological conditions, in addition to the therapeutic effects of their agonists/antagonists.

Keywords

Neurotransmission · Neurotransmitter · Neuroreceptors · Agonists · Antagonists

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Abbreviations

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
Ach	Acetylcholine
AchE	Acetylcholinesterase
AD	Alzheimer's disease
BuchE	Butyrylcholinesterase
cAMP	Cyclic adenosine monophosphate
CBD	Cannabidiol
CNS	Central nervous system
DAO	Diamine oxidase
ECs	Endogenous cannabinoids
EOPs	Endogenous opioid peptides
GABA	γ-Aminobutyric acid
GPCRs	G-protein-coupled receptors
HDC	L-histidine decarboxylase
HNMT	Histamine N-methyltransferase
KO	Knockout
MAO- A	Monoamine oxidase A
NO	Nitric oxide
NOP	Nociceptin opioid receptor
NOS	Nitric oxide synthase
PKG	Protein kinase G
PNS	Peripheral nervous system
TCA	Tricarboxylic acid
THC	Tetrahydrocannabinol
VGAT	Vesicular GABA transporter

1.1 Introduction

Neurotransmitters are endogenous chemical messengers that are responsible for neuronal communication throughout the body. These compounds serve to provide various functions facilitated by the brain via a process known as chemical synaptic transmission (Rizo 2018). Neurotransmitters play essential roles in many processes of early human development, which include differentiation, neurotransmission, the growth of neurons, as well as the neural circuitry development (Herlenius and Lagercrantz 2001, 2004). A compound is referred to as a neurotransmitter when it meets four criteria. First, being synthesized in the neurons. Second, being present in the presynaptic terminal and released in sufficient amounts enough for exerting a certain action on the postsynaptic neuron or targeting receptors in effector organs. Third, the exogenous administration has to mimic the endogenously produced neurotransmitter's action, and last, the presence of intrinsic mechanisms responsible for its removal from the site of action (Kavalali 2015).

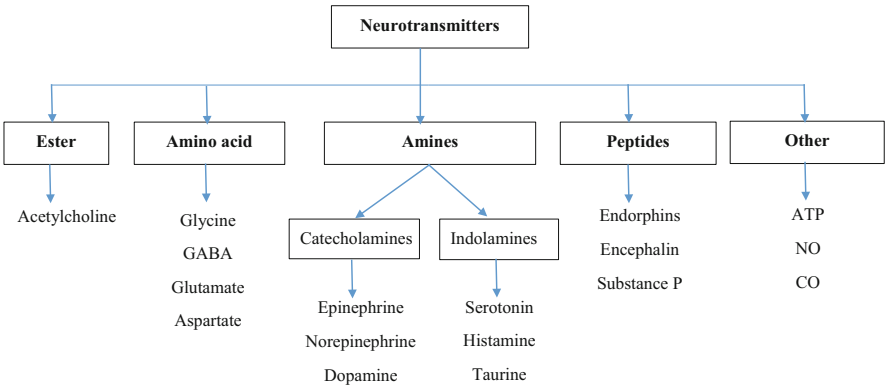


Fig. 1.1 Chemical classification of neurotransmitters

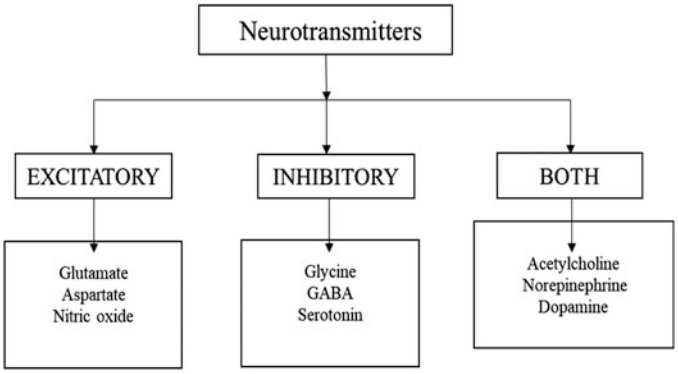


Fig. 1.2 Functional classification of neurotransmitters

The discovery of various types of neurotransmitters has taken place over the past years where the neurotransmitters have been classified based on their chemical (Fig. 1.1), functional (Fig. 1.2), and molecular properties along with their location in the body (Fig. 1.3) (Zhou and Danbolt 2014). Neurotransmitters are classified into (a) biogenic amines that include serotonin, dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline), (b) neuropeptides that include substance P, as well as (c) amino acids that include glutamate and γ-aminobutyric acid (GABA) (Rangel-gomez and Meeter 2016). Neurotransmitters show their action via two classes of receptors possessing distinctive modalities of synaptic transmission. The first class is known as ionotropic receptors, which comprise the ligand-gated ion channels that are responsible for eliciting fast synaptic transmission. The second class is metabotropic receptors, which consist of GPCRs that bind to neurotransmitters causing slow synaptic transmission by intracellular signaling pathways in addition to inducing gene expression (Komatsu 2015). The aforementioned neurotransmitters

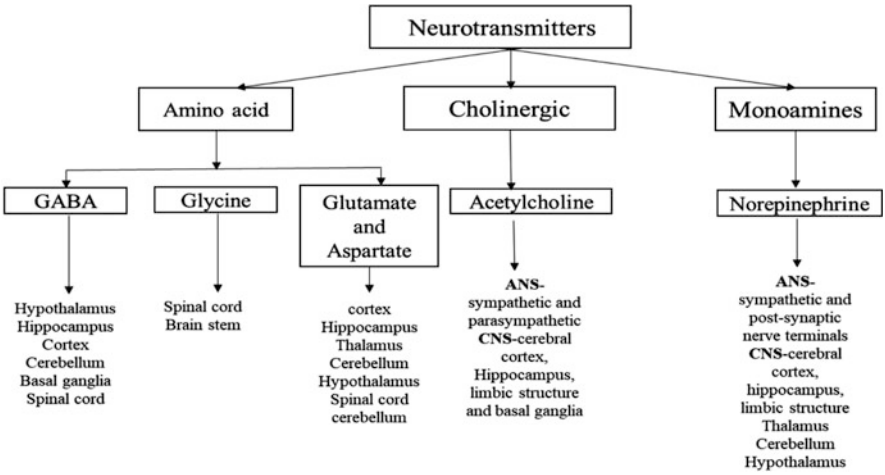


Fig. 1.3 Classification of neurotransmitters based on location

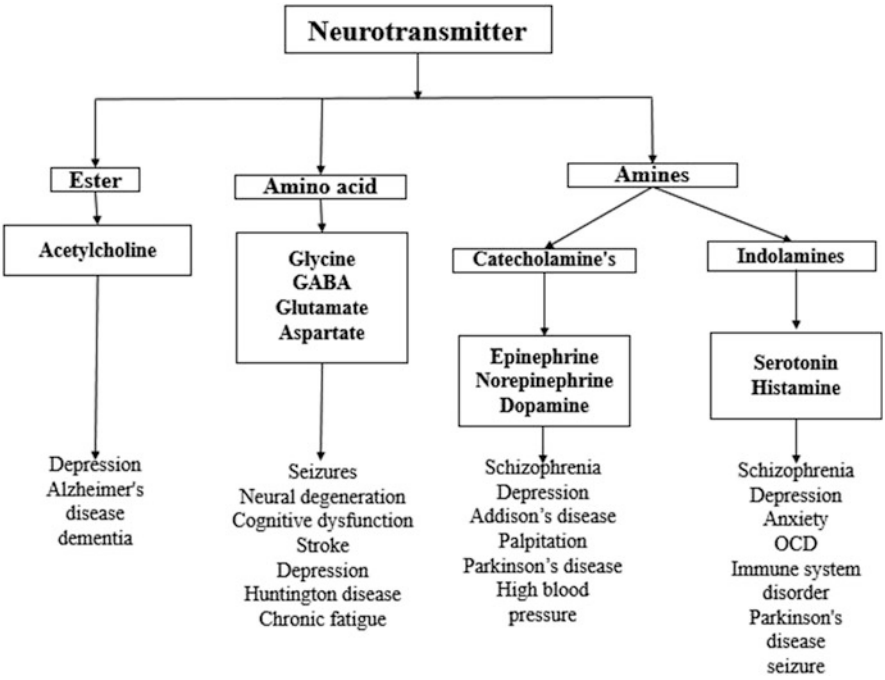


Fig. 1.4 Classification of neurotransmitters based on disease association

along with other important ones are briefly discussed in this chapter, taking into account the roles they serve in the physiological/pathological conditions, including their mechanisms of action and their pharmacological properties (Fig. 1.4).

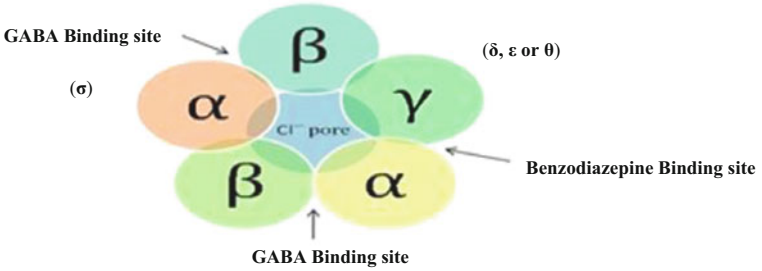
1.2 GABA

The γ-aminobutyric acid (GABA) is a non-protein amino acid and serves as a key inhibitory neurotransmitter in the adult brain. GABA is widespread in early embryonic life and has been shown to have an excitatory synaptic transmission activity in the immature brain (Luján et al. 2005). The neurotransmission served by GABA is known to be present in nearly all organisms, starting from bacteria to human beings (Owens and Kriegstein 2002). GABA is synthesized by the GABAergic neurons from L-glutamate, and the catalysis of this reaction is done by the glutamic acid decarboxylase (GAD) enzyme (Olsen 2002; Bouche et al. 2003). Once GABA is synthesized, it is then stored in GABAergic synaptic vesicles which is facilitated by a vesicular GABA transporter (VGAT) and upon depolarization, it undergoes calcium ion-dependent release. The release of GABA from the presynaptic terminals will be then followed by the action on GABA receptors ($GABA_A$ and $GABA_B$), (Markwardt and Overstreet-wadiche 2008; Li et al. 2012). $GABA_A$ receptors are ion channels, whereas $GABA_B$ receptors are G-protein-coupled receptors (GPCR). Stimulating $GABA_A$ receptor was shown to increase the permeability to chloride ions which therefore resulted in inhibition or hyperpolarization of neurons by an increase in the potential of the postsynaptic membrane. Stimulating $GABA_B$ receptor leads to modulating cyclic adenosine monophosphate (cAMP) production as well as increasing the conductance of potassium and hence resulting in either hyperpolarization or inhibition of the voltage-gated calcium channels (Olsen 2002; Bouche et al. 2003).

GABAergic neurotransmission has been shown to play a vital role in proliferating, migrating, and integrating other neuronal progenitors that are of key importance for modulating the brain patterns, and deliberates various trophic functions along with its contribution in the synaptic plasticity (Schmidt and Mirnics 2015). Disruptions in the GABAergic neurotransmission mostly result in sequential events in the brain development, which was revealed in numerous *in vitro* and *in vivo* studies by using various receptor modulators and knockout (KO) mice models (Fig. 1.5) (Owens and Kriegstein 2002).

1.3 Glutamate

Glutamate is an excitatory amino acid neurotransmitter that is highly distributed in the brain tissue (Danbolt et al. 2016). Glutamate is biosynthesized from glucose-determined tricarboxylic acid (TCA) derivatives in the mitochondria (Stanley et al. 2017). In the neurotransmission of glutamate, four enzymes are involved, where glutamate may be acting either as a substrate or a final product. Glutamate dehydrogenase, aminotransferase, glutamine synthetase as well as glutaminase are the enzymes involved in the neurotransmission of glutamate (Walker and van der Donk 2016). When the ionotropic glutamate receptors (NMDA, AMPA, and Kainate) get activated, they open membrane channels that allow ions to pass through. AMPA and Kainate receptors increase the permeability of sodium and



A) The $GABA_A$ receptor consists of two α, two β, and one γ subunit arranged in αβαβγ way. The γ subunit may be replaced with either δ, ε, or θ.

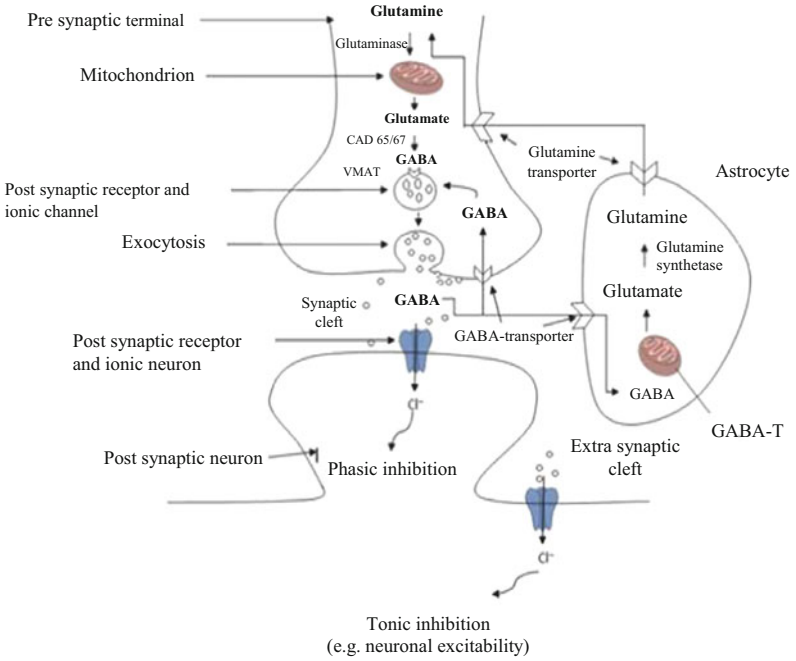


Fig. 1.5 Downstream regulating pathway of GABA

potassium, whereas NMDA receptor increases membrane permeability for calcium as shown in Fig. 1.6.

Glutamate has been shown to serve a major role in the learning as well as memory of the normal brain that include cognition, behavioral patterns, and sensation (Stanley et al. 2017). Glutamate mainly serves a role in the CNS development including synapse's induction and elimination; migration, differentiation as well as apoptosis process of the cell. Moreover, glutamate also has a key role in the peripheral tissues and endocrine cells. Despite the important roles of glutamate as a neurotransmitter, it is also known for its lethality to neurons causing a phenomenon known as "excitotoxicity" which causes excessive firing of the neurons in the brain

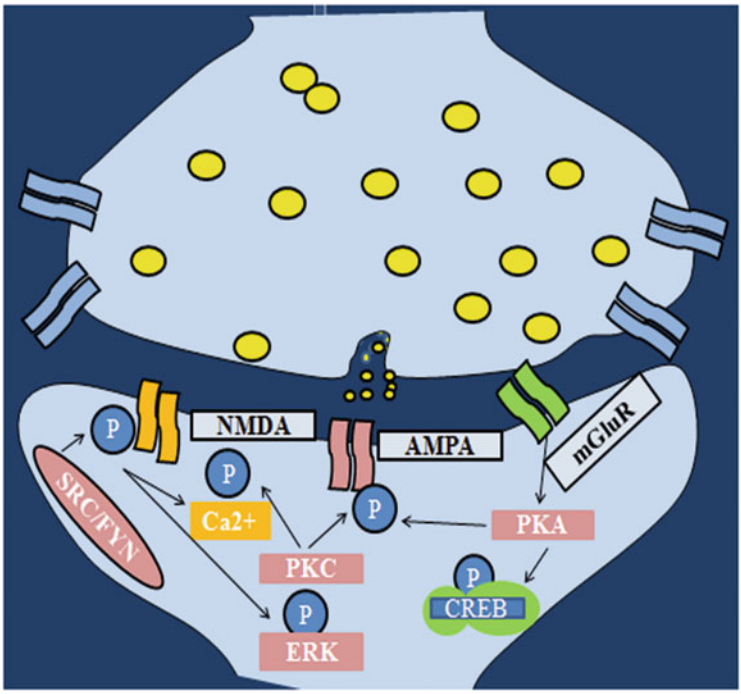


Fig. 1.6 Downstream regulating pathways of glutamate

and triggers development of the brain disorders such as hyperkinetic progressive neurodegenerative disorder (Huntington disease), progressive loss of memory, cognitive decline (Alzheimer's disease), progressive muscle weakness (amyotrophic lateral sclerosis), stroke, and epilepsy (Murrough et al. 2017).

1.4 Dopamine

Dopamine is considered as the major neurotransmitter in the brain from the catecholamine group. Dopamine is responsible for various functions in the brain including controlling the voluntary actions, reward, consciousness, circadian rhythm as well as cognition (Hasbi et al. 2011). The dopaminergic neurons are present in the midbrain and the associated regions that include the basal ganglia, ventral tegmental area, and the retrorubral field (Haber 2016). The mediation of dopamine physiological actions is done by its interaction with its receptors over dopaminergic synapses. There are mainly five types of dopamine receptors that are categorized under two subclasses, D1 and D2 dopamine receptors (Beaulieu et al. 2015). Dopamine receptors are typically stable, however sharp and sometimes prolonged. The increase or decrease in dopamine levels can downregulate or upregulate the number of dopamine receptors as shown in Fig. 1.7.

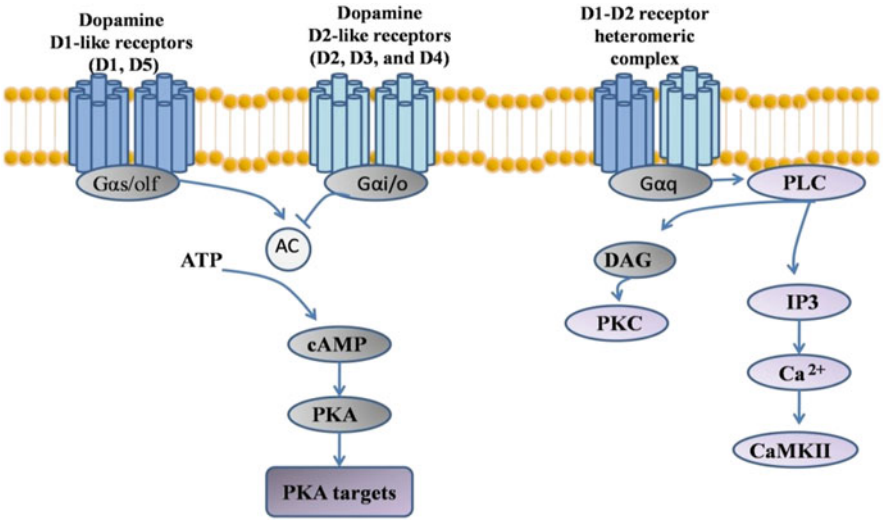


Fig. 1.7 Regulation of dopamine

The distribution of D1 receptors takes place in nigrostriatal, mesolimbic, and mesocortical areas, like the caudate-putamen (striatum), substantia nigra, olfactory bulb, amygdala and at small level in the hippocampus, cerebellum, thalamic, and hypothalamic areas. However, D2 receptors are expressed in the striatum, the substantia nigra, ventral tegmental area, hypothalamus, cortical areas, septum, amygdala, hippocampus, nucleus accumbens, and the olfactory tubercle regions (Rangel-Barajas et al. 2015). These dopamine receptors serve diverse functions. D2 auto-receptors that are localized in the presynaptic region are responsible for inhibiting the release of dopamine, hence decreasing locomotor activity, whereas D2 receptors in the postsynaptic region are responsible for enhancing the release of dopamine and hence stimulating the locomotion. Therefore, dopamine agonists show biphasic response by the activation of both the presynaptic and postsynaptic dopamine receptors (Sulzer et al. 2016). Likewise, D3 receptors exhibit a biphasic response, however at a moderate level. D3 receptors are responsible for regulating reward and reinforcement like functions of the human behavior. On the other hand, D4 and D5 receptors are less expressed at motor control regions; therefore, they only provide a low contribution to movement control (Beaulieu et al. 2015). Furthermore, D1 and D2 receptors are also responsible for regulating learning, working memory as well as executive functions. However, D3, D4 as well as D5 were shown to provide less influence over cognition, because of the lower expression they have in the hippocampus (Nyberg et al. 2016). Nowadays, clinical psychotic drugs show their action by blocking D2 actions to treat diseases like schizophrenia and bipolar disorder, due to the contribution of dopamine in the psychotic behavior. Dopamine also serves other functions outside CNS including D2-mediated prolactin secretion from the pituitary gland, D1-mediated renin secretion from kidney, and adrenal

gland-mediated D1-directed aldosterone secretion (Lv et al. 2018). Some specific agonists and antagonists of dopamine are available for D1 and D2 class of receptors. Selective agonists for D1 include A77636, SKF38393, SKF81297, and dihydrexidine, while D2 selective compounds include quinpirole and N-0437. On the other side, antagonists of D1 class involve SCH23390, SCH39166, and SKF35566, while D2 antagonism is caused by domperidone, nemonapride, raclopride, and sulpiride (Smee and Overstreet 1976).

1.5 Melatonin

Melatonin or 5-methoxy-*N*-acetyltryptamine was discovered and isolated from bovine pineal in 1958 by Aaron Lerner (Lerner et al. 1958). Melatonin is considered as the main hormone secreted by the pineal gland. However, it has been reported that melatonin is also obtained from extrapineal sources including the retina, bone marrow cells, platelets, skin, lymphocytes, harderian gland, cerebellum, as well as the gastrointestinal tract of vertebrate species (Lerner et al. 1958; Dubbels et al. 1995; Hattori et al. 1995; Manchester et al. 2000; Reiter et al. 2014; Shi et al. 2016; Tan et al. 2016). Melatonin was identified in various animals and plants (Lerner et al. 1958). The initial function of melatonin was thought to be the detoxification of the free radicals resulting from processes like photosynthesis and metabolism (Tan 1993; Manchester et al. 2015; Galano et al. 2018). Later, melatonin became a pleiotropic molecule that is responsible for resisting oxidation-related stress; however, it was also shown to influence the biological rhythms, suppression of inflammation, as well as other actions (Tan et al. 2010; Lochner et al. 2018; Onaolapo and Onaolapo 2018; Tamtaji et al. 2018). The molecular structure of melatonin has determined its high efficiency in the detoxification of free radicals due to its ability to donate an electron or a hydrogen atom, or in other means, depending on the radical type (Reiter et al. 2014; Tan et al. 2015; Galano et al. 2018). The melatonin's superior antioxidant capacity of limiting the oxidative stress is attributed to the cascade reaction that occurs as a result of generating derivatives that are likewise free radical scavengers (Tan et al. 2013, 2015; Jou and Peng 2018). Furthermore, melatonin was also shown to play a role in some age-related processes such as retardation, anti-inflammatory activity, neurodegenerative changes resistance, preventing apoptosis in normal cells, as well as the preservation of mitochondrial and chloroplast physiology (Poeggeler 2005; Jou et al. 2007; Manchester et al. 2015; Majidinia et al. 2018; Nabavi et al. 2019).

Melatonin is formed specifically from the amino acid tryptophan (Hardeland 2015). Tryptophan is consumed in the diet along with being synthesized via the shikimic acid pathway that starts with D-erythrose-4-phosphate, phosphoenolpyruvate, or carbon dioxide in certain species (Bochkov et al. 2012). Tryptophan is initially converted to serotonin in a reaction that involves both a decarboxylation and a hydroxylation reaction. Darkness and light are the two factors that are responsible for melatonin release as well as its synthesis as shown in Fig. 1.8.

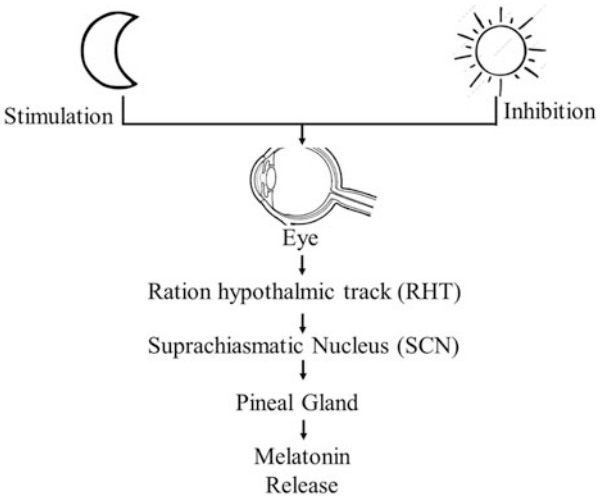


Fig. 1.8 Mechanism of melatonin release

Melatonin demonstrates its activity via different molecular pathways; however, the activation of two types of membrane-specific receptors, high-affinity ML1 sites and low-affinity ML2 sites, was shown to be the best characterized pathways (Morgan et al. 1994; Dubocovich 1995). Melatonin receptors have been reported in both the central and the peripheral tissues, which include the heart and arteries, adrenal gland, kidney, lung, liver, gallbladder, small intestine, adipocytes, ovaries, uterus, breast, prostate, and skin (Ekmekcioglu 2006). Melatonin is responsible for regulating circadian rhythms like the sleep-wake rhythm, neuroendocrine rhythms, or body temperature cycles via its action on both MT1 and MT2 receptors (Axelrod 1974; Dahlitz et al. 1991; Morgan et al. 1994; Zisapel 2001; Von Gall et al. 2002; Jin et al. 2003; Ekmekcioglu 2006; Karasek and Winczyk 2006; Liu et al. 2016). Ingesting melatonin prompts fatigue, sleepiness, and a diminution of sleep latency (Zhdanova et al. 1997). Besides, melatonin also plays a role in the regulation of blood pressure and autonomic cardiovascular activity, as well as regulating the immune system. Melatonin has been also reported for its various therapeutic potentials in several disorders including certain tumors, cardiovascular or psychiatric disorders (Tordjman et al. 2017).

1.6 Adrenaline and Noradrenaline

Adrenaline (epinephrine) is a hormone that is produced by the adrenal glands as well as by a small number of neurons in the medulla oblongata of the CNS. Adrenaline serves as a neurotransmitter that regulates visceral functions, e.g., respiration. Adrenaline also plays a significant role in the fight-or-flight response by increasing the blood flow to muscles, the heart output, pupil dilation, as well as the levels of blood sugar as it binds to alpha and beta receptors (Goldstein 2010). Noradrenaline (norepinephrine) shows its action as a neurotransmitter in both the CNS and PNS

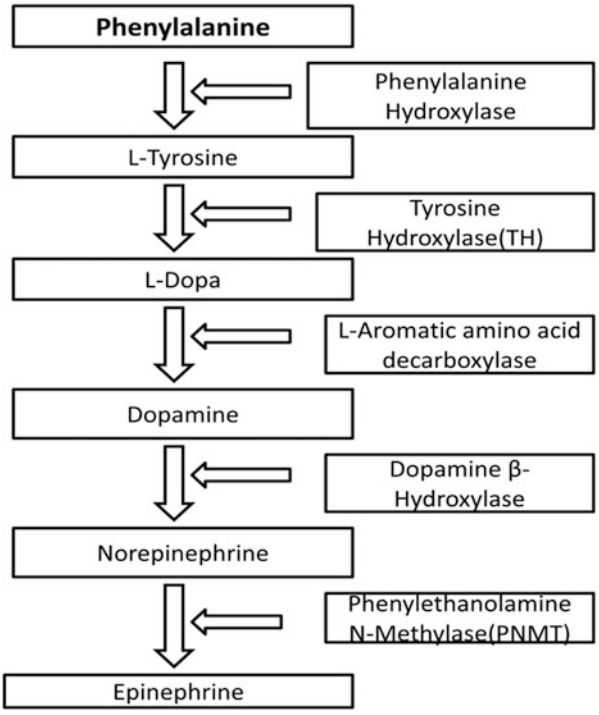


Fig. 1.9 Conversion of phenylalanine to adrenaline

(Wassall et al. 2009). Under stressful conditions, adrenaline is released into the blood and therefore transmits signals to the organs of the body and creates a specific response (Daubner et al. 2011). Adrenaline is synthesized from dopamine by the action of phenylethanolamine *N*-methyltransferase as shown in Fig. 1.9.

The effects of both noradrenaline and adrenaline were shown to be mediated by seven transmembrane G-protein-coupled receptors "adrenergic receptors" or "adrenoceptors" (AR) which can be categorized into two major groups: α (α_1, α_2) and β (β_1, β_2, β_3) receptors (Strosberg 1993). The α_1-ARs are activated by adrenergic agonists stimulating G_q protein and phospholipase C (PLC). This activation promotes the phosphatidylinositol bisphosphate (PIP_2) hydrolysis, thereby producing inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Cotecchia 2010). The α_2-AR is referred to as the inhibitory receptors which, upon activation, stimulates the G_i protein, thus inhibiting adenylate cyclase (AC) and reducing cAMP (Civantos Calzada and Aleixandre De Artiñano 2001). The β_1-AR is responsible for mediating the cardiovascular responses to the circulating adrenaline as well as noradrenaline that is released from the sympathetic nerve terminals. The β_2-AR is present in the airway smooth muscle cells including the bronchial muscles. The β_3-AR is primarily present in the adipocytes and can bind to G_i as well as G_s proteins. When these receptors are stimulated, protein kinase A (PKA) will be activated, L-type Ca^{++} channels will be phosphorylated, and Ca^{++} entry will be in the state of relaxation (Lefkowitz 2000; Ma and Huang 2002; Kohout and Lefkowitz 2003). AR ligands

Table 1.1 Subtypes of α and β receptors

Receptor	Location	Post receptor mechanism
α_1	Contraction: most vascular smooth muscle, pupillary radical muscle, pilomotor smooth muscle, prostate, sphincters (urinary bladder and anal)	IP_3/DAG cascade (G_q)
α_2	Presynaptic CNS adrenoreceptors: decreases sympathetic flow, GIT (heterotrophic receptors—relaxation), pancreas	cAMP inhibition (G_i)
β_1	Heart, juxtaglomerular cells	cAMP activation (G_s)
β_2	Relaxation: smooth muscle of GIT, urinary bladder, uterus and bronchial, ciliary epithelium, vascular smooth muscle (skeletal), liver	cAMP activation (G_s)
β_3	Fat cells	cAMP activation (G_s)

have been tested and applied as drug therapeutics for the treatment of different cardiovascular diseases including hypertension, angina pectoris, and congestive heart failure. AR ligands have been also shown to be useful in the treatment of diseases like asthma, depression, benign prostatic hypertrophy, glaucoma, shock, premature labor, opioid withdrawal, and adjunct medications in general anesthesia (Wassall et al. 2009). Agonists of β_2-AR are used as first-line therapeutic agents for the treatment of asthma and chronic obstructive pulmonary disease (COPD) as shown in Table 1.1.

1.7 Acetylcholine

Acetylcholine (Ach) is a natural substance and the first neurotransmitter which was identified by Otto Loewi. Ach alters many functions in the brain like neuronal excitability, influences transmission, and coordinates the firing of neurons. Ach also plays an important role in processing memory and learning behavior (Thorne 2010). In Alzheimer's disease (AD), there is a decrease in the concentration as well as the function of acetylcholine. Loss of Ach contributes to memory and attention deficit. It is the major neurotransmitter in the parasympathetic nervous system (PNS). It acts as a neurotransmitter at various sites, i.e., autonomic ganglia, neuromuscular junction, CNS, blood vessels, and postsynaptic receptors in the PNS (Oddo and Laferla 2006). Acetylcholine is synthesized in cholinergic nerve terminal from choline. Choline is actively taken up by the axonal membrane by a Na^+: choline transporter. Ach is hydrolyzed by acetylcholinesterase (AchE) (true cholinesterase) and butyrylcholinesterase (BuchE) (pseudocholinesterase) into choline and acetate. Acetylcholine acts on cholinergic receptors (muscarinic and nicotinic receptors). Muscarinic receptors are of five types—M1, M2, M3, M4, M5; and nicotinic receptors are of two types—Nm and Nn. All muscarinic receptors are G-protein coupled receptors. The odd-numbered muscarinic receptors act through IP_3/DAG

pathway, whereas the even-numbered muscarinic receptors act by inhibiting cAMP production (Sarter and Parikh 2005). Nicotinic receptors are ion channel-gated receptors. The M1 receptor is present on gastric ganglia and CNS. It stimulates the secretion of HCl in stomach. The M2 receptor is present in the heart and has an inhibitory effect on it. The M3 receptor is present in the eye, GIT, secretion glands, and bladder and stimulates the secretion of glands and results in increased salivation and lacrimation. The Nm receptor is present at the neuromuscular junction, and the Nn receptor is present in autonomic ganglia, adrenal medulla, and the brain. M2 receptors act as autoreceptors on cholinergic terminals. The cholinergic neuron is mainly present in basal forebrain and known as CNS cholinergic clusters. In the substantia innominate of the basal forebrain, nucleus basalis of Meynert with neuron projection is located throughout the cortex and amygdala. Degradation of these neurons is the main cause of Alzheimer's disease (AD). Muscarinic-mediated inhibition is caused by the increase in K^+ influx and inhibition of glutamergic excitatory neuron which is present in the postsynaptic neuron and decreases Ca^{++} influx (Ishii and Kurachi 2006). From the aforementioned information, we conclude that Ach is a major neurotransmitter with a modulatory function which acts by inhibiting or stimulating the receptors, depending on which type of receptor is present. It is distributed all over the brain and plays an important role in many physiological functions such as learning, attention, sleep, wakefulness, and sensory information as well as regulating sleep cycle (Boonstra et al. 2007). AD is treated by giving cholinesterase inhibitors like donepezil, rivastigmine, and physostigmine, which act by inhibiting NMDA receptors as shown in Fig. 1.10.

1.8 Histamine

Histamine (2-[3*H*-imidazol-4-yl]ethanamine) is a significant mediator that plays a role in vasodilation, increasing vascular permeability and contributing to the anaphylactic reactions (O'Mahony et al. 2013). It has been shown that histamine also plays other physiological functions like cell differentiation, proliferation, hematopoiesis, as well as cell regeneration. Histamine is synthesized by a decarboxylation reaction of the amino acid histidine with the help of L-histidine decarboxylase (HDC) enzyme, which is naturally expressed in neurons, parietal cells, gastric mucosal cells, mast cells, as well as basophils. Histamine is degraded by the enzymes diamine oxidase (DAO) and histamine N-methyltransferase (HNMT), which catalyze histamine deamination as shown in Fig. 1.11 (Biegański 1983; Yoshikawa et al. 2013). HNMT is expressed in the CNS and has a critical regulatory role, and its deficiency was reported to lead to aggressive behavior and abnormal sleep-wake cycles in mice (Naganuma et al. 2017).

Histamine was also shown to play a crucial role in the pathogenesis of several allergic diseases, including atopic dermatitis, allergic rhinitis, as well as allergic asthma via differential regulation of T helper lymphocytes (Jutel and Akdis 2007). The characterization of histamine receptors (Table 1.2) (H1R–H_4R) depends on their function, structure, distribution, as well as their affinity to histamine (Leurs et al.

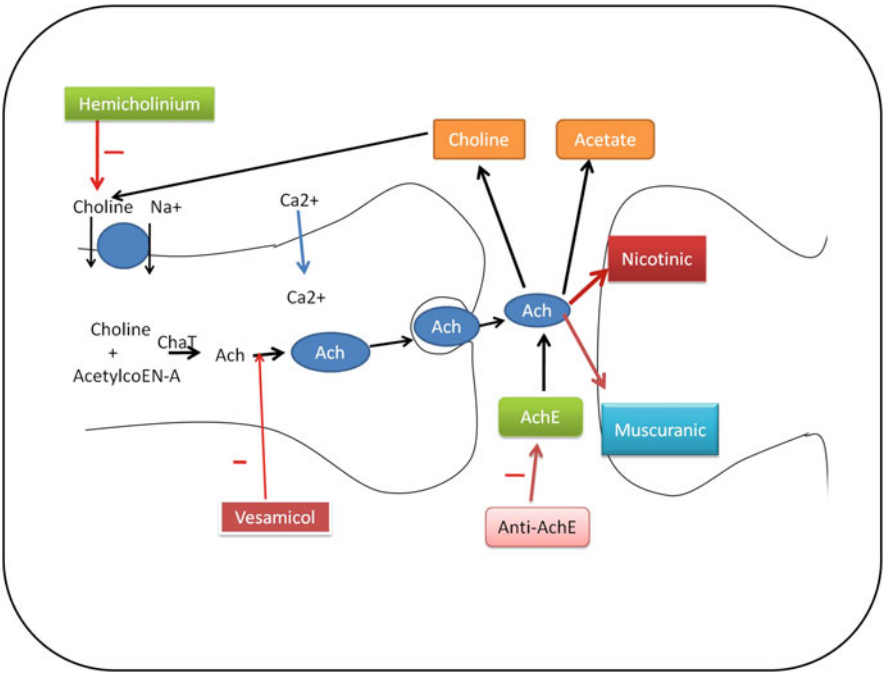


Fig. 1.10 Mechanism of synthesis, storage, and release of acetylcholine

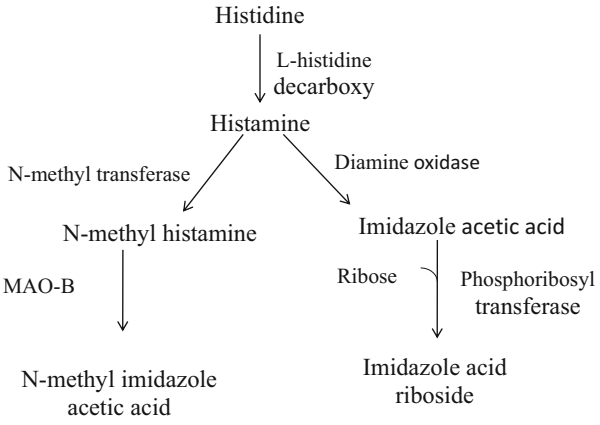


Fig. 1.11 Synthesis and metabolism of histamine

2009; Singh and Jadhav 2013). The H_1 receptor is responsible for mediating cellular migration, nociception, vasodilatation, and bronchoconstriction (Bakker et al. 2001). On the other hand, the H_2 receptor is responsible for modifying the secretion of gastric acid, production of airway mucus, and vascular permeability (Seifert et al. 2013). The H_3 receptor was reported to have a role in neuro-inflammatory diseases

Table 1.2 Characteristics of histamine receptors

	H_1	H_2	H_3	H_4
G-protein coupling (secondary messenger)	$G_{q/11}$ ($\uparrow Ca^{2+}$ $\uparrow$cAMP)	G_s ($\uparrow$cAMP)	$G_{i/o}$ ($\downarrow$cAMP)	$G_{i/o}$ ($\downarrow$cAMP, $\uparrow Ca^{2+}$)
Distribution	Smooth muscle, endothelial cells, CNS	Gastric parietal cells, cardiac muscle, mast cells, CNS	CNS: presynaptic, myenteric plexus	Cells of hematopoietic origin
Representative agonist	2-CH_3-histamine	Amthamine	(R)α-CH_3-histamine	4-CH_3-Histamine
Representative antagonist	Chlorpheniramine	Ranitidine	Tiprolisant	JNJ7777120

(Singh and Jadhav 2013), whereas the H_4 receptor has been shown to have a role in allergy and inflammation (Tiligada 2012; Thurmond 2015).

H_1-antihistamines like azatadine, cetirizine, as well as mizolastine are useful for treating mast cell activated diseases (Church and Church 2013). Other agents like cimetidine, ranitidine, famotidine, and nizatidine are known as H_2R selective antihistamines that cause a reduction of gastric acid secretion (Shim and Kim 2017). H_3R antihistamines include thioperamide, clobenpropit, BF2649, PF-03654746, JNJ-17216498, and MK 0249. It has been evidenced that histamine binding to H_4 receptors cause exacerbation of allergy and inflammation. It has been also demonstrated that mast cells have H_4 receptors which, upon stimulation, enhance degranulation and cytokine production. Consequently, antihistamines that target both the H_1 and H_4 receptors might be effective treatments for mast cell-mediated allergic reactions (Mishra et al. 2011).

1.9 Serotonin

Serotonin is a chemical that is found in almost all types of human tissues as well as in plant and aerobic organisms like bacteria. Serotonin is a ubiquitous monoamine that acts as both a neurotransmitter and hormone (Mohammad-Zadeh et al. 2008), which is also known as 5-hydroxytryptamine (5-HT) (Shad 2017). The cell bodies of serotonergic neuron are localized in the brainstem midline with broad axonal ridge which extends to all the regions of the CNS (Peterlin and Rapoport 2007). 5-HT is involved in various diseases like schizophrenia, anxiety, depression, hypertension, migraine, carcinoid diarrhea, vomiting, irritable bowel syndrome (IBS), pulmonary hypertension, eating disorders, and others (Pauwels 2003; De Ponti 2004).

5-HT is secreted by the nuclei in the median raphe of the CNS, and then transferred to the spinal cord and other parts of the brain including the hypothalamus. The synthesis of 5-HT is initiated through an active uptake of tryptophan in neurons and enterochromaffin cell by a specific amino acid transporter (Upadhyay 2003;

Pytliak et al. 2011). Initially, in the presence of tryptophan hydroxylase, tryptophan is converted into 5-hydroxytryptophan, which is finally converted into 5-hydroxytryptamine (serotonin) with the help of aromatic L-amino acid decarboxylase (Berger et al. 2009). 5-HT is mainly degraded through oxidative deamination, catalyzed by monoamine oxidase A (MAO- A), and followed by oxidation to 5-hydroxyindoleacetic acid (5-HIAA) (Ni and Watts 2006). In the brain, 5-HT is secured from degradation by the storage in synaptic vesicles of the neurons (Hamel and Current 2007).

5-HT and its receptors were shown to contribute to brain functioning. Therefore, the dysregulation of serotonin system will in most cases end up with psychiatric and neurological disorders (Berger et al. 2009). Serotonin has been also shown to play a significant role in peripheral tissues, in addition to its contribution to the immune system (Wu et al. 2019).

1.10 Adenosine

Adenosine is an endogenous nucleoside molecule which is ubiquitously present throughout the body, mediating various pathophysiological functions (Borea et al. 2018). It is an integral component of ATP; therefore its synthesis is highly dependant on the metabolic conditions of the cells. Extracellularly, adenosine concentration ranges from 20 to 300 nM under physiological conditions, whereas metabolic stressful conditions like hypoxia and inflammation might increase the adenosine levels to micromolar concentrations (up to 30 μM) (Borea et al. 2016). Intracellularly, adenosine is mainly produced from adenosine monophosphate (AMP) and *S*-adenosyl-homocysteine (SAH) with the help of hydrolyzing enzymes endo-5-′-nucleotidase and/or SAH hydrolase, respectively. Adenosine is also generated extracellularly due to the dephosphorylation of ATP, ADP, and AMP under metabolic demand by ectonucleosidase triphosphate diphosphohydrolase (CD39) and ecto-5′-nucleotidase (CD73), respectively (Chen et al. 2013; Zimmermann 2000). Once generated, adenosine transportation across the cell membrane takes place either by concentrative nucleoside transporters (CNTs) or by equilibrative nucleoside transporters (ENTs). However, there are three isoforms of CNTs which are cation linked (Na^+) energy dependent and four isoforms of energy independent ENTs assisting the adenosine transportation across the cell membrane (Deussen 2000, Deussen et al. 1993, 1999).

In physiological conditions, adenosine kinase (AK) is the principal enzyme involved in the biotransformation of adenosine inside the cell by phosphorylation to AMP. On the other hand, adenosine deaminase (ADA) can cause deamination of adenosine to inosine both intracellularly and extracellularly. Extracellular clearance of adenosine is also facilitated by influx through ENTs. It should be noted that under pathological conditions, ADA plays a major role in the intracellular clearance of adenosine (Gracia et al. 2013; Pacheco et al. 2005).

Biological functions of adenosine are mediated by interactions with four GPCRs: A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ARs) (Fredholm et al. 2001; Fredholm

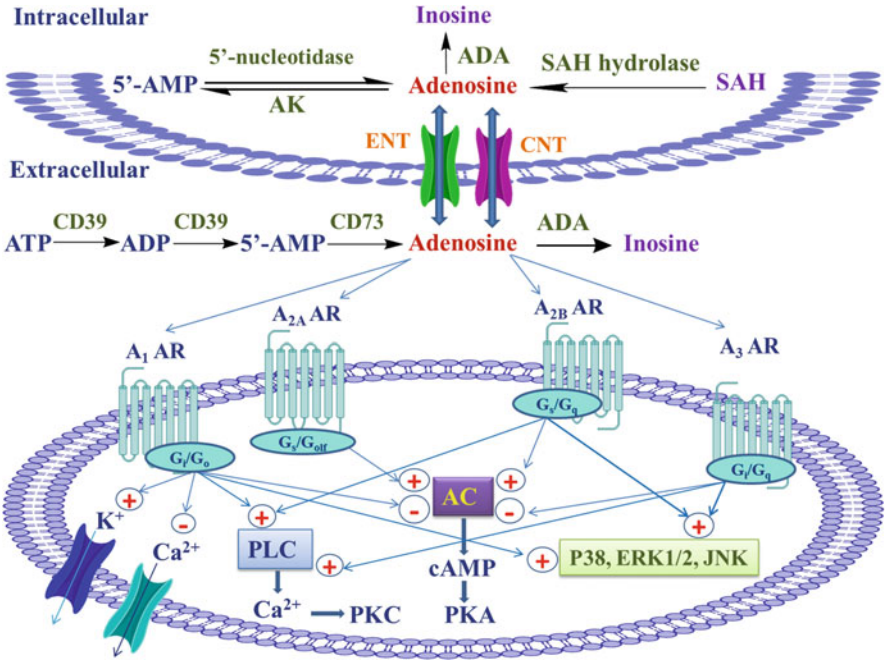
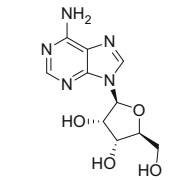


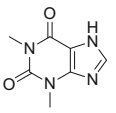
Fig. 1.12 Synthesis, biotransformation, and signal transduction mechanism of adenosine and A_1, A_{2A}, A_{2B}, and A_3 receptors (reprinted with permission from Pran Kishore Deb 2019a)

et al. 2011; Borea et al. 2018). The primary signal transduction mechanism of ARs involves the modulation of adenylyl cyclase (AC). Activation of A_1 and A_3 ARs causes inhibition of AC activity leading to the reduction of cAMP and inhibition of protein kinase A (PKA), whereas A_{2A} and A_{2B} ARs activation stimulates the AC activity and consequently increases cAMP and PKA levels (Fredholm 2014; Merighi et al. 2018). Figure 1.12 represents the synthesis, biotransformation, and signal transduction mechanism of adenosine and its four receptor subtypes (Deb 2019a).

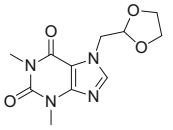
Ubiquitous distributions of ARs in the form of homomers, heteromers, or oligomers have made them interesting drug targets for the therapeutic interventions of various pathological conditions (Borea et al. 2018; Fredholm 2014; Merighi et al. 2018). In the last two decades a large number of agonists, antagonists, and allosteric modulators of ARs have been discovered, some of which are under various phases of clinical trial investigations, including those already in the market as shown in Fig. 1.13 (Deb 2019a, b, c; Deb et al. 2019a, 2019b, 2018, 2011; Chandrasekaran et al. 2018; Chandrasekaran et al. 2019; Shaik et al. 2019; Mailavaram et al. 2019; Al-Attraqchi et al. 2019; Borah et al. 2019; Baraldi et al. 2008). In particular, recent FDA approval of istradefylline as an A_{2A} AR antagonist for the treatment of Parkinson's disease further boosted the research on ARs (Hoffman 2019; Voelker 2019).



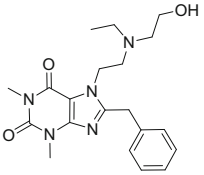
Name: Adenosine
1. MoA: A_1 AR agonist
Application: Paroxysmal supraventricular tachycardia (PSVT)
2. MoA: A_{2A} AR agonist
Application: Myocardial perfusion imaging



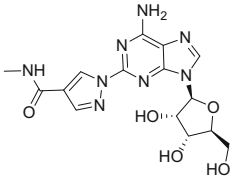
Name: Theophylline
MoA: A_1 AR antagonist
Application: Asthma



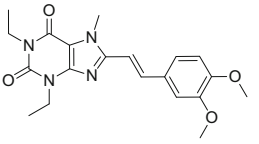
Name: Doxofylline
MoA: A_1 AR antagonist
Application: Asthma



Name: Bamifylline
MoA: A_1 AR antagonist
Application: Asthma



Name: Regadenoson
MoA: A_{2A} AR agonist
Application: Myocardial perfusion imaging



Name: Istradefylline
MoA: A_{2A} AR antagonist
Application: Parkinson's disease

Fig. 1.13 Therapeutic applications of clinically approved drugs targeting ARs (reprinted with permission from Pran Kishore Deb 2019a)

1.11 Angiotensin

The renin-angiotensin-aldosterone system (RAAS) is a critical regulator of blood volume and systemic vascular resistance. Organ systems that are involved in RAAS are kidneys, lungs, vasculature system, and brain (Liu et al. 2019). A powerful effect of the RAAS is the obstruction in the pathophysiological condition like hypertension and CHF. Kidney diseases, such as renal artery stenosis, secretion of a kidney tumor, or other damage to the kidneys can lead to excessive levels of renin and angiotensin I as well as high blood pressure (Hall et al. 2019). The renin-angiotensin system is considered as a cardiovascular control system as it controls the extracellular fluid volume as well as the blood pressure. Angiotensin I is considered as the active product of peptide hormones from cascade renin enzymes. This hormone maintains blood pressure and volume through various stages, including vasoconstriction, stimulation of aldosterone secretion by the adrenal glands, increased absorption of renal sodium activation of the sympathetic nervous system, and contractility of the heart. Renin-angiotensin I plays an important role in the heart and kidney, with a decrease in cardiac output in heart failure and an increase in the reactive angiotensin-I and sodium retention in the kidneys due to blockage of blood circulation as shown in Fig. 1.14.

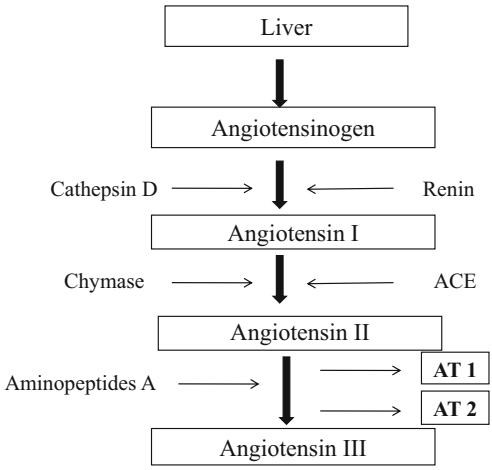


Fig. 1.14 Renin-angiotensin system

Angiotensin II is the main stimulant for the production of aldosterone in the adrenal glomeruli. It is most sensitive to the kidneys and also affects its function. It is believed that some effects of ACE inhibitors and angiotensin receptor antagonists, such as an increase in renal plasma velocity and a decrease in glomerular pressure, are associated with antagonism of arterial vasoconstriction induced by selective angiotensin (Bauer and Reams 1986). In the systemic circulation, a decrease in resistant arterioles leads to an increase in blood pressure which causes vein contraction to worsen heart failure. The effect of angiotensin II on afterload (arterial vasoconstriction) is more important in terms of CHF sensitivity than the effect on preload (reduced nutrition). Angiotensin II can also act as a factor in the growth of blood vessels, causing arterial hypertrophy of smooth muscles and increasing peripheral resistance; this effect may be associated with vascular damage (Sasamura et al. 1992). Angiotensin II can also contribute to the expansion of the extracellular matrix by stimulating the production of TCF-β. Angiotensin II has a direct effect on the renal tubules, thereby increasing proximal sodium reabsorption and subsequent sodium retention (Cogan 1990; Kagami et al. 1994). It also acts directly on the proximal renal tubules and acts against the normal functioning, causing contraction of glomerular cells, reducing glomerular surfaces, and reducing glomerular filtration rate. It also promotes the growth of myocytes and fibroblasts, a process that has a direct effect on the heart and is believed to play an important role in the development of left ventricular hypertrophy (Baker et al. 1992). There are two types of angiotensin II receptors, i.e., AT_1 and AT_2 receptors, which have different mechanisms of signaling pathways and tissue distribution. The AT_1 receptor binds with phospholipase A_2 and phospholipase C and negatively to adenylate cyclase as shown in Table 1.3. These enzymes exert their action on specific substrates for generating second messengers that will cause a transduction of the surface signal into a series of biochemical events within the cell whereas AT_2 inhibits guanylate cyclase (Sasamura et al. 1992).

Table 1.3 Characteristics of AT_1 and AT_2 receptors

	AT_1 receptor	AT_2 receptor
Distribution	Widely distributed in adult tissues, e.g., blood vessels, kidney, adrenal gland, heart, liver, brain	Widely distributed in fetal tissues, expression in the adult brain, adrenal glands, ovary endothelium, uterus, myocardium
Function	Vasoconstriction, cardiac contractility, aldosterone release, glomerular filtration, renal blood flow, cardiac and vascular hypertrophy, central osmoregulation	Possible role in growth and development (antiproliferation, inhibition of neointima, cell differentiation)
Structure	Seven transmembrane receptor, G-protein coupling	Seven transmembrane receptor, G-protein coupling
Ligands	Losartan, Valsartan, Irbesartan, Candesartan, Tasosartan	PD 123177, CGP 42112A, PD 123319
Isoforms	AT_{1a}, AT_{1b}	

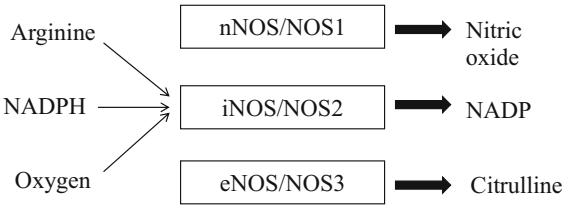


Fig. 1.15 Synthesis of nitric oxide

1.12 Nitric Oxide

Nitric oxide (NO) production results from the oxidation of the terminal guanidine nitrogen of the amino acid arginine. The catalysis of this reaction occurs by the action of NADPH-dependent enzyme, nitric oxide synthase (NOS). When NO is synthesized, it gets diffused outside the cell. It is produced by a group of enzymes called nitric oxide synthases. These enzymes convert arginine into citrulline, producing NO in the process as shown in Fig. 1.15 (Virarkar et al. 2013).

The concentration of NO differs under physiological conditions (Tieu et al. 2003). As a neurotransmitter, NO was shown to play a role in the activation of the cGMP-dependent protein kinase G (PKG) pathway which is responsible for phosphorylating synaptophysin, which is of significant importance for the fusion of presynaptic vesicles, therefore leading to the potentiation and facilitating of neurotransmission (Wang et al. 2005). NO was also shown to play a role in the inhibition of GABAergic synaptic transmission by cGMP-dependent pathways in addition to ion channels and exchangers (Yamamoto et al. 2015). NO plays a role in supporting vascular homeostasis in endothelium-dependent vasodilatation; however, its over- or underproduction was shown to be related to pathological conditions (Džoljić et al. 2015). NO is therefore considered to be a critical mediator under certain pathological conditions. For example, in brain ischemia-reperfusion injury,

the formation of NO is first increased serving a protective function by the induction of collateral perfusion resulting from its powerful stimulatory effect on vasodilatation as well as angiogenesis (Su et al. 2014). Nitric oxide, as mentioned above, is considered as a key molecule for regulating physiological brain homeostasis (Nathan 1997).

1.13 Opioids

In general, any compound or substance that is related to opium is known as opioid. Endogenous opioid peptides (EOPs) are naturally available endogenous substances or ligands that mainly bind to the opioid receptors (Borg and Kreek 2003; Yaksh and Wallace 2011). Opioid receptors are the receptors that are associated with opioid analgesic activity. Mainly three classes of opioid receptors are found, namely, MOP (μ), KOP (kappa), and DOP (delta). Additionally, subtypes of these receptors were also identified that include epsilon, iota, lambda, and zeta. Nociceptin opioid receptor (NOP) was also identified due to the structural similarities with opioid receptors. Opioids do not have a high affinity to the NOP receptor but nociceptin, which is structurally related to dynorphin, appears to be a ligand for NOP receptor. The μ receptors are subdivided into two types, namely, μ1 and μ2. Endogenous ligands for μ receptor are endomorphin1, endomorphin2, and enkephalin. The antagonists for μ receptor include CTOP and beta-funaltrexamine. Upon stimulation of μ receptor, relevant responses like analgesia (supraspinal and spinal), respiratory depression, euphoria, miosis, sedation, physical dependence, and reduction in gastrointestinal motility were reported (Gentilucci and Tolomelli 2004). Kappa receptors have higher affinity to ketocyclazocine and dynorphin A. Kappa receptors are also subdivided into three types, kappa1, kappa2, and kappa3. Dynorphin A is the endogenous ligand for kappa receptor and norbinaltorphimine, a bivalent derivative of naltrexone and specific antagonist for kappa receptors. Upon stimulation of kappa receptors, relevant responses like analgesia (spinal), miosis, sedation, respiratory depression, hallucination, and others were reported. Delta receptors have a higher affinity for enkephalin and leuenkaphalin. Delta receptors are also subdivided into two types, delta1 and delta2. Enkephalins are the endogenous ligands for the delta receptors and naltrindole is the selective antagonist for delta opioid receptors. Upon stimulation of delta opioid receptors, relevant responses like analgesia (supraspinal and spinal), respiratory depression, feeding, inhibition of dopamine release, and increase in the release of growth hormones have been observed.

Mostly, opioid receptors are present on the peripheral sensory nerve, and they are upregulated during the inflammation of the cell. EOPs are derived from the immunocytes and occupied by the receptors on the sensory nerves that cause analgesia by inhibiting the nerve excitability or inhibiting the release of pro-inflammatory neuropeptides (Rasakham 2008). The opioid analgesic agonists include morphine, codeine, dextropropoxyphene, fentanyl, heroin, methadone, tramadol, and others. Morphine is known as a prototype opioid agonist that is widely

used for pain control. Well-known opioid antagonists are nalorphine, naloxone, and naltrexone. Opioids like morphine and others produce their activity by the release of the EOPs through direct action on opioid receptors (Koneru et al. 2009).

1.14 Cannabinoids

Cannabinoids are naturally occurring compounds found in the *Cannabis sativa* plant, of which Δ^9-tetrahydrocannabinol (THC) is the principal compound. Cannabidiol (CBD) is another important component, which makes up to 40% of the plant resin extract (Appendino and Chianese 2011). Cannabis has been used for a variety of therapeutic applications such as pain, stimulation of appetite, nausea, fever, infection, and gynecological disorders. Cannabinoids are a group of chemicals which activate the cannabinoid receptors (CBRs) in the body such as endogenous cannabinoids (ECs) which are present in human and animals, herbal cannabinoids which are present in the cannabis plant, and synthetic cannabinoids which are synthesized in the laboratory. The cannabinoid receptors are a class of cell membrane receptors under the GPCRs group. The activation of CBRs occurs by ligands, endocannabinoids, plant cannabinoids, and synthetic cannabinoids. There are currently two known cannabinoid receptors, namely, CB_1R and CB_2R. Both the receptors are 7-transmembrane GPCRs, which inhibit the accumulation of cAMP within the cells (Grotenhermen 2003). Like many other GPCRs, the CB_1R is primarily localized in the cell membrane. CB_1Rs are abundantly present in the brain and the periphery (i.e., liver, blood vessels, GIT, and peripheral nerve endings).

CBD serves as an antagonist at the central CB_1R causing inhibition of several CB_1-mediated THC effects. CBD causes a considerable reduction in the receptor activation of a potent classical CB_1R agonist. CBD causes inhibition of both the uptake and hydrolysis of the endocannabinoid anandamide, hence causing an increase in its concentration (Mackie 2006). The first identified EC was anandamide and the second was 2-AG (2-arachidonoyl glycerol). Additional endocannabinoids include virodhamine, noladin ether, and NADA (Alexander et al. 2018). Endocannabinoids are retrograde synaptic messengers that are released from postsynaptic neurons and travel backward across synapses which activate the CB_1 receptors on presynaptic neurons and suppress the transmitter release (Ahluwalia et al. 2000).

The physiological role and pharmacological effects of cannabinoids emphasize that the activation of the cannabinoid system through THC, phytocannabinoids, synthetic cannabinoids, and endocannabinoids causes numerous actions that include effects on the central nervous system (anti-nociceptive, neuroprotection, alleviation of painful spasms and spasticity, antiemetic, regulation of food intake, and energy expenditure), reproductive system , immune system, vasodilation, anti-proliferative, bronchodilation, as well as effect on intraocular pressure. Endocannabinoids are agonists for CB_1 receptors. CB_2 receptor agonists have potentially useful effects in many models of inflammatory and neuropathic pain and possibly involving the release of endogenous opioids and can inhibit the growth of CB_2 receptor expressing

glioma *in vivo* (Howlett 2002). The neuroprotective effects include short-term adaptation to neuronal stress, limiting excitotoxicity, long-term adaptation, enhancing neurogenesis, and decreasing excitotoxicity (Jinwala and Gupta 2012).

1.15 Conclusion

Neurotransmitters are endogenous chemical messengers that are responsible for neuronal communication throughout the body. Neurotransmitters have been classified based on their chemical, functional, and molecular properties as well as their location in the body. Each neurotransmitter plays different roles to maintain the proper functioning of the body. However, efforts are still under progress to gain a complete understanding of the pathophysiological basis of various neurological disorders in order to discover and develop new drugs for the treatment of various diseases including inflammation, mental diseases, Alzheimer's disease, Parkinson's disease, cancers, and other disorders.

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Drug-Receptor Interactions

2

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Abstract

Conventional treatment of any disease can be achieved by the administration of drugs of natural and synthetic origin. The drug exhibits its pharmacological action by altering cellular signaling or the biochemical events associated with the respective target proteins such as receptors or enzymes. Functional groups/pharmacophores of the drug interact with functional groups present in the receptor's binding site, complementarily thereby producing effective binding interactions. Key interactions that occur between the drug and the receptor will decide the potency and intrinsic activity of the drug. Major interactions observed in the drug-receptor complexes are mostly of reversible type which consist of electrostatic interactions, ion-dipole and dipole-dipole interactions, hydrogen bonding, charge-transfer interactions, hydrophobic, and Van der Waals interactions. In this chapter, we have discussed the types of receptors, theories and types of drug-receptor interactions, the role of functional groups, and stereo-

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chemical aspects involved between the drug and the receptor. Further, we have shed light on the development of adenosine receptor antagonists through *in silico* interactions as a case study.

Keywords

Drug-receptor complex · H-bonding · VdW interaction · Antagonist · Agonist

Abbreviations

3D	Three dimensional
Ach	Acetylcholine
ARs	Adenosine receptors
ATP	Adenosine triphosphate
CRC	Concentration-response curve
DBD	DNA-binding domain
DRC	Dose-response curve
GPCRs	G-protein-coupled receptors
H-bond	Hydrogen bonding
LBD	Ligand-binding domain
PPARγ	Peroxisome proliferator-activated receptor gamma
RTK	Receptor tyrosine kinase
TK	Tyrosine kinase
vdW	Van der Waals

2.1 Introduction

Upon drug administration, it gets absorbed, transported to the site of action through general circulation, and elicits a pharmacological response. The target site for the drug action is ultimately a bio-macromolecule, known as a receptor. Receptors are mostly membrane-bound proteins, consist of various amino acids, and receive signals from outside the cell through a ligand molecule. When such small molecules or ligands bind to the receptor and interact, subsequently they elicit some form of cellular/tissue responses (Uings and Farrow 2000). There are three different ways wherein the receptor responds to the chemical messenger/ligand such as relay of signal, amplification, and integration. Most of the receptors are integral proteins connected to the protein-lipid bilayer of the cell membrane. The two functionally important components of receptors include the recognition component (ability of recognizing specific molecules) and the amplification component (an ability of the intermolecular complex formation between the ligand and the receptor) to initiate a pharmacological response (Pierce et al. 2002). Based on the complementarity between the ligand and binding site of a receptor, each receptor is connected to a specific biochemical pathway by specific binding towards a particular ligand. Upon ligand binding to its binding site present in the receptor, it may activate or inhibit various receptor-associated biochemical events. Further, this single receptor will

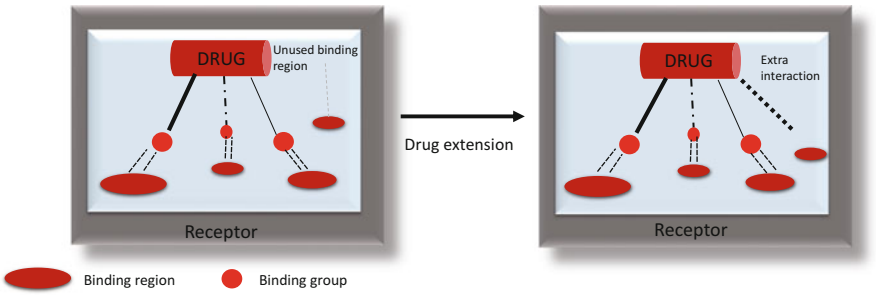


Fig. 2.1 The binding of a drug to the binding site of the receptor based on shape complementarity

send chemical responses as signals to the surrounding receptors or nearby proteins, thereby allowing them to amplify according to the first response (Pramanik 2015). Figure. 2.1 presents points of binding of the drug to the receptor based on shape complementarity.

2.2 Types of Receptors

The structure of receptors varies which depends entirely on the diversity of the binding site and functions of the receptors. Principally, four types of receptors are identified, namely ligand-gated ion channel receptors, guanine nucleotide binding regulatory protein (G-protein)-coupled receptors (GPCRs), tyrosine kinase-linked receptors, and nuclear receptors.

2.2.1 Type-I: Ligand-Gated Ion Channel Receptors or Ionotropic Receptors

Various neurotransmitters such as acetylcholine, nicotine, and GABA will bind to type-I receptors. As a result of this binding, activation of the movement of ions takes place across the membrane. The general structure of these receptors is normally heteromeric, and these receptors include an extracellular binding domain for ligands and a transmembrane domain bearing α-helices. They contain three important domains such as pores (for transport of ions), gates (open or close based on stimuli), and sensors to receive the stimuli. There are specific channels for ions such as Na^+, K^+, Ca^{2+}, and Cl^-. Three integral functions of these receptors include opening, closing, and activation according to the conformational changes. Figure 2.2 indicates the signaling process of ligand-gated ion channel receptors wherein the ligand binds to an extracellular domain of the receptor; thereby the channel opens for the influx of Ca^{2+} ions into the cell. Membrane permeation is another way in which ligands can enter through these receptors (Sansom et al. 1998). Cation-permeable "nicotinic" acetylcholine (Ach) receptors, ionotropic glutamate-gated receptors, ATP-gated P2X receptors, and the anion-permeable γ-aminobutyric acid-gated $GABA_A$ receptor are few examples of type-I receptors.

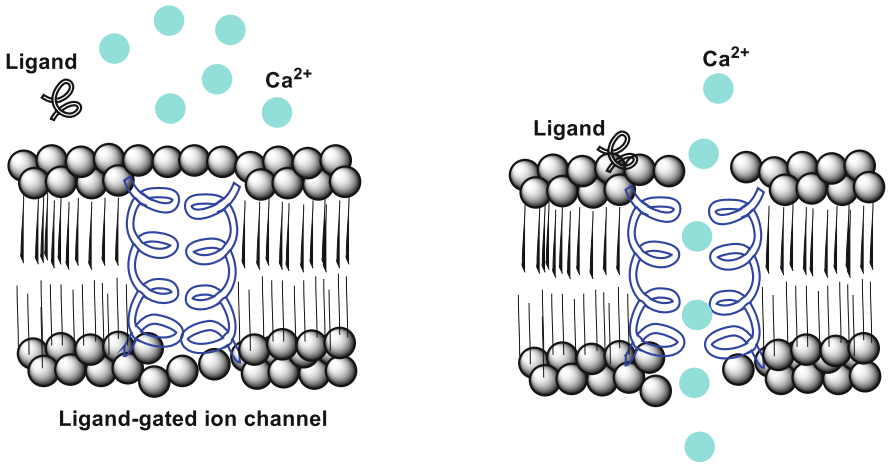


Fig. 2.2 The signaling process shown by ligand-gated ion channel receptors

2.2.2 Type II: G Protein-Coupled Receptors (GPCRs; Metabotropic Receptors)

GPCRs are the largest family of receptors for some transmitters (metabotropic glutamate and dopamine), light-sensitive compounds, various hormones, lipids, glycoproteins, small molecules, peptides, and larger proteins. Upon binding of these ligands to the GPCRs, they facilitate the interaction with members of the G-protein receptor family. GPCRs are mostly identified in eukaryotes, yeasts, and animals (Saengsawang and Rasenick 2015). The structure of GPCRs includes seven transmembrane α-helices, and loops for their integration. As the name indicates, these receptors are coupled to diversified intracellular-effector systems incorporating both extracellular (a binding site for large ligand) and intracellular (separate binding site) involving the activation of signal transduction pathways resulting in a cellular response, accordingly (Rosenbaum et al. 2009). Most of the modern medicines (about 40%) act through modulating the signaling processes associated with GPCRs (Chandrasekaran et al. 2019). Since they occur as transmembrane proteins, they are difficult to isolate, purify, and crystallize. To date, bovine rhodopsin, β-adrenoceptors, and A_{2A} adenosine receptors were crystallized in their pure forms (Deb et al. 2019a). A typical orientation of a type II receptor involved in the signal transduction process is shown in Fig. 2.3.

2.2.3 Type III: Kinase-Linked and Related Receptors

Tyrosine kinase (TK) and enzyme-linked receptors have an extracellular domain bearing a binding site for ligands (normally glycosylated) and an intracellular domain associated with the enzymatic activity, connected by a transmembrane

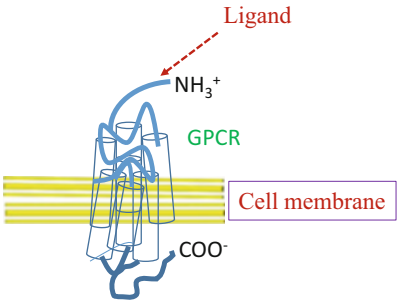


Fig. 2.3 The orientation of type II GPCRs

α-helix. The TK receptor is located on the surface of the cell exhibiting higher affinity for many of the ligands such as growth factors, hormones, and cytokines (Cadena and Gill 1992). They are the key regulators for a number of cellular biochemical processes including their critical role in the progression of cancer. There are almost twenty different classes of TK-linked receptors and the insulin receptor is one such example. The main function of TK-linked receptors is the phosphorylation of tyrosine amino acid residue of target proteins by transferring a phosphate group from high energy donor molecule (ATP) (Huang and Reichardt 2003). The receptor tyrosine kinase (RTK) pathway is regulated through a number of positive and negative feedback loops. Since RTKs are involved in many cellular functions (cell proliferation, differentiation, survival, metabolism, migration, and cell cycle regulation), they must be regulated to avert cellular abnormalities like fibrosis and cancer (Gschwind et al. 2004). Figure 2.4 illustrates a typical signaling process mediated by kinase-linked receptors.

2.2.4 Type IV: Nuclear Receptors

They are normally present in the cytoplasm when there is no ligand binding to the receptor. Once the ligand binds to such receptors, they migrate to the nucleus of the cell, accordingly. They are composed of different binding domains such as N-terminal regulatory domain, DNA-binding domain (DBD) containing two zinc fingers, hinge region, ligand-binding domain (LBD), and C-terminal domain. In particular, the N-terminal domain interacts with other cellular transcription factors in a ligand-independent manner (Robinson-Rechavi and Laudet 2003). Depending on such interactions, the binding/activity of the receptor can be affected, suitably. Nuclear receptors are accountable for the recognition of steroidal and thyroid hormones, fatty acids, bile acids, prostaglandins, and certain vitamins. As they regulate the gene expression by binding directly to DNA, they are classified as transcription factors and all these processes depend on the availability of a ligand. Upon ligand binding to a nuclear receptor, conformational changes occur in the receptor which activates the receptor; thereby upregulation or downregulation of gene expression has been effected. Hence, they control the general embryonic development, homeostasis, and metabolism (Aranda and Pascual 2001). Lipophilic

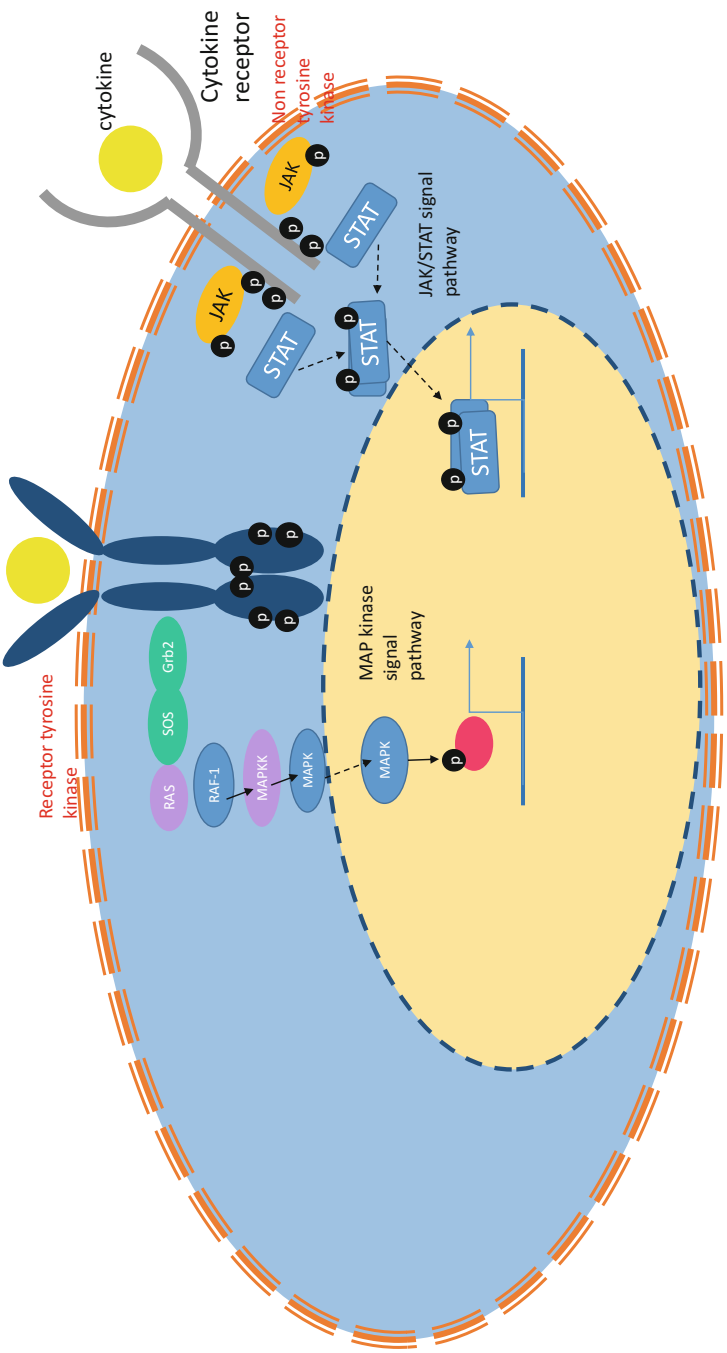


Fig. 2.4 Signal transduction process mediated by kinase-linked receptors

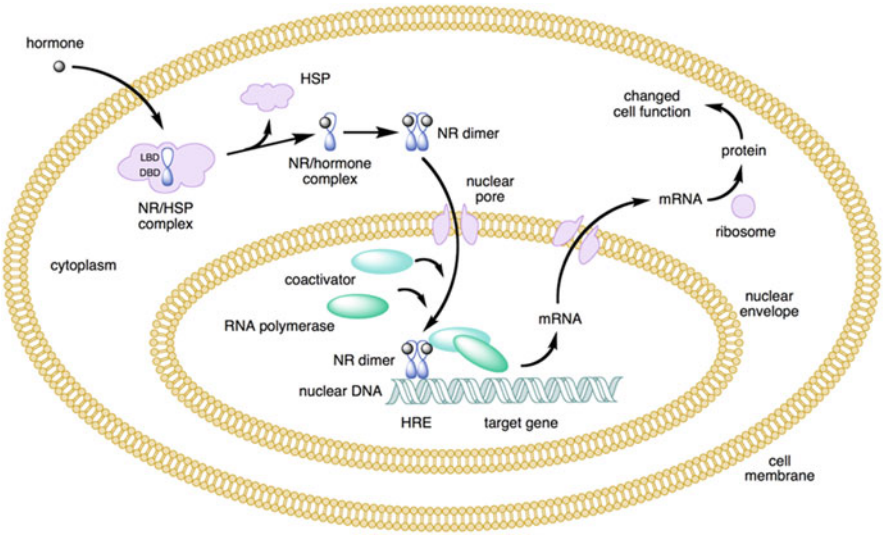


Fig. 2.5 Signal transduction process mediated by nuclear receptor

components like xenobiotics, endogenous hormones, endocrine disruptors, and vitamin D are the examples of common ligands binding to the nuclear receptors. Figure 2.5 represents a typical signaling process mediated by a nuclear receptor.

2.3 Drug-Receptor Complex

Drug targets are molecules such as proteins (enzymes, receptors, and transport proteins), lipids, carbohydrates, or nucleic acids (as RNA or DNA) wherein the drug is going to reach and bind. An important step for a drug action is the binding and subsequent interaction with the target. While drug is traveling throughout the body, it reaches its target and identifies the correct binding site in a receptor protein and interacts to elicit a biological response. The binding site in a receptor also known as a pocket or canyon is at the surface of the macromolecular target. It is very important to gain an insight into the forces involved in the receptor-drug binding in order to determine the mode of drug action (Hase et al. 2009). A receptor has unique amino acids with specific side chains, so the functional groups in drugs have the complementary shape characteristics to fit into the binding site or pocket. There are points for the attachment between a drug and the receptor selectively to provoke the drug action, and this binding always exists in an equilibrium state. The drug should have strong interaction with the receptor for association and to allow signal transduction; at the same time, it should also show weak interaction for dissociation and to allow the drug to depart, after completion of its action. Hence, a fine balance between strong and weak interactions is required for the better activity of the drugs (Bs Suvarna 2011).

The three-dimensional (3D) structural determination of the receptors using X-ray crystallography facilitated the identification of the binding site for the small organic molecules binding process. Prior to the structural determination, the receptor should be isolated, cloned, sequenced, purified, and crystallized using suitable methods. It is a very challenging task, as the receptors have higher molecular weight, traverse the cell membrane, are complex, and exist in a tiny amount or lower concentration (Topiol and Sabio 2009). Furthermore, drug-target interactions are classified into two types, namely, irreversible interaction (a permanent interaction where covalent bonds are involved) and reversible interaction (a transient interaction where different types of bonding involved except covalent bond). If weaker and noncovalent interactions exist between the drug-receptor complex, it leads to the reversible pharmacological response (Marc 2008). However, the drug must detach from the binding site to terminate the pharmacological action to stay away from the toxicity and adverse side effects. In such responses, the drug becomes inactive as soon as the concentration of the drug decreases in the extracellular fluid. These reversible interactions are often very useful in drugs acting on the CNS (stimulants and depressants). Sometimes, irreversible interactions are anticipated between the drug and receptor to elicit the drug action for a prolonged period of time (e.g., anticancer drugs) (Baillie 2016). When a drug interacts with the binding site of a receptor, the bonding will usually be intermolecular between the drug and receptor to elicit the desired pharmacological actions. Drugs bind to the receptor and form a complex (drug-receptor), and the low energy state of this complex is the reason for the driving force that exists in such complex (Eq. 2.1).

$$\text{drug} + \text{receptor} \underset{K_{\text{off}}}{\overset{K_{\text{on}}}{\rightleftharpoons}} \text{drug-receptor complex} \tag{2.1}$$

where, K_{on} is the rate constant for the drug-receptor complex formation and depends on the concentrations of both the drug and the receptor and K_{off} is the rate constant for dissociation or the breakdown of the complex and entirely depends on the concentration of the drug-receptor complex. The pharmacological response of a drug is associated with its affinity towards the receptor. Moreover, the stability of the drug-receptor complex can be determined based on the dissociation of the complex as represented in Eq. 2.2.

$$K_{\text{d}} = \frac{[\text{drug}][\text{receptor}]}{[\text{durg-receptor complex}]} \tag{2.2}$$

where, K_{d} is dissociation constant for the drug-receptor complex at equilibrium and the lower K_{d} value indicates the higher concentration and stability of the complex which in turn explains the higher affinity of the drug to the receptor.

The formation of a drug-receptor complex is an entropically unfavorable process which ultimately generates lowering of conformational degrees of freedom in both ligand (drug) and protein (receptor), at the same time decreasing rotational and translational degrees of freedom, correspondingly. The interaction between a drug

and the receptor is usually consists of solvation, attractive-repulsive forces, conformational changes, and long-range interactions (Tallarida 2007). Drugs can binds to the binding site of the receptor and exhibit different types of interactions or bonds such as covalent bonding, ionic or electrostatic interactions, ion-dipole and dipole-dipole interactions, hydrogen bonding, charge-transfer interactions, hydrophobic interactions, cation-π interactions, halogen bonding, and van der Waals (vdW) interactions. Many theories were proposed for the binding between the ligand and a receptor.

2.4 Theories of Drug-Receptor Interactions

After studying the different types of interactions between the drugs and their receptors, scientists have proposed and developed a set of hypothesis over the years, known as theories of drug-receptor interactions. These theories can explain the ability of the drug to interact with the receptor to induce or stimulate a suitable biological response (Kenakin 2008).

2.4.1 Occupancy Theory

This theory was proposed by Gaddum and Clark in 1926 (Clark 1926). According to this theory, the drug binds to its specific receptor (complementary structures like lock and key) and gives a cellular response. Based on the study, scientists concluded that the intensity of cellular response is directly proportional to the number of receptors occupied which means that a maximum response occurs when all receptors are occupied. Drugs exhibit all or no response on each receptor, so it will be either fully activated or not at all activated. Moreover, there are no partial activation because once the drug-receptor complex dissociates, the response will halt, accordingly. This concept is illustrated in Fig. 2.6.

As this theory does not cover the concept of partial agonist, Ariens and Stephenson (Stephenson 1956) have modified the occupancy theory to incorporate the concept of partial agonist. They have used the main concept of the drug-receptor interactions that generally involves two stages, namely, affinity and efficacy. Affinity is a feature that determines the capacity of a drug to bind to the receptor for activating and producing a desired response. The degree of affinity between the drug and receptor depends on molecular complementarity. If drugs have different affinity

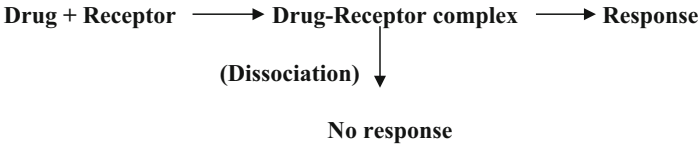


Fig. 2.6 The association and dissociation of the complex

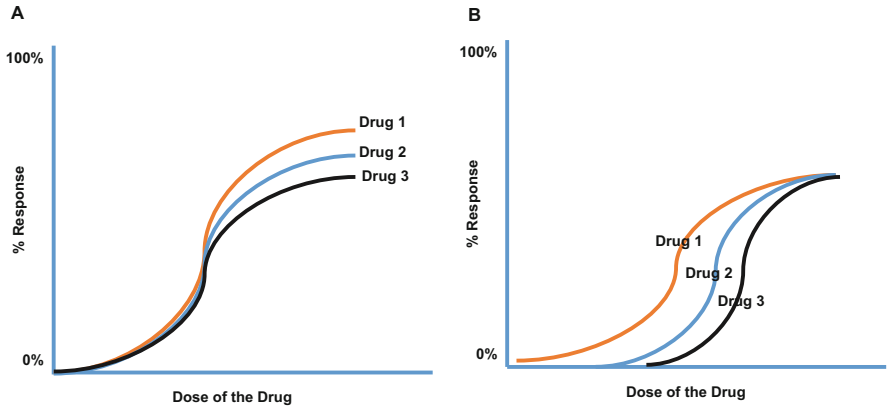


Fig. 2.7 (**A**) Drugs have similar affinities but different efficacies; (**B**) drugs have similar efficacies but different affinities

to specific receptors, then it is difficult to figure out which one is going to bind to the receptor. The answer is when a drug with high binding capacity comes around the receptor, it will inevitably bind to the receptor, even if it is associated with another drug. In this situation, the former drug will remove another drug which has lower affinity from the active site of the receptor. The nature of the binding or the interaction between the receptor and the drug molecule relies mainly on the degree of affinity (Shuker et al. 1996). On the other hand, the maximum response can be achieved by the dose of the drug, termed as efficacy and intrinsic activity (Nelson et al. 2016). Figure 2.7 elucidates the exact difference between the affinity and the efficacy.

Figure 2.7A illustrates the percentage of biological response relative to the dose of three different drugs, and all of them have similar affinities to the same receptor. At the same time, they have quite different efficacies ranging from 100% and ended with approximately 70% of the maximum. By taking into consideration that the drug which reached 100% of the maximum response is known as a full agonist while others are partial agonists. On the other hand, Fig. 2.7B presents the dose-response curve for three drugs, all of which exhibit an equal efficacy but the affinities of each one vary. It is also interesting to know that those three drugs are full agonists. Moreover, both agonist and antagonist will bind to receptors but each one will produce a different response. Agonist will activate the receptor, while antagonist will halt the receptor action. Also, a drug may be an agonist when it is bound to one of the receptors and exerts a positive response, while the same drug could be antagonist for another receptor. The reason why it is possible for two drugs that can fit a specific receptor exert different effects is not clarified by this modified occupancy theory (Maehle et al. 2002). The concept of efficacy has been displayed in Fig. 2.8.

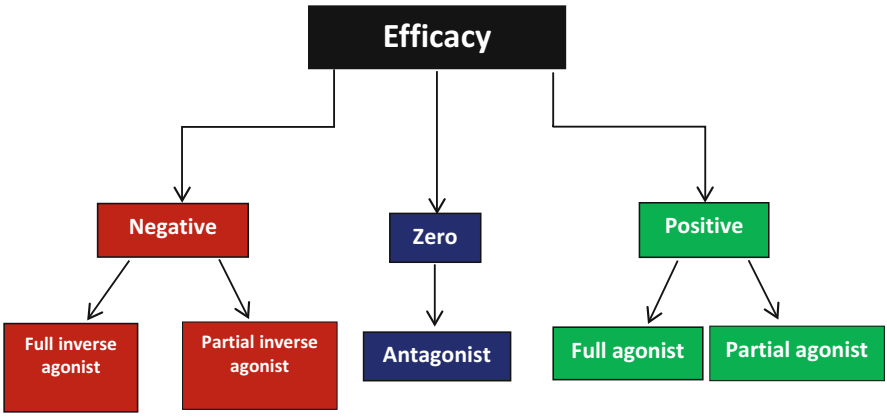


Fig. 2.8 The concept of drug efficacy

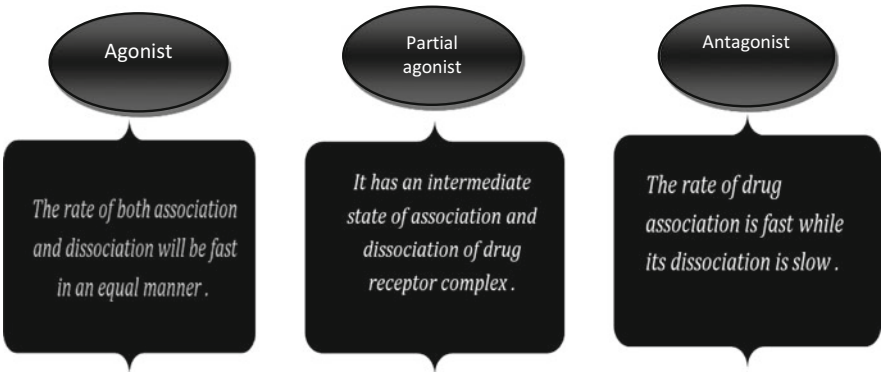


Fig. 2.9 The concept of ligands (agonist, partial agonist, and antagonist)

2.4.2 Rate Theory

The rate theory was proposed by the scientist Paton in 1961 (Paton 1961). This theory suggested that the pharmacological activity of any drug depends on the rate of association and dissociation of the drug with the receptor and will differ based on agonist, partial agonist, or antagonist. Further, the intensity of the pharmacological response depends on the number of the drug-receptor interactions per unit time, and this is directly proportional to the rate of association and dissociation between the drug and the receptor and the total number of molecules involved in such interactions. Like in the occupancy theory, the rate theory was also unable to justify why various types of compounds display their own features. The concept of agonist, partial agonist, and antagonist has been presented in Fig. 2.9.

2.4.3 Induced-Fit Theory

Induced-fit theory is another expression of the "lock-key hypothesis," proposed by Koshland in 1958 (Koshland 1958). The concept of this theory is the fitting of the substrate at the active site of the receptor or an enzyme after causing conformational changes in that receptor or an enzyme to make it convenient in forming the essential interactions or the bonds with the drug in the right sequence to obtain the desired response. Also, the drug may undergo a process to change its shape and fit into the active site. Once the substrate reaches the surface of the enzyme, many conformational changes could take place to make the catalytic groups closer to the substrate and react to yield products which are released from the surface of the enzyme and the enzyme being free for a new turn, subsequently. On the other hand, if the non-substrate molecules reach the active site, it will change the shape in an undesirable way and the catalytic groups will be misaligned; even if the molecule is in an exact match with the receptor, the response will not be achieved, adequately. The elasticity of the proteins (receptors) is very crucial because it facilitates its return to its normal shape after the drug has dissociated from the active site. For example, the noncompetitive inhibitor molecules produce a response from substrate-protein binding without preventing the binding of the substrate to the active site of the enzyme; these inhibitors bind to an allosteric site and make deformations of the active site and lead to preventing the occurrence of the desired response. According to this theory, the agonist will bind to the receptor and affect different conformational changes to produce a response; due to these modifications the molecule binds less tightly to the receptor, thereby easily dissociating the product. The partial agonist will produce a partial conformational change once it binds to the corresponding receptor (Koshland and Neet 1968). While in the case of antagonist, it binds without causing any of the conformational changes. If there is no conformational change in the receptor, then the resultant drug-receptor complex will be stable, and the antagonistic action will be produced. From the induced-fit theory, other theories have also been developed such as macromolecular perturbation theory, activation-aggregation theory, and multistate theory.

2.4.4 Macromolecular Perturbation Theory

Based on the consideration of the flexibility of the receptor, two general types of macromolecular perturbation were proposed by the scientist Belleau (Belleau 1964). Either one or both of them could result into the receptor functions once the interaction between the drug and the receptor has occurred. According to the macromolecular perturbation theory, the intensity of pharmacological response is directly proportional to the rate of formation of perturbations. One type of perturbation is a specific conformational perturbation which contributes to generate a biological response when specific molecules bind to the receptor's binding site (agonist). The other type is nonspecific conformational perturbations. In this case, no response will be produced because the receptor binds with other types of molecule (antagonist).

However, if the molecule has both molecular perturbations, the result will be a mixture of two complexes (partial agonist). This theory illustrates the physicochemical properties of the molecules binding to the receptor, but does not cover the significance of the concept of inverse agonism.

2.4.5 Activation-Aggregation Theory

Activation-aggregation theory and macromolecular perturbation theory are based on the concept of induced-fit theory as mentioned previously. Activation-aggregation theory was proposed by Monod et al. (1965) and Karlin (1967) (Monod et al. 1965; Karlin 1967). According to this theory, if receptors are free from ligands (agonists, partial agonists, and antagonists), they will be in an equilibrium state. It is a state between the activated (elicits a biological response) and inactivated forms of the receptor. As per this theory, agonists will move towards the receptor that exists in an activated form, antagonists migrate to a receptor in an inactivated form, while partial agonist will bind to both of these conformations. So, it can be concluded from this model that the binding sites of receptors in an active form differ from those in an inactive form, and these structural differences justify why the agonist is responsible for giving the desired biological response. This theory also illustrates that partial agonists can have both the agonist and antagonist characteristics. For example, when partial agonists exist, they will interact with free receptors and this will enhance the response and it could reach to the maximum. Further, they compete with other molecules (like neurotransmitters) at the respective binding sites and displace them. As a result, the amount of receptor forms will be affected in the following way, the inactivated form will increase, and the response will decrease, substantially. Nevertheless, the concept of inverse agonist is not explained by this theory.

2.4.6 The Two-State Model of Receptor Activation Theory

This theory has been developed because the activation-aggregation theory mentioned previously did not sufficiently explain the activation of a receptor. Hence, the two-state model of receptor activation theory was developed which depends on the competitive and noncompetitive kinetics. In addition, it is based on the performance and results of experiments applied by direct binding to the receptor's binding-site. In this model, scientists relied on the previously proposed models but explained the process of activation in a comprehensive way (Bridges and Lindsley 2008). Simply, the theory assumes the presence of the receptor in two conformational shapes. The first one makes the receptor in the active state (R$*$) and then can give a downstream effect. While the second concept keeps the receptor in an inactive (R) state; therefore no pharmacological effects can be exerted. The receptor will be in the equilibrium state if the ligand is not available in the binding site. This is a state between R$*$

and R, which is known as the basal activity of the receptor. Depending on the constant need for the formation of a drug-receptor complex (K_d and K_d*), the drug will either bind to one conformational state or to both of them (Colquhoun 1998). Ligands such as full agonist, partial agonist, full inverse agonist, and antagonist will bind with a specific state and give a completely different effect. In the following subsections, the behavior of different ligands is discussed briefly.

2.4.6.1 The Behavior of Agonist in the Two-State Model

Full agonists bind selectively to the active state of the receptor and the resulting binding is responsible for the liberation of free energy, and this will trap a portion of the receptor in the active site. The remaining portions of the unbound receptor will proceed further to reach the equilibrium between active and inactive state (Leff 1995). Moreover, the binding with the active conformation will lead to the full shifting of the receptor state from inactive equilibrium to active and yield a maximum response, effectively. Figure 2.10 presents the agonist binding to the active state of the receptor model.

2.4.6.2 The Behavior of Partial Agonist in the Two-State Model

Partial agonists are ligands which have the features of both agonists and antagonists. They play an important role in regulating the activity of the receptor. Partial agonists also play a significant role of competitive antagonists when both full agonistic and partial agonistic properties co-exist together. In this case, it will minimize the activity of the receptor because it competes with full agonists at the binding site of the receptor. On the other hand, it can increase the activity of a receptor and produce a submaximal response when there is a lack in the sufficient amounts of endogenous ligands. The partial agonist prefers to bind with the active state receptors but not as much to the extent observed for the full agonist. As the partial agonist has lower efficacy than the full agonist, the maximum response will not be attained.

2.4.6.3 The Behavior of Full Inverse Agonist in the Two-State Model

Full inverse agonists are ligands that bind to the receptor as an agonist but give a completely different effect; this binding stimulates the receptor to contribute the opposite response. Most of the time, it prefers to bind to the inactivated state of the

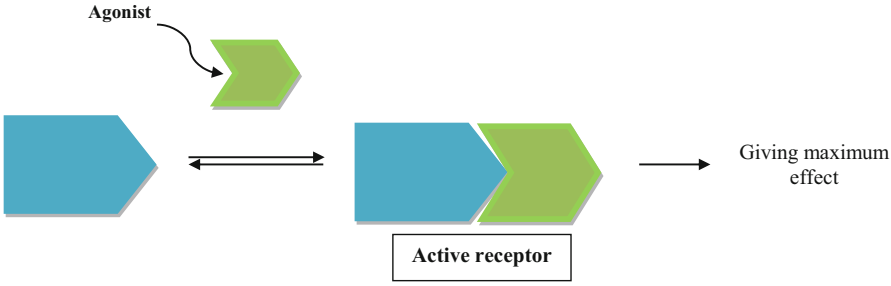


Fig. 2.10 The agonist binding selectively to the receptor in the active state

receptor and shift the equilibrium to inactive state, thus decreasing the basal activity of the receptor and a negative efficacy was observed (<0% efficacy).

2.4.6.4 The Behavior of Antagonist in the Two-State Model

Antagonists are two types, namely, competitive and noncompetitive antagonists. Specifically, competitive antagonists compete with the same binding site wherein the natural ligand or the agonist normally binds. If a competitive antagonist exists alone, then it will interact with the receptor reversibly; however, agonists can replace the antagonist from the binding site and will completely abolish the pharmacological effect of antagonists. Conversely, noncompetitive antagonists interact either allosterically or irreversibly to the receptor and prevent the effect of the agonist irrespective of the agonist concentration (Lew and Ziogas 2004). In general, noncompetitive antagonists have the same affinity towards both the states of receptor, and they do not have any impact on the equilibrium or the basal activity of the receptor. As a result, they will never associate with positive or negative efficacy. Moreover, the noncompetitive antagonist molecule can replace either the agonist or inverse agonist from the receptor binding site Figure 2.11 presents the antagonist binding to the receptor model.

2.4.6.5 Three-State Receptor Model of Agonist Action

This theory has been developed by Leff and coworkers and proposed initially that the model involves three states, two of them are in the active state and the third is in the inactive state. Later stages the model has been extended to include more than two active states which is known as multi-state model of receptor activation. This model gives us a clear idea about why the agonist and inverse agonist have different behaviors in both affinities and efficacies, while they are at the same receptor binding site According to this theory (Leff et al. 1997), the differentiation that occurs between different agonists in their efficiencies is due to their different affinities for various active states.

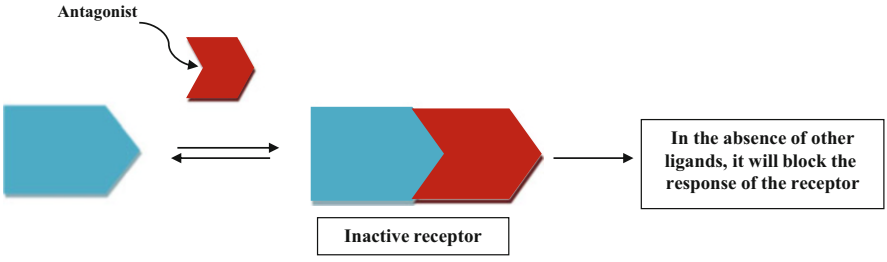


Fig. 2.11 The antagonist binding to the receptor model

2.5 Types of Drug-Receptor Interactions

The driving force for drug-receptor interaction is the low energy state of the drug-receptor complex. The biological activity is related to the drug affinity for the receptor, i.e., the stability of the complex. Dissociation constant of the drug-receptor complex gives an idea about how potent is the drug. The binding interactions occur through points of attachment; for a chemical compound they are the functional groups. Functional groups use their electronic and shape characters in the binding process. If we talk about reversible binding, binding of the drug to receptor should be in equilibrium state. Drug-target interactions can be grouped into two types: Permanent (irreversible)—covalent bonding and reversible interactions. All plausible interactions existing between a receptor and the ligand are discussed in the following subsections.

2.5.1 Covalent Interactions

A covalent bond is produced between two species by mutual sharing of electrons and is the strongest bond, irreversible, and exhibits a stability of −40 to −110 kcal/mol. This type of covalent bond is mostly observed in drug-enzyme or drug-DNA complexes rather than the drug-receptor complex. It is also beneficial in avoiding toxic effects of drugs through an irreversible inhibition of the receptor. This covalent bond usually occurs between a nucleophile (molecule having negative charge, rich in electrons) such as hydroxyl or thiol group in the receptor amino acids and an electrophile (molecule having positive charge, deficient in electrons) such as epoxide or allyl group in drug structure (Kumalo et al. 2015). Two types of covalent bonds, namely polar and nonpolar, are possible during interactions. Water molecule is an example of a polar covalent bond, whereas peptide bond is an example of noncovalent bond. The antibiotic penicillin is an irreversible inhibitor of the enzyme glycopeptide-transpeptidase, the enzyme which catalyzes an essential step in bacterial cell wall synthesis. Penicillin covalently blocks the active-site amino acid serine present inside the glycopeptide-transpeptidase through the formation of a covalent bond.

2.5.2 Ionic or Electrostatic Interactions

Ionic bond is also known as electrostatic interaction which is weaker than the covalent bond and can provide an ionic interaction energy of −5 kcal/mol. This bond appears between two opposite (negative and positive) charges of amino acids of a receptor and ionized species of the ligand (Klebe 2013). Under the normal physiological pH, some of the basic amino acids like arginine, lysine, and histidine bearing amino group in their side chain get protonated, thereby providing a cationic environment. On the other hand, acidic amino acids such as aspartic acid and glutamic acid having carboxylic group get deprotonated and become anionic in

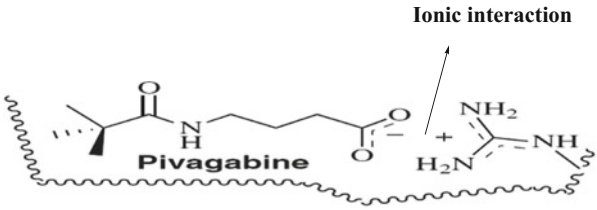


Fig. 2.12 The ionic interaction between carboxylate anion (pivagabine) and protonated amino group (receptor)

nature. Thus, both positive (cationic) and negative (anionic) charges available in the receptors take part in an ionic bonding with ionized groups of drugs. Ionic bond is a most prevalent bond in drug-receptor interaction which depends entirely on the extent of ionization and the distance between two opposite charges. An example of ionic interaction is presented in Fig. 2.12.

2.5.3 Ion-dipole and Dipole-Dipole Interaction

Due to the electronegativity of hetero-atoms (O, N, S, and halogens over the carbon atom), an electric dipole is formed subsequently generating the polarization in bonds. In the polarized bond, one of the pole will be partially positive and the other confers partially negative charge, respectively. These partially positive or negative charges can form an electrostatic bond with either partially charged atoms or ionized elements. As a result of higher electronegativity of one atom, an asymmetric distribution of the electrons was observed. Hence, ion-dipole interaction involves an ion (side chain amino acids of receptors) and a dipole (drug) or vice versa, but dipole-dipole interaction occurs between two dipoles of the drug and receptor, respectively. This bond is polar and electrostatic; also the dipole-dipole interaction is weaker than ion-dipole interaction. This type of interaction involves the energy of −1 to −7 kcal/mol (Du et al. 2016). An example for the drug is zaleplon (Fig. 2.13) which is indicated for the treatment of insomnia.

2.5.4 Hydrogen Bonding Interactions

It is a type of dipole-dipole interaction in which hydrogen atom is a linker between two electronegative atoms, of which one electronegative atom donates the available hydrogen, while the other electronegative atom (bearing a pair of non-bonded electrons) accepts it (Varma et al. 2010). Consider atoms such as N-H and O are electronegative atoms, in which the "N" removes the electron density from "H," rendering "H" a partial positive charge, allowing it to attract towards other partially negative atom "O." Thus, N-H acts as a proton donor and "O" as a proton acceptor. If two atoms had equivalent electronegativity and degree of ionization, then the proton can be shared equally between them, thereby generating a low-barrier hydrogen bond. In general, the distance of hydrogen bond between a carbonyl oxygen (C=O)

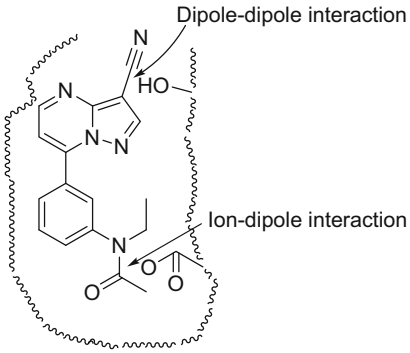


Fig. 2.13 The dipole-dipole (top) and ion-dipole (bottom) interactions of zaleplon

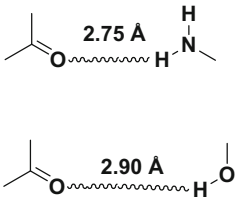


Fig. 2.14 Hydrogen bond distance between heteroatoms

and a hydroxyl group proton (H-O) is 2.75 Å, whereas a carbonyl oxygen (C=O) and proton of N-H is 2.90 Å (Fig. 2.14).

A hydrogen bond is very important and unique to hydrogen atom, exclusively as it is the only atom that can confer a positive charge at physiological pH (Balakumar et al. 2010). Hydrogen (H)-bonding includes two types of interactions, that is, intermolecular and intramolecular H-bonds, respectively. However, compounds that tend to make intramolecular H-bond will be less active and unable to interact with the receptor. The possibility of hydrogen bonding involves the orientation of hydrogen atom in donor and acceptor group and depends on the distance between two atoms (1.5–2.2 Å). Hydrogen bond acceptor has electron-rich atom and slightly negative (carboxylate ion) charge, whereas the donor has an electro-deficient hydrogen and slightly positive charge (alkyl ammonium ion or secondary and primary amines) (Kuhn et al. 2010). Two types of hydrogen bonding interactions are presented in Fig. 2.15.

2.5.5 Charge Transfer Interactions

Charge transfer interactions happen between electron donor group in one molecule (alkene) and an electron acceptor in another group (aromatic ring). Some amino acids in the receptor have electron donor groups like -OH group in aromatic amino acid tyrosine and carboxylate group (-COO) of aspartate (Zhang et al. 2016). Similarly, few amino acids bearing electron acceptor group (sulfur-containing amino acid cysteine) and amino acid residues like histidine, tryptophan, and

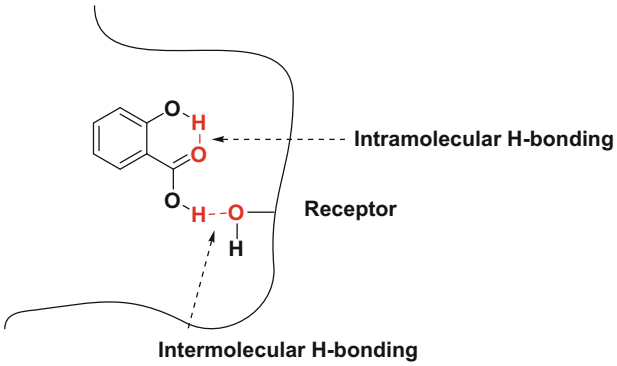


Fig. 2.15 Intramolecular and intermolecular hydrogen bonding interactions

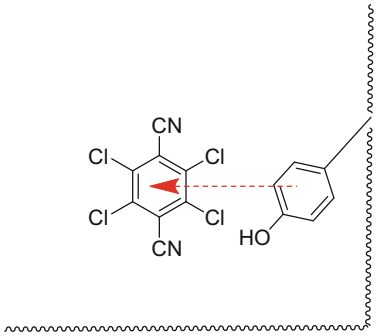


Fig. 2.16 Chlorthalonil interacting with tyrosine residue of the receptor through charge transfer process

asparagine have both electron donating and accepting capabilities. An example of charge-transfer interaction is illustrated using the drug, chlorthalonil, in Fig. 2.16.

2.5.6 Hydrophobic Interactions

Hydrophobic interaction is a type of noncovalent interaction that occurs in an aqueous solution. This type of interaction is due to the stabilization of the receptor-drug complex originated from higher entropy and lower free energy (Varma et al. 2010). When a nonpolar lipophilic group on a drug and nonpolar group in a receptor surrounded by ordered water molecule, it will be disordered to associate each other leading to the stabilization of the drug-receptor complex (Fig. 2.17).

Further, it includes π-π interaction involving a face-to-face arrangement of aromatic ring of drug and another aromatic ring of amino acids in the receptor, both of which have π electron system (Fig. 2.18).

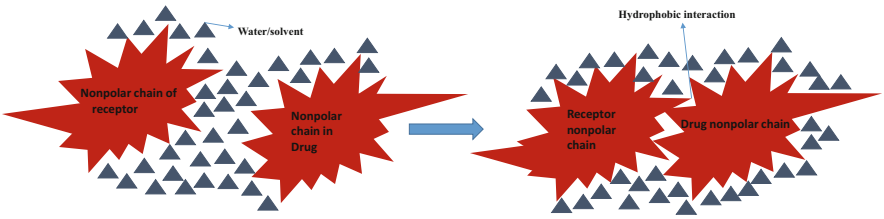


Fig. 2.17 Hydrophobic interaction between drug and receptor

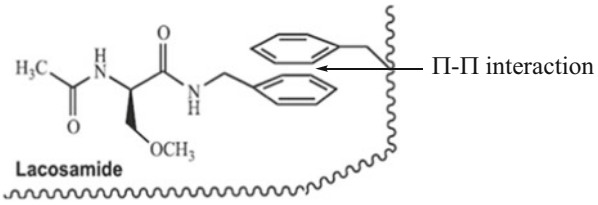


Fig. 2.18 Aromatic system of lacosamide drug interacting with the aromatic ring of the receptor through π-π interaction

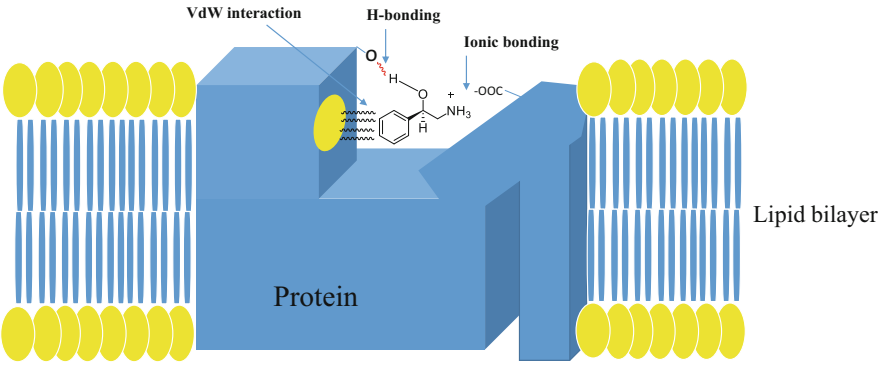


Fig. 2.19 Van der Waals interaction between the drug and the receptor is shown for epinephrine including H-bonding and ionic bonding

2.5.7 Van der Waals Interactions

Sometimes a temporary nonsymmetrical distribution of electron density occurs in a molecule generating a temporary dipole which interacts with another dipole in the receptor. These bonds are much weaker in comparison to other types of bonds, bearing −0.5 kcal/mole energy, and is known as Van der Waals interactions (Barratt et al. 2005). An example of Van der Waals interactions is illustrated in Fig. 2.19.

An example of multiple interactions exhibited by Dibucaine (local anesthetic drug) is illustrated in Fig. 2.20.

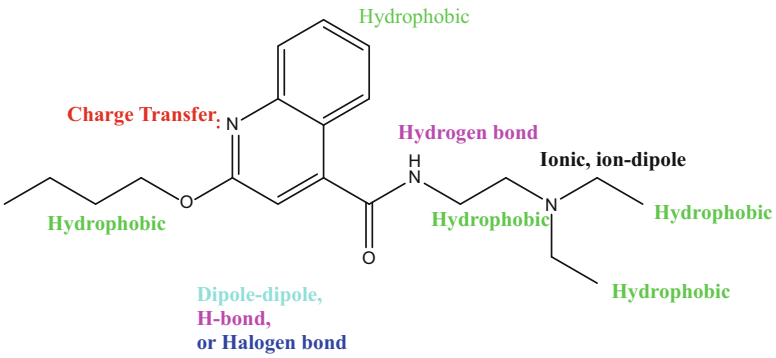


Fig. 2.20 Multiple interactions shown by Dibucaine towards the receptor

2.6 Determination of Drug-Receptor Interactions

2.6.1 Agonists

Some endogenous molecules are responsible for the regulation of certain physiological functions through binding with receptors and interact in the tissues which lead to a specific physiological response. Like a natural messenger to the receptor, an agonist is a chemical messenger that can activate the receptor as a result of binding to it. Due to the structural similarity with the natural messenger, agonists can also exhibit intermolecular interactions/bonds by employing the same induced fit similar to natural messenger performs. However, the binding of agonist to the receptor should be a reversible binding. The knowledge about the properties of the binding site including the geometry and topography, the chemical structure of the normal substrate that binds to the receptor, the correct binding group of agonists, position of interaction with complementary binding region, and the shape and size of agonists is required for the design of novel agonists so as to fit the binding site of the receptor (Auerbach 2016).

There are two terms which describe the agonists as partial and full based on dose-response curve (DRC) or concentration-response curve (CRC). This curve is obtained initially by administering endogenous compounds like Ach to animal muscle tissue and allowing the contraction of muscle as a measure of response. Initially, the concentration of Ach is low; therefore, only a small number of molecules interact with the receptor, and at the time they had linear relationship between the concentration and the response because all of the receptors are occupied at 100%. If a similar kind of response is obtained by the use of an exogenous compound to this muscle tissue, then such compound is known as a full agonist. However, 50% response indicates that the administered compound is a partial

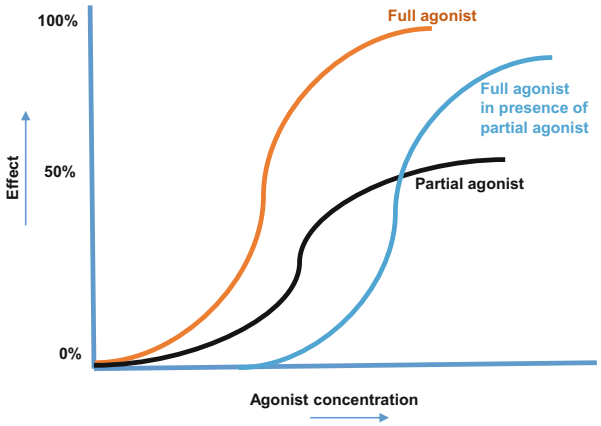


Fig. 2.21 CRC of different types of agonists

agonist (Lambert 2004). The CRC pattern of different types of agonists is shown in Fig. 2.21.

2.6.2 Antagonists

Receptor antagonists are compounds that can inactivate the receptor after binding to it. The antagonist must also have the complementary shape and binding group to orient towards the binding site of a receptor. In general, the chemical structure of antagonists exhibits slightly higher size than the endogenous compound and is classified into competitive and noncompetitive antagonists. If the administration of Ach does not initiate a response in the presence of another exogenous compound or there is a need for a higher concentration of Ach, it indicates that the exogenous compound could be a competitive antagonist. It directly demonstrates that the agonist and the antagonist compete each other for the same binding site. The competitive antagonist possesses structural similarity to agonists in terms of size, shape, and functional groups to allow binding on the same agonist binding site on the receptor. As mentioned earlier, the response will be tardy after adding an increased amount of the agonist; consuming the binding is reversible. The predominant binding to a receptor depends on the higher concentration of either agonist or an antagonist (Buchwald 2017). If the unknown drug exhibits 50% of response and no further enhanced response obtained even with the addition of excess amount of Ach, it is a noncompetitive antagonist. Figure 2.22 represents the CRC of both competitive and noncompetitive antagonists.

There are two strategies to design the noncompetitive antagonists such as allosteric antagonists (Fig. 2.23) and antagonists using umbrella effect (Fig. 2.24). If the designed compound binds to an allosteric binding site (another site of the normal agonist binding site) that may be located beside the major binding site, then it leads to geometric changes in the binding site, thereby preventing the agonist binding is called as noncompetitive antagonist. It could be a drug or an endogenous compound,

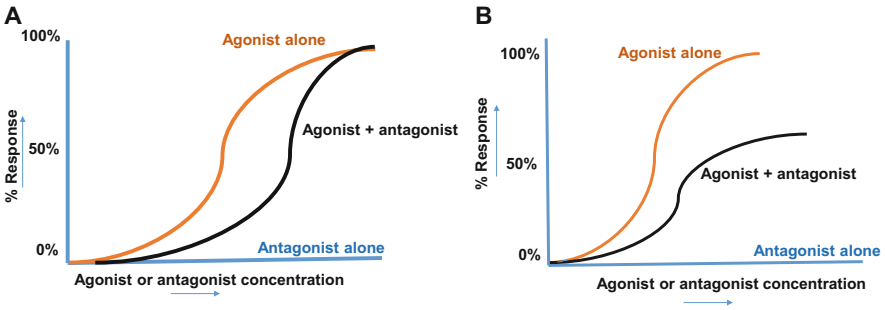


Fig. 2.22 (**A**) Competitive antagonist; (**B**) noncompetitive antagonist

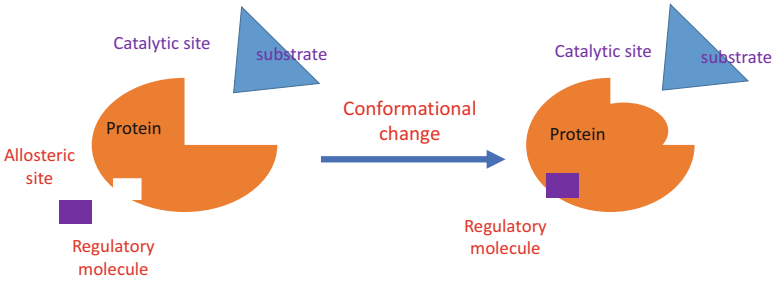


Fig. 2.23 Noncompetitive antagonism by allosteric effect

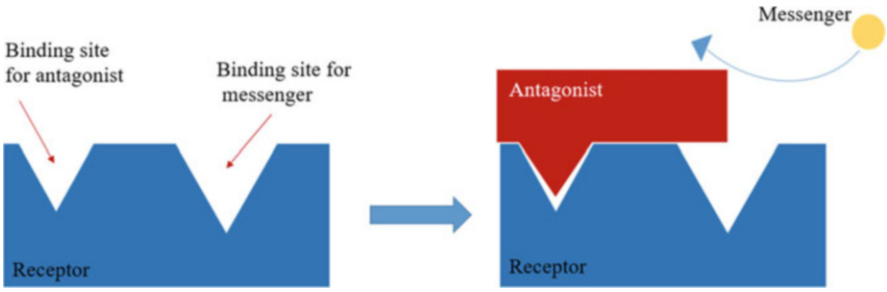


Fig. 2.24 Noncompetitive antagonism by umbrella effect

and there will be little or no response to agonist, so maximum achievable response will be reduced in this type. The percentage of antagonism in the noncompetitive type does not depend on the amount or the concentration of the agonist or antagonist (Schwartz and Holst 2007). Agonists will take much more time to give a response when it is mixed with a competitive antagonist, but in case of a noncompetitive antagonist, the response will be lowered due to the allosteric effect.

The second strategy in the design of an antagonist is by umbrella effect, in which the noncompetitive antagonist will bind closely to the agonist binding site, but the

antagonist displays a part of its structure like a tail that will cover the opening of the binding site, thus preventing the binding of an agonist (Karschin et al. 1988). In this case, the percentage of antagonism depends on the amount of both the agonist and the antagonist.

2.7 Contribution of the Functional Groups to Drug-Receptor Interactions

The strength of the association between the receptor and a drug can be determined by total free energy of interaction. Nevertheless, it does not clarify about the quality of interaction or the effect of the addition of new functional group. Hence, it is very important to estimate the contribution of an individual functional group to drug-receptor interactions. To understand drug-protein interactions, functional group additives (Eq. 2.3) or the additivity of free enthalpy components (Eq. 2.4) is majorly employed (Andrews et al. 1984).

$$\Delta G = \Delta G_{Me} + \Delta G_{OH} + \Delta G_{Ph}(Ph) + \ldots\ldots \tag{2.3}$$

$$\Delta G = \Delta G_{H-bridge} + \Delta G_{solvation} + \Delta G_{conformation} + \ldots\ldots T\Delta S \ldots\ldots \tag{2.4}$$

The free energy of binding is defined in terms of the binding energies for the individual functional groups that construct a drug molecule according to Eq. 2.5.

$$\Delta G = T\Delta S_{t,r} + n_r E_r = E\, N_x E_x \ldots\ldots \tag{2.5}$$

where $T\Delta S_{t,r}$ is the loss of overall translational and rotational entropy related to the drug binding, n_r is the number of internal degrees of conformational freedom lost on binding the drug molecule, and Er is the energy equivalent of the entropy loss associated with the loss of each degree of conformational freedom on receptor binding (Andrews et al. 1984).

2.7.1 Intrinsic Binding Energy

If the specific functional group of a drug aligned to the specified functional group of the receptor without any strain, the E_x is known as intrinsic binding energy or apparent binding energy. In general, E_x is the combination of the various enthalpic and entropic interactions including enthalpy of interaction between the drug and receptor binding site. The change in enthalpy is associated with the removal of water of hydration (the functional group and its target binding site), subsequent bond formation between the displaced water molecules, and the corresponding entropy terms associated with the displacement and subsequent bonding of water molecules. Thus, intrinsic binding potentials can be used reasonably in an additive manner in the determination of the drug-receptor interaction (Jencks 1981).

2.7.2 Anchor Principle

Based on Eq. 2.5, the binding energy E_x can be determined by comparing the binding energies for pairs of compounds that differ only in terms of functional group "X." This concept was employed by the scientist "Page" who declared it as "Anchor Principle" (Page 1977). It is based on the fact that the difference in binding of a drug molecule by the presence or the absence of the particular functional group is mainly due to the number of factors associated with that functional group. In other words, the binding energy E_x with loss of any degrees of conformational freedom arose due to the binding of group "X." The magnitude of the binding energies deduced by the anchor principle will vary according to the quality of the interaction. If the functional groups are unable to align correctly, then small or even repulsive interaction may occur.

2.7.3 Average Binding Energy

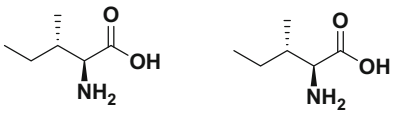
Andrews et al. studied the average contributions of individual functional groups to the observed binding energies of about 200 different ligand-protein interactions in aqueous solution (Andrews et al. 1984). In this, the average loss of rotational and translational entropy $T\Delta S_{t,r}$ (Eq. 2.5) was determined as 58.5 kJ/mol at 310 K. The outcome of their study indicated that the loss of entropy associated with each internal rotation (ΔGr) on receptor binding is equivalent to a decrease in the free energy of binding by an average of 3 kJ/mol. The averages calculated were smaller than the respective intrinsic binding energies.

2.7.4 Contribution of Methyl Group and Nonpolar Groups

The scientist Page (1977) determined that the intrinsic binding energies for the CH_2 group (methylene) fall in the range of 12–14 kJ/mol based on the data of the selectivity of amino acid-tRNA synthetases. Under physiological conditions, the value of Gibb's free energy is approximated (in kJ/mol) by Eq. 2.6.

$$\Delta G = -5.85 \log K_d \ldots \tag{2.6}$$

For example, the calculated binding energies for isoleucine and its desmethyl analog (Fig. 2.25) to isoleucyl-tRNA synthetase are 29.7 and 15.9 kJ/mol,



Isoleucine Desmethyl isoleucine

Fig. 2.25 2D structures of isoleucine and desmethyl analog

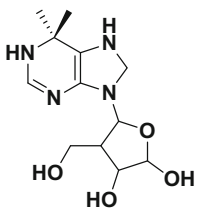
respectively, demonstrating that the methyl group contributes a total of 13.8 kJ/mol to overall interactions. In case of long chain hydrocarbons, the positive contribution is observed mainly because of dispersion forces and hydrophobic interactions generating the loss of conformational entropy on binding. Hence, 3 kJ/mol binding energy average derived for sp^2 and sp^3 carbons is same to the "average" decrease in free energy of binding. While comparing with the unsaturated or cyclic analogs, this effect will be higher in case of saturated hydrocarbons (Andrews et al. 1984).

2.7.5 Contribution of Hydroxyl Group or Hydrogen Bonding Groups

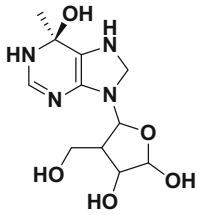
The contribution of hydrogen bonds mediated by hydroxyl groups in transition-state analogs was explained by Wolfenden et al. Based on the experiments conducted and by the application of the anchor principle, it was observed that apparent binding energies for single hydroxyl groups ranged from 20 to 42 kJ/mol (Wolfenden and Kati 1991). By comparing the binding of 1,6-dihydropurine ribonucleoside and its 6-hydroxy derivative (Fig. 2.26) towards the protein adenosine deaminase, the authors witnessed a binding energy value of 41 kJ/mol (Kati and Wolfenden 1989). Based on this particular observation, the authors perceived that the hydroxyl group at sixth position has limited movement and had a proper alignment within the active site, thereby generating a hydrogen bond. This was further supported by the X-ray solved crystal structure of the inhibitory complex between adenosine deaminase and 6-hydroxy-1,6-dihydropurine ribonucleoside. In this crystal structure, interactions between the 6-hydroxyl group with a zinc atom, protonated histidyl residue, and aspartic acid residue at the active site were observed (Wilson et al. 1991).

2.7.6 Acidic and Basic Substituents

Some acidic and basic entities of charged groups influence the binding interaction between the ligand and its receptor. In particular, the phosphate (cation) binds to



1,6-Dihydropurine ribonucleoside



6-Hydroxy 1,6-dihydropurine ribonucleoside

Fig. 2.26 2D structures of 1,6-dihydropurine ribonucleoside and 6-hydroxy 1,6-dihydropurine

Table 2.1 Contributions of functional groups to overall binding energies

Type of functional groups	Determination of interaction energy methods		
	Anchor principle	Site-directed mutagenesis	Average energy
Nonpolar (each carbon)	12–14	1–3	3–6
H-bonding (uncharged)	16	2–6	5–14
H-bonding (charged)	20–42	15–19	-
Carboxyl, amine groups (charged)	18–28	12–25	34–48
$T\Delta S_{t,r}$	12–60	-	58.5
ΔG_r (internal rotation)	5–6	-	3

alkaline phosphatase (Levine et al. 1969) and showed a ΔG value of 33 kJ/mol. By considering the loss of rotational and translational entropy related to this interaction, Eq. 2.5 resulted in a lower estimate for binding (45 kJ/mol) for the phosphate ion. If the same value of $T\Delta S_{t,r}$ is applied to the binding of oxalate ion (anion) to the enzyme transcarboxylase, Eq. 2.5 yields an apparent binding energy of 24 kJ/mol per carboxylate group after the minimal loss of conformational entropy (3 kJ/mol). These values are in accordance with the average values estimated by Andrews et al. (34–48 kJ/mol) (Andrews et al. 1984). The contributions of some functional groups and/or bond types to overall binding energies are collected in Table 2.1.

2.8 Stereochemical Considerations in Drug-Receptor Interactions

2.8.1 Diastereomerism and Binding Interaction

They are the type of stereoisomers, but not mirror images of each other. Diastereomers are actually the complexes formed between two enantiomers, thereby yielding different energies and chemical properties consequently resulting in different dissociation constants for drug-receptor complexes of enantiomeric drugs. There are a special case of diastereomers such as geometric isomers (*E* and *Z*) and epimers (compounds having the same chemical formula but differing in their spatial arrangements around the single carbon atom). They can be separated conveniently using chromatography or recrystallization techniques than the enantiomers. Diastereomers exhibit different energies and stabilities due to the fact that they demonstrate different interactions with the same receptor after binding to the binding site (Kier 1997). For example, the neuroleptic potency of the *Z*-isomer of the chlorprothixene (an antipsychotic drug) is 12 times greater than that of the corresponding *E*-isomer. Conversely, the *E*-isomer of the diethylstilbestrol (an anticancer drug) had 14 times better estrogenic activity than the corresponding *Z*-isomer (Fig. 2.27).

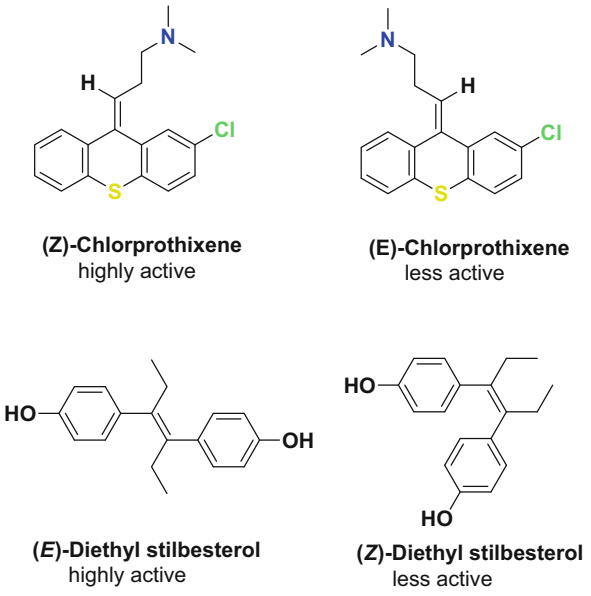


Fig. 2.27 Structures of diastereomers of chlorprothixene and diethylstilbestrol

2.8.2 Enantiomerism and Binding Interaction

According to the nomenclature of Ariëns (1987), the potent isomer is called as the eutomer and the weaker one is known as distomer. The potency ratio of higher affinity enantiomer to lower affinity is termed as eudismic ratio. The distomer can be regarded as an impurity in the mixture, which may contribute to undesirable side effects or toxicity. In some cases, the distomer will be responsible for the biological activity and the eutomer attributable to the side effects. D-ketamine (Fig. 2.28), a hypnotic and analgesic agent, is responsible for the pharmacological actions, whereas the isomer L-ketamine is known for the undesired side effects. It is also possible that both isomers are active biologically, but one of them causes toxicity (e.g., the local anesthetic prilocaine). In some cases, it is required to have the two isomers for better pharmacological activity. Both isomers of bupivacaine (Fig. 2.28) act as local anesthetic, but only the L-isomer shows vasoconstrictive activity (Aps and Reynolds 1978). On the other hand, the D-isomer is responsible (i.e., eutomer) for both the diuretic activity and the side effect (uric acid retention).

Enantiomers may have different therapeutic actions, for example, Darvon, an analgesic drug and its enantiomer, Novrad is an antitussive drug. Thus, these enantiomers are marketed (Darvon and Novrad) separately under different trade names. Another case for enantiomers is that they may display opposite effects. The (*R*) enantiomer of 1-methyl-5-phenyl-5-propylbarbituric acid (Fig. 2.28) acts as a narcotic, while the (*S*) enantiomer works as a convulsant. Hence, the receptor has an ability to select and recognize the isomers through the chiral nature. Enantiomers may have different biological activities depending on the fact that one isomer may fit into the receptor binding site much better than its counterpart to demonstrate better

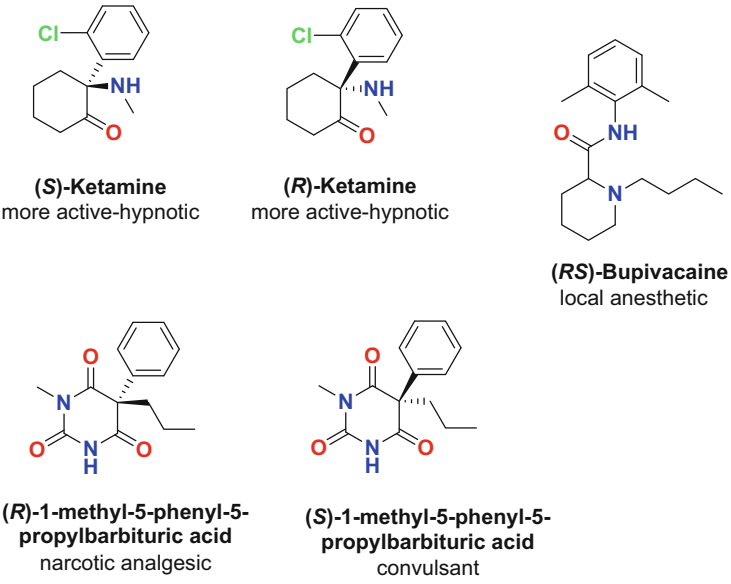


Fig. 2.28 Structures of enantiomers of Ketamine and 1-methyl-5-phenyl-5-propyl-barbituric acid with racemic mixture of Bupivacaine

pharmacological activity profile (Arthur 1927). If a receptor has two binding site points (Fig. 2.29 A, B), then it is difficult to recognize the specific enantiomer (epinephrine); on the other hand, if a receptor has three binding site points (Fig. 2.29 C, D), it can recognize a particular enantiomer and distinguishes between pairs of enantiomers (Arthur 1927).

R-(−)-isomer of epinephrine has three points of interaction due to the specific conformation to maximize molecular complementarity. However, the *S*-(+)-isomer showed two sites of interaction (the hydroxyl group cannot interact with the binding site) and exhibited lower binding energy (Fig. 2.29A, B).

Similarly, Talapatra et al. studied the crystal structure of the Eg5-K858 complex and its implications in structure-based design of thiadiazole-containing inhibitors as anticancer agents (Talapatra et al. 2018). In their study, the inhibitor molecule K-858 (Fig. 2.30) exists in a racemic mixture, which was resolved using chiral HPLC. Interestingly, *S*-enantiomer of K-858 showed higher inhibition of Eg5 protein, while *R*-enantiomer was unable to inhibit the protein. Figure 2.30 describes the favorable interaction for the *S*-enantiomer of K-858 displaying the correct orientation of methyl group to solvent accessible region, while the phenyl ring is involved in aromatic π-π interaction (Trp127) and hydrophobic interactions (Arg119 and Pro137). Thus, the *S*-enantiomer demonstrated good inhibition of Eg5 protein and acts as a potential anticancer agent. On the other hand, the phenyl group with a significantly larger hydrophobic character than the methyl would be placed towards the solvent region resulting in larger unfavorable interactions for the *R*-enantiomer which is responsible for the lack of inhibition of Eg5 protein.

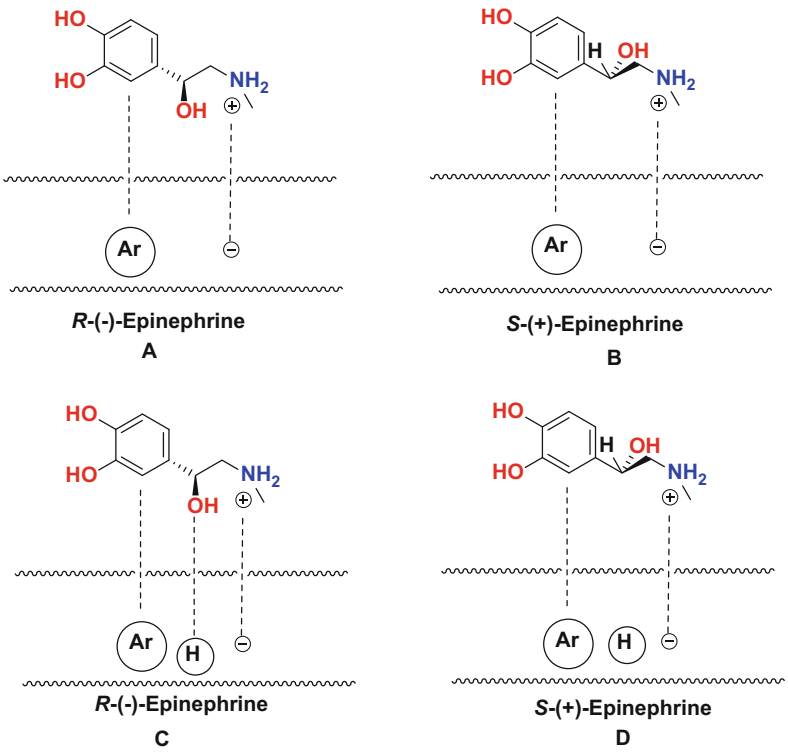


Fig. 2.29 Binding of epinephrine enantiomers to two-site receptor (A, B **a**, **b**) and three-site receptor (C, D **c**, **d**)

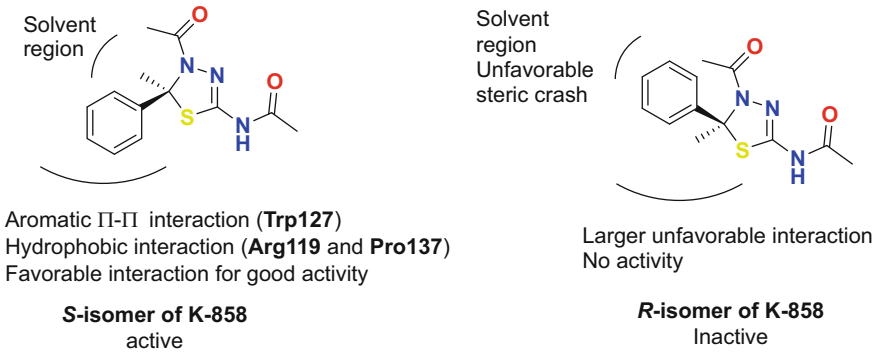


Fig. 2.30 Binding interactions of enantiomers of K-858 with crucial amino acids in the binding site of Eg5 protein

2.8.3 Conformational Isomerism and Binding Interaction

Conformational isomers are the type of isomers formed due to the free rotation of single bonds and cannot be separated. The concept of pharmacophore is best described in terms of the configuration of a set of atoms and the bio-active conformation as well (Balakumar et al. 2018). The crucial amino acid residues in the binding site of the receptor can bind to only one specific conformer. The conformer that binds to the receptor's binding site should have adequate energy which can be determined by sophisticated instrumentations such as X-ray crystallography and NMR spectrophotometry or by computation through molecular mechanics calculations. It is very imperative to identify the bioactive conformation which is an active conformation of the drug that is involved in binding to the receptor for the design of ideal drug candidates. If there is a lead compound exhibiting low potency, then it is mainly due to the existence of a low amount of the active conformer in a solution. For example, the antidiabetic drug rosiglitazone (Fig. 2.31) binding to peroxisome proliferator-activated receptor gamma (PPARγ); the favorable orientation must be in a "U" shaped conformation for better activity (Gampe et al. 2000). In this particular conformation, the thiazolidinone moiety was buried into the binding site so that it can display H-bonding interactions with crucial amino acids (Ser289 and Tyr473). Thus, the bioactive conformer of rosiglitazone demonstrated good inhibition of PPARγ.

Li and Biel demonstrated the correlation between the conformers and the tranquilizing action of 4-(4-Hydroxypiperidino)-4′-fluorobutyrophenone (Fig. 2.32A) with mild anti-emetic property (Li and Biel 1969). The compound had 2 chair (Fig. 2.32B,E) and 2 twisted-boat (Fig. 2.32C, D) conformations. Initially, a relative compound, *N*-methyl-4-piperidinol (R = Me), was considered, and the difference in free energy between the axial and equatorial hydroxyl conformers was determined to be 0.94 ± 0.05 kcal/mol at 40 °C (the equatorial conformer is most favorable by a factor of 4.56 over axial conformer). The energies for the twist-boat conformers are 6 kcal/mol higher due to hydrogen bonding; thus C was considered as more stable than B. Based on this assumption, three

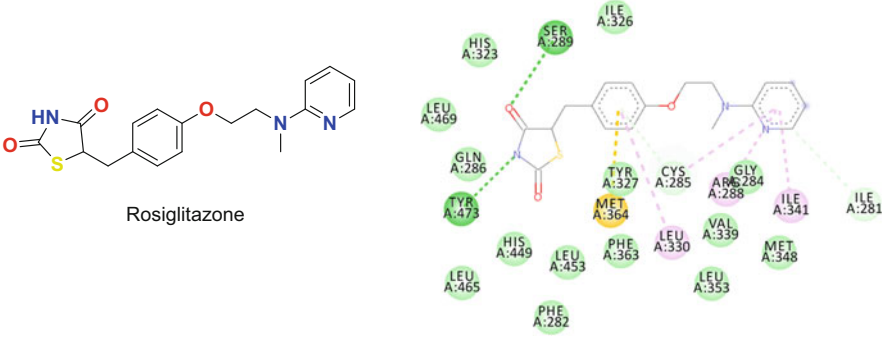


Fig. 2.31 2D structure of rosiglitazone and 3D interaction plot of rosiglitazone with PPARγ

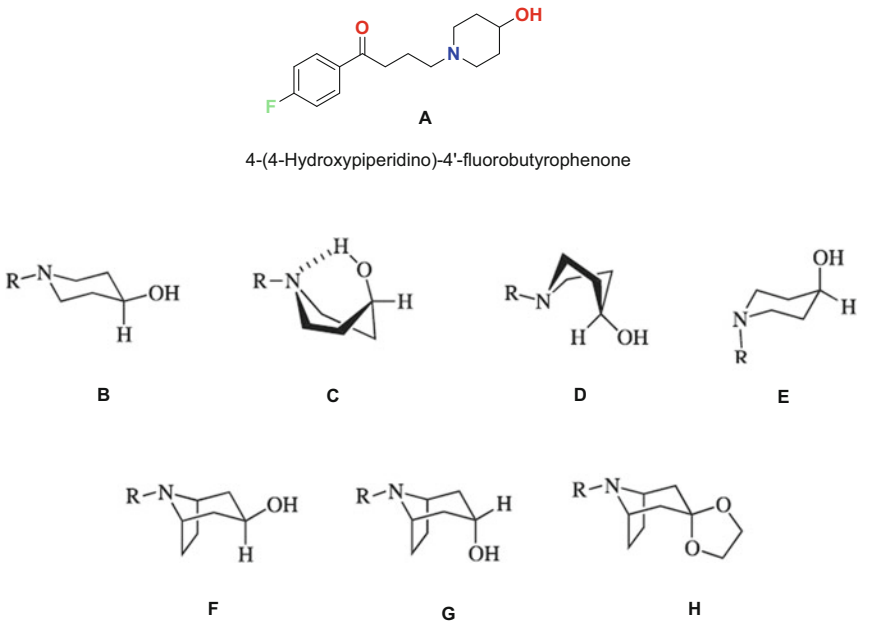


Fig. 2.32 Chemical structure of 4-(4-Hydroxypiperidino)-4′-fluorobutyrophenone (A **a**) and its conformers (B-H **b**–**h**)

conformationally rigid chair analogs (F-H **f**–**h**) were synthesized and evaluated for their muscle relaxant activity. The structure G was found to be conformationally less stable with the axial hydroxyl group resulting in a better molecular complementarity with the receptor, thereby yielding good pharmacological activity.

This is a distinguished approach that can be used to determine the bioactive conformation of a drug molecule in the drug-receptor complex. This involves the synthesis of conformationally rigid analogs, followed by biological evaluation. The highest potent analog can be used as a prototype. The major disadvantage in this approach is that in order to form the analogs, additional atoms must be added to the original compound and this may affect both chemical and physical properties. In such cases, it is essential that the drug and the analog must be similar in size, shape, and mass.

2.9 Case study in the Design of Adenosine Receptor Antagonists Through Interactions

Adenosine receptors (ARs) are classified into four subtypes A_1, A_{2A}, A_{2B}, and A_3 ARs belonging to the superfamily of GPCRs (Kaur et al. 2011; Agrawal et al. 2019; Deb 2019a). At present, about 40% of modern medicines function through the mediation of various signaling processes associated with GPCRs (Deb et al.

2019b; Deb 2019c; Mailavaram et al. 2019). In particular, two important AR subtypes, namely A_{2B} AR and A_3 AR signaling pathways, are implicated mainly in asthma and COPD (Pran Kishore et al. 2011). Hence, the design of antagonists (Deb 2019b) for these types depends on the availability of crucial amino acids in the binding site and the interacting functional groups of receptor amino acids with the designed antagonists (Banda et al. 2013; Chandrasekaran et al. 2018). Earlier, fluorinated fused quinazolines were designed as A_{2B} AR antagonists and investigated in silico by molecular docking with the developed homology model of A_{2B} AR (Chandrasekaran et al. 2017). Recently, a number of potential AR antagonists were discovered (Shaik et al. 2019).

Due to the lack of availability of X-ray crystal structures of the A_{2B} and A_3 ARs, homology models were constructed and subsequently employed for the docking studies (Balakumar et al. 2012; Chandrasekaran et al. 2017). We describe here the case study involving a homology model of A_3 AR interacting with different ligands through an *in silico* molecular docking and molecular dynamics simulations. For the structure-based modeling study, Deb et al. developed a homology model of A_3 AR using X-ray crystal structure of human A_{2A} AR (PDB ID: 4EIY) and conducted docking (GLIDE XP, IFD-Schrodinger) of novel thieno[2,3-*d*]pyrimidine derivatives (Deb et al. 2018). The results of the docking study revealed majorly hydrogen bonding (H-bonding) and hydrophobic interactions. The most active compound **8** was subjected to molecular dynamics simulation (Desmond software) for 50 ns with hA_{2A} and hA_3 ARs to study the stability and binding interaction. The crucial amino acid residues Phe168, Val 169, Asn236, and Leu232 exhibited significant interaction with the ligand through π–π stacking and H-bonding to impart good binding affinity towards hA_3 AR binding site. A 2D interaction plot of the most active compound **8** in the binding site of hA_3 AR (Deb et al. 2018) is shown in Fig. 2.33.

2.10 Conclusion

Certainly, for the drug to function, it must interact with the target protein such as receptors. The interactions between a drug and its receptor are mediated through the structural features, mainly by functional groups. It is very imperative that probing the real receptors for the establishment of essential binding features can offer crucial information about the mode of drug action. Of equal importance, the binding orientation of the drug structure inside the binding site of a receptor can influence the pharmacological activity. Theories of drug-receptor interactions can provide more useful information to deduce the probability of proposing the mode of action of any drug under biological environment. In particular, noncovalent interactions mostly are weaker and they work in conjunction with other types of interactions. This is mainly due to the loss in translational entropy (first interaction) followed by lower entropy (second interaction). Finally, the effect of this cooperativity causes the conversion of weak interactions to yield strong interactions.

The selectivity of the drug is primarily decided by the strength and existence of good interactions between the functionalities in drug-receptor complexes. Many

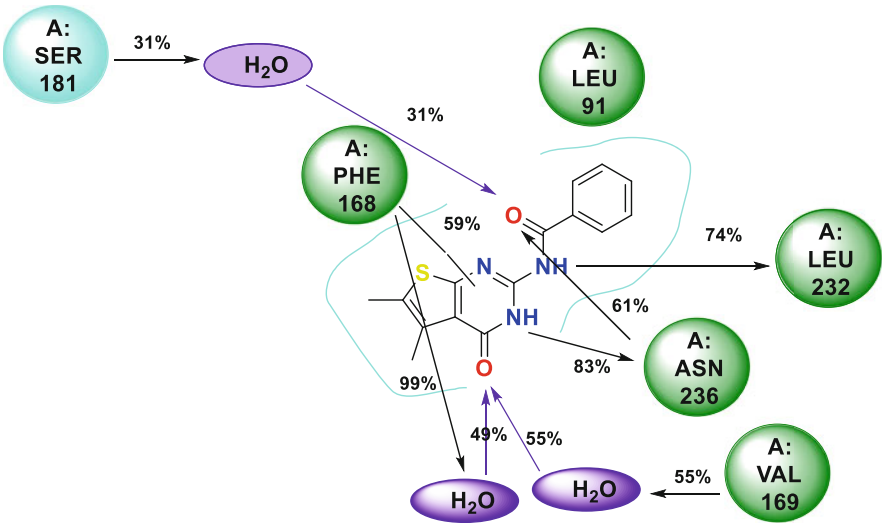


Fig. 2.33 Ligand interaction pattern showing percentage of contacts with crucial amino acid residues of A_3 AR

times charged functional groups tend to bind more effectively than polar functional groups, which bind more tightly than nonpolar groups. In case of electrostatic interactions, ammonium ions are efficient followed by phosphate ion, and then carboxylate anion. In order to exhibit good pharmacological activity of the ligands, they must have higher binding energy than the calculated average binding energy. Conversely, compounds demonstrating less binding energy can fit into receptors poorly. Hence, the determination of drug-receptor interactions is very essential for the safety and efficacy of a drug.

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Pharmacology of Acetylcholine and Cholinergic Receptors

3

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Abstract

Acetylcholine is a neurotransmitter that plays a significant role in a variety of physiological functions. Cholinergic neurons synthesize, store, and release acetylcholine and are also responsible for sympathetic and parasympathetic responses of the autonomous nervous system. The wide range of functions that the cholinergic system plays explains the diverse range of therapeutic potential that targets this system. Over the decades, cholinergic and anticholinergic drugs are utilized as treatment options for various conditions including ophthalmology, neurogenic bladder, myasthenia gravis, dementia, postoperative urinary retention, xerostomia, anticholinergic overdose, snakebites, Parkinson's disease, and

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Alzheimer's disease. Alongside, they are also investigated for various promising therapeutics. This chapter provides an overview of the cholinergic system pharmacology, functions in the body, cholinergic and anticholinergic compounds, and their potential role in the medical field. Further, the chapter highlights the updates on the cholinergic compounds currently used to treat various conditions as well as compounds under investigation.

Keywords

Acetylcholine · Cholinergic system · Nicotinic receptors · Muscarinic receptors · Acetylcholinesterase inhibitors · Cholinergic ligands

Abbreviation

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
ANS	Autonomic nervous system
ASDs	Autism spectrum disorders
BQCA	Benzyl quinolone carboxylic acid+
BTX	Botulinum toxin
BuChE	Butyrylcholinesterase
ChAT	Choline acetyltransferase
ChE	Cholinesterase
CNS	Central nervous system
CoA	Coenzyme A
COPD	Chronic obstructive pulmonary disease
DMN	Default mode network
DS	Down syndrome
ECT	Electroconvulsive therapy
EDHF	Endothelium-derived hyperpolarizing factor
EDRF	Endothelium-derived relaxing factors
EPSP	Excitatory postsynaptic potential
FDA	Food and Drug Administration
GPCR	G-protein-coupled receptor
LAMA	Long-acting muscarinic receptor antagonist
mAChR	Muscarinic acetylcholine receptor
MCI	Mild cognitive impairment
MTL	Medial temporal lobe
nAChR	Nicotinic acetylcholine receptor
NAL	Neutral allosteric ligand
NAM	Negative allosteric modulator
NMJ	Neuromuscular junction
NO	Nitric oxide

OPC	Organophosphate compound
OSCC	Oral squamous cell carcinoma
OSF	Oral submucous fibrosis
PAM	Positive allosteric modulator
PD	Parkinson's disease
PLA2	Phospholipase A2
PNS	Peripheral nervous system
UAB	Underactive bladder

3.1 Introduction

Autonomic nervous system (ANS) is a collection of ganglia (motor nerves) situated in pelvis, abdomen, thorax, neck, and head, in addition to motor neurons axonal connections. Acetylcholine (ACh) is the neurotransmitter found in sympathetic and parasympathetic preganglionic autonomic neurons (Blessing and Gibbins 2016). ACh also elicits activity on the postsynaptic dendrite and nerve cell body of nicotinic receptor subclass that innervate the ganglia (Taylor 2012). The first neurotransmitter to be identified was ACh. It has also been detected in primitive plants, fungi, algae, protozoa, and bacteria, which indicates the wide distribution of cholinergic system in living organisms before its identification as a part of the nervous system (Greig et al. 2013). Acetylcholine was first synthesized in 1867. However, its biological importance was only discovered 50 years later (Bylund 2016). Cholinergic neurons are responsible for ACh synthesis, storage, and release. The ionotropic neuronal nicotinic acetylcholine receptors (nAChRs) and the muscarinic metabotropic receptors are the two primary receptors that transduced the signal of ACh (Picciotto et al. 2012). Moreover, cholinergic neurons control peripheral sympathetic and parasympathetic responses of the ANS. The "rest and digest" functionalities are mediated by the release of ACh by the parasympathetic terminals in the ANS (Tiwari et al. 2013). While in the central nervous system (CNS), ACh acts as a neuromodulator and neurotransmitter when released from cholinergic neurons and interneurons in the brain and spinal cord (Naser and Kuner 2018). In the ANS, ACh is released from postganglionic parasympathetic and sympathetic nerves and preganglionic neurons. In the somatic system, Ach is released at the neuromuscular junction. ACh is a quaternary ammonium parasympathomimetic compound; it produces a transient action due to its rapid destruction by cholinesterase enzyme, which limits the therapeutic application of ACh. Another limiting factor is the fact that acetylcholine possesses no specificity as it interacts with all nicotinic and muscarinic receptors making it particularly not a useful therapeutic agent (Bylund 2016). Nevertheless, ACh chloride is used topically after cataract surgeries to reduce a possible postoperative increase in intraocular pressure (Drudi et al. 2017). Many compounds that target the cholinergic system are currently used for the treatment of various conditions, and these compound include muscarinic antagonists (e.g., darifenacin, fesoterodine, oxybutynin, and tolterodine) for the treatment of urinary incontinence, scopolamine for motion sickness, and aerosol ipratropium for COPD management (Ehlert 2019a). Acetylcholinesterase inhibitors have also shown great therapeutic importance in the treatment of diseases such as myasthenia gravis, Alzheimer's

disease, and glaucoma (Potter and Kerecsen 2017). Nicotinic receptors stimulation has shown to improve cognitive functioning after several studies on animal models. Besides, some preclinical and clinical studies suggested that nicotinic receptors also play a role in depression, mood, and anxiety (Aboul-Fotouh 2015; Quik et al. 2015). In this chapter, we present an overview of the cholinergic system pharmacology, functions in the body, cholinergic and anticholinergic compounds, and their role in the medical field. Finally, an update on the cholinergic compounds currently used to treat various conditions as well as compounds under investigation are discussed.

3.1.1 Chemistry of Acetylcholine

Acetylcholine (2-acetoxy-*N*,*N*,*N*-trimethylethanaminium) is a small molecule with the chemical formula $CH_3COOCH_2CH_2N^+(CH_3)_3$ and a molecular mass of 146.2074 g/mol. It has a simple chemical structure composed of an ester of choline and acetic acid as shown in Fig. 3.1 (Tunç et al. 2016; Ueda et al. 2016).

ACh plays a crucial role in maintaining homeostasis in the brain by acting as a neurotransmitter in both the peripheral nervous system (PNS) and CNS. Choline acetyltransferase is the enzyme responsible for synthesizing acetylcholine from the substrates choline and acetyl CoA (coenzyme A) (Fig. 3.2) (Akaike and Izumi 2018). Pyruvate that results from glucose breakdown serves as the key source of acetyl CoA inside the neurons. The rate-limiting factor of ACh synthesis is the choline uptake by neurons. Choline acetyltransferase is found as a particulate membrane-bound enzyme in cholinergic neurons and as a soluble enzyme in the cytoplasm. Acetylcholine storage takes place in small synaptic spherical vesicles

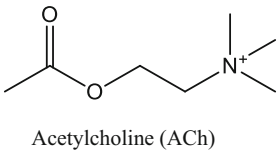


Fig. 3.1 Chemical structure of ACh

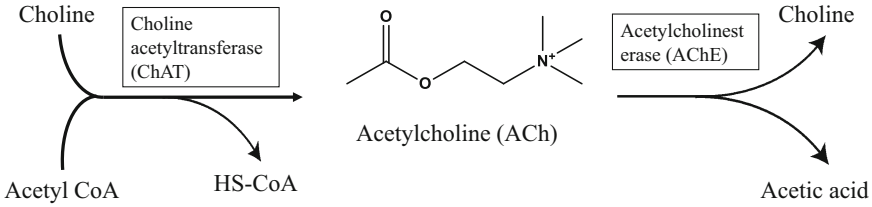


Fig. 3.2 Synthesis of acetylcholine by choline acetyltransferase (ChAT) using choline and acetyl coenzyme A, releasing coenzyme A (HS-CoA). Metabolism of acetylcholine is catalyzed by acetylcholinesterase (AChE) producing acetic acid and choline

where it is protected from being destroyed by the enzyme acetylcholinesterase (Browning 2010; Colzato et al. 2017).

3.1.2 Acetylcholine Functions

Acetylcholine (ACh) is a neurotransmitter that functions in both the PNS and the CNS. The ANS (sympathetic and parasympathetic) uses acetylcholine to generate a nerve impulse. In PNS, ACh mainly acts on the muscular system by activating muscle contraction after being released in the neuromuscular junction. In the central nervous system, ACh has various effects on cognitive functions, alertness, learning, and memory (Haerter and Eikermann 2016; Maurer and Williams 2017; Panus et al. 2009). When ACh gets released from somatic nerve endings into the neuromuscular junction, it causes the nicotinic ligand-gated ion channels to open, leading to sodium entrance into muscle cells. The sodium ions produce an excitatory postsynaptic potential (EPSP) that generates an action potential which eventually stimulates muscle contraction (Pappano 2018). Also, serum ACh has shown to produce vasodilation by activating vascular endothelial muscarinic receptors, which causes the release of endothelium-derived relaxing factors (EDRF) including prostanoids, endothelium-derived hyperpolarizing factor (EDHF), and nitric oxide (Tangsucharit et al. 2016). The chapter further presents the role of acetylcholine and cholinergic system together with few specific ligands and their clinical utility.

3.2 Cholinergic Receptors

Cholinergic receptors were named "cholinergic" due to their activation by Ach, and these receptors are mostly parasympathetic and transduce signal in the autonomic and somatic nervous system (Wehrwein et al. 2016). Based on the stimulation by muscarine or nicotine, cholinergic receptors are classified into muscarinic and nicotinic receptors, respectively. Nicotinic receptors are ionotropic ligand-gated channels, unlike muscarinic receptors, which are G-protein-coupled receptors (GPCRs) (Kruse et al. 2014; Papke 2014). Both receptor types are present within the CNS; however, nicotinic receptors are also found at the neuromuscular junction. The difference in signal transduction upon activation of the two receptor types results in distinctive physiological functions.

3.2.1 Muscarinic Receptors

Muscarinic receptors are mostly present at parasympathetic target organs, but it can also be found at specific sympathetic target organs such as the eccrine sweat glands that produce copious secretion for thermoregulation purpose, and in blood vessels of the skeletal muscles. In the PNS, muscarinic receptors are mainly found on autonomic effector cells, which are innervated by postganglionic parasympathetic nerves

Table 3.1 Classification of muscarinic receptors, their distribution and role

Subtype	Distribution	Role
M_1	Brain (cortex, hippocampus), salivary glands	Cognitive functioning and memory, salivary secretion
M_2	Heart, brain, smooth muscle	Regulation of heart rate and heart rate variability, behavioral flexibility
M_3	Smooth muscle, glands, eye	Smooth muscle contraction, gland secretion, iris contraction
M_4	Brain (forebrain, striatum)	Modulation of several important dopamine-dependent behaviors
M_5	Brain (substantia nigra), eye	Regulation of striatal dopamine release

Table 3.2 Muscarinic receptors subtypes localization and role in the brain

Subtype	Presence in brain	Functional aspect
M_1	All major areas of the forebrain including cerebral cortex, hippocampus, thalamus, and corpus striatum. Cellular localization at striatum nigrum neurons and glutamatergic pyramidal neurons	Highly responsible for cholinergic functions. Synaptic plasticity, learning and memory (cognition), neuronal differentiation during development, and neuronal excitability
M_2	Throughout the brain especially in hippocampus and neocortex. Abundant in non-cholinergic neurons in these areas	Inhibitory effect on dopaminergic action. Antinociceptive effect reported
M_3	Highly expressed in hypothalamus, lesser expression in hippocampus	Major role in food intake, body growth
M_4	Major presence in corpus striatum	An important role in psychosis, an involvement in pathology of Parkinson's disease, inhibits D1 receptor of dopamine signaling
M_5	Pars compacta of substantia nigra, ventral tegmental region	Rewarding effect of abusive drugs

(Tiwari et al. 2013). Muscarinic receptors are classified into five subtypes; each subtype distribution and role are listed in Table 3.1 (Glavind and Chancellor 2011).

These five muscarinic receptor subtypes are expressed throughout the human brain and are involved in various functional processes, such as learning, memory, attention, sleep-wake cycles, sensorimotor processing, and arousal (Lebois et al. 2018). Table 3.2 summarizes the details concerning muscarinic receptors distribution and function in the brain (Verma et al. 2018). Muscarinic receptors are GPCRs in which M_1, M_3, and M_5 are Gq/11 G-proteins which mediate excitatory neuromodulatory acetylcholine actions while M_2 and M_4 receptors are Gi/o G-proteins that produce inhibitory neuromodulatory acetylcholine actions (Brown 2019; Felder 1995; Lebois et al. 2018).

All five muscarinic receptor subtypes express throughout the mammalian brain. However, different regions in the brain contain different receptor subtype concentrations exemplified by M1 and M2 receptors. In the major forebrain areas,

the expression of these receptors is comparatively higher to that of other subtypes. M1 receptor is the highest muscarinic receptor expressed in the cortex, striatum, and hippocampus. M2 receptor is highly expressed in the occipital cortex and nucleus basalis, while M4 receptor is more prominent within caudate putamen and the striatum. The least muscarinic receptor expressed compared to the other subtypes is M5 receptor (Carruthers et al. 2015; Scarr et al. 2016).

3.2.2 Nicotinic Receptors

Nicotinic acetylcholine receptors (nAChRs) are hetero- or homo-pentameric structured ligand-gated ion channels. Nicotinic receptors possess an essential role in various biological processes such as learning, memory, locomotion, anxiety, and attention. Recent researches showed that nicotinic receptors play a role in regulating inflammation by α7 nicotinic acetylcholine receptors activation in macrophages (Egea et al. 2015). The nAChRs are mainly divided into two subclasses: neuronal and muscular. In the neuromuscular junctions, the muscular nAChRs contribute to the neuromuscular transmission, and the neuronal nAChRs are located in both the PNS and the CNS. The nAChRs have a large number of homologous subunits that can form many different combinations of pentamers which produce various receptors with diverse functionalities (Kulbatskii et al. 2018).

Composition of nicotinic acetylcholine receptors (nAChRs) consists of five subunits surrounding a water-filled pore. Neuronal nAChRs are classified based on the presence of adjacent cysteine groups within the extracellular part of the α subunits into two types: the alpha and beta. The alphas are α2, α7, α9, and α10, while the betas are β2–β4 (Dani 2015; Fasoli and Gotti 2015). There have been 17 subunits of nAChRs identified (α1–10, β1–4, γ, δ, and ε). All those subunits were found in mammals except for α8, which is found in avian species. In muscular nicotinic receptors, the binding sites are at the interfaces of the δ or γ subunits and the α subunit, while in neuronal nicotinic receptors, the binding sites are found at the interfaces of the β subunit and α subunit or at two adjacent α subunits. Alpha subunits are the only ones containing two cysteine residues near the binding site of acetylcholine (Lukas et al. 1999; Melroy-Greif et al. 2016). The characteristics of nAChRs are listed in Table 3.3 (Akaike and Izumi 2018; Dani 2015).

In the brain, the predominant subtypes expressed are α7 subunit containing receptors which are either homo- or heteromeric. Different subtypes of nAChRs have shown the ability to modulate synaptic transmission in various brain parts, including thalamic nuclei, cortical interneurons, the visual cortex, and supraoptic nuclei. nAChR subtypes are distributed differently in the CNS (Table 3.4) (Akaike and Izumi 2018; Dineley et al. 2015).

Table 3.3 Nicotinic acetylcholine receptors characteristics

Subtype	Primary subunit composition	Ca^{2+} permeability	Major location
α1	$(\alpha1)_2\beta1\gamma\delta$, $(\alpha1)_2\beta1\delta\varepsilon$	Low	Neuromuscular junction
α2	α2β2, α2β4	Low	CNS
α3	α3β2, α3β4	Low	CNS, autonomic ganglion
α4	$(\alpha4)_3(\beta2)_2$, $(\alpha4)_2(\beta2)_3$	Low	CNS
α5	α3β2α5, α3β4α5, $(\alpha4)_2(\beta2)_2\alpha5$	High	CNS, autonomic ganglion
α6	α6β2β3, α6α4β2β3	High	CNS
α7	$(\alpha7)_5$	High	CNS, non-neuronal cells
α8	$(\alpha8)_5$	High	CNS
α9	$(\alpha9)_5$, α9α10	High	Mechanosensory hair cells
α10	α9α10	High	Mechanosensory hair cells

3.3 Pharmacology of Cholinergic Receptors

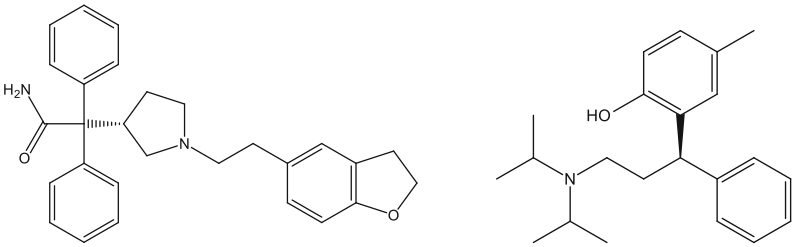
Cholinergic ligands provide a wide range of therapeutic applications, many are currently on the market for various medical conditions including scopolamine, neostigmine, tacrine, oxybutynin, donepezil, tolterodine, ipratropium, ambenonium, and edrophonium (Bukala et al. 2019; Potter and Kerecsen 2017; Ramaswamy et al. 2018; Vozmediano-Chicharro et al. 2018). Other compounds are under investigation for further therapeutic options in the future such as xanomeline, cevimeline, tazomeline, benzyl quinolone carboxylic acid (BQCA), and bispyridinium oximes (Antonijevic et al. 2016; Lorke and Petroianu 2018; Verma et al. 2018).

3.3.1 Muscarinic Receptor

Muscarinic acetylcholine receptors play a significant role in modulating physiological processes related to mental health, respiration, salivation, excretion, and motion perception. In recent years, investigations on the mAChR ligands have accelerated the discovery of various novel chemical entities and some were approved for certain conditions, including psychosis (Foster and Conn 2017), Alzheimer's disease (Bradley et al. 2017), asthma, motion sickness, and incontinence. An ideal therapeutic agent for these diseases should not exert any adverse effects that can be caused by nonspecific interaction with other mAChR subtypes. However, the orthosteric sites of the mAChR subtypes have high amino acid sequence similarities, which explains the current difficulties in designing drugs that target specific receptor subtype (Korczynska et al. 2018). For example, darifenacin and tolterodine (Fig. 3.3) are mAChR antagonists that treat incontinence by targeting M3 receptors, often cause adverse effects such as dry mouth by interacting with glandular M1 and M3 receptors, and increase heart rate due to effects on M2 receptors and increase drowsiness (Glavind and Chancellor 2011; Korczynska et al. 2018; Naicker et al.

Table 3.4 Distribution of nicotinic receptors in CNS

α2	α3	α4	α5	α6	α7
Cortex		Cortex	Cortex		Cortex
Hippocampus	Hippocampus	Hippocampus	Hippocampus		Hippocampus
		Striatum	Striatum	Striatum	
Amygdala		Amygdala			Amygdala
		Thalamus			
Hypothalamus		Hypothalamus			Hypothalamus
	Substantia nigra	Substantia nigra	Substantia nigra	Substantia nigra	Substantia nigra
	Cerebellum	Cerebellum			Cerebellum
	Spinal cord	Spinal cord			Spinal cord



Darifenacin Tolterodine

Fig. 3.3 Chemical structures of darifenacin and tolterodine

2017). Such adverse effects of muscarinic receptor antagonists have reduced their convenience as treatment options; nevertheless they are highly active compounds (Korczynska et al. 2018) with scope for further structural optimization.

In CNS disorders, the majority of studies are focused on compounds targeting M1 and M4 mAChRs subtypes. On the other hand, M5 mAChRs expresses itself in highly variable regions of therapeutic interest, and it is the least mAChR expressed in the brain by 2% of the total mAChRs population. Despite the fact, M5 receptors are the only subtype with an identifiable mRNA transcript in dopamine-containing neurons. This factor makes it a viable target for drug addiction treatment by supporting the hypothesis that mAChR subtype may be responsible for regulating the transmission of midbrain dopamine and reward mechanisms (Berizzi et al. 2016; Gunter et al. 2018).

3.3.1.1 Muscarinic Agonists

Arecoline (methyl-1,2,5,6-tetrahydro-1-methyl-nicotinate), an alkaloid, is considered the primary active constituent of A. *catechu.* In recent years, many studies have been carried out to further investigate arecoline's pharmacological and toxic effects (Bhat et al. 2017). Several pharmacological activities produced by arecoline have been reported such as anti-parasitic effects, in addition to effects on the digestive, cardiovascular, nervous, and endocrine systems. The main toxic effects of arecoline are genotoxicity, oral squamous cell carcinoma, and oral submucous fibrosis. However, arecoline has an agonistic effect on muscarinic receptors which upon further investigation have shown to reverse memory impairment and scopolamine-induced memory loss in male rats model of Alzheimer's disease (Kuca et al. 2016; Liu et al. 2016).

In preclinical studies, muscarinic receptor agonists have exhibited atypical antipsychotic effects. Xanomeline is a mAChR agonist that can reverse certain dopamine-mediated behaviors. Cevimeline, milameline, sabcomeline, and xanomeline have all progressed into different clinical development stages for possible treatment of Alzheimer's disease (AD) and schizophrenia. Phase II clinical trials of xanomeline have demonstrated its effect and efficacy for various cognitive symptoms domains such as hallucinations and behavioral disturbances associated

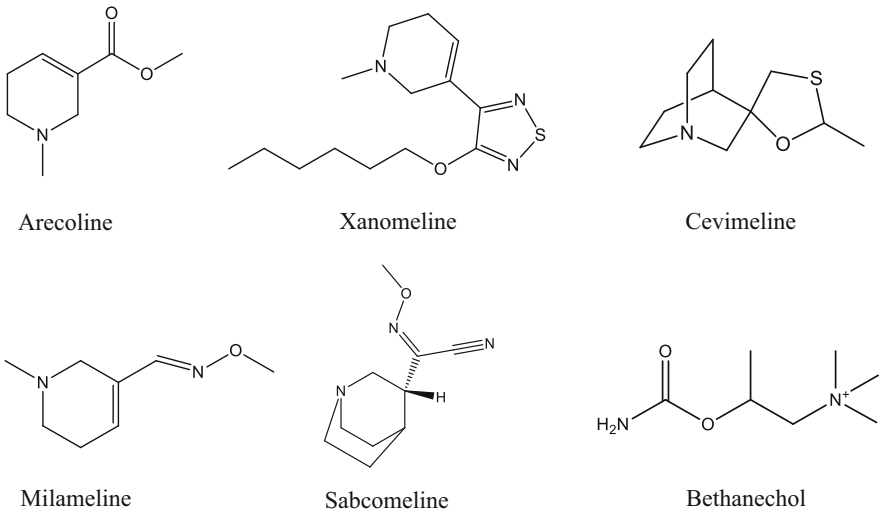


Fig. 3.4 Structures of mAChR agonists

with AD (Congreve et al. 2018; Sivaraman et al. 2019). Figure 3.4 depicts the chemical structures of some mAChR agonists mentioned in this section.

Bethanechol is a cholinergic agonist that acts on M1 and M2 muscarinic receptors. It is a methyl analogue of acetylcholine and used clinically for women with underactive bladder (UAB). Stimulation of mAChRs at the neuromuscular junction of smooth muscle with bethanechol can induce the contraction of the detrusor muscle, which eventually improves bladder emptying. However, various clinical evidence supporting bethanechol efficacy for UAB is limited and poor (Sivaraman et al. 2019; Gaitonde et al. 2018). Bethanechol has also been investigated in the treatment of tracheomalacia. However, further trials are required to ensure the safety and efficacy of bethanechol as a treatment regimen (Bass et al. 2018).

BuTAC ([5*R*-(exo)]-6-[4-butylthio-1,2,5-thiadiazol-3-yl]-1-azabicyclo-[3.2.1]-octane) is a muscarinic receptor agonist that produces full agonist activity on M2 receptor and partial agonist activity on both M1 and M4 receptors. Contrarily, it exhibits full antagonist activity on M3 and M5 receptors. BuTAC has shown to produce antipsychotic activity on schizophrenic animal models (Andersen et al. 2015; Watt et al. 2013). Another muscarinic agonist is cevimeline, which activates both M1 and M3 receptors, and it was originally developed for the treatment of Alzheimer's-type senile dementia. Currently, this compound is used to treat xerostomia, which is a condition that causes numerous disorders in oral functions, including swallowing, taste, speech, and mastication. Other than pilocarpine, cevimeline is the only agent available for the therapeutic intervention of xerostomia (Kishimoto et al. 2016; Mitoha et al. 2017). Table 3.5 summarizes various

Table 3.5 Muscarinic receptor agonists in preclinical and clinical phases

Agonist	Muscarinic receptor	Status	Therapeutic application	Reference
AF102B, AF150, AF267B, AF292	mAChR	*In vivo* passed	Alzheimer's disease	Ferreira-vieira et al. (2016); Fisher (2012); Sehgal et al. (2018)
77-LH-28-1	mAChR	*In vivo* passed	Alzheimer's disease and schizophrenia	Langmead et al. (2008a, b); Zhao et al. (2019)
VU0357017 and VU0364572	M1 mAChR	*In vivo* passed	Cognitive deficits (schizophrenia, Alzheimer's disease)	Digby et al. (2010); Lebois et al. (2017); Rogers and Gray (2012)
Xanomeline	M1/M4 mAChR	Phase 2 trials	Schizophrenia	Bender et al. (2017); Khatwal et al. (2014)
Arecoline	M1, M2, M3, M4 mAChR	*In vivo*	Wide pharmacological activities (nervous, cardiovascular, endocrine, digestive system, and anti-parasitic effects)	Langmead et al. (2008a, b); Liu et al. (2016)
CI-1017	M1 mAChR	*In vivo*	Cognitive deficits (Alzheimer's disease and schizophrenia)	Brady et al. (2008); Weiss et al. (2000)
Cevimeline	M3 mAChR	Passed in *in vitro* and *in vivo* study but failed in clinical trial	Primary Sjögren's syndrome (dry mouth)	Garlapati et al. (2019); Langmead et al. (2008a, b)
Tazomeline, talsaclidine, milameline, and arecoline	M1 mAChR	Passed in *in vitro* and *in vivo* study but failed in clinical trial	Schizophrenia	Langmead et al. (2008a, b)
AF102B	mAChR	Clinical trial phase 2/3	Alzheimer's disease	Digby et al. (2010); Kumar and Kumar (2018)
SB202026	M1, M2, M3 mAChR	Discontinued due to cholinergic adverse effects	Alzheimer's disease	Digby et al. (2010)
Benzyl quinolone carboxylic acid (BQCA)	M1 mAChR	*In vivo* passed	Alzheimer's disease	Digby et al. (2010); Hepnarova et al. (2018)

(continued)

Table 3.5 (continued)

Agonist	Muscarinic receptor	Status	Therapeutic application	Reference
MK-7622	M1 mAChR	Discontinued after phase 1 trials	Alzheimer's disease	Cummings et al. (2016)
AC-260854	M1 mAChR	*In vivo* study	Schizophrenia and Alzheimer's disease	Berizzi et al. (2016)
VU0152099, VU0152100	M4 mAChR	*In vivo* study	Alzheimer's disease	Lebois et al. (2017)
TBPB	M1 mAChR	*In vivo* study	Schizophrenia	Conn et al. (2009)

muscarinic receptor agonists in different developmental stages (Berizzi et al. 2016; Cummings et al. 2016; Verma et al. 2018).

3.3.1.2 Muscarinic Antagonists

As discussed earlier, muscarinic receptors are present in autonomic ganglia, peripheral tissues, and various regions of the brain innervated by parasympathetic nerves. Antagonism of muscarinic receptors can produce therapeutic effects in several clinical conditions. For instance, during eye examination, muscarinic receptor antagonist is applied topically to the eye inducing relaxation in the pupillary constrictor muscles and circular ciliary, which results in pupil dilation, thereby making it easier to view the retina and measure refractive errors of the lens (de Linder Henriksen et al. 2019; Yi et al. 2015). Muscarinic antagonists have also been considered for the treatment of COPD and asthma due to the constricting effect in vagal tone in the pulmonary airways (Busse et al. 2016; Oba et al. 2016). Muscarinic antagonists are sometimes used to treat diarrhea associated with inflammatory bowel conditions and dysenteries due to their ability to reduce gastrointestinal tract mobility (Aleem and Janbaz 2018). Muscarinic antagonists (trospium, oxybutynin, tolterodine, solifenacin, fesoterodine, and darifenacin) can also relieve symptoms of frequency, urgency, and incontinence of an unstable bladder, which reduces micturition frequency, and are currently used for the treatment of overactive bladder and urge incontinence (Andersson 2019). Muscarinic antagonists inhibit the vestibular apparatus of the inner ear, which reduces motion sickness. Scopolamine has been used topically as a patch to be placed on the skin behind the ear for the treatment of motion sickness (Zhang et al. 2016). Muscarinic antagonists can also act as antidotes in cases such as muscarine poisoning from mushrooms, insecticides poisoning, and war gases that contain cholinesterase (ChE) inhibitors. Pharmacokinetic properties of the muscarinic antagonist and the route of administration are the main factors affecting which muscarinic antagonist is of choice to treat each health condition. Table 3.6 summarizes the currently used muscarinic antagonists (Ehlert 2019a, b).

Long-acting muscarinic receptor antagonists (LAMA) such as tiotropium has been used for years in the management of COPD. LAMAs antagonize

Table 3.6 Muscarinic receptor antagonists that are currently used and their therapeutic applications

Agent	Use
Tertiary amines	
Atropine	Treatment of anticholinergic poisoning
Scopolamine	Treatment of motion sickness
Homatropine	Mydriatic and cycloplegic; for mild uveitis
Dicyclomine	Alleviates GI spasms, pylorospasm, and biliary distention
Darifenacin	Treatment of urinary incontinence
Fesoterodine	Treatment of urinary incontinence
Oxybutynin	Treatment of urinary incontinence
Tolterodine	Treatment of urinary incontinence
Oxyphencyclimine	Antisecretory agent for peptic ulcer
Cyclopentolate	Mydriatic and cycloplegic
Tropicamide	Mydriatic and cycloplegic
Benztropine	Treatment of Parkinson's and Huntington's diseases
Trihexyphenidyl	Treatment of Parkinson's and Huntington's diseases
Pirenzepine	Antisecretory agent for peptic ulcer
Quaternary ammonium derivatives	
Methylatropine	Mydriatic, cycloplegic, and antispasmodic
Methylscopolamine	Antisecretory agent for peptic ulcer, antispasmodic
Ipratropium	Aerosol for COPD
Glycopyrrolate	Antisecretory agent for peptic ulcer, antispasmodic
Tolterodine	Treatment of urinary incontinence
Propantheline	GI antispasmodic
Tiotropium	Aerosol for COPD

parasympathetic bronchoconstriction in the airways reversing airflow obstruction. Recently, several new agents have been developed and studied for COPD management such as aclidinium bromide, umeclidinium bromide, and glycopyrronium bromide. For COPD management, many LAMAs are under development, and some are already available, including bencycloquidium, V0162, CHF 5407, AZD8683, AZD9164, and TD-4208 (Mark A. Mastrodicasa et al. 2017). Tiotropium was the only LAMA in clinical use for almost two decades, and it was the first LAMA prepared as daily single-dose treatment. Tiotropium exerts its anticholinergic effects by binding to M1 and M3 receptors (Alvarado-Gonzalez and Arce 2015). Tiotropium has shown to decrease hospitalization, symptoms, and exacerbations and improve health status in COPD patients. It also showed an improvement in the effectiveness of pulmonary rehabilitation. As compared to salmeterol, tiotropium has proven to show a higher increase in the time to first exacerbation occurrence, thus decreasing exacerbation rate in general (Han and Lazarus 2016).

3.3.1.3 Allosteric Modulators

Many efforts were made to produce specific M1 and M4 receptor targeting agents to maintain therapeutic efficacy and reduce adverse side effects (Foster et al. 2014; Jones et al. 2012). The targeting of a specific subtype of muscarinic receptors is difficult due to the highly conserved orthosteric binding site between the receptor subtypes. Allosteric modulators have been developed to overcome this problem since the allosteric binding sites are considered less conserved among the receptor subtypes. Allosteric modulators show higher selectivity and can modulate receptor activation either by acetylcholine or other agonists, or it can activate the receptor. Allosteric activators can either be allosteric agonists or positive allosteric modulators (PAMs). Allosteric agonists act on the allosteric site of the receptor, hence activating the receptor without the presence of acetylcholine, while PAMs cannot activate the receptor directly but only potentiate the activation by acetylcholine (Jakubik and El-Fakahany 2016). One compound can also act as both an allosteric agonist and PAM. However, since allosteric mechanisms are affected by cooperativity factors and affinity, they present challenges and practical implications for drug discovery and development (Conn et al. 2014; Yohn and Conn 2018).

Positive Allosteric Modulators

As mentioned before, PAMs possess higher selectivity than muscarinic agonists because the allosteric sites of the muscarinic receptors have higher sequence divergence as compared to orthosteric site. Generally, PAMs are considered as compounds with an excellent safety profile because they do not cause the induction of independent agonistic activity, which means they cannot exert their effect without the presence of the endogenous agonist. Thiochrome is a natural compound that results from the oxidation and metabolism of thiamine. Thiochrome acts as a PAM to M4 muscarinic receptors (Takai and Enomoto 2018). Recently, there has been much successful development of selective mAChR PAMs, especially M1 and M4 selective compounds that have shown promising results including excellent brain penetration and pharmacokinetic profiles. After the discovery of the M4 selective PAMs (VU10010 and LY2033298), many efforts were directed towards developing M4 selective compounds. M4 mAChR possesses a potential role in dopaminergic systems' regulations, which are highly related to the positive symptoms of schizophrenia, and these receptors are also significant in reducing negative symptoms and cognitive impairment in AD patients (Chan et al. 2008; Foster and Conn 2017). M4 receptor PAMs are also considered as a potential therapeutic approach for drug addiction, Huntington's disease, and Parkinson's disease (PD). The thieno[2,3-*c*] pyridazine analogues received most of the attention due to the limited chemical diversity of M4 PAMs discovered, and one of the promising analogue for further preclinical trials is VU6005806/AZN-00016130 (Engers et al. 2019). Recently, the discovery of selective M1 receptor PAMs showed their ability to enhance both hippocampal and prefrontal cortex-dependent forms of cognitive functions in non-human primates and rodents, which agrees with many studies suggesting that activating M1 receptors produce cognitive-enhancing effects (Digby et al. 2012; Gould et al. 2015; Lange et al. 2015; Vardigan et al. 2015). For example, MK-7622,

a potent and selective M1 PAM, is under preclinical and clinical trials to reduce cognitive dysfunction in AD patients (Uslaner et al. 2018).

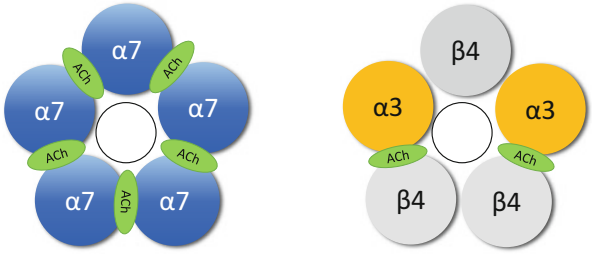
Negative Allosteric Modulators

Allosteric ligands for individual muscarinic receptors subtypes are currently under investigations. Allosteric ligands exhibit selective cooperativity between the ligand and the receptor at the receptor after targeting allosteric site(s) (Berizzi et al. 2018). Lately, ML375 (VU0483253) became the first negative allosteric modulator (NAM) that displayed selectivity for M5 muscarinic receptor (Gentry et al. 2013). Studies have shown that there is a functional interlink between M5 receptor and addiction/reward pathways, and further substantiated by the attenuation of cocaine addiction liabilities in a rodent model with cocaine addiction by ML375 (Gunter et al. 2018).

However, ML375's elimination half-life is excessively long, with approximately 80 h in rats. An equipotent M5 receptor NAM (VU6008667) has been developed later with a more appropriate shorter elimination half-life (2.3 h) in rats, excellent selectivity, and high CNS penetration (Bender et al. 2019; Gentry et al. 2013; McGowan et al. 2017).

3.3.2 Nicotinic Receptor

The nAChRs are a part of the "Cys-loop" receptor superfamily, which are multi-subunit transmembrane receptors. nAChRs mediate excitatory neurotransmission in autonomic ganglia, specific synapses in both the spinal cord and brain, and at the vertebrate neuromuscular junction (Albuquerque et al. 2009; Alcaino et al. 2017). These nAChRs generate complex calcium signals that influence neuronal processes and signaling molecules. The combination of the nicotinic receptor subtype, Ca^{2+} signaling pathway, neuronal type, and developmental stage all together translate the stimulation of the nicotinic receptors into a neuronal response and eventually into a physiological outcome (Dajas-Bailador and Wonnacott 2004; Kabbani and Nichols 2018). As stated before, nicotinic receptors are ligand-gated ion channels, which are pentamers assembled from five subunits either as homomeric or heteromeric pentamers (Fig. 3.5) (Improgo et al. 2010; Millar and Gotti 2009; Wu et al. 2016).



Homomeric nAChR (e.g., α7)

Heteromeric nAChR (e.g., α3β4)

Fig. 3.5 Schematic representation of homomeric and heteromeric nicotinic receptors with the ACh binding site location

During the inactivated state, the pentamers exist as a funnel shape structural motif with a central core, and it remains closed. When activated by ACh, the channel undergoes a conformational change in all subunits, and the core opens to allow Na^+ and K^+ flow according to electrochemical gradients leading to cellular response (Svorc 2018). The nAChRs in the CNS are responsible for regulating various processes including neuronal integration, neurotransmitter release, and cell excitability and can also affect certain physiological functions including cognition, pain, arousal, sleep, and mood (Rahman et al. 2015).

Nicotine, a prototypic tobacco alkaloid, had received interest as a potential therapeutic compound 30 years ago, which was the time when tobacco smoking was discovered to affect the performance of a particular cognitive task positively. Further investigations substantiated that nicotine administration indeed affects pro-cognitive performances (Terry and Callahan 2018; Heishman et al. 1994; Sherwood 1993). Nowadays, growing pieces of evidence corroborate nicotine's effect in enhancing cognitive function and information processing in both experimental animals and human nonsmokers (Levin et al. 2013). Additionally, other effects of nicotine have been reported from *in vivo* and *in vitro* studies such as sustained attention, working memory (Newhouse 2019) and recognition memory in rats (Nikiforuk et al. 2015), attention, processing of visual information, and short term memory in humans. *In vivo* and *in vitro* studies have also shown that nicotine possesses neuroprotective activity in specific disease models which proposes that nicotine can be used not only for symptomatic control but also to produce effects that enhance cognition in neurodegenerative diseases such as AD and PD (Newhouse 2019). However, nicotine has limited therapeutic potentials for psychiatric and neurologic conditions due to several factors including cardiovascular side effects, abuse potentials, and its relatively short half-life. Nicotine transdermal patches used for smoking cessation have shown no cardiovascular side effects while exhibiting improved cognition in patients with mild cognitive impairment (MCI) (Newhouse et al. 2012). Various types of nicotine formulations have been developed for smoking cessation such as tablets, patches, nasal sprays, gums, inhalers, and lozenges to reduce cravings and withdrawal symptoms (Shahab et al. 2013). Nicotinic acetylcholine receptor ligands other than nicotine have various therapeutic potentials which are listed in Fig. 3.6 (Terry and Callahan 2018).

Nicotinic cholinergic receptors (nAChRs) are pentameric proteins that are activated by acetylcholine at central and peripheral synapses. Neuronal nicotinic receptors in the brain, autonomic ganglia, adrenal gland, and immune cells are composed of either α subunits or both α and β subunits. One of the primary phenomena that nicotinic receptors exhibit upon continued nicotinic agonist exposure is desensitization, which occurs in both muscular and neuronal nicotinic receptors.

3.3.2.1 Nicotinic Agonists

Nicotinic agonists are any compound that can mimic ACh action on nAChRs. Nicotine (Fig. 3.7) is the most popular nicotinic agonist. After the discovery of the positive effects of nicotine, the approach to investigate and design more nicotinic

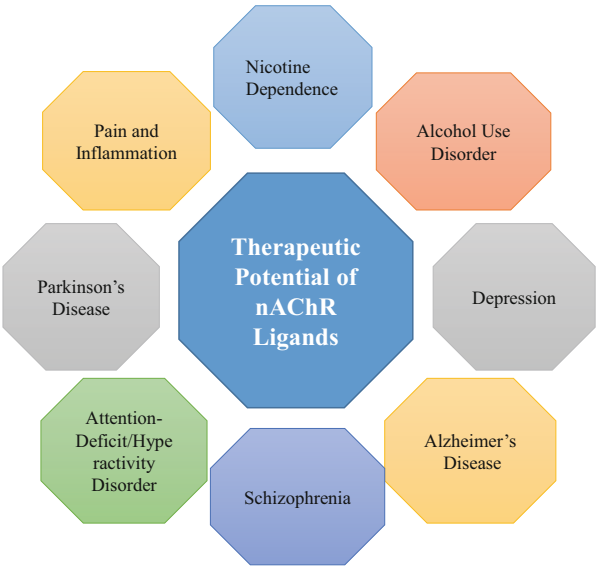


Fig. 3.6 Potential therapeutic applications of nicotinic acetylcholine receptors (nAChRs) ligands

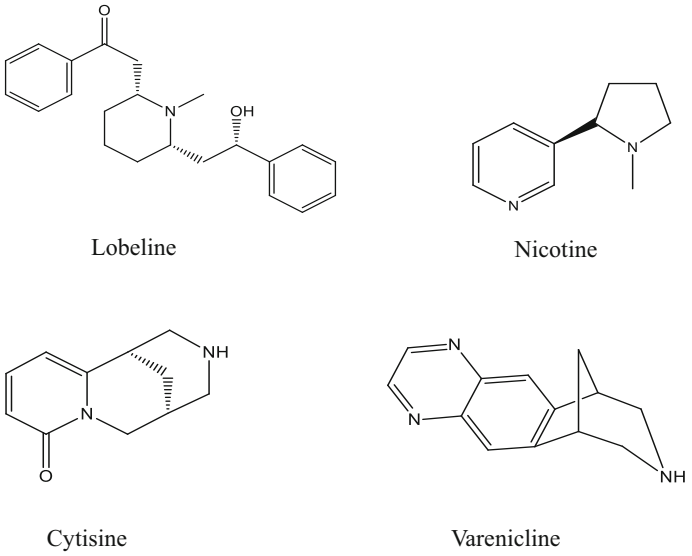


Fig. 3.7 Chemical structures of nicotinic agonists

agonists started in the early 1990s. ABT-418 was one of the first nicotinic agonists developed by Abbott Labs. Further investigations of ABT-418 have shown cognitive-enhancing effects that are three to ten times more potent than nicotine. Clinical trials on ADHD patients with ABT-418 have shown promising results in

reducing ADHD symptoms (Buccafusco 2004; Takechi et al. 2016). Lobeline (Fig. 3.7) like nicotine is a naturally occurring alkaloid and has similar effects with less potency as compared to nicotine. Both nicotine and lobeline are full agonists; they lead to nicotinic receptor activation in sympathetic and parasympathetic ganglia, on immune cells, in the brain, and at the adrenal medulla which leads to a wide range of physiological effects. Lobeline has been investigated for drug abuse treatment such as cocaine, alcohol abuse, opioid, and methamphetamine (Martin et al. 2018; Zheng and Crooks 2015). Both the cytisine (natural plant alkaloid) and varenicline (a synthetic compound) act as full agonists on (α3β4 and α7) nicotinic receptor subtypes and as partial agonists on (α4β2) receptors. Both the compounds were investigated for smoking cessation (Walker et al. 2018).

Nicotinic receptors are involved in various behavioral and cognitive functions, which makes nicotinic ligands good candidates for the treatment of conditions with cognitive dysfunction, such as schizophrenia. Postmortem studies of schizophrenic patients have shown that both protein expression and binding levels of α7 nicotinic acetylcholine receptor (α7 nAChR) were decreased in prefrontal cortex and hippocampus (cognition-related areas). A study was conducted on sub-chronic schizophrenic rats, by using α7 nAChR partial agonist (A-582941) for treatment. Results showed improvement in cognitive dysfunctions and social deficits on spatial and visual memory (Unal and Aricioglu 2017; Verma et al. 2018).

ABT-126, an α7 nAChR agonist, showed high potency and affinity in mouse, rat, and human in nonclinical studies. 25 mg once daily of ABT-126 in a phase 2a study resulted in cognitive improvement in subjects with both mild and moderate Alzheimer's dementia (AD) (Gault et al. 2016; Valle's et al. 2014).

3.3.2.2 Nicotinic Antagonists

Nicotinic antagonists block acetylcholine action at the autonomic ganglia, adrenal medulla, and neuromuscular junction. Neuromuscular blockers act on muscle-type nicotinic receptors, and ganglionic blockers act on neuronal ganglionic nicotinic receptors. Neuromuscular blockers are categorized based on the mechanisms of action and their use into two categories: depolarizing and non-depolarizing agents (Ding 2017). Neuromuscular blockers produce skeletal muscle relaxation which makes them useful in specific clinical situations such as electroconvulsive therapy (ECT), scoping procedures, endotracheal intubation, orthopedic procedures, and as a surgical adjunct (Keating 2016). Ganglionic blockers, on the other hand, can be used to control hypotension during procedures and for the treatment of hypertensive emergencies. Non-depolarizing neuromuscular blockers include benzylisoquinolines (doxacurium, mivacurium, atracurium), aminosteroids (rocuronium, pipecuronium, pancuronium), and natural alkaloids like d-tubocurarine. All these compounds are considered relatively large and contain multiple rings, making them bulky molecules that block acetylcholine from binding to the receptor. Besides, these compounds are competitive antagonists that produce flaccid paralysis and increasing acetylcholine concentration at synapsis by acetylcholinesterase inhibitors reverses their effect (D'Souza and Johnson 2019; Smetana et al. 2017).

Similarly, depolarizing neuromuscular blockers such as succinylcholine that binds and activates the receptor causes muscle relaxation that also leads to flaccid paralysis. Although succinylcholine activates the receptor, it resists hydrolysis by acetylcholinesterase, remaining bound to the receptor for a longer time than acetylcholine leading to blockage (Bertrand and Terry 2018; Hager and Burns 2018). Trimethaphan and mecamylamine are ganglionic blockers with different structures and mechanisms of action. While mecamylamine is a voltage-dependent noncompetitive antagonist that prevents activation by blocking the open cation channel, trimethaphan is a competitive antagonist that competes with ACh for receptor binding. Trimethaphan is used rarely for hypertensive emergencies during surgeries to produce controlled hypotension (Petrakis et al. 2018; Sear 2019).

3.3.2.3 Nicotinic Allosteric Modulators

Acetylcholine binds at a site on the interface of two subunits from the extracellular part of the nicotinic receptors. Many compounds were discovered that compete on the same site and exert either an agonistic or antagonistic effect. The site where acetylcholine binds is called the orthosteric binding site. However, researchers have identified compounds that show a modulatory effect on nicotinic receptors by binding to different sites other than the orthosteric-binding site, including the transmembrane domain. These compounds are termed as allosteric modulators, and the sites where they bind to are called allosteric sites (Abdel-Magid 2015; Chatzidaki and Millar 2015).

Allosteric modulators do not all exert the same effect, as mentioned in Sect. 3.3.1.3. The allosteric modulators that initiate an agonist-mediated response are termed as positive allosteric modulators (PAMs), while negative allosteric modulators (NAMs) are those that inhibit the effects of agonist activation. PAMs are different from allosteric agonists, as allosteric agonists can activate the receptor directly when binding to the allosteric site. Conversely, neutral allosteric ligands (NALs) do not cause changes in the receptor activity or its binding ability to the ligands (Cecchini and Changeux 2015; Chatzidaki and Millar 2015; Gentry et al. 2015).

PAMs binding to the receptor causes potentiation of agonist-evoked responses, which means they neither activate the receptor nor compete with acetylcholine binding. PAMs acting on α7 nicotinic receptors show therapeutic potentials for the treatment of inflammatory and neurological disorders. PAMs possess advantageous characteristics that make them more promising as compared to exogenous agonists, such as higher target selectivity, less tolerance due to desensitization, and their ability to maintain the spatial and temporal characteristics of the endogenous activation processes (Corradi and Bouzat 2016; Uteshev 2014). Based on the effect on macroscopic currents of receptors, PAMs are classified into two types, type I and II. Type I PAMs such as NS-1738, genistein, 5-HI, and ivermectin do not significantly affect current decay but enhance the agonist-induced peak currents. Type II PAMs, on the other hand, show slow desensitization onset and reactivate receptors that are desensitized (Bouzat and Sine 2018; Corradi and Bouzat 2016).

3.3.3 Cholinesterase Inhibitors

There are two types of cholinesterases in the body, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), which differ in substrate specificity and their distribution within the body. While AChE terminates the action of ACh at synapses in the nervous system, BuChE concentrates in non-neuronal sites such as liver and plasma. BuChE is also responsible for metabolizing certain drugs (e.g., ester-type local anesthetics, succinylcholine) (Colovic et al. 2013; Kishore et al. 2012; Mehrpouya et al. 2017).

Most compounds used clinically to inhibit cholinesterase work on both the enzymes without discrimination. Cholinesterase inhibitors are classified into two main types based on the nature of the compound-enzyme binding, *viz.* reversible and irreversible. Reversible compounds can either be covalent or non-covalent inhibitors. Edrophonium is a non-covalent inhibitor that binds reversibly to the anionic part of the acetylcholinesterase enzyme. Drug-enzyme bond characteristics mainly determine the duration of these compounds. For instance, edrophonium binds weakly to the enzyme and is rapidly cleared by the kidney, which results in a short duration of action that is approximately 10 min long (Romano et al. 2017; dos Coelho et al. 2018). Other examples of non-covalent ChE inhibitors are donepezil and tacrine used for the treatment of AD. These two compounds possess a longer duration of action due to their partition into lipids and their higher affinities (Birks and Harvey 2018; Sameem et al. 2017). Carbamic acid ester derivatives such as neostigmine and physostigmine are covalent inhibitors, sometimes termed as carbamate inhibitors. After their binding to the AChE enzyme, they get hydrolyzed, which results in carbamylation of the serine residue in the active site of the enzyme (Arens and Kearney 2019; Lauretti 2015).

On the other hand, irreversible ChE inhibitors act on the serine group in the active site of the enzyme and phosphorylate it. Nerve gases such as tabun, sarin, and soman are examples of irreversible ChE inhibitors. Other examples include insecticides (malathion, parathion) and therapeutic agents such as isofluorophate and echothiophate referred to as organophosphate or organophosphorus ChE inhibitors. The reason why these compounds are considered irreversible is that the resultant phosphorylated enzyme is exceptionally stable, which means that the dephosphorylation process can take hours to occur. The possibility of dephosphorylation of phosphorylated enzyme by secondary and tertiary alkyl-substituted phosphates (soman and isofluorophate) cannot be achieved to restore the enzyme activity until further biosynthesis of new enzyme molecules (Sánchez-Santed et al. 2016; Thapa et al. 2017).

Inhibition of ChE enzyme by anticholinesterase results in augmentation of acetylcholine and the effects get relayed throughout the whole nervous system leading to both therapeutic actions and associated adverse effects. Hence those compounds lead to a nonselective indirect activation of both muscarinic and nicotinic receptors. Therefore, the receptors in the peripheral nervous system and the brain get activated, including peripheral tissues innervated by parasympathetic nerves, sympathetic and parasympathetic ganglia, and at the neuromuscular junction

(Ehlert 2019b). Many acetylcholinesterase inhibitors are available on the market for the treatment of various conditions (Table 3.7) (Potter and Kerecsen 2017).

3.3.4 Release Inhibitors

Release inhibitors' primary mechanism of action is preventing ACh release from the presynaptic end of the neuron. Botulinum toxin (BTX) is produced by *Clostridium botulinum* bacteria that target cholinergic receptors at neuromuscular junctions in skeletal muscles, inhibiting ACh release leading to neuromuscular blockage and paralysis. The inhibition of ACh occurs after the toxin enters the nerve and reaches the cytoplasm, then cleaves the (SNARE) proteins that are responsible for vesicle fusion mediation. Prevention of vesicle fusion means that the ACh vesicles can no longer bind into the intracellular cell membrane and release ACh into the synaptic cleft (Dressler and Saberi 2005; Zhao et al. 2016). Some snake venoms produce an irreversible blockade of neuromuscular transmission such as Crotoxin (CTX). CTX is a heterodimeric phospholipase A2 (PLA2) neurotoxin produced by a **Brazilian rattlesnake** (*Crotalus durissus terrificus*). This neurotoxin causes prevention of acetylcholine release from presynaptic ends, which depends on intrinsic PLA2 activity (Cavalcante et al. 2017). Blockage of ACh release leads to interruption of neuromuscular transmission and eventual muscle paralysis. Crotoxin exerts postsynaptic level effects too by stabilizing desensitized acetylcholine receptors (Faure et al. 2017).

3.4 Therapeutic Applications

Cholinergic and anticholinergic drugs have a wide range of therapeutic application including myasthenia gravis, dementia, ophthalmology, neurogenic bladder, xerostomia, anticholinergic overdose, snakebites, and postoperative urinary retention including tensilon test. Further explanation will be presented later in this section.

3.4.1 Nicotinic Receptor Ligands

The most established therapeutic outcomes of nicotinic stimulation are cognitive improvements. Nicotine was the first compound to bring attention to this since it can improve performance cognitively and attention requiring vigilance tasks, which suggests that nicotine possesses an optimizing ability to response and attention mechanisms and enhances working memory (Gandelman et al. 2018; Grundey et al. 2015). Figure 3.8 illustrates a potential model explaining how nicotinic stimulation can enhance network function leading to improved cognitive performance. Nicotine has been shown to enhance the efficacy of cognitive/function control networks in frontal attention while decreasing the activity in default mode network nodes in parietal regions, frontal lobe, and cingulate. These effects

Table 3.7 Currently used acetylcholinesterase inhibitors (AChEIs) for various therapeutic applications

Drug	Brand name	Class/type of binding	Duration	CNS effect	Uses
Physostigmine	Eserine	Carbamate, reversible	0.5–2 h	Yes	Treatment of atropine poisoning, glaucoma
Neostigmine	Prostigmin	Carbamate, reversible	0.5–4 h	No	Reversal of NMJ blockade, myasthenia gravis
Pyridostigmine	Mestinon, Regonol	Carbamate, reversible	4–6 h	No	Reversal of NMJ blockade, myasthenia gravis, prevention of organophosphate poisoning
Ambenonium	Mytelase	Carbamate, reversible	3–8 h	No	Reversal of NMJ blockade, myasthenia gravis
Tacrine	Cognex	Pyridine, reversible	4–6 h	Yes	Alzheimer's disease (discontinued)
Donepezil	Aricept	Piperidine, non-covalent, reversible	>6 h Yes	Yes	Alzheimer's disease
Galantamine	Reminyl, Razadyne	Tertiary alkaloid, reversible	>6 h Yes	Yes	Alzheimer's disease
Rivastigmine	Exelon	Carbamate, pseudo-irreversible	10–12 h	Yes	Alzheimer's disease
Edrophonium	Tensilon	Electrostatic, rapidly reversible	5–15 min	No	Diagnosis of myasthenia gravis, reversal of NMJ blockade
Echothiophate	Phospholine	Organophosphate, irreversible	>24 h	N/A (topical)	Glaucoma

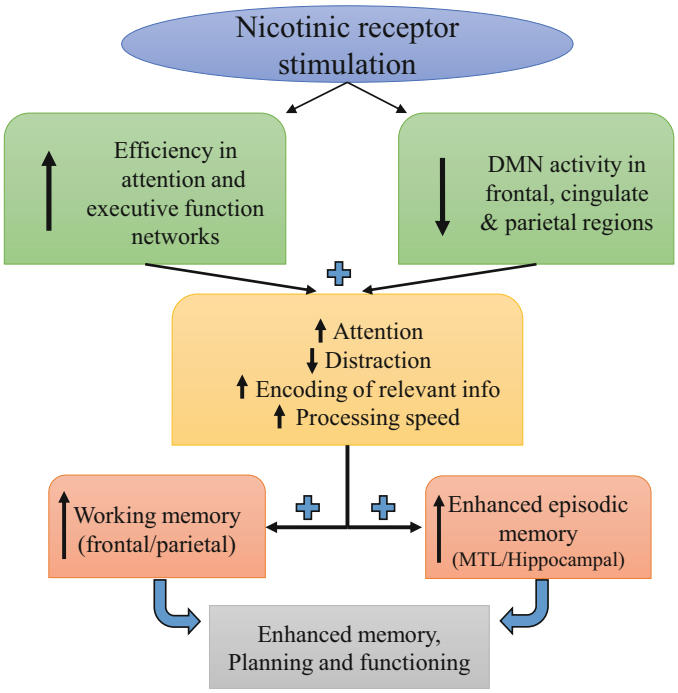


Fig. 3.8 Simplified model of nicotinic receptor stimulation effect in the enhancement of cognitive functioning. Abbreviations: MTL: medial temporal lobe; DMN: default mode network

altogether may enhance processing speed, attention, and response inhibition, eventually leading to improved memory and attention to external stimuli and decreased internally focused processing (Hahn 2019; Newhouse 2019).

Nicotine and nAChRs ligands have shown a different level of enhancement in cognitive functions after many studies carried out on animal models, including attention, memory, and learning. Further clinical trials have shown that these cognitive effects are also present when varenicline has improved cognitive impairment in schizophrenic patients and increased lapses in attention in smokers. Recent clinical trials have shown that activating α7 nAChRs has procognitive effects on patients with AD and schizophrenia. Likewise, a randomized placebo-controlled double-blinded study with TC-1734, a β2-selective nicotinic agonist, had shown an improved cognition when given to patients with age-associated memory impairment (Hoskin et al. 2019; Quik et al. 2015). Some preclinical and clinical studies supported that nicotinic receptors have a role in anxiety, mood, and depression. A study revealed that the treatment of Tourette's disorder with mecamylamine, a nAChR antagonist, showed considerable improvement in depression symptoms (Aboul-Fotouh 2015; Silver et al. 2001).

Nicotinic receptors are the principal receptors affecting the regulatory pathways of nicotinic and cholinergic signaling, as suggested by recent studies. These signals

regulate both cell growth and migration, as well as regulation of angiogenesis of endothelial cells during pathological and physiological conditions (Schaal and Chellappan 2016). The nicotinic receptor that showed most of the oncogenic responses in cancer is α7-nAChR. However, in breast cancer cells (estrogen receptor-positive), the α9-nAChR was found to be upregulated. α9-nAChR coalition with estrogen receptors stimulates both the initiation and progression of breast cancer. Accordingly, these recent studies suggest that nicotinic receptors-mediated oncogenic signaling has a significant role in cancer initiation and progression, which supports that cytotoxic and mutagenic effects of tobacco smoke promote growth and angiogenesis of tobacco-related cancers. Hence, nicotinic receptors are presenting promising new targets for diagnosis, treatment, and prevention of cancers associated with tobacco consumptions (Dang et al. 2016; Zhao 2016).

Currently, there has not been a medication developed that can target defects in social communication and repetitive, restrictive patterns of behaviors in patients with autism spectrum disorders (ASDs). Although individuals with Down syndrome (DS) exhibit a progressive decline in adaptive functioning, that is also associated with AD patients. Unfortunately, for DS patients and people with ASDs, there has not been any effective medication strategy to stop or retard the worsening of adaptive functions. However, α7-nAChRs present a potential therapeutic target in the treatment of these disorders due to their involvement in the pathophysiology of these disorders (Deutsch et al. 2014, 2015).

3.4.2 Muscarinic Receptor Ligands

Xanomeline, an M1/M4 receptor muscarinic agonist, exhibited positive effects on psychotic and cognitive-like symptoms, including delusions and hallucinations in patients with AD, which also makes it a therapeutic option to treat patients with schizophrenia. Studies supported this notion when xanomeline had an antipsychotic effect on rodent models (Yohn and Conn 2018). These studies and trials have revealed the importance of muscarinic receptors in the modulation of neuronal activity and release of neurotransmitter in various brain regions. Muscarinic receptors additionally play a crucial role in shaping neuronal plasticity and affect a wide range of functions from sensory and motor function to cognitive processes. In recent years, with the development in gene targeting technology, knockout mice offer valuable insight into the physiology and pathophysiology of the brain, thus accelerating the efforts to develop new medications for analgesia and certain conditions such as schizophrenia, addiction, PD, and AD (Thomsen et al. 2017). Analgesic therapy by muscarinic agonists has been proposed in preclinical and clinical models long ago. In a recent study, an M1 selective agonist has shown analgesic activity *in vivo*. However, the pharmacokinetic/pharmacodynamic measures have shown that to have the productive analgesic activity, a tenfold of higher exposure is needed as compared to cognitive effects in a rodent model (Wood et al. 2017).

3.4.3 Cholinesterase Inhibitors

Acetylcholinesterase (AChE) inhibitors prevent acetylcholine breakdown. There are already many AChE inhibitors currently used, such as galantamine, donepezil, tacrine, and physostigmine in the treatment of AD (Fig. 3.9) (Verma et al. 2018). These compounds have shown to positively affect psychosis, visual hallucinations, and cognitive dysfunction in schizophrenia. Additionally, preclinical studies showed that clinically used AChEIs could improve memory and learning in rodent models for schizophrenia. However, clinical trials on schizophrenic patients treated with AChEIs showed dose-limiting adverse effects due to their intrinsic ability to activate the peripheral receptors (Thakurathi et al. 2013; Yohn and Conn 2018).

The two popular hypotheses, the cholinergic and the amyloid hypothesis, explain the pathological mechanism for AD progression based on observed biochemical and morphological changes in the brain of AD patients (Huang et al. 2014). The amyloid hypothesis focuses on deposits accumulation in extracellular space formed from β-amyloid peptides and tau proteins, which causes oxidative stress on neurons causing inflammation leading to neurons degeneration (Jiang et al. 2014). On the other hand, the cholinergic hypothesis is characterized by alterations in the biochemical state of the brain due to ACh decrease in the cholinergic neurons present in the cerebral cortex and the hippocampus. AD patients have a stunted Meynert nucleus basalis, which is responsible for producing the enzyme ChAT that plays a central role in acetylcholine synthesis and results in acetylcholine deficiency (Kuhn et al. 2015; Liu et al. 2015). The last treatment approved by the FDA for AD was memantine, an NMDA (*N*-methyl-D-aspartate) glutamate receptor antagonist. Memantine is currently indicted for moderate to severe phases of AD (Matsunaga et al. 2015; de Souza et al. 2016). Postmortem studies have shown that patients with PD have concomitant degeneration of nigrostriatal and cholinergic pathways. This impairment in both neurotransmitter systems is related to specific motor and non-motor features of the disease, such as cognitive and gait dysfunction (Aarsland et al. 2017). Although the neurochemical trait in patients with PD is depletion in dopamine, cognitive decline in PD is also associated with defects in the activity of cortical acetylcholinesterase. Concomitant of dementia and classical gait

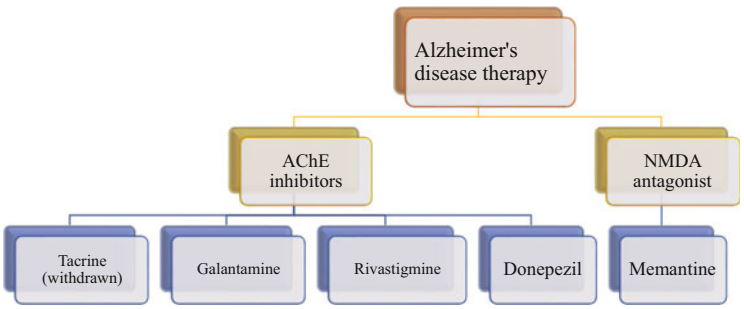


Fig. 3.9 Currently approved therapy regiment for Alzheimer's disease

dysfunction in those patients increases the risk of falls and hospitalizations. 80% of PD patients develop dementia after 20 years, which makes dementia the leading risk factor for falls. Treatments that interfere with both dopaminergic and cholinergic imbalance can improve long-term outcomes of PD patients (Hiller et al. 2015; Pagano et al. 2015).

Organophosphorus compounds (OPCs) are primarily a group of pesticides that inhibit acetylcholinesterase, and they are considered a severe health hazard. Current therapy for OPCs exposure is oxime-type enzyme reactivators such as obidoxime and pralidoxime, while atropine is administered for symptomatic treatment sometimes with unsatisfactory therapeutic outcomes. Better therapeutic results are observed when AChE inhibitors (reversible) are administered before the exposure to organophosphorus compounds. Recent studies showed that bispyridinium oximes K027 and K203, AChE inhibitors under development, are highly effective (K027 more than K203) in protecting against OPCs when administered pre-exposure. This strategy makes it a promising prophylactic agent and preventing possible occupational hazard (Antonijevic et al. 2016; Lorke and Petroianu 2018).

3.5 Conclusion

Cholinergic ligands possess great importance for various medical conditions. Over the years, studies and research have provided substantial evidence of positive outcomes from cholinergic ligands, such as nicotinic stimulators and muscarinic agonist and their ability to improve cognitive performance. Currently, there are potential cholinergic ligands approved for medical applications such as acetylcholinesterase (AChE) inhibitors including galantamine, donepezil, and rivastigmine for the treatment of AD. Furthermore, several AChE inhibitors displayed promising and appreciable therapeutic benefit for glaucoma, atropine poisoning, myasthenia gravis, and reversal of NMJ (neuromuscular junction) blockade. Another example is muscarinic receptor antagonists (tertiary amines, quaternary ammonium derivatives) used for conditions such as urinary incontinence, motion sickness, GI antispasmodic, COPD, Parkinson's disease, Huntington's disease, and as an antisecretory agent for peptic ulcer. The involvement and pathophysiology of cholinergic receptors in certain medical conditions are subsequently investigated to provide more potential therapeutic targets in the future. For example, α9-nAChR upregulation in estrogen-positive breast cancer suggests that nicotinic receptors can mediate oncogenic signaling, making them a potential new target in treating and preventing certain cancers. Gene knockout technology is currently being used to provide a further understanding of the cholinergic receptors, especially in the brain to present new potential therapeutic agents for various conditions.

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Pharmacology of Adrenaline, Noradrenaline, and Their Receptors

4

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Abstract

Adrenaline and noradrenaline are important catecholamines of the biological system, responsible for the regulation of major functions of the body *via* their action on the brain. This noradrenaline is the chief neurotransmitter of the sympathetic nervous system, whereas adrenaline is an important metabolic hormone, known to play a vital role in the cardiovascular system and a mediator of the fight-or-flight response. These catecholamines act in the system through the membrane-bound GPCRs, adrenergic receptors (ARs). Two major classes of ARs, α-ARs and β-ARs, facilitate a number of functions at central and peripheral sites. There are two subtypes of α-ARs (α1-AR and α2-AR), whereas three different subtypes of β-ARs have been identified—β1-AR, β2-AR, and β3-AR. Based on their role, different AR modulators have been introduced clinically for their therapeutic application. In this chapter, we focus on the pharmacology of the two catecholamines through their action on different ARs within the biosystem

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and the modulators of ARs towards the treatment of potentially life-threatening conditions.

Keywords

Adrenaline · Noradrenaline · Adrenergic receptors · Agonists · Antagonists · Biological function

Abbreviations

AR	Adrenergic receptor or adrenoceptor
BP	Blood pressure
BPH	Benign prostatic hyperplasia
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CHF	Congestive heart failure
CNS	Central nervous system
GIT	Gastrointestinal tract
GPCRs	G-protein-coupled receptors
HT	Hydroxytryptamine
KO	Knockout
L-DOPA	L-dihydroxyphenylalanine
MDMA	Methylenedioxymethamphetamine
mRNA	Messenger ribonucleotide
NO	Nitric oxide
PKA	Protein kinase A
TM	Transmembrane

4.1 Introduction

Adrenaline and noradrenaline are two important catecholamines, which are responsible for foremost activities in the maintenance of the "inner world" of the brain body. The existence of these substances in the adrenal gland, which turns red upon oxidation, was first discovered by Vulpian (Gaddum and Holzbauer 1957); however, the pressor effect of the adrenal extracts was first recognized by Oliver and Schafer (Oliver and Schäfer 1895). Later, adrenaline was isolated and within a few years, adrenaline was synthesized chemically (Szymonowicz 1896). Later, the component was known by two different names due to progress in research in two different countries where Takamine referred to it as "adrenalin" (Takamine 1902) and "epinephrin" was preferred by Abel (1899). Later, adrenaline is considered as its official name in Britain, whereas in the United States, epinephrine is preferred.

Simultaneously, Addison revealed the necessity of the adrenal gland for life, and in continuation to that some of the researchers thought cortisol from adrenal medulla is the necessary principle responsible for the pressor effect (Addison 1855; Davenport 1982). Following researches over decades, noradrenaline is recognized as the chief neurotransmitter from the postganglionic sympathetic nerve fibers (Gaddum and Holzbauer 1957). Noradrenaline is also recognized as norepinephrine (similar to adrenaline), which is the chief sympathetic neurotransmitter. It is known to control tonic and reflexive changes in cardiovascular tone. Alternatively, adrenaline is a key factor for the metabolic responses or for global challenges to homeostasis, including expression of emotional distress. This adrenomedullary hormone is also involved in preserving homeostasis in emergencies (Goldstein 2010).

4.1.1 Noradrenaline

4.1.1.1 Synthesis

Tyrosine, the circulatory neutral aromatic amino acid, is obtained biologically from the hepatic hydroxylation of phenylalanine or from diet. This amino acid, upon uptake into the para-aortic enterochromaffin cells of adrenomedullary cells, sympathetic neurons, and specific centers in the brain, begins in synthesis of catecholamines (Fig. 4.1). Tyrosine is then converted to L-dihydroxyphenylalanine (L-DOPA) by the catalytic effect of tyrosine hydroxylase, where ionized iron, tetrahydrobiopterin, and molecular oxygen potentiate this rate-limiting step of catecholamine synthesis (Wassall et al. 2009). Later this L-DOPA is rapidly converted to dopamine in the cytoplasm of neuronal cells by the help of DOPA decarboxylase (L-aromatic-amino-acid decarboxylase), found mainly in the brain, liver, gut, and kidney, in the presence of the cofactor pyridoxal phosphate. Finally, noradrenaline is synthesized from dopamine by the catalytic action of dopamine-beta-hydroxylase, the enzyme mostly confined to the noradrenergic cell vesicles, in the presence of copper and ascorbic acid cofactor (Wassall et al. 2009). During increased adrenomedullary and sympathetic outflow, may be due to stressor effect, the rate of synthesis and presence of tyrosine hydroxylase increase simultaneously (Daubner et al. 2011).

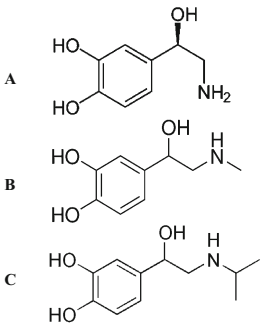

Fig. 4.1 Synthesis and release of noradrenaline from the sympathetic nerve terminals and metabolic pathway of noradrenaline by uptaking into the nerve terminal and to the vesicle or *via* enzymatic degradation to form vanillyl mandelic acid and 3-methoxy-4-hydroxy phenylglycol

According to the exocytic theory, depolarization of the terminal nerve cell membrane results due to the action of acetylcholine, which increases permeation of sodium into the cell. Increased permeation of sodium into the cell directly or indirectly influences voltage-gated calcium channels to increase transmembrane calcium influx (Awatramani et al. 2005). Thereafter, an increased level of cytoplasmic calcium induces a series of biochemical reactions to bring fusion of axoplasmic and vesicular membranes. Thus, diffusion of the intra-vesicular contents occurs in the extracellular environment (Müller and Schier 2011). There are a huge variety of components that act on specific noradrenergic receptors at the nerve terminals to stimulate release of noradrenaline during cellular activation. Alternately, up- or downregulation of extracellular receptors or modulation of intracellular biochemical reactions following receptor activation also affects the responses of the agonists (Collins et al. 1991).

4.1.1.2 Metabolism

Noradrenaline is inactivated by taking up by the nerve cells, followed by storage of the neurotransmitter or consequent intracellular metabolism. In this process, reuptake by the noradrenaline transporters (uptake-1) on the nerve terminal cell membrane plays the major pathway for terminating the action of noradrenaline (Trendelenburg 1991). This uptake-1 process is carrier mediated and thus requires energy to fulfill this action. Binding of adrenaline to the uptake-1 transporter does not require catechol backbone; only one phenolic hydroxyl group containing drugs are substrate to that site. Furthermore, the transport efficiency of the uptake-1 transporter decreases with increase in alkylation of the primary amino group of the neurotransmitter. For example, reuptake efficiency of noradrenaline, adrenaline, and isoprenaline (Fig. 4.2) is different, where reuptake of noradrenaline is highest followed by adrenaline and isoprenaline (the extensively alkylated catechol) (Burgen and Iversen 1965; Graefe and Bönisch 1988; Trendelenburg 1991).

As mentioned earlier, the uptake of noradrenaline *via* cell membrane mediated to the axoplasm may undergo two fates, deamination to degrade by the action of monoamine oxidases (Fig. 4.1) or may translocate into storage vesicles (Burgen and Iversen 1965).

Thus, vesicular storage and degradation of the axoplasmic noradrenaline result in an intra-neuronal "sink" with very low concentration of noradrenaline in cytoplasm. The metabolism of catecholamines through oxidative deamination by the monoamine oxidases is common in neural and non-neural tissues. Rapid uptake of the axoplasmic noradrenaline in the vesicles maintains the concentration of monoamine oxidases onto the surface of mitochondria for continuous functioning of catecholaminergic systems (Goldstein 2010). Anything that prevents these monoamine oxidases can increase the cytoplasmic concentration of catecholamines, stimulate the outward transport of noradrenaline, and thereby promote cardiac smooth muscles, vasoconstriction, leading to hypertension.

These monoamine oxidase enzymes are the chief intracellular enzyme that is involved in metabolizing xenobiotic or biogenic amines throughout the biological system (Westlund et al. 1988). Over a half of a century ago, Johnston brought two

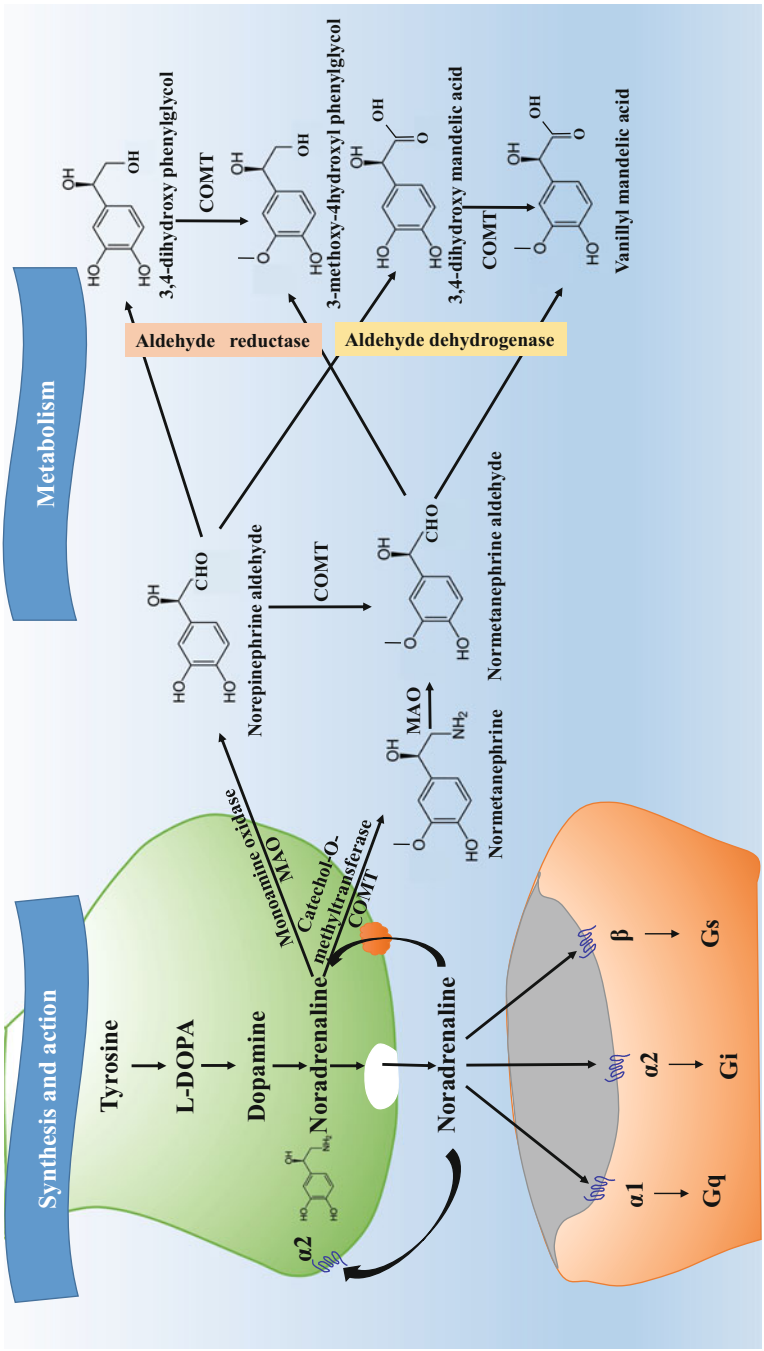

Fig. 4.2 Chemical structure of (**a**) noradrenaline, (**b**) adrenaline, (**c**) isoprenaline

different isoforms of monoamine oxidases in the human system, monoamine oxidase A and monoamine oxidase B (Johnston 1968). It has been postulated that monoamine oxidase A is expressed mostly in sympathetic neurons, whereas extraneuronal cells are known to produce both forms of the enzyme (Westlund et al. 1988).

Alternatively, uptake-2 process is involved in metabolism of noradrenaline by non-neuronal cells, where the carrier protein has low specificity and affinity for catecholamines. Concurrently, the uptake-2 system involves catechol-O-methyltransferase for degrading the uptaken catecholamines (Babin-Ebell and Gliese 1995). Accordingly, the catalytic action of catechol-O-methyltransferase leads the conversion of noradrenaline to normetanephrine, where S-adenosyl methionine acts as the methyl group donor. Further process of the parent and product in the presence of monoamine oxidase and aldehyde reductase or aldehyde dehydrogenase forms the two end products of noradrenaline metabolism, vanillylmandelic acid and 3-methoxy-4-hydroxy phenylglycol (Fig. 4.1) (Babin-Ebell and Gliese 1995; Wassall et al. 2009).

4.1.2 Adrenaline

Adrenaline is one of the important adrenomedullary hormones in humans, which maintains the function of different body organs. Adrenomedullary secretion of this adrenomedullary hormone covers prominently in neuroendocrine patterns attending distress. Adrenaline is only sourced from adrenal medulla, and thus its role in the maintenance of physiological functions is more explored for endogenous adrenaline than for endogenous noradrenaline (Hillarp and Hokfelt 1955).

4.1.2.1 Synthesis

Adrenaline synthesis follows the similar synthetic pathway from tyrosine, as described in the synthesis process of noradrenaline. Following the synthesis of noradrenaline, it is converted to adrenaline by the catalytic action of phenylethanolamine-N-methyltransferase, where adrenocortical steroidal hormones (e.g., cortisol) act as trophic for this catalytic enzyme (Hillarp and Hokfelt 1955; Wurtman and Axelrod 1966).

It has been postulated that the uptake-1 in sympathetic nerve fibers usually takes up noradrenaline along with the circulatory adrenaline. Upon stimulation of the sympathetic fibers, the nerve terminals release noradrenaline along with the up taken adrenaline. This coreleased adrenaline binds to specific β-adrenoceptors on the nerve terminal, subsequently increasing the release of noradrenaline from the terminal (Iversen 1971). This hypothetical phenomenon brings up a model where the endogenous components are taken up into the nerve terminals, coreleased with the neurotransmitter, and finally exaggerate or prolong the release pattern through binding at facilitatory presynaptic receptors. However, confirmation of this hypothetical assumption in isolated tissue preparation in *in vitro* models has failed (Goldstein 2010).

4.1.2.2 Metabolism

The presence of catechol-O-methyltransferase in the medullary cells of adrenal cortex leads to the formation of adrenaline from noradrenaline, simultaneously resulting in inactivation of adrenaline following a similar pathway of noradrenaline. As a result of metabolism, catechol-O-methyltransferase produces metanephrine, thereby decreasing cytosolic concentration of adrenaline. Thus, the production of it is continuous in the cytosol, whereas the release of adrenaline from the adrenomedullary vesicles is episodic, upon receiving the impulse. Pheochromocytoma is a type of chromaffin cell tumors, where synthesis of catecholamines increases heavily and thus, increases the metanephrines in the body. Therefore, the detection of the level of metanephrines in plasma provides information on increased levels of catecholamines, to aid effective treatment of the disease (Lenders et al. 2002; Spence et al. 2018).

4.2 Pharmacological Responses of Catecholamines Acting Through Specific Receptors

Understanding the action of the catecholamine, particularly adrenaline and noradrenaline, is crucial because of the diverse physiological responses of the catecholamines acting through different categories of adrenoceptors or adrenergic receptors (ARs) (alpha (α) and beta (β) adrenoceptors) (Strosberg 1993). All these adrenoceptors are G-protein-coupled receptors (GPCRs), where these receptors are linked to heterotrimeric G proteins. Each major type of receptors is preferentially linked to a specific class of G protein, for example, Gq is observed in α1 adrenoceptors, Gi can be found in α2 adrenoceptors, whereas β adrenoceptors are linked to Gs (Fig. 4.1). Although these receptors are structurally related, individual receptors possess particular response to the physiological and biochemical pathways through the production of particular second messengers. Thus, the activities following activation of these adrenoceptors are solely dependent on the G protein-mediated generated second messengers and on the action of different ion channels. Hereafter, individual adrenoceptors are elaborated in detail.

4.2.1 Alpha (α) Adrenoceptors

Studies in the last few decades enabled developing concepts regarding different receptors that mediate actions owing to the binding of endogenous catecholamines. These were previously divided into only α- and β-adrenoceptors or ARs, while later in the 1970s the α adrenoceptors are further divided into α1 and α2 ARs. The α1 ARs are generally designated to mediate the responses in the effector organ. The α2 ARs are generally localized in the presynaptic knob to regulate the release of neurotransmitters, while reports also suggest their postsynaptic locations. Both α1 and α2 ARs are important in regulating the vascular tone, while none are known to include homogenous groups. The initial division of the α1- and the α2-AR subtypes

was purely based upon the pharmacological properties of the same, but later detailed structural analyses were performed by isolation and identification via cloning methods. Today, α1-ARs have been classified into α1A-AR subtype (formerly α1a/c, cloned α1c), α1B-AR subtype (cloned α1b), and α1D-AR subtype (formerlyα1a/d, cloned α1d). It may convincingly be suggested that the α1A-AR subtype is more prominently designated for vascular basal tone and arterial blood pressure (BP) regulation, while α1B-AR subtype is better implicated for responses towards exogenous agonists. The α1B-AR subtype expressions may also get altered or modified owing to various pathophysiological modifications. The α2-AR classifications include α2A/D-AR subtype, α2B-AR (cloned as α2b), and α2C-AR (cloned as α2c). The α2A and α2B ARs are mainly used to mediate arterial contraction, while α2C-ARs are mainly designated for venous vasoconstriction. The functions of the α-ARs are subtype specific as well as common, depending upon the required responses of the effector tissue. In most of the cases, the responses evoked different α-AR subtypes do overlap due to the lack of receptor specificity of some ligands. Thus, it is essential to design drugs that will be specific in their mode of actions *in vivo*, for therapeutic intervention using these receptors.

4.2.1.1 α1-Adrenoceptors

Structural Biology

α1-ARs mediate the essential role to regulate the physiological action of norepinephrine and epinephrine. The α1A, α1B, and α1D represent the three α1-AR subtypes. They belong to the GPCR family and act via the Gq/11 signaling pathway. They display individualistic patterns of tissue distributions and pharmacological properties (Cotecchia 2010).

The pioneer hypothesis of Easson-Stedman had initiated bulk of research directed towards drug designing for the ARs. Once ARs were cloned and the subtypes had surfaced, the studies on drug design had oriented to reveal the detailed structure of the receptors and new interactions were found. The α1 subtype and β-ARs were found to greatly differ in their ligand-binding pocket which accounted for selective drug design (Easson and Stedman 1933). Ahlquist, in later years, had used a series of agonists to reveal the AR subtypes as α and β, and further into α1 and α2 receptors as post- and pre-junctional ARs respectively (Starke et al. 1989; Ahlquist 1948). The development of advanced pharmacological techniques, such as the radioligand binding assay, leads to the classifications of α1-ARs into α1A and α1B-subtypes (Morrow and Creese 1986). This was followed by the interventions of Art Hancock who revealed the structure-function association of α1-ARs (Hancock et al. 1988) and led to the synthesis of the first synthesized selective agonist α1A-AR, A-61603 (Knepper et al. 1995).

Initially four subtypes of α1-AR were revealed by cloning techniques. The α1b-AR subtype was the first cloned α1-AR subtype, which displayed similar binding properties like that of α1B-AR. Besides this, there were α1a from rats (Cotecchia et al. 1988), bovine α1c- (Schwinn et al. 1990) and rat α1d-AR (Perez et al. 1991), which had 99.8% homogeneity and considered a same subtype. At present, it is

conceived that the α1a/α1d clones refer to a novel α1D subtype. The latest classification includes α1A (former α1a/c), α1B (former α1b), and α1D (former α1a/d) (Perez et al. 1994).

Biological Distribution

The tissue distribution for α1-AR varies with their subtypes and depends on the specific functions. Experiments in human cells, rats, and rabbits have showed that the α1A/c-ARs are highly expressed in the heart, liver, salivary gland, lung, and vas deferens (Table 4.1) (Schwinn et al. 1990; Perez et al. 1994). The mRNA expression and receptor protein expressions also revealed the expression of this receptor subtype in rat kidney, mediating vasoconstriction. *In situ* hybridization analysis of α1-AR-mRNAs in rat brain displayed substantial levels of α1A-ARs expressions in the olfactory system, hypothalamus, brainstem, and spinal cord, especially in the structures that play roles in motor functions. The expression of α1A-ARs has also been reported in glial cells, thus suggesting their non-neuronal mode of action in mediating several brain functions (Day et al. 1997).

The α1B-AR has been reported to be highly expressed in rat liver and heart while less in the hippocampus, aorta, and salivary gland. The expression of this receptor subtype is high in the pineal gland, the lateral nucleus of the amygdala, most of the thalamic nuclei, and in the raphe nuclei (Day et al. 1997).

The α1D-AR has been found to be abundant in the deferens and aorta and less expressed or absent in the liver, spleen, kidney, and salivary gland. In neural tissues, the distribution of this subtype is the most unique. It is highly expressed in the olfactory system, hippocampus, cortical layers II–V, amygdala, the reticular thalamic nucleus, the motor nuclei of the brainstem, and in the spinal cord (Day et al. 1997).

Role in Biological System

The α1-AR subtypes carry out innumerable biological effects by binding their ligands, epinephrine and norepinephrine. There is still a gap in the understanding of the exact mechanism and specificity of the α1-AR subtypes. Besides their discreet functions, many functions of the α1-ARs overlap with the α2-ARs.

Subtype-Specific Functions

Functions Mediated by α1A-ARs

The α1A-ARs have been suggested to play a vital role in mediating contractions in several tissues such as in the vas deferens (Moriyama et al. 1997; Burt et al. 1998), renal artery (Villalobos-Molina et al. 1997), rabbit ear artery (Fagura et al. 1997), tail artery in rats (Lachnit et al. 1997), right atrium (Yu and Han 1994), internal anal sphincter (Mills et al. 2008), and in the prostate (Marshall et al. 1995). α1A-ARs in the rat vas deferens display two responses. One of these responses is phasic, may be due to calcium ion release from ryanodine-sensitive compartments, and the other one is tonic response which may be mediated *via* the pathway involving protein kinase C, diacylglycerol, and calcium ion influx through L-type and may be also the T-type channels (Burt et al. 1998). The rat submandibular gland may contain

Table 4.1 Classifications, functions, and phenotypes of α1-adrenoreceptor subtype in mice with genetic modifications

AR group	Present	Previous pharmacology	Cloning	Functional responses	Genetic modification	Phenotype
α1H[a]	α1A (α1A/c)	α1A	α1c	Control of blood pressure; vasoconstriction; smooth muscle contraction	Overexpression/ heart-specific promoter	Increased contractile response, no hypertrophy
	α1B	α1B	α1b	Regulatory; minor contractile role	Gene deletion	Decreased resting blood pressure, decreased vasoconstriction
	α1D	α1D	α1a, α1d, α1a/d	Control of blood pressure; vasoconstriction; smooth muscle contraction	Gene deletion	Decreased resting blood pressure, decreased vasoconstriction
α1L[b]	α1N[c]	α1N	–			
	α1L	α1L	–	Vasoconstriction		

[a]High affinity for prazosin vs α1L-AR group
[b]Members of this group have not yet been cloned
[c]High affinity for HV 732 vs α1L receptors. Poorly classified with available probes

both α1A- and α1B-ARs, but in several experimental setup, it serves as a model for the α1A-AR ligand-binding sites (Bruchas et al. 2008). It has been observed that positive inotropic effects of phenylephrine in mouse are mediated *via* its binding to the α1A-ARs (Ross et al. 2003). Moreover, noradrenaline-mediated contractions in the prostate are also evident to occur via the α1A-AR (Gray et al. 2008). It is suggested that overexpression of the α1A-AR elevates β-AR-supported cardiac muscle contractility, thereby ameliorating the outcome from myocardiac infarction (Woodcock 2007).

Functions of α1L-ARs: α1A-ARs

Under the classification of α1-ARs, based on the high and low affinity for prazosin, the receptors have α1H, α1N, and α1L subtypes (Table 4.2). α1L-ARs have been found to be expressed in longitudinal but not circular smooth muscle (Amobi et al. 2002) in the aorta, mesenteric, and carotid arteries (Muramatsu et al. 1990), rat anococcygeus muscle (Civantos Calzada and Aleixandre de Artiñano 2001), and vas deferens (Ohmura et al. 1992). Contractions of rat vas deferens in response to exogenous agonists are suggested to be arbitrated by the α1A-ARs (Cleary et al. 2004). α1L-ARs are also found in the rabbit cutaneous resistance arteries (Smith et al. 1997), rabbit iris (Nakamura et al. 1999), prostatic arteries in pigs (Recio et al. 2008), small mesenteric artery in rats (Graaf et al. 1996), guinea-pig aorta (Muramatsu et al. 1990), rabbit bladder neck (Kava et al. 1998), pig internal anal sphincter (Mills et al. 2008), in human, rat, and dog urethra, and in human prostate (Muramatsu et al. 1990; Gray et al. 2008). α1-ARs are suggested to show the ligand

Table 4.2 Summary of tissue distribution of α1-adrenoreceptor subtype

	mRNA abundance					
	Humans			Rats		
Tissue	α1A/c	α1B	α1D	α1A/c	α1B	α1D
Brain	ID	ND	ND	Yes	Yes	Yes
Cerebellum	Yes	ID	ID	ID	ID	ID
Cerebral cortex	Yes	ID	ID	Yes	Yes	Yes
Hippocampus	ID	ID	ID	Yes	Yes	Yes
Pituitary	Yes	ND	ND	ID	ID	ID
Heart	Yes	Yes	Yes	Yes	Yes	Yes
Aorta	Yes	Yes	Yes	Yes	Yes	Yes
Vena cava	ID	ID	ID	Yes	ID	ID
Lung	Yes	Yes	Yes	Yes	Yes	Yes
Kidney	Yes	Yes	Yes	Yes	Yes	Yes
Vas deferens	ND	ND	ND	Yes	Yes	Yes
Liver	Yes	Yes	Yes	Yes	Yes	Yes
Spleen	Yes	Yes	Yes	Yes	Yes	Yes
Salivary gland	ND	ND	ND	Yes	Yes	Yes
Prostate	Yes	Yes	Yes	ID	ID	ID
Epididymis	ND	ND	ND	Yes	Yes	Yes

ND not determined; *ID* insufficient data

binding properties of α1A-AR, as well as the functional characteristics of α1L-AR (Daniels et al. 1999). Using mRNA-based techniques, it has been shown that α1L-AR expressions are predominant in the tissues that mostly express α1A-ARs (Martí et al. 2005). It is evident that human α1A-AR splice variants (Shibata et al. 1996) have similar pharmacological features to that of the homo- and heterodimers of human α1A variants (Ramsay et al. 2004). Thus, it can be inferred that α1L-ARs represent a functional phenotype of α1A-AR, however, identification of these functional phenotypes remain contentious.

Functions Mediated by α1B-ARs

Research regarding the functions of α1B-AR is scant owing to the unavailability of selective antagonists. In several tissues, these receptors reportedly are involved in mediating contractions, such as in mouse spleen (Eltze 1996), rat spleen (Noble et al. 1997), rat right atrium (Yu and Han 1994), rabbit cutaneous resistance arteries (Smith et al. 1997), and in corpus cavernosum (Noble et al. 1997) and human prostate (Teng et al. 1994). Knockout (KO) technology could put forth certain functional aspects of α1B-ARs; for example, α1B-KO mice showed reduced or no noradrenaline or phenylephrine potency in aorta, while combined α1B/α1D-KO led to complete absence of noradrenaline and phenylephrine-mediated contractions in aorta (Hosoda et al. 2004). Daly et al. (2002) used knockout technology to show that α1B-ARs participate in mediating contractions in mouse arteries (such as aorta and tail artery). α1B-AR overexpression has been associated with reduced β-AR-mediated contractility in the heart (Woodcock 2007) and leads to cardiac muscle hypertrophy and hypotension (Zuscik et al. 2001), increasing the risk of heart failure. The α1B-AR overexpression also may diminish the positive inotropic effects on phenylephrine in the heart owing to decrease in α1A-ARs. This suggests rather a regulatory role of these receptors than contractile effects (Ross et al. 2003).

Functions Mediated by α1D-ARs

In several tissues, contractions of vascular smooth muscle are suggested to be regulated at least partly by α1D-ARs. These tissues include human prostate, rat aorta, mesenteric artery, pulmonary artery, iliac artery, renal artery, carotid artery, rabbit ventricle and aorta. In certain occasions, α1D-ARs may also be the cause of endothelium-dependent relaxation, as found by binding of phenylephrine to α1D-AR in rat mesenteric vascular bed. The stimulation of α1D-AR may exert trophic effects on endothelial cells. The ARs that are responsible for nerve stimulation-mediated contractions are found to be predominantly α1D, while exogenous noradrenaline-mediated contractions are predominantly functions of α1A-AR. Experiments in sympathectomized rats have led to the conclusion that α1D-ARs mostly mediate phasic contractions while α1A-ARs are attributed mainly for tonic contractions (Cleary et al. 2004). These observations suggest that α1D-ARs are mostly localized at the junctional region to stimulate nerve activities, but in case of absence of the nerves, their locations extend along the smooth muscles.

Physiological Functions

Control of Blood Pressure

α1-ARs in the vascular system mainly mediate contractions and contribute greatly in blood pressure regulation and in the baroreceptor reflex response. Piascik et al. (1990) had put forth that in the conscious rat, α1A-AR subtype mediates tonic maintenance of blood pressure, while α1B-AR subtype mediates responses towards the exogenous agonists. In the unconscious or pithed rat, it has been reported that both the blood pressure reflex and actions of exogenous noradrenaline involve both α1A- and α1D-ARs, while blood pressure regulation via noradrenaline is mostly α1-AR mediated (Vargas and Gorman 1995).

Temperature Control

Temperature regulation is another essential function of vascular α1-ARs since vasoconstriction of superficial blood vessels is the primary mechanism for the core body heat conservation. Methylenedioxymethamphetamine (MDMA) is an extensively used recreational drug, which may lead to life-threatening hyperthermia. In animal experimentations, MDMA reportedly affects thermoregulation, often leading to hypothermia or hyperthermia in cold or hot ambient temperatures, respectively. The α1-AR (α1A/D-ARs) antagonists could potentially covert the MDMA-mediated monophasic hyperthermic response to a biphasic response characterized by hypothermia followed by hyperthermia (Docherty 2010).

Depression

Depression, a multifactorial disorder, involves innumerable neural circuits and neuronal processes. Noradrenaline plays significant roles in depression *via* the ARs (Morilak et al. 2005). The ARs were found to undergo alterations followed by the administration of antidepressant drugs (Blier 2003). Brain α1-adrenergic neurotransmission impairment has been reported in certain depressive illnesses. It is not yet clearly understood what is the exact mechanism by which individual α1-AR subtypes mediate antidepressant effects. It had been suggested that α1-AR is specifically associated with antidepressant action via a study using chronic treatments with imipramine and electroconvulsive shock. The observations revealed an increase in α1A-AR and not in the α1B-AR mRNA land receptor expressions in the rat hippocampus and cerebral cortex (Stone and Quartermain 1999).

Nociception

The noradrenergic system is well known for its analgesic effects (Jinushi et al. 2018). A majority of the studies that are focused on noradrenaline-mediated analgesic effects have reported the antinociceptive role of α2-AR, while studies on the role of α1-AR are mostly behavioral ones (Wei et al. 2016; Di Cesare et al. 2017). The α1-AR binding modulations due to pain mostly take place in the areas of the central nervous system (CNS) involved in pain processing. The receptor modulations, including those of the α1B-AR subtype in acute pain phase, have been suggested to be often lateralized and to vary according to the pain phases, whereas α1-ARs involved in the late phases of pain were other than the α1B. Research on α1D-AR-deficient mice [19] that focused on responses to different noxious stimuli put forth the concept that α1D-ARs in the spinal cord participate in mediating responses of thermal pronociception (Dogrul et al. 2006).

Genitourinary Functions

Three α1 AR subtypes have been found to be localized in the lower urinary tract: α1A, α1B, and α1D. α1A receptors are predominant (about 70%) in the stroma of the prostate gland, α1B in the prostate gland epithelium, and α1D mostly in the prostate blood vessels as well as in the stroma. The α1ARs are also located in the urinary bladder and urethral smooth muscle, as well as in the spinal cord and ganglia. The extraprostatic sites of α-receptors lead to reduced organ selectivity and cause side effects of treatments that focus on modulations of these receptors for lower urinary tract symptoms. The α-blockers are used to treat benign prostatic hyperplasia (BPH) owing to direct α-adrenergic antagonism of smooth muscle tonicity in prostatic stroma. Current research has revealed longer-term effects of α-blockers on prostate cellular differentiation and apoptosis (Cavallo 2018).

Receptor Modulators

Selective α1-adrenergic modulators retain a tiny low market share among all antihypertensive drug medications within the United States (Griffith 2003). Prazosin, introduced in 1976, was the primary marketed drug in this category. Afterwards, two more α1-adrenergic blockers, doxazosin and antihypertensive drug, were available on the market, and their potential for once-daily dosing has provided extra treatment flexibility (Xie et al. 2005). Within the last decade, sustained release formulations for alpha-blocker and doxazosin are revealed. Tamsulosin and alfuzosin are so-called uroselective agents with a better affinity for prostate α1-ARs and are unremarkably employed in the management of patients with BPH (White and Moon 2005).

α1-adrenergic antagonists, both selective and nonselective, are clinically available. Phenoxybenzamine, a nonselective, noncompetitive blocker, is currently reserved for the preoperative treatment of pheochromocytoma-associated high blood pressure. Nonselective α-blockade means that presynaptic α2-receptors, which cut back the discharge of noradrenaline, are downregulated because of inhibited feedback mechanism. Phentolamine could be a short-acting, competitive, nonselective α-blocker parenterally administered and used for severe sorts of high blood pressure prompted by extreme release of catecholamines. Prazosin, the pioneer selective α blocker, contains high affinity for the α1-AR associated with an immediate-release formulation and has speedy onset of action. These features most likely account for its comparatively higher rate of syncope and postural hypotension compared with terazosin and doxazosin. Syncopal episodes may be decreased by limiting the initial dose to 1 mg, administering the primary dose at night before sleep, and gradually increasing the dosage. Doxazosin and terazosin have lower lipid solubility than prazosin and thus bear lesser affinity for α1 receptors.

Different α1-adrenergic antagonists are pharmacologically discrete. Prazosin features a comparatively short period of action and should be provided in a minimum of double doses daily (Stanaszek et al. 1983). Doxazosin and terazosin with longer half-lives might be given once daily. Doxazosin should be administered at bedtime, which appears to be safe and effective in reducing morning hypertension (Pickering et al. 1994; Fulton et al. 1995). α1-adrenergic antagonists ought to be used

cautiously in pregnant women and in children, since the effectualness and/or safety of those compounds have not been estimated.

α1-adrenergic-specific antagonists hinder the noradrenaline-mediated vasoconstriction via selective inhibition of postsynaptic α1 receptors activation by catecholamines (Lund-Johansen and Omvik 1991). The presynaptic α2-ARs are unblocked with these selective compounds, so inhibition of vasoconstriction by a feedback mechanism of α2-AR stimulation is preserved.

The norepinephrine inhibition may be the cause of the absence of tachycardia, higher cardiac output, and increased renin levels by the antagonists of both presynaptic α2 ARs and the postsynaptic α1 ARs such as phentolamine (Baez et al. 1986). As these drugs do not affect the renin-angiotensin-aldosterone system, they are suited to be employed for hypertension regulation in patients with disorders related to this axis (Webb et al. 1987). These drugs have ameliorative effects on hemorheology as well including blood cell disorders, blood viscosity, and endothelial function (Gomi et al. 1997).

The α1-AR antagonists may cause orthostatic hypotension in patients with either volume-depletion disorders or susceptible to the loss of α1-ARs-induced vasoconstriction. The actions of the α1-AR antagonists are at per with the sympathetic activation, which is the reason for unchanged blood pressure after administration of α1-AR antagonists in normotensive persons with normal activities of sympathetic nervous system.

4.2.1.2 Alpha 2 (α2) Adrenergic Receptor

The α2-ARs belong to GPCR family and is linked to Gi heterotrimeric G protein. Its vastly homologous subtypes include α2A, α2B, and α2C ARs. Few species excluding humans possess an extra α2D AR subtype. α2-AR mediates the actions of its ligands, such as the catecholamines (noradrenaline and adrenaline), in the peripheral and central nervous systems (Civantos Calzada and Aleixandre De Artiñano 2001a, b).

Structural Biology

As discussed earlier, ARs were first classified as α- and β-receptors by Raymond Ahlquist in 1948 on the basis of their pharmacological actions in various tissues (Ahlquist 1948). With the advent of research in this realm, the adrenergic or "adrenotropic" receptors were divided into three primary classes and into nine different mammalian subtypes (Ahlquist 1948). The recent classification of receptors into three main classes, α1-, α2-, and β-ARs, mainly relies on their amino acid sequences along with pharmacological characteristics. Each of these classes of the ARs has again been sequestered into three subtypes (Hieble et al. 1995). For α2-adrenoceptor, three subtypes were previously referred to as α2-C10 (Kobilka et al. 1987), α2-C2 (Lomasney et al. 1990), and α2-C4 (Regan et al. 1988) according to their gene locations on human chromosomes. These are now better recognized as AR subtypes α2A, α2B, and α2C.

α2-ARs are membrane glycoproteins whose most structural features bear resemblance and are common to other GPCRs. The common feature includes the presence of seven transmembrane (TM) domains with extracellular amino terminus and intracellular carboxyl terminus. The receptor binding sites must be accessible to the specific ligands like adrenaline and noradrenaline. Thus, the binding sites of α2-

ARs are positioned within the core of the receptor proteins comprising the seven α-helical TM domains (Laurila 2011).

The unique structural characteristics have rendered the α2-ARs-ligand interactions to be highly specific. The ligand binding cavity in these receptors has been reported to be structured by the residues in the third, fifth, sixth, and seventh TM as well as the second extracellular loop. The relative lower affinity of dopamine at α2-ARs than that of noradrenaline may be due to the absence of a specialized β-hydroxyl moiety in dopamine molecule (Nyrönen et al. 2001). Besides these, the other residues those supposedly have hydrophobic interactions with the N-methyl group (positively charged) of catecholamine ligands are two phenylalanines positioned at 7.38 and 7.39 in the seventh TM. In the fifth TM of the human α2A-adrenoceptor, two serine residues expose sites for hydrogen bonding to two catecholic hydroxyl groups of the catecholamine ligands. These interactions may be altered by conformational changes through receptor activation. They are also vital for ligand orientations within the ligand-binding site of the α2-ARs. Some residues mediate π-π stacking interactions with the aromatic ring present in the phenylethylamine-type ligands (Nyrönen et al. 2001).

Unlike the agonists of α2-ARs, the concepts regarding receptor structural characteristics of antagonists binding for these receptors are less clear. The α2-AR antagonists bear way higher chemical diversity than that of agonists. Thus, the antagonists are much complex and possess more divergent modes of binding. There is no report that could specify the "antagonist binding site" for adrenoceptors or for other GPCRs. However, in α2-ARs, a monoamine-binding GPCR, antagonists have been predicted to bind almost at the same orthosteric agonist-binding sites (Nyrönen et al. 2001).

Biological Distribution

The distributions of α2-AR subtypes in the mammalian tissues, especially in humans and in rodents, have been detected using various techniques. Receptor radioligand binding assays and autoradiography were employed to detect ligand binding (Boyajian and Leslie 1987); *in situ* hybridization and other mRNA quantification methods were used to determine receptor gene expression (Nicholas et al. 1993), and antibody-based techniques such as immunohistochemistry and Western blotting were used for receptor characterization (Tran et al. 2004). The three α2-AR subtypes have exclusive tissue distribution patterns in the CNS, peripheral nervous system, and in the peripheral tissues. The expressions of α2A-AR have been found extensively in peripheral tissues and in the CNS, i.e., brainstem (especially the locus coeruleus), midbrain, hypothalamus, hippocampus, spinal cord, cerebral cortex, cerebellum, and septum. The expressions of α2B-adrenoceptor have been observed mostly in the peripheral tissues and in lower levels in the CNS, mainly in the thalamus, olfactory system, pyramidal layer of the hippocampus, and cerebellar Purkinje layer. The α2C-ARs seem to be expressed mostly in the CNS, with different expression patterns from those of α2A-ARs. They are most abundant in the cerebral cortex, midbrain, amygdala, thalamus, olfactory system, dorsal root ganglia, hippocampus, basal ganglia substantia nigra, striatum, and ventral tegmentum (Brodde et al. 2001) (Table 4.3).

Table 4.3 Tissue distributions of α2-adrenoceptor subtypes in the central nervous system and peripheral tissues with their functions

Receptor subtype	CNS	Peripheral tissues	Physiological functions
α2A	Amygdala	Kidney	Analgesia
	Locus coeruleus	Vasculature	Bradykinesia and hypotension
	Lateral septum	Urethra	Hypothermia
	Brainstem	Heart	Inhibition of epileptic seizures
	Cerebral cortex	Platelets	Presynaptic inhibition of neurotransmitter release
	Thalamus	Spleen	Anxiety like behavior
	Hypothalamus	Salivary glands	Sedation and anesthesia
	Hippocampus	Pancreas	Regulation and blood glucose and insulin
	Spinal cord	Fat cells	Decrease in intraocular pressure
	Olfactory nucleus		Inhibition of gastrointestinal motility
	Retina		
α2B	Thalamus	Kidney	Placental angiogenesis
		Placenta	Salt-induced hypertension
		Liver	Vascular smooth muscle contraction
		Vasculature	
α2C	Striatum	Kidney	Presynaptic inhibition of catecholamine release
	Olfactory tubercle	Adrenal gland	Modulation of motor behavior
	Locus coeruleus	Vasculature	Regulation of dopamine and serotonin balance in the brain
	Hippocampus	Pancreas	Vascular smooth muscle contraction
	Cerebral cortex		
	Amygdala		
	Substantia nigra		

Role in Biological System

The α2-AR is a GPCR, which is classically localized on vascular prejunctional nerve terminals, while several of its postsynaptic and extrasynaptic actions are also evident. Its biological functions are mainly based on its ability to inhibit norepinephrine (noradrenaline) release *via* a negative feedback mechanism (Starke et al. 1989) (Table 4.3). It is also localized on the vascular smooth muscle cells in specific blood vessels for example on skin arterioles as well as on veins alongside other ARs. The α2-AR comprises inhibitory G protein—Gi, whose α subunit dissociates from itself upon activation and binds with adenylyl cyclase, thereby inhibiting the same. This causes

reduction in intracellular cAMP level and thereby protein kinase A (PKA) remains inactivated. The PKA-mediated phosphorylation of downstream proteins such as phosphorylase kinase, an enzyme for glycogen metabolism, is thus inhibited (Starke et al. 1989).

The α2-ARs have slightly higher affinity in binding noradrenalin released from the sympathetic postganglionic fibers than adrenaline from the adrenal medulla (Boron and Boulpaep 2003). These receptors mediate several common functions as the α1-AR, while having few specific physiological roles as well, including sympathetic inhibition analgesia, sedation, hypotension, increased opioid and alcohol withdrawal symptoms, pupil control, body temperature regulation, seizure susceptibility modulation, and blood glucose homeostasis. The α2-AR agonists are often used as veterinary anesthetics owing to its functions to cause analgesia, sedation, and muscle relaxation *via* acting upon the CNS (Khan et al. 1999).

Presynaptic Regulation

In central adrenergic nerves as well as in sympathetic nerves, α2A- and α2C-ARs regulate the neurotransmitter release by acting as inhibitory autoreceptors. α2B-receptors on postsynaptic cells are activated by catecholamines released *via* the sympathetic nerves. By doing so, they mediate various physiological functions such as vasoconstriction. Functional differentiation of the presynaptic α2A- and α2C-ARs has been proposed such that α2A-ARs have been found to inhibit the release of norepinephrine from sympathetic nerves initially at high stimulation frequencies, while the operational frequency for the α2C receptor can be very low in order to regulate basal norepinephrine release (Philipp et al. 2002).

Blood Pressure Regulation

α2A-ARs mediate blood pressure regulation by impeding central sympathetic outflow and release of norepinephrine (Altman et al. 1999). On the contrary, the α2B-ARs may counteract this function of α2A-Ars by inducing direct hypertension and vasoconstriction. α2C-ARs play a role in α2-induced vasoconstriction on exposure to extreme cold temperatures (Philipp et al. 2002).

Analgesia

All the three α2-AR subtypes play vital roles in the regulation of pain perception (Bücheler et al. 2002). α2-ARs present in high levels in the spinal cord, specifically in the superficial layers of the dorsal horns, are reported to regulate incoming nociceptive impulses. α2A-ARs are essential to mediate the analgesic effect of α2-AR agonists, the spinal α2C-ARs are reported to effectuate the moxonidine-mediated analgesia, while the α2B-ARs are needed for spinal antinociception caused by nitrous oxide (Philipp et al. 2002).

Sedation

α2-ARs agonists serve as potent sedatives and hypnotic agents in intensive care as well as in the postoperative phase. The hypnotic effects mediated by α2-ARs are

suggested to be regulated by the locus ceruleus, since its neurons express high levels of α2A-ARs (Philipp et al. 2002; Nguyen et al. 2017).

Behavior

α2-ARs mediate several behavioral functions owing to their high levels of expressions in multiple sites in the CNS (Schramm et al. 2001; Frances Davies et al. 2004). They have been shown to impede sensory information processing by the CNS. They may also cause locomotor inhibition. α2A- and α2C-ARs have complementary actions in the integration of CNS function and behavior (Björklund et al. 2001; Philipp et al. 2002).

Other Physiological Functions

α2-ARs are potent mediators of body temperature regulation and control of seizure threshold owing to their high antiepileptogenic effect (Janumpalli et al. 1998). α2A and α2C-ARs have been suggested to bear hypothermic effects (Hunter et al. 1997). They also mediate downregulation of intraocular pressure as suggested by the effects of their agonists, apraclonidine and brimonidine (Liu et al. 1991; Kanagy 2005). The α2-ARs have been reported to inhibit lipolysis in adipose tissue and thus are target receptors to modulate conditions in obesity (Lafontan and Berlan 1980; Lafontan et al. 1997; Morigny et al. 2016).

Receptor Modulators

α2-blockers represent the alpha blocker subset of drugs that are antagonists to the α2-ARs (Rang et al. 1999). These drugs are mostly used in research arena, still not widely applied in clinical interventions for humans. Their functions are based on blockade of α2-ARs and thereby increase in the release of noradrenaline. These α2-AR antagonists have been shown to significantly increase the release of dopaminergic and serotonergic besides the adrenergic neurotransmitters, trigger insulin secretion, and reduce blood sugar levels (Rang et al. 1999).

Yohimbine is the most commonly used antagonist of α2-ARs (Rang et al. 1999). It still finds immense applications in veterinary medicine, alongside its potent alternative, atipamezole (Haapalinna et al. 2003; Lemke 2004). They operate to reverse the effects of α2-AR agonists (such as medetomidine) that are used as sedatives during surgery (Lemke 2004). Other α2-AR antagonists are efaroxan, idazoxan, phentolamine, and rauwolscine (Chopin et al. 1999).

α2-AR antagonists find implications in the treatment of depression. The tetracyclic antidepressants mirtazapine and mianserin are two of the antidepressants belonging to this class of antagonists, but their antidepressant effects are also supported by activation of other receptor sites as well. Sudden withdrawal from use of the α2-AR antagonist drugs can prove detrimental because an immediate global downregulation of release of the neurotransmitters may lead to various neurological problems, depressions and sudden hyperglycemia, and reduction in insulin sensitivity triggering diabetes. Moreover, downregulation of microcirculation as well as adrenaline hypersensitivity in organs like the liver may also result if there is a sudden withdrawal of a regular α2-AR treatment (Rang et al. 1999).

4.2.2 Beta (β) Adrenoceptors

Three different types of β adrenoceptors (β1, β2, and β3) are identified so far with a homology of approximately 60% amino acid sequence. Ligands, such as adrenaline and noradrenaline, bind to the ligand-binding pocket of the receptor site to control various functional responses including contractility and heart rate, relaxation of smooth muscle, and numerous metabolic events. As discussed earlier, all these receptor subtypes are coupled to G-protein Gs and simultaneously activate adenylyl cyclase (Table 4.4). However, research findings suggest variance in moderation of events and signals, such as receptor downregulation or desensitization, following activation of three different β-ARs (Lefkowitz 2000; Ma and Huang 2002; Kohout and Lefkowitz 2003). However, the magnitude of such regulation largely depends on the affinity of the receptor to bind with the ligand, and thus, β2 ARs are found more susceptible. Overall, the activation process of β-receptors leads to the accumulation of cyclic adenosine monophosphate (cAMP) followed by activation of PKA and phosphorylation, thereby alteration of function of cellular protein (Table 4.4).

4.2.2.1 β_1-Adrenoceptor

Structural Biology

One of the major subtypes of the β-ARs is the β1 receptor (Bylund et al. 1994). The receptors consist of seven membrane-spanning domains, three intra- and three extracellular loops, one extracellular N-terminal domain, and one intracellular C-terminal tail.

Structural determination of β1 ARs was challenging because of its purification difficulty and instability in detergent (Warne et al. 2008). However, β1 ARs from frog and turkey red blood cells were estimated to have a molecular weight of 54,000 and 43,500, whereas β1 ARs obtained from mammalian lungs were shown to have a molecular weight of 60,000–65,000 (Shorr et al. 1985). In fact, the first high resolution picture of the ARs is obtained from β ARs, where the ligand binding pockets were clear to fit a selective ligand (Suryanarayana et al. 1992).

Biological Distribution

β1-ARs are dominantly present in the cardiac tissue, coronary artery, adipose tissue, and juxtaglomerular cells of kidney (Wallukat 2002). The presence of β1-AR has also been found in the central nervous system, in brain (Gingrich and Caron 1993).

Table 4.4 Beta (β) adrenergic receptors and their effector system

Receptor subtype	G protein	Examples of some biochemical effectors
β1	Gs	↑ adenylyl cyclase,
		↑ L-type Ca^{2+} channels
β2	Gs	↑ adenylyl cyclase,
β3	Gs	↑ adenylyl cyclase,

Role in Biological System

β1-ARs act through GPCRs to stimulate adenylyl cyclase that hydrolyzed ATP to cAMP. Then, the generated cAMP activates cAMP-dependent PKA, which phosphorylates troponin that interacts with calcium ion to increase the rate and force of contraction in cardiac muscle (Wallukat 2002).

The stimulation of β1-ARs leads to an increased heart rate and force of contraction (positive inotropic action) as well as to an increased heart rate (positive chronotropic action) and rhythm for enhanced conduction velocity (positive dromotropic action) (Sakuma et al. 2001; Wallukat 2002). It also mediates to raise markedly the cardiac output and consumption of oxygen in the heart. An increase in BP also occurs upon its activation but significant enhancement in BP directed to reflex bradycardia mediated by the stimulation of vagus nerve. β-AR triggers the release of rennin and ghrelin (Zhao et al. 2010). It also stimulates the secretion of amylase from the salivary gland. Isoprenaline/isoproterenol, adrenaline/epinephrine, and noradrenaline/norepinephrine display agonism (sympathomimetics) towards β_1-ARs where the order of potency is as follows: Isoprenaline>adrenaline>noradrenaline (Rang et al. 1999).

Receptor Modulators

β1-AR agonists are used for cardiac stimulation in the treatment of cardiogenic shock and severe congestive heart failure (CHF) (Wallukat 2002). Dobutamine, denopamine, and xamoterol are the important members in this category. Though dobutamine acts on both α and β-ARs, its specificity towards β1-AR is relatively higher for which it has been considered as a selective β1-AR agonist (Parker et al. 2008). Denopamine is also a β1-AR agonist and is used in the treatment of angina (Ishide 2002; Nakajima et al. 2006). It can also be used to treat CHF and pulmonary edema (Nishio et al. 1998; Sakuma et al. 2001). In contrast, xamoterol is a partial agonist to β-AR where it stimulates the heart at rest and blocks during exercise (Rang et al. 1999). On the other hand, β-AR blockers are classified into β1-AR blocker (cardioselective) and nonselective blocker, i.e., they block both β1 and β2-AR. In all cases associated with β-AR blockers, 'selectivity' is a relative term and not absolute. Therefore, selective β1-AR blocking may be associated with β_2-AR-mediated bronchoconstriction but less likely to produce these side effects (Baker et al. 2017). Metoprolol, atenolol, acebutolol, bisoprolol, esmolol, betaxolol, celiprolol, etc. are the more potent blockers of β1-AR than β2-AR and produce better cardioselective actions depending upon the dose. These drugs are used to treat patients depending upon the associated properties of their own viz. with intrinsic sympathomimetic action, without sympathomimetic action, with additional α-AR blocking ability, membrane stabilizing action etc. as well as patient's viz. diabetes, asthmatics, etc. (Rang et al. 1999). On this basis, there are numerous clinical uses of β-AR antagonists like hypertension, angina pectoris, myocardial infractions, cardiac arrhythmias, glaucoma, etc. Metoprolol is the classical molecule as a selective β1-AR blocker. It shows weak membrane stabilizing activity but devoid of intrinsic sympathomimetic activity. Acebutolol is another cardioselective drug with partial agonistic effects, i.e., it activates β1 and/or β2 submaximally and membrane stabilizing actions. It is preferred for patients prone to bradycardia. It has also intrinsic sympathomimetic activity. Diacetolol is also a

β-adrenergic blocker which is the primary metabolite of acebutolol and used as an anti-arrhythmic agent (Basil and Jordan 1982). Atenolol and bisoprolol are selective β1-AR blockers without any intrinsic sympathomimetic action and primarily used to treat high blood pressure as well as heart-associated chest pain. Bisoprolol has a higher degree of β1-AR selectivity compared to other selective β1-AR β blockers like atenolol, metoprolol, and betaxolol. However, nebivolol has better selectivity than bisoprolol. Esmolol is an ultrashort acting β1-AR blocker and preferably used during cardiac surgery to control heart rate and BP (Jaillon and Drici 1989; Deng et al. 2006). Betaxolol is a selective β1 AR blocker, which is used for the treatment of hypertension as well as glaucoma. Its therapy may be advantageous over timolol with respect to fewer β2-AR blocking mediated side effects. The levo form of betaxolol is also available on the market. Celiprolol is a selective β1-AR blocker with additional β2-AR partial agonistic activity that is beneficial for asthmatic patients to avoid worsening of disease conditions. It is also a weak α2 receptor antagonist. It has orphan drug status for the treatment of vascular Ehlers–Danlos syndrome through promoting normal collagen synthesis in the blood vessels and thereby shifting the pressure load away from the vessels prone to rupture (Beridze and Frishman 2012). Flusoxolol is also a selective β-AR blocker. Landiolol is a selective β1-AR antagonist that reduces heart rate effectively with less negative effect on blood pressure or myocardial contractility (Ikeshita et al. 2008; Wada et al. 2016). This is also an ultra short-acting drug like esmolol but shows better cardioselectivity (Baker 2005; Okajima et al. 2015).

4.2.2.2 β_2-Adrenoceptor

Structural Biology

β2 ARs are another key subtype of β-ARs which also belong to GPCRs superfamily (Bylund et al. 1994). The representation of the binding pockets of β2 ARs from humans is made in Fig. 4.3, where only one antagonistic binding site differs between β1 ARs and β2 ARs constituting 15 amino acids. The presence of tyrosine in β2 AR at the top of TM7 differs by the presence of phenylalanine in β1 AR (Suryanarayana et al. 1992). The represented extracellular surface of β2 AR in Fig. 4.3b shows the differences with β1 AR in yellow. The interior of the β2 AR has been represented in Fig. 4.3c, where the binding pockets are split, with TM6-TM7 on the left and TP-TM5 on the right (Kobilka 2011). The figure represents the identical nature between the structures of β1 AR and β2 AR from the binding pocket to the cytoplasm. Thus, it had been inferred that the structural diversity of the amino acids are much higher into the binding pockets (Kobilka 2011).

Biological Distribution

β2-ARs are located mainly in the smooth muscles of the biological system, which include vascular, airway, gastro-intestinal tract, uterus, bladder sphincter, seminal tract, iris (radial muscle), and ciliary muscle (Bylund et al. 1994).

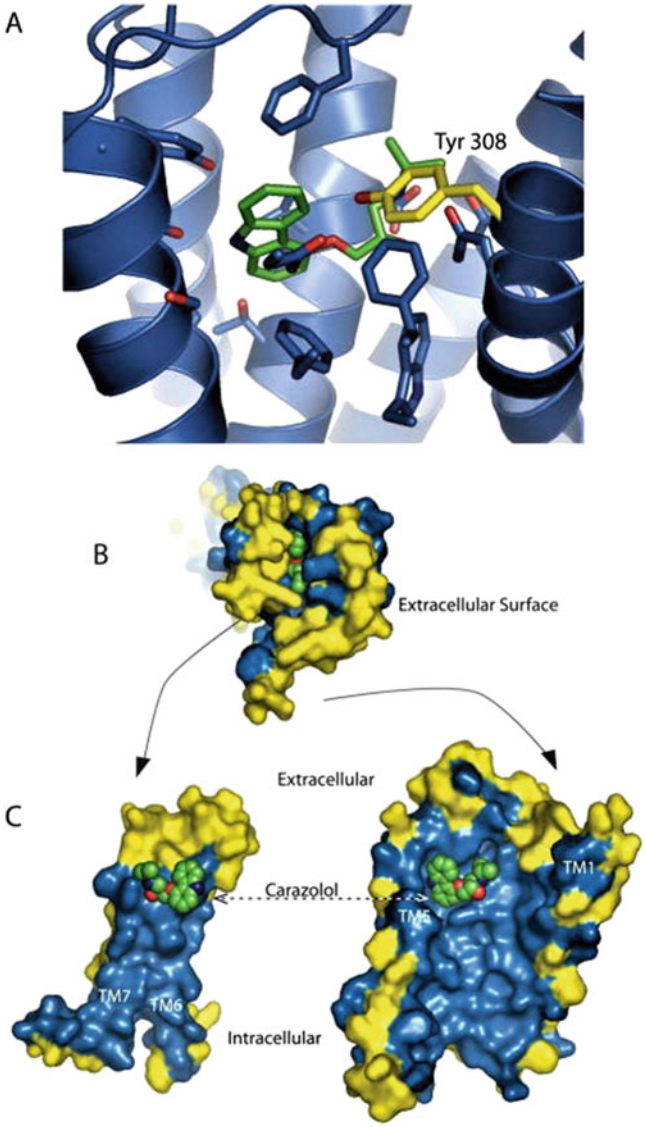

Fig. 4.3 Subtype-specific ligand binding in the β2AR. Amino acids that differ between β1AR and β2AR are shown in yellow. The inverse agonist carazolol is shown with green carbons. (**a**) The binding pocket of the human β2AR. Only one of the 15 amino acids that constitute the antagonist binding pocket differs between β1AR and β2AR. Tyr 308 at the top of TM7 in the β2AR is Phe in the β1AR. (**b**) The extracellular surface of the β2AR. (**c**) The interior surface of the β2AR that has been split along the plane of the binding pocket (Kobilka 2011)

Role in Biological System

The pathway to exert the pharmacological response through this receptor is similar to β1 ARs except PKA-phosphorylated phospholamban that interacts with calcium ion for faster relaxation of the smooth muscle. The stimulation of selective β2-ARs causes relaxation of smooth muscle without affecting the function of the heart. The activation of β2-ARs leads to glycogenolysis in both liver and skeletal muscle. An increase in release of histamine occurs through stimulation of β2-ARs. The agonism to β2-ARs augments the secretary action of ciliary muscle (Bylund et al. 1994). Isoprenaline/isoproterenol, adrenaline/epinephrine, and noradrenaline/norepinephrine display agonism (sympathomimetics) towards β2-ARs where the order of potency is as follows: Isoprenaline>adrenaline>noradrenaline which is similar to β1-ARs-mediated agonism.

Receptor Modulators

Many selective β2-ARs agonists such as salbutamol, terbutaline, salmeterol, and formoterol have been developed for their bronchodilator action for the relief of reversible airway obstruction like asthma. These drugs have lower incidence of side effects mediated by β_1-ARs whereas long-term use of it led to muscle tremor through change in β_2-ARs-mediated rate and force of contraction. The activation of β2-ARs in the α-cells of pancreatic islets causes increase in glucagon secretion. Isoxsuprine is a selective β2 ARs agonist that causes direct relaxation of uterine and vascular smooth muscle via β_2-ARs. Isoxsuprine is used as a tocolytic for the treatment of premature labor and as a vasodilator for the treatment of cerebral vascular insufficiency. Ritodrine is a short-acting β2-ARs agonist and is also used to suppress threatened abortion. Butaxamine/butoxamine is a selective antagonist to β2-ARs and has no clinical use. It is used for experimentation where selective blocking of β2-ARs is required to estimate the effect of other receptors. In contrast to cardioselective β-AR blocker, nonselective β-blockers are widely used to treat hypertension, myocardial infraction, cardiac arrhythmia, glaucoma, angina pectoris, CHF, atrial fibrillation etc. The blockade of facilitatory effect of presynaptic β-ARs on noradrenaline release may also contribute to the antihypertensive effect. Propanolol is the classical example as the nonselective category of β-AR blocker. Prior to treating patients with β-blocker, associated adverse effects of these drugs should be considered, viz. bronchoconstriction for patients with airway disease like asthmatics, or obstructive pulmonary disease; bradycardia leading to heart block for coronary disease patients; adequate cardiac output for cardiac failure patients to impart a degree of sympathetic drive to the heart; hypoglycemia for diabetic patients; and cold extremities due to block of beta receptor mediated vasodilation in cutaneous vessels. Topical β-blockers are the first-choice drugs for open angle glaucoma because these drugs lower intraocular tension by reducing aqueous formation without affecting pupil size, tone of ciliary muscle, or outflow facility. Timolol is a nonselective β-AR blocker. It has no local anesthetic or intrinsic sympathomimetic activity. Cartiolol is a β blocker with intrinsic sympathomimetic activity. It can also act as HT_{1A} and 5-HT_{1B} receptor antagonist. It has the ability to preserve ocular perfusion and increase retinal blood flow. This may prevent optic nerve damage independent of intraocular tension reduction. Hydroxycarteolol is the active metabolite of carteolol. Levobunolol is an alternative to timolol for the management of ocular hypertension

and open-angle glaucoma. Metipranolol and mepindolol are also similar to timolol but have different corneal anesthetic character. Befunolol is a β-AR blocker with intrinsic sympathomimetic activity. This also acts as a β-ARs partial agonist. Pindolol is a nonselective β-ARs blocker. It preferentially blocks 5-HT_{1A} receptor as well as acts as a weak partial agonist to this receptor. It also shows intrinsic sympathomimetic activity and membrane-stabilizing effects. Tertatolol also displays similar action like propanolol and pindolol to serotonin receptor (Langlois et al. 1993). Carvedilol is a nonselective β-AR blocker as well as α_1 blocker. Both S(−) and R(+) enantiomers are responsible for later action whereas S(−) enantiomer is responsible for former action (Ruffolo et al. 1990). Like carvedilol, labetalol also possesses α_1 and β-ARs antagonistic activity. It has intrinsic sympathomimetic activity as well as membrane stabilizing activity (Craig and Stitzel 2004; Mottram and Erickson 2009). Nebivolol is generally categorized as a nonselective β-AR antagonist, but this unique drug shows cardioselectivity at a dose of 5 mg and loses β1-ARs selectivity at a dose of above 10 mg (Nuttall et al. 2003). Moreover, nebivolol is devoid of cardioselectivity in patients who are genetically poor metabolizer or who are coadministering this agent with CYP2D6 inhibitors (Nuttall et al. 2003). Alprenolol is a nonselective β-AR blocker with additional 5-HT_{1A} and 5-HT_{1B} receptor antagonist (Langlois et al. 1993). This is used for the treatment of angina pectoris (Hickie 1970). The activity as well as application of oxprenolol is closely related to alprenolol (Langlois et al. 1993). Alprenoxime is a prodrug to alprenolol and also block β-ARs (Prokai et al. 1995). Adimolol is a nonselective α1, α2, and β-AR antagonist (Palluk et al. 1986). Bevantolol is a β-AR blocker as well as a calcium channel blocker (Vaughan Williams 1987). Bopindolol is a β-adrenergic blocker which acts as a prodrug for its active metabolite 4-(3-t-butylamino-2-hydroxypropoxy)-2-methylindole (Nagatomo et al. 2001). Bufuralol is a potent β-AR antagonist with partial agonist activity (Pringle et al. 1986). Bupranolol is a nonselective β-AR blocker with strong membrane stabilizing activity but devoid of intrinsic sympathomimetic activity. Sotalol is a nonselective β-AR blocker with both class II and class III antiarrhythmic properties. Like other nonselective blockers, it has also potential side effects but used generally to treat abnormal heart rhythms associated with ventricular arrhythmias, atrial fibrillation, or atrial flutter (Bertrix et al. 1986). Nipradilol is a β-AR antagonist and also acts as a nitric oxide donor (Inoue et al. 2011). Medroxalol is a dual inhibitor for both α and β ARs. Flestolol is a short-acting β-AR antagonist. Other than those mentioned above, here are some β-AR blockers which have been developed for desired therapeutic applications with lesser side effects like adaprolol, alfurolol, amosulalol, ancarolol, arnolol, bornaprolol, brefonalol, bucumolol, bufetolol, bunitrolol, Butidrine, carpindolol, cicloprolol, cinamolol, cloranolol, cyanopindolol, dalbraminol, ecastolol, epanolol, ericolol, ersentilide, exaprolol, eugenodilol, falintolol, indenolol, indopanolol, isoxaprolol, levomoprolol, moprolol, nadolol, nadoxolol, nifenalol, pacrinolol, pafenolol, pamatolol, pargolol, primidolol, procinolol, ridazolol, ronactolol, soquinolol, spirendolol, sulfinalol, talinolol, tazolol, tienoxolol, tilisolol, tiprenolol, tolamolol, toliprolol, xibenolol, and xipranolol.

4.2.2.3 β3 Adrenoceptors

The existence of β3 ARs was first revealed in 1984, where the author reported the existing β ARs (β1 and β2) are not specific to the novel β-adrenergic ligands (BRL 35135A, BRL 33725A, BRL 26830A). These ligands were shown to produce remarkable anti-obesity actions on experimental animals with severe diabetes and obesity (Arch et al. 1984). Later, this novel AR was cloned by Emorine and team (Emorine et al. 1989).

Structural Biology

This β3 ARs, similar to β1 and β2, belong to serpentine seven transmembrane GPCRs. Each segment of the transmembrane consists of 22–28 amino acids, with three extracellular and three intracellular loops. Thus, altogether, these β3 receptors contain 396 amino acids, where the C-terminal is intracellular and the N-terminal is extracellular. This N-terminal ending is glycosylated, whereas the intracellular C-terminal ending is phosphorylated by the action β-receptor kinases or PKA (Coman et al. 2009). The interaction of specific ligands to this receptor and its subsequent activity depend on the disulfide linkage between the two amino acids (Cys110 and Cys361) in the second and third extracellular loops (Coman et al. 2009).

Additionally, four transmembrane domains are also reported crucial for the interaction between receptors and ligands, such as transmembrane 3, 4, 5, and 6. Further, transmembrane 2 and 7 are associated with the activation of G protein to promote the formation of a second messenger (Skeberdis 2004).

Biological Distribution and Role

The distribution of β3-ARs is observed in the myocardium, where the activation of the atrial myocytes results in the phosphorylation of the calcium channels. Such phenomenon gives rise to the transmembrane current of calcium (Kaumann 1996). It has also been postulated that β3-ARs are also known to produce negative ionotropic action on the ventricles. It is established that the stimulation of ARs in the ventricular region activates endothelial NO synthase, thereby increasing nitric oxide production. This increased nitric oxide in turn increases cyclic guanosine monophosphate (cGMP) with a subsequent activation of phosphodiesterase 2 or inhibition of phosphodiesterase 3; thus there is a reduction in myocardium contractility (Gauthier et al. 1996).

The presence of β3-ARs was found in the isolated canine pulmonary rings, where *in vitro* stimulation of this receptor has indicated the production of cAMP-dependent vasodilation (Tagaya et al. 1999). Similarly, the presence of β3-ARs was also evidenced in peripheral microvascular muscle of dogs and anesthetized rhesus monkeys, where stimulation of the receptors resulted in vasodilator effect to decrease the blood pressure of the respective animals (Berlan et al. 1994; Hom et al. 2001).

The presence of β3-ARs has also been established in the respiratory system, particularly in the nasal epithelium in rabbits to maintain salt and water movement through epithelium (Danner et al. 2001). The presence of this AR in dog's bronchi

muscle is reflected by the increase in cAMP production, leading to bronchodilation (Tamaoki et al. 1993). The presence of β3-ARs in the respiratory system is species dependent, as a specific agonist to this receptor showed relaxation of bronchial muscle in dogs without producing any effect in sheep, guinea pig, and even in humans (Martin and Advenier 1995).

The presence of β3-ARs has also been observed in different areas of the brain, particularly lower in cerebellum, brain stem, and hypothalamus and higher in striatum, cortex, and hippocampus (Summers et al. 1995). The administration of β3-AR agonists directly to the CNS of experimental animals results in neuronal activation, leading to increase in appetite (Castillo-Meléndez et al. 2000).

The presence of β3-AR in the human retinal endothelial cell lysate has been established, and its role in the migration and proliferation of human retinal endothelial cells and choroidal endothelial cells is investigated (Steinle et al. 2003, 2005).

The existence of β3-ARs has been reported in the gastrointestinal tract (GIT), pancreas, and gall bladder. Agonists to this receptor have shown a decrease in gastrointestinal motility, and thus a decrease in gastric emptying and intestinal transit of the gastric contents. Stimulation of β3-ARs in the GIT of guinea pig showed cAMP-dependent relaxation of stomach fundus and cAMP-independent relaxation of the duodenum (Arch and Kaumann 1993; Horinouchi et al. 2002). It has also been reported that the stimulation of β3-ARs produces a gastro protective action, may be by increasing mucous production or by inhibition of gastric secretion (Vinay et al. 2002; Adami et al. 2003).

The role of β1 and β2 ARs is well assumed and established in the management of obstructive airway diseases or coronary heart disorders, and the role of β3-ARs is focused on overactive bladder syndrome (Chapple et al. 2008). Apart from the described distribution of β3-ARs, it has also been observed in genital apparatus (Berkowitz et al. 1995; Cirino et al. 2003) and skeletal muscles (Chamberlain et al. 1999).

Receptor Modulators

Although β3-AR modulators along with noradrenaline-serotonin uptake inhibitor have been focused towards the treatment of obesity, there is no β3-AR-selective agonist being approved so far for this purpose currently (Jesudason et al. 2011). Alternatively, the activation of β3-ARs through brown adipose tissue recently focus on moderate sleep, eating habit and metabolic responses (Szentirmai and Kapás 2017). Several β3-ARs agonists, such as vibegron and mirabegron, are presently approved by regulatory agencies and introduced clinically to overcome problems associated with overactive bladder syndrome, where solabegron and ritobegron are still under clinical investigation towards approval (Warren et al. 2016; Schena and Caplan 2019). Amibegron has also crossed the barrier of laboratory, but discontinued during progression in clinical research for application as antidepressant and antianxiolytic (Schena and Caplan 2019).

4.2.3 Conclusion

The role of catecholamines in the biological system is eminent and cannot be compromised for a healthy life. Their action largely depends on engagement to the particular receptor followed by conformational change and generation of the second messenger. Understanding the activities of different receptors-based modulatory action has led to the development of several modulators in the treatment of different physiological ailments, and several are yet to be explored.

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Pharmacology of Dopamine and Its Receptors

5

Sunpreet Kaur, Shamsher Singh, Gagandeep Jaiswal, Sandeep Kumar, Wafa Hourani, Bapi Gorain, and Puneet Kumar

Abstract

Dopamine (DA) is the major catecholamine neurotransmitter in the brain which regulates multiple functions including the control over voluntary action, reward, circadian rhythm, consciousness, and cognition. The synthesis of DA involves two events, i.e. hydroxylation of L-tyrosine to DOPA catalysed by tyrosine hydroxylase (TH) and further, DOPA gets decarboxylated to final product DA via aromatic L-amino acid decarboxylase (AADC) enzyme. Metabolism involves monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) enzymes, which degrade dopamine finally into 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). DA acts through two different subclasses of receptors including D1-like (D1 and D5) and D2-like (D2, D3 and D4) dopamine receptors. Dopamine performs various functions through its receptors like regulation of growth, reward, sleep, locomotion, emotions, renal functions, gastrointestinal motility, etc. Furthermore, dopaminergic system plays

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an important role in the pathogenesis of various neurological diseases like Parkinson's disease (PD), Huntington's diseases (HD), Alzheimer's disease (AD), schizophrenia, anxiety, epilepsy, traumatic brain injury (TBI), and multiple sclerosis (MS). Also, high pace discoveries that occurred in the research field pave the way for recent advancements in the dopaminergic system. Currently, with the help of molecular cloning, two D1-like and three D2-like receptor genes have been successfully identified. In the current chapter, various roles of dopamine and dopaminergic receptors have been highlighted but there is still a need to understand a lot of functions and specific roles of the receptors. Hence, the high pace of research along with newly developed advancements in the field of neuroscience and pharmacology will be useful to get more knowledge about dopamine receptor signalling in devastating disorders.

Keywords

Catecholamines · Dopaminergic receptors · Circadian rhythm · Parkinson's disease · Huntington's disease · Alzheimer's disease · Schizophrenia · Anxiety · Epilepsy · Traumatic brain injury · Multiple sclerosis

Abbreviations

5-HT	5-Hydroxytryptamine
AADC	Aromatic amino acid decarboxylase
AC	Adenyl cyclase
ACC	Anterior cingulated
AD	Alzheimer's disease
ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ALLO	Allopregnanolone
AMP	Adenosine monophosphate
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BLA	Basolateral amygdala
cAMP	Cyclic adenosine monophosphate
COMT	Catechol-O-methyl-transferase
CREB	cAMP response element binding protein
D1R	D1 receptor
D5R	D5 receptor
DA	Dopamine
DARPP-32	Dopamine- and cAMP-regulated neuronal phosphoprotein
DAT	Dopamine transporters
DHX	Dihydrexidine
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
DOPAL	3,4-Dihydroxyphenylacetaldehyde

DOPET	3,4-Dihydroxyphenylethanol
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
EPS	Extrapyramidal symptoms
FAD	Flavin adenine dinucleotide
GABA	Gamma-aminobutyric acid
GAD	Generalized anxiety disorder
GPCRs	G-protein-coupled receptors
GPe	External globus pallidus
GPi	Globus pallidus internal
$G_{\alpha i}$	G_i alpha subunit
$G_{\alpha s}$	G_s alpha subunit
HD	Huntington's disease
HVA	Homovanillic acid
L-DOPS	L-Dihydroxyphenylserine
MAO	Monoamino oxidase
MAPK	Mitogen-activated protein kinase
MS	Multiple sclerosis
MSN	Medium spiny neuron
NAc	Nucleus accumbens
NMDA	N-methyl-D-aspartate receptor
O_2	Molecular oxygen
OB	Olfactory bulb
OCC	Orbitofrontal cortical
OCD	Obsessive compulsive disorder
PD	Parkinson's disease
PFC	Prefrontal cortex
PKA	Protein kinase A
PKC	Protein kinase C
PP1	Protein phosphatase
PRMS	Progressive relapsing multiple sclerosis
PTSD	Post-traumatic stress disorder
RNA	Ribose nucleic acid
RRMS	Relapsing remitting multiple sclerosis
SCZ	Schizophrenia
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulata
SPD	Sensory processing disorder
SPMS	Secondary progressive multiple sclerosis
STN	Subthalamic nucleus
TBI	Traumatic brain injury
TBZ	Tetrabenazine
TH	Tyrosine hydroxylase
VMAT2	Vesicular monoamine transporter 2
VTA	Ventral tegmental area

5.1 Introduction

Dopamine is the major catecholamine neurotransmitter in the brain. It regulates a variety of functions including the control over voluntary action, reward, circadian rhythm, consciousness, and cognition (Hasbi et al. 2011). Indeed, it is a neurotransmitter that controls emotions, motor, and mental functions of the brain. The adequate balance of dopamine assures a state of happiness while overexcitation of dopamine system leads to manic and psychotic responses. Dopamine plays a major role in controlling various brain functions including voluntary movement. It not only participates in movements but also predominantly regulates cognition, consciousness, and addiction. The role of dopamine is unique and complex as dopamine serves as a key unit in the reward system of the brain. The reward system provides the evidence regarding drug addiction, dependence, and reinforcement. To understand the functions of dopamine, it is necessary to understand the widespread distribution of dopamine neurons and its projections in the brain.

The dopaminergic neurons are specifically localized in the midbrain and its associated regions including substantia nigra pars compacta, the ventral tegmental area, and the retrorubral field like regions (Haber 2016). The pathways formed by these projections are known as nigrostriatal, mesolimbic, mesocortical, and tuberoinfundibular pathways which individually coordinate distinct functions like nigro for substantia nigra and striatal for striatum; this pathway initiates from substantia nigra and terminates in dorsal striatum. The dopaminergic neurons of these pathways coordinate motor control, thereby implicit in motor coordination disorders like Parkinson's disease (PD) and Huntington's disease (HD). Similarly mesolimbic pathway as the name suggests originates from the ventral tagmented area of midbrain and terminates towards ventral striatum and its nearby regions like nucleus accumbens, olfactory tubercle, amygdala, and hippocampus. The function of these pathways remains motivation, drug addiction, apathy, and psychiatric illness, so it is also well known as a reward pathway. Further, the mesocortical pathway travels from the ventral tegmental area to the prefrontal cortex (PFC) and other cortical areas and known to coordinate the executive functions including cognition, motivation, and emotional responses (Jučaitė 2002). This pathway usually associates with the pathogenesis of schizophrenia and also with behavioural symptoms in Alzheimer's disease (AD), HD, and PD. At last, the tuberoinfundibular pathway originates from the arcuate nucleus of the hypothalamus and ends in medium eminence of the pituitary gland. It regulates the secretion of prolactin from anterior pituitary gland so implicit in menstrual disorders and other sexual disorders. In this way, dopamine is broadly distributed in the brain to mediate a variety of functions in neurodegenerative and psychiatric disorders.

5.2 History

Historical development in the discovery of dopamine and its receptor is provided in Table 5.1.

Table 5.1 History of dopamine discovery

Year	Important events	Reference
1910	Synthesized dopamine at Wellcome labs in London, England	Barger and Dale (1910)
1911	Synthesized L-dopa	Hornykiewicz (2002)
1952	Current name of dopamine suggested	Barger and Dale (1910)
1950	Function of dopamine was discovered as a neurotransmitter at the Laboratory for Chemical Pharmacology of the National Heart Institute of Sweden	Carlsson et al. (1962)
1956	Effect of dopamine on blood pressure had found	Hornykiewicz (1958)
1957–59	Dopamine as a neurotransmitter in the brain was shown and it was found that the highest regional concentration existed in the basal ganglia and in the striatum in high concentrations	Carlsson (1959)
1959	It was found that dopamine is responsible for PD	Hornykiewicz (2006)
1960	The enzyme tyrosine hydroxylase that converts L-tyrosine to L-dopa was discovered	Nagatsu et al. (1964)
1965	Dopamine has the potential to excite or inhibit neurons	Bloom et al. (1965)
1966	Dopamine hypothesis of schizophrenia	Van Rossum (1967)
1972	The existence of dopamine receptors was revealed	Brown and Makman (1972)
1976	Two dopamine receptors proposed: inhibitory and excitatory	Cools and Van Rossum (1976)
1978	Two dopamine receptors: coupled and uncoupled to adenylate cyclase	Spano et al. (1978)
1979	Names of D1 and D2 used	Kebabian and Calne (1979)
1990–91	Dopamine D1 and D5 receptors were cloned	Sunahara et al. (1990)
1990	Dopamine D3 receptor was cloned	Sokoloff et al. (1990)
1991	Dopamine D4 receptor was cloned	Van Tol et al. (1991)
1992	D2 receptor is more than 80% block by the antipsychotics associated with parkinsonism	Farde et al. (1992)
1996	Amphetamine-induced release of dopamine is higher in schizophrenia	Laruelle et al. (1996)
1998	D2 short receptors located mostly in nigral neurones	Khan et al. (1998)
1999	Therapeutic doses of antipsychotics block 60–80% D2	Kapur and Mamo (2003)

(continued)

Table 5.1 (continued)

Year	Important events	Reference
2003	Antipsychotics occupy more D2 in limbic areas than striatum	Bressan et al. (2003)
2005	Dopamine supersensitivity correlates with elevated D2 high states	Seeman et al. (2005)
2005	Higher D2 density in healthy identical twins of schizophrenia patients	Hirvonen et al. (2006)
2006	Markedly elevated D2 high receptors in all animal models of psychosis	Seeman et al. (2006)
2008	Dopamine neurons implanted into people with Parkinson's disease survive without pathology for 14 years	Mendez et al. (2008)
2011	Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease	Kriks et al. (2011)
2013	Dopamine modulates reward-related vigour	Beierholm et al. (2013)
2014	Protective and toxic roles of dopamine in Parkinson's disease	Segura-Aguilar et al. (2014)
2016	Unexpected rewards induce dopamine-dependent positive emotion–like state changes in bumblebees	Perry et al. (2016)
2018	Activity of dopamine neurons is more closely associated with the drug's reinforcing property	Wei et al. (2018)
2019	Dorsal striatum dopamine levels fluctuate across the sleep–wake cycle and respond to salient stimuli in mice	Dong et al. (2019)

5.3 Structure of Dopamine

Chemically, dopamine is a catecholamine consisting of a catechol ring and one ethylamine group. Various substituted phenethylamine analogues have been formed leading to the development of many therapeutic drugs including agonists and antagonists as shown in Fig. 5.1.

Dopamine behaves like an organic base which becomes protonated in the acidic environment like other amines. Water solubility and stability occur relatively in protonated form and is degraded when exposed to oxygen or other oxidants. Only in acidic environment dopamine is protonated but in basic form it is highly reactive and less soluble in water. Dopamine hydrochloride is a fine colourless powder in dry form which is used in various pharmaceutical applications.

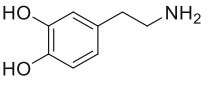

Fig. 5.1 Structure of dopamine

5.4 Synthesis, Reuptake, and Metabolism of Dopamine

The classical pathway of DA biosynthesis was initially stated by Blaschko in the late 1930s. It involves two events, proceed with hydroxylation in L-tyrosine to dihydroxyphenylalanine (DOPA) catalysed by tyrosine hydroxylase (TH) in the presence of tetrahydrobiopterin, O_2, and iron (Fe^{2+}) as cofactors (Juárez Olguín et al. 2016). Further, DOPA gets decarboxylated to final product DA via enzyme aromatic amino acid decarboxylase (AADC) where pyridoxal phosphate (PP) is present as a cofactor. The tyrosine utilized in this pathway formed by the enzyme phenylalanine hydroxylase in the presence of molecular oxygen (O_2) and tetrahydrobiopterin as cofactors (Meiser et al. 2013) (Fig. 5.2). Two other alternative pathways of dopamine synthesis can be described in the form of flow charts as shown in Figs. 5.3 and 5.4.

After formation, dopamine gets readily sequestered inside the synaptic vesicles through vesicular monoamine transporter 2 (VMAT-2) and remains stored under slightly acidic pH to prevent oxidation of dopamine. This vesicular transport irreversibly inhibited by reserpine and amphetamine like drugs (Meiser et al. 2013). Further upon excitation of dopaminergic neurons, synaptic vesicles get degranulated, hence releasing dopamine in the synapse where it interacts with dopamine receptors for subsequent actions and dopamine transporters (DAT) remove excess of dopamine from synapse.

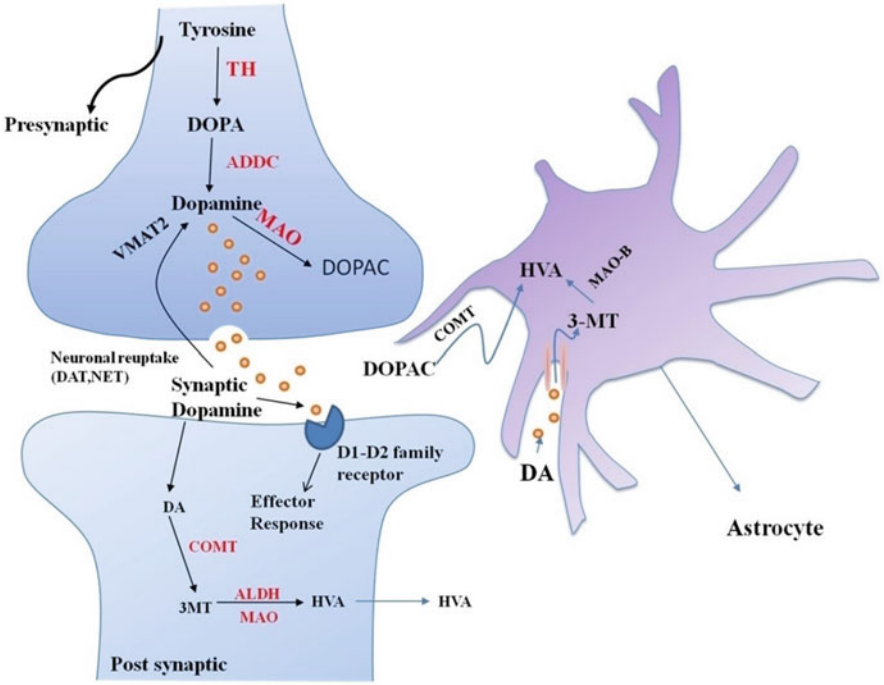

Fig. 5.2 Dopamine biosynthesis in the brain

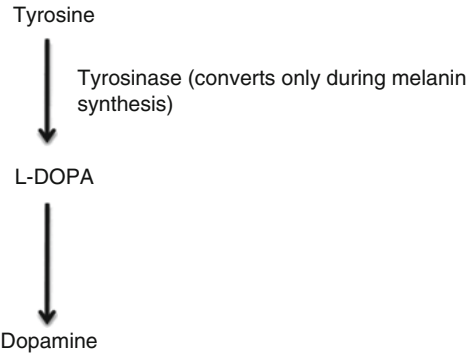

Fig. 5.3 First alternative pathway

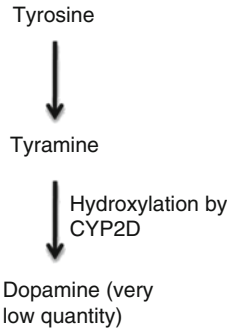

Fig. 5.4 Second alternative pathway

Reuptake is mediated either by the dopamine transporter or by the plasma membrane monoamine transporter. Once it comes back in the cytosol, dopamine can either be broken down by a monoamine oxidase (MAO) or repackaged into vesicles by vesicular monoamine transporter 2 (VMAT-2), making it available for future release.

The remaining excess of dopamine is degraded by enzymatic action. The enzyme MAO degrades dopamine to hydrogen peroxide and 3,4-dihydroxyphenylacetaldehyde (DOPAL) in the presence of flavin adenine dinucleotide (FAD). This DOPAL either gets frequently oxidized by aldehyde dehydrogenase (ALDH) or reduced by the action of alcohol dehydrogenase (ADH) to 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethanol (DOPET). The enzyme MAO exists in two types: MAO-A and MAO-B that reside in the outer mitochondrial membrane in neurons, microglial cells, and astrocytes. Even glial cells degrade dopamine by MAO or catechol-*O* methyltransferase (COMT). COMT, in Mg^{2+}-dependent manner, converts the DOPAC to homovanillic acid (HVA) but the activity of COMT is seen only in glial cells and not in striatal neuronal cells (Meiser et al. 2013). This way, well-regulated machinery works to maintain an adequate level of dopamine in the brain through highly controlled synthesis, reuptake, and degradation mechanism as shown in Fig. 5.5.

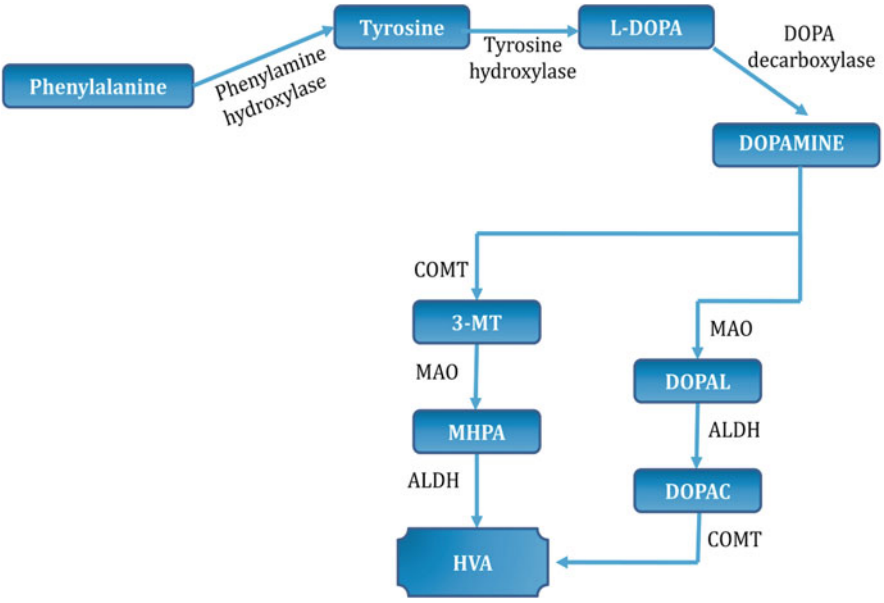

Fig. 5.5 Metabolism of dopamine

5.5 Dopamine (DA) Receptors and Their Localization

The physiological actions of dopamine are mediated by its interaction with dopamine receptors that are localized over dopaminergic synapses. There are five types of dopamine receptors categorized into two different subclasses including D1-like and D2-like dopamine receptors (Beaulieu et al. 2015). The D1-like class includes D1 and D5 receptors (D1R and D5R), whereas D2-like class constitutes of D2, D3, and D4 receptors (D2R, D3R, and D4R). Both classes of receptors differ in their mechanism of action. The D1-like receptors act by coupling to $G_{\alpha s}$ that causes adenylyl cyclase (AC) activation for cAMP production while D2-like couples to $G_{\alpha i}$ to negatively regulate AC. Here, the D1-like receptors are found to be expressed only postsynaptically but D2-like receptors are expressed both presynaptically and postsynaptically.

The D1 receptors are distributed over nigrostriatal, mesolimbic, and mesocortical areas, like the caudate-putamen (striatum), substantia nigra, olfactory bulb, amygdala and at small level in the hippocampus, cerebellum, thalamic areas, and hypothalamic areas (Rangel-Barajas et al. 2015). On the other side, D5 receptors are found to be expressed at a very low level in the prefrontal cortex, premotor cortex, cingulated cortex, entorhinal cortex, substantia nigra, hypothalamus, hippocampus, and dentate gyrus. A small proportion is also reported over medium spiny neurons of the caudate nucleus and nucleus accumbens.

The D2 receptors are expressed on the striatum, the substantia nigra, ventral tegmental area, hypothalamus, cortical areas, septum, amygdala, hippocampus, the nucleus accumbens, and the olfactory tubercle like regions (Rangel-Barajas et al. 2015). The distribution of D3 receptor is considered smaller in the limbic areas, striatum, the substantia nigra, pars compacta, the ventral tegmental area, hippocampus, septal area, and various cortical areas. Low expression of D4 receptor is reported upon the frontal cortex, amygdala, hippocampus, hypothalamus, globus pallidus and substantia nigra pars reticulata (Rangel-Barajas et al. 2015). Apart from this, D1, D2, and D4 receptors have been reported in retina whereas D2 receptors are detected in pituitary gland.

5.5.1 D1-Like Dopamine Receptor Expression

The striatum or caudo-putamen, nucleus accumbens (NAc), SN pars reticulata (SNpr), and olfactory bulb (OB) consist of a higher amount of D1-like receptor family. The D1-like receptor family includes the D1 and D5 receptors that are G-protein-coupled receptors (GPCRs) (Mishra et al. 2018). Entopeduncular nucleus, cerebral aqueduct, and ventricles have moderate expression while the dorsolateral prefrontal cortex, cingulated cortex, and hippocampus show lower expression of D1 receptors (Lud Cadet et al. 2010). This receptor plays a pivotal role in the reward system regulation, locomotor activity, learning and memory (Arias-Carrión et al. 2010). The adenylyl cyclase (AC) stimulation is induced by D1 receptors and results in the activation of guanosine nucleotide-binding proteins (G proteins) and production of cyclic AMP as a secondary messenger. Typically, signal transduction pathways are involved in different neuropsychiatric disorders causing activating the phospholipase C and inducing intracellular calcium release due to the involvement of D1 receptors. Usually, calcium is involved in the modulation of neurotransmitter release by exocytosis and regulating signalling pathway that causes the activation of proteins, such as calcium-dependent protein kinase C (PKC) (Ha et al. 2012). The electrochemical gradient is regulated by the Na^+K^+-ATPase and activation of this pump is inhibited through D1 receptors by protein kinase A (PKA) and PKC signalling pathways in the striatum (Pivovarov et al. 2019) and the kidney (Arnaud-Batista et al. 2016) (Fig. 5.6).

5.5.2 D2-Like Dopamine Receptor Expression

It includes subfamilies of D2, D3, and D4, whereas D2R subtypes possess 2 isoforms: the D2-short and D2-long type receptors. Most brain regions like striatum, external globus pallidus (GPe), amygdala, cerebral cortex, hippocampus, and pituitary possess D2 receptor and its subtypes (Mishra et al. 2018). Usually the prefrontal, temporal, and entorhinal cortex, and the septal region along with the VTA and SNpc of DAergic neurons show the expression of messenger RNA D2R (Villanueva 2015). The activity of AC and the production of cAMP levels and

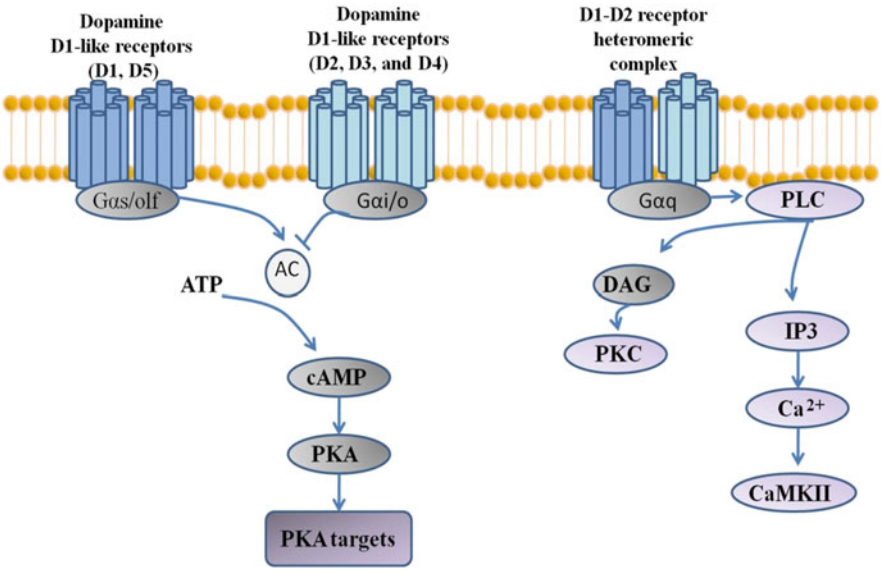

Fig. 5.6 Regulation of the signalling networks by DA in D1-like receptors, D2-like receptors, and D1-D2 receptor heteromers. DA D1-like family and D2-like family receptor homodimers signal through Gαs/olf and Gαi/o protein to regulate cyclic AMP through adenylyl cyclase (AC) activity. D1-like receptors activate AC through Gαs/olf, thereby increasing intracellular cAMP and stimulating PKA. D2-like receptors inhibit AC through Gαi/o, thereby suppressing cAMP and inhibiting PKA. The DA D1-D2 receptor heterodimer signals through Gαq, phospholipase C, enhancing the production of IP_3, mobilization of intracellular calcium and of DAG with subsequent activation of PKC

PKA are inhibited by this class of receptors (Zhang et al. 2006). Behavioural and extrapyramidal activities are mediated by the D2-type postsynaptic receptor. D2 receptors are known as autoreceptors acting via somato dendritic auto-receptors, which are known to decrease neuronal excitability (Chiodo and Kapatos 1992; Lacey et al. 1987) or inhibit dopamine release by terminal auto-receptors, which mostly reduce DA synthesis and packaging (Onali et al. 1988; Pothos et al. 1998). DA neuronal development is due to D2 auto-receptor during the embryonic stage (Baik 2013) and mediate various responses like cell proliferation–related pathways, such as the mitogen-activated protein kinase (MAPK) (Yoon and Baik 2013) and Akt (thymoma viral proto-oncogene also knows as protein kinase B) signalling pathways (Collo et al. 2013) (Table 5.2).

5.6 Function of Dopamine Receptors

The dopamine receptors are widespread across the brain and their crucial localization makes them play a variety of actions. The presynaptic localization of D2 auto-receptors inhibits dopamine release that decreases locomotor activity. On the other

Table 5.2 Dopamine receptors

Receptors	D1	D2	D3	D4	D5
Types	Gs-couple	Gi-couple	Gi-couple	Gi-couple	Gs-couple
Mechanism	Increased intracellular level of cAMP by activated adenylate cyclase	Increased intracellular level of cAMP by activating adenylate cyclase	Adenylate cyclase is decreased	Adenylate cyclase is decreased	Adenylate cyclase is increased
Location	Striatum, nucleus accumbens, olfactory bulb, amygdala hippocampus, substantia nigra, hypothalamus, frontal cortex	Striatum, VTA olfactory bulb, cerebral cortex	Striatum, VTA olfactory bulb, cerebral cortex	Frontal cortex, amygdala, hypothalamus, nucleus accumbens	Cortex, substantia nigra, hypothalamus
Function	Locomotion, learning and memory, attention, impulse control, sleep, regulation of renal function	Locomotion, learning and memory, attention, sleep, reproductive behaviour	Locomotion, cognition, attention, impulse control, sleep, regulation of food intake	Cognition, impulse control, attention, reproductive behaviour, sleep	Cognition, attention, decision making, motor learning, renin secretion
Selective agonists	KF-38393 SKF-81297 Fenoldopam (SKF-82526)	Bromocriptine Pergolide Cabergoline Ropinirole	5-OH-DPAT Pramipexole, Rotigotine, PD-128907, A 412997 ABT-670 PD-168077	A-412997 ABT-670 PD-168077	SKF81297
Selective antagonists	SCH-23390 SCH-39166 SKF-83566	Haloperidol, Raclopride Sulpiride Spiperone Risperidone	Nafadotride GR-103691, GR-218231, SB 277011A, NGB-2904 PG 01037 ABT-127	A-381393 FAUC213 L-745870 L-750667	SCH39366

side, postsynaptic D2 receptors enhance dopamine release to stimulate locomotion. This way dopamine agonists exhibit biphasic response by activating both presynaptic and postsynaptic receptors (Sulzer et al. 2016). Similarly, D3 receptor exhibits biphasic response but at a moderate level, it regulates reward and reinforcement like functions of human behaviour. The D4 and D5 are less expressed at motor control regions so they provide a low contribution to movement control (Beaulieu et al. 2015). Further, D1 and D2 receptors also regulate learning, working memory, and executive functions due to abundance in prefrontal cortex. But D3, D4, and D5 give less influence over cognition due to their lower expression in the hippocampus. The other major action of dopamine receptors is contributed by D2 receptors that regulate aggression, emotion, motor control, and food intake like human behaviour (Nyberg et al. 2016). These functions often contribute to psychotic behaviour, so today most clinical psychotic drugs act by blocking D2 actions for the treatment of schizophrenia and bipolar disorder. Other actions of dopamine include D2-mediated prolactin secretion from the pituitary gland, D1-mediated renin secretion from kidney, and adrenal gland-mediated D1-directed aldosterone secretion (Lv et al. 2018).

The major function of the dopamine system is to regulate voluntary movements which are carried out through the direct and indirect pathway in the basal ganglia. These both pathways are regulated from striatum that carries both D1 and D2 receptors. The D1 receptors are excitatory and send GABAergic inhibitory neurotransmission to globus pallidus internal (GPi). As D1 receptor activates GABA, so globus pallidus get inhibited. Further, the GPi sends GABAergic inhibitory projections to the thalamus that itself sends excitatory message via glutaminergic projections to motor cortex for proceeding movement. But as the GPi is already inhibited here, so cannot inhibit thalamus and voluntary movement generated. The alternate pathway is indirect pathway coordinated by D2 receptor which itself is inhibitory in nature. The D2 receptor sets up inhibitory control over glutaminergic projections going towards the globus pallidus external (GPe), and this GPe remains unaffected and sends GABAergic projections to the subthalamic nucleus (STN) which activates the thalamus and motor cortex via glutaminergic projections. In this way, the inhibited STN cannot activate the thalamus and voluntary movement stops to initiate. Both direct and indirect pathways act frequently to regulate the control over voluntary muscles while the entire dopaminergic system that lies in the basal ganglia is responsible for coordinating the motor activity in human body. Hence, motor coordination remains a predominant function of this neurotransmitter and any irregularity can predispose the human body towards movement disorders (Fig. 5.7).

Apart from this, dopamine receptors also play a vital role in addiction which reflects from changes in expression of dopamine receptors in the basal ganglia. As already mentioned, dopamine neurons that project from VTA to NAc are well known to implicit in reward-associated stimuli and addiction. Usually the abuse of drugs potentiates dopamine release and mimics the phasic response that results in fast dopamine release via the activation of D1 receptors (Atcherley et al. 2015). Changes in dopamine firing patterns are modulated by tonic response rather than phasic. It results in lowering the dopamine release which is sufficient to activate the

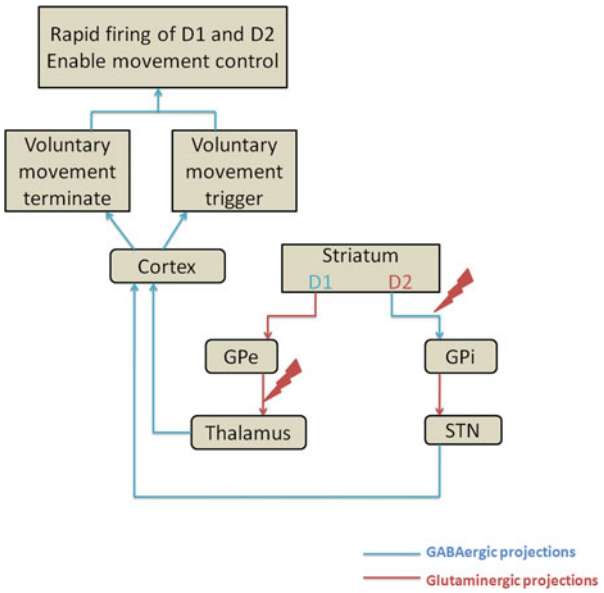

Fig. 5.7 Direct and indirect pathway for voluntary movement control

D2 receptor for motivation. Alterations that occur in phasic and tonic responses result from interaction with cortico-striatal glutaminergic synapses that modulate the functional signalling of D1 and D2 receptors expressing GABAergic medium spiny neurons (MSN). The striatal excitatory D1 receptors expressing MSN act through direct pathway while D2-MSN receptors act in an inhibitory manner through indirect pathway (Paladini and Roeper 2014). Both the ventral striatal pathways are evidenced to participate in reward and punishment respectively.

Let cite an example of abused drug which firstly activates the D1 and D2 receptors via enhancing the dopamine release. When dopamine decreases rate dependently, only D2 receptors remain active and justify the reward phenomenon in patients (Zweifel et al. 2009). Moreover, the identical pathways also justify associative learning which brings out the involvement of glutaminergic projections emerging from the hippocampus, amygdala, and prefrontal cortex. These projections uniquely regulate activation of D1 receptor that remains responsible for emotion-associated learning, hippocampal-dependent learning, and cortex associative learning. Besides this, the role of D2 receptor is confirmed in addiction as the expression of the receptor in striatum gets downregulated on repeated exposure of abuse drugs (Volkow and Morales 2015). The downregulation of D2 receptor in striatum mediates the suppression of the indirect pathway which further distorts the thalamo-cortical activity in prefrontal cortex. Moreover, decreasing availability of D2 receptors in both dorsal and ventral regions had been reported in PFC of abused patients specifically in anterior cingulated (ACC) and orbitofrontal cortical (OCC) regions. Altered functioning of dopamine receptors in these two regions is observed to be responsible for impulsive and compulsive behaviour in drug abusers. Hence, in

this way, it is the ratio and imbalance in between the D1- to D2-mediated direct and indirect pathway that uniquely contribute to addiction, learning, and other behavioural changes.

5.7 Agonists and Antagonists of Dopamine

Specific agonists and antagonists of dopamine are available for D1 and D2 class of dopamine. Selective agonists for D1 class include A77636, SKF38393, SKF81297, and dihydrexidine, while D2 selective compounds include quinpirole and N-0435. On the other side, antagonists of D1 class involve SCH23390, SCH39166, and SKF35566, while D2 antagonism is caused by domperidone, nemonapride, raclopride, and sulpiride (Smee and Overstreet 1976). Moreover, some compounds show receptor-specific actions and participate in dopamine mediated functioning as enlisted in Table 5.2.

D1 receptor family agonists and antagonists and their functions:

1. Agonists:
   (a) Dihydrexidine: It is a selective D1 agonist. Dihydrexidine gives its action through stimulating inspiratory motor output and by depressing medullary expiratory neurons. Dihydrexidine has been used in clinical trials for studying the treatment of SPD, cocaine-related disorders, and schizotypal personality disorder (Lalley 2009).
   (b) Fenoldopam: Fenoldopam is a fast-acting vasodilator. It is an agonist for D_1-like dopamine receptors and has a moderate affinity towards binding to the α_2-adrenoceptors. Fenoldopam is a racemic mixture and the R-isomer is responsible for the biological activity. The R-isomer has approximately 250-fold higher affinity for D_1-like receptors than does the S-isomer. It helps lowering down the blood pressure through arteriolar vasodilation. Thus, this agonist is also used as an antihypertensive agent (Felder et al. 1993).
   (c) SKF38393: It is a selective D1 agonist. It improves motor function in PD (Robertson et al. 1990).
   (d) SKF81297: It is a selective D1/D5 receptor agonist. It is believed to reduce long-term potentiation to prevent synaptic failure (Reavill et al. 1993).
2. Antagonists:
   (a) SCH23390: It is a D1 antagonist and believed to improve motor function in PD and has anxiolytic effects on the hippocampus, VTA (ventral tegmental area) (Lidow et al. 1991).
   (b) SCH39366: It is a classical benzazepine D1/D5 antagonist. It has been used in human clinical trials for studying different diseases including schizophrenia, cocaine addiction, and obesity (Wu et al. 2005).

D2 receptor family agonists, antagonists, and their functions:

1. Agonists:
   (a) Quinpirole: It is a selective D2 agonist. It has been observed that quinpirole has shown to increase locomotion and sniffing behaviour in mice. One study has found that quinpirole induces compulsive behaviour symptomatic of obsessive compulsive disorder in rats (Szechtman et al. 1998).
   (b) Bromocriptine: It is a selective D2 agonist. It is used in the treatment of pituitary tumours, type 2 diabetes, hyperprolactinemia, Parkinson's disease, and neuroleptic malignant syndrome (Kimberg et al. 1997).
   (c) Cabergoline: It is a long-acting dopamine agonist and also an inhibitor of prolactin. It is used in the treatment of hyperprolactinemic disorders and parkinsonian syndrome. Cabergoline possesses potent agonistic activity on dopamine D2 receptors (Colao et al. 1997).
   (d) Carmoxirole: It is a peripherally active selective agonist for D2-like receptors. It has a role as an antihypertensive agent, a dopamine agonist, and a platelet aggregation inhibitor (Rump et al. 1992).
   (e) Pramipexole: It is a D2-like receptor agonist. Pramipexole is a useful medicament in the treatment of the symptoms of PD. It is a *non-ergot dopamine agonist* drug that is effective in treating various symptoms of PD such as tremor, bradykinesia, and rigidity (slow movement) (Constantinescu 2008).
2. Antagonists:
   (a) Clozapine: It is a dopamine antagonist specifically for D4 receptor. Serotonin antagonist, with strong binding to 5-HT 2A/2C receptor subtype. It is used as an antipsychotic drug.
   (b) Haloperidol: It is a typical D2 antagonist. Haloperidol is an inhibitor of dopamine neurotransmitters and it increases its turnover. It is a traditional antipsychotic drug and is primarily used to treat schizophrenia and other types of psychoses. It is also used for the management of schizoaffective disorder, ballism, delusional disorders, and Tourette syndrome (a drug of choice) and used occasionally as an adjunctive therapy for mental retardation and the chorea associated with Huntington's disease. It is also a potent antiemetic and is used in the treatment of intractable hiccups (Seeman 2010) (Table 5.3).

5.8 Role of Dopaminergic System in Parkinson's Disease and Potential Therapeutic Drug Targets Under Research

Parkinson's disease (PD) is the rapidly growing neurodegenerative disorder troubling the ageing population with motor and non-motor complications. The motor complications in PD include bradykinesia, rigidity, tremor, and postural abnormalities caused by triggering cell death of dopaminergic neurons in substantia nigra pars compacta (SNpc), whereas the non-motor complications involve

Table 5.3 Agonists and antagonists of dopamine

Compound	Mechanism for dopamine
Dopamine	Dopamine agonist
A77636, SKF38393, SKF81297, dihydrexidine	Selective D1 agonists
Quinpirole and N-0437	Selective D2 agonists
Chlorpromazine	D2 blocker
Clozapine	Dopamine antagonist specifically for D4 receptor
Haloperidol	Dopamine antagonist specifically for D2 receptor
Apomorphine	Nonselective dopamine agonist
Fenoldopam	Selective D1-like partial agonist
Bromocriptine, cabergoline	Selective D2-like agonist
Carmoxirole	Peripherally active selective agonist for D2-like receptors
Dihydroergotamine	Partial D2-like agonist
Dihydroergocristine	Partial dopamine receptor agonist
Piribedil	Dopamine agonist
Quinilorane	Selective D2-like agonist
Roxindole	D2 auto-receptor agonist
Pimozide	D2-like antagonist
LE-300, SKF83566, SCH23390, SCH39166, and SKF35566	D1 selective antagonists
Melperone, risperidone, zipseridone, L-741, L-626, domperidone, nemonapride, raclopride, and sulpiride	D2 selective antagonists
Eticlopride, GR-103691, nafadotride, NGB-2904, U99194	D3 selective antagonists
Fananserin, PNU96415E, L-870, L-742	D4 selective antagonists
Bupropion	Nonselective inhibitor for dopamine and norepinephrine transporters
Cocaine	Inhibitor of monoamine transporters
GBR-12783, GBR-12909, GBR-12935, GBR-13069, indatraline	Selective dopamine uptake inhibitors
Reserpine	Inhibitor of vesicular monoamine transporter
Rimacazole	DAT inhibitor
Tetrabenazine	Potent inhibitor of vesicular monoamine transporter and depletes dopamine store

autonomic disturbances, olfactory dysfunction, and sleep problems which may originate due to neuron loss in other regions. The aetiology of PD is complex as multiple factors contribute to it which makes it a multifactorial disease. The contributing factors in PD are selected on the basis of neuronal loss accompanied by mitochondrial dysfunction, excitotoxicity, enhanced oxidative stress, abnormal protein folding and deposition, loss of trophic functions, and neuroinflammation like pathogenic mechanisms (Maiti et al. 2017).

As PD is a motor disorder and dopamine neurons are major motor coordinating neurons in the brain, dopamine contribution in PD is not controversial. The loss of dopaminergic neurons in midbrain remains a prominent feature of PD. Physiologically, the motor circuit of the basal ganglia constitutes direct and indirect pathways. The direct pathway regulates the excitatory input over thalamus via SNpc, SNpr, and GPi like key regions. On the other side, the indirect pathway regulates the inhibitory control over the thalamus to restrict movement by modulating GPe and STN. The dopaminergic regulation on SNpc gets defective due to the loss of dopaminergic neurons, causing PD by inhibiting the direct pathway, while the indirect pathway gets upregulated because compensatory mechanism of D1/D2 disrupts that result in overactivity of STN to mediate over inhibitory responses (Fig. 5.8).

On this basis, currently most of the approved drugs for PD potentiate dopamine for neuroprotective approach (Zinger et al. 2011). These drugs include levodopa, selegiline, bromocriptine, apomorphine, and ropinirole-like molecules that enhance dopamine levels in the brain. But these drugs exhibit several side effects that limit their use (Maiti et al. 2017).

This limitation leads to the assessment of receptor-specific drugs for therapeutic benefit. As discussed above, there are different subclasses of dopamine receptors belonging to D1 and D2 class. Untreated PD patients having dense D2-like receptors as compared to D1 receptors. The remaining D2 receptors generally gets supersensitive and contribute to tremor-like symptoms of PD. The ongoing research studies for receptor-specific drugs include evaluation of molecules like D1/D2 full agonist dihydrexidine; CY-208-243, A86929, and dihydrexidine (DHX) give positive results over symptoms of PD (Malo et al. 2012). Not only this, several partial agonists and agonists revealed to have good ligand activity for beneficial effects in PD (Table 5.4).

5.9 Role of Dopaminergic System in Huntington's Disease (HD) and Enlisting Potential Therapeutic Drug Targets Under Research

HD is a devastating slowly progressive motor disorder that results in chorea (uncontrolled dancing movements), cognition loss, and psychiatric problems (Rubinsztein and Carmichael 2003). The aetiology behind HD associates with autosomal genetic mutation in Huntingtin gene that causes medial spiny neuron loss in the striatum and cortex. Other regions affected include hypothalamus, globus pallidus, subthalamic nucleus, and substantia nigra.

The striatum remains a major regulatory unit for motor control and its degenerative changes contribute to different pathogenic mechanisms in HD. The dopaminergic neurons of striatum principally coordinate movement control via direct and indirect pathways. The direct pathway expresses D1 receptors that project towards substantia nigra pars reticulate and internal globus pallidus to regulate disinhibition of thalamus for motor function, whereas the D2 pathway coordinates its inhibition

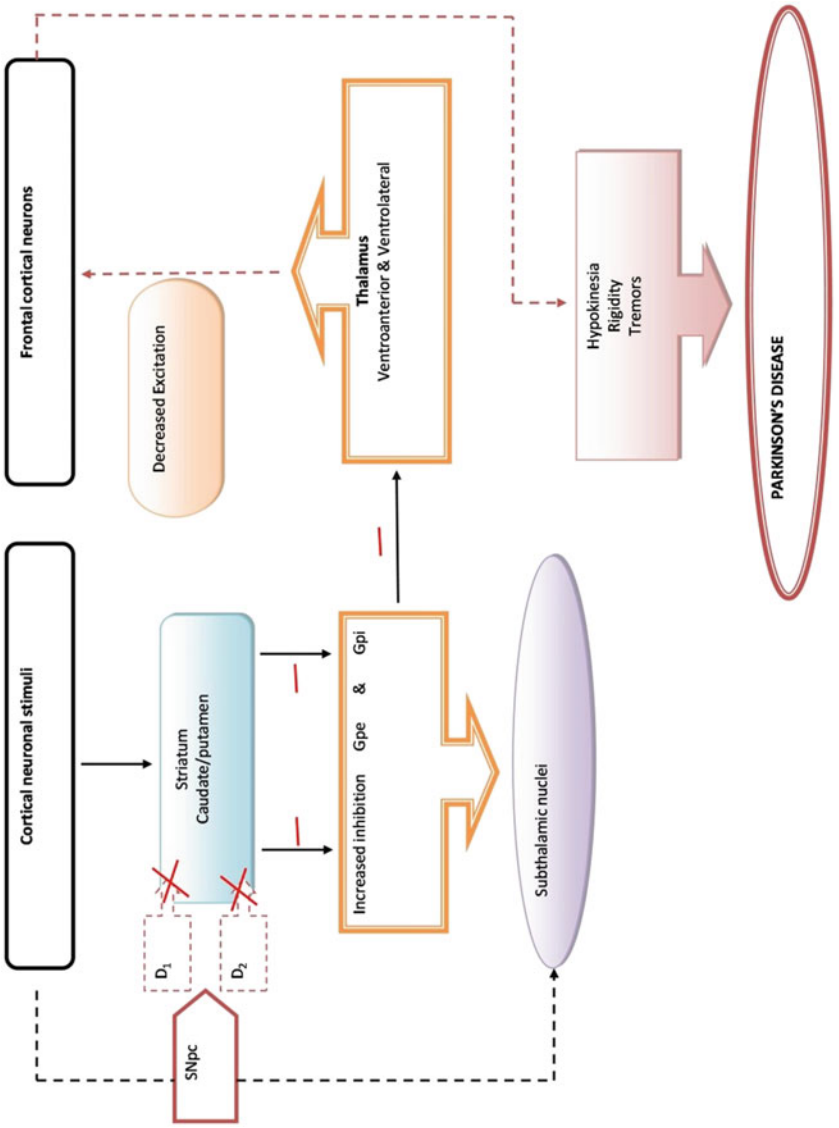

Fig. 5.8 Dopamine deficiency and motor symptoms

Table 5.4 Dopaminergic drugs under research for therapeutic benefits in PD

Drugs	Mechanism of action	Pharmacological effects
Apomorphine	Non-selective dopamine agonist	Improves motor function in PD
SKF38393	D1 agonist	Improves motor function in PD
SCH23390	D1 antagonist	Improves motor function in PD
D-512	D2/D3 agonist	Improves 6-OHDA-induced neurotoxicity in PD Improves motor function in MPTP induced PD
Quinpirole	D2-like receptor agonist	Improves motor function in PD
Pramipexole	D2-like receptor agonist	Improves motor function in PD
Piribedil	D2/D3 agonist	Reduce symptoms of PD
D-440	Highly D3-receptor-selective agonist	Improves motor function in PD
BMY-14802	Sigma-1 receptor antagonist, modulates dyskinesia like side effects that occur due to dopamine agonism	Improves dyskinesia induced by L-DOPA treatment
SKF81297	D1 receptor agonist	Improves motor symptoms of PD

over cortical activity by subthalamic nuclei and globus pallidus external. It has been reported that this system gets dysregulated in HD and loss over movement control occurs in the form of chorea. The early symptoms of HD associate with the loss of D2 receptors which initiate uncontrolled dance-like movements by creating a deficit in inhibitory regulation of the dopamine system, whereas in later stages of HD, on the other hand, the lack of D1 receptors also results in thalamus inhibition inducing dystonia, rigidity, and akinesia-like symptoms. Furthermore, evidence-based justification lies behind the degeneration of this system. It has been analysed that dopamine treatment differently modulates NMDA and AMPA receptors. These responses occur due to the localization of dopamine receptor on glutaminergic terminals. The involvement of D2 receptors in glutaminergic terminals through excitotoxicity may exacerbate neurotoxicity in HD, whereas D1 receptor itself mediates potentiation of NMDA receptor that contributes to neurotoxicity (Chen et al. 2013). Further, agonists of D1 enhance neurotoxicity via NMDA receptor activation whereas the activation of D2 receptor reduces NMDA activation for neuroprotection. Hence, D1 receptors remain neurotoxic whereas D2 receptors prove to be neuroprotective in HD.

Moreover, cell death of dopamine neurons also proceeds by free radical production as they remain more prone to oxidation due to their distinct features. Changes in dopamine levels also contribute to cognitive loss in earlier HD as cholinergic neurons reside in striatum also degenerate, thus deteriorating cognitive functions like attention, execution, learning, and cognition (Wang et al. 2006). The initial phase of HD reveals to be in a hyperdopaminergic state, so reducing dopamine remains a potential treatment strategy. The only approved drug in HD is tetrabenazine (TBZ) that inhibits vesicular monoamine transporter to reduce dopamine in presynaptic vesicles which reduce chorea and other symptoms of HD. Aripiprazole is a partial D2 receptor agonist that improves symptoms of chorea

Table 5.5 Enlisting the compounds targeting dopamine for therapeutic efficacy in HD

Drug	Mechanism of action	Status in HD
Tetrabenazine	Vesicular monoamine transporter to reduce dopamine level	Clinically approved drug
Haloperidol	Typical D2 antagonists	Improves symptoms of disease
Olanzapine, risperidone	Atypical D2 antagonists	Improve chorea and behavioural disturbances
Bromocriptine	D2 agonist	Improves chorea and other motor symptoms
Lisuride	D2 agonist	Provides symptomatic relief
Aripiprazole	Partial agonist of D2	Improves chorea but cognition not improved
L-DOPA	Dopamine agonist	Provides relief over rigidity
Pridopidine	DA stabilizer	Improves motor dysfunction
SCH23390	D1 antagonist	Shows positive result in animal studies
SKF38393	D1 agonist	Shows positive result in animal studies
Haloperidol	D2 antagonist	Shows positive result in animal studies
Quinpirole	D2 agonist	Shows positive result in animal studies

in HD whereas dopamine stabilizer pridopidine also provides relief over motor symptoms of HD (Coppen and Roos 2017). On the other side, different D2 antagonists like olanzapine, risperidone, quetiapine, and ziprasidone improve functional disabilities of HD along with reduced side effects. Thus, numbers of dopamine agonists, antagonists, and stabilizers of dopamine are going to be evaluated for therapeutic efficacy in HD to ensure a reliable target (Table 5.5). This way targeting striatal dopamine dysfunction could be a beneficial approach for therapeutic efficacy in HD (Fig. 5.9).

5.10 Role of Dopaminergic System in Alzheimer's Disease (AD) and Enlisting Potential Therapeutic Targets Under Research

AD today becomes the most severe neurodegenerative disorder resulting in memory loss, cognition impairments, and functional abnormalities caused by neuronal loss in the hippocampus, cerebral cortex, and neocortex inside the brain (Jahn 2013). The loss of neuron gradually spreads throughout the brain but other regions like striatum, amygdala, and prefrontal cortex do not remain spared from AD pathology (Braak and Del Tredici 2015). No doubt, mainly cholinergic neurons get lost in AD but recent studies showed the loss of other neurons which also contributing in neurochemical alterations in AD.

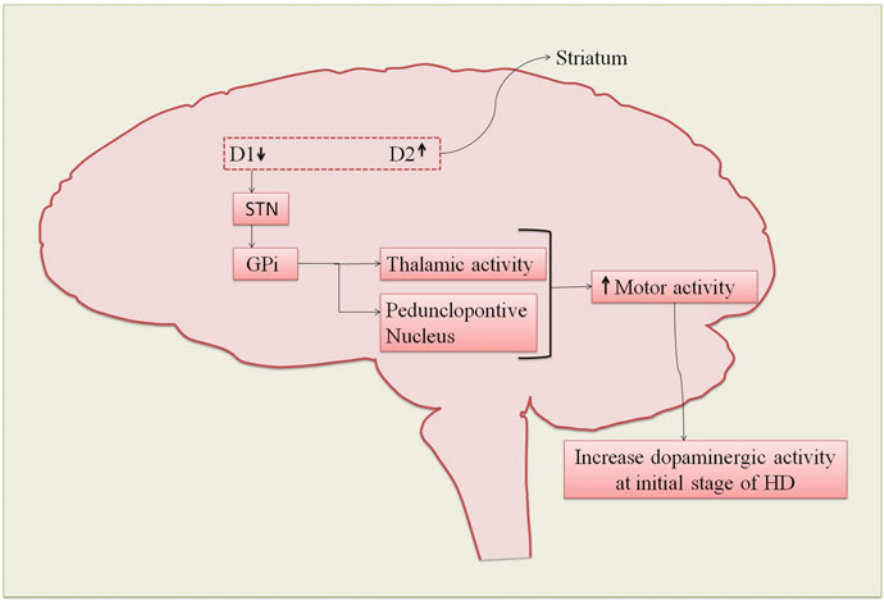

Fig. 5.9 Excessive dopaminergic activity in HD

The contribution of the dopaminergic system in AD gathers specific attention for the last few years. Dopaminergic neurons present in ventral tagmental area regulate functions like memory, cognition, and synaptic plasticity. In early AD, degeneration of these neurons occurs which may contribute to cognition impairment and memory loss whereas extrapyramidal symptoms (EPS) occur in late AD which are associated with the loss of dopaminergic neurons in the striatum (Nobili et al. 2017). About 35–40% of AD patients are affected by EPS in their late age of disease (Martorana and Koch 2014). On this basis, the treatment strategy adopted to counteract the loss of dopamine, which includes administration of dopamine and selegiline, shows to improve memory and neuron loss in the hippocampus (Nobili et al. 2017). This beneficial effect of dopamine is attributed to the mesocorticolimbic pathway that contributes to cognition and memory by projecting dopaminergic projections to hippocampus, cerebral cortex, and nucleus accumbens and symptoms of late AD associates with mesostriatal pathway that regulates voluntary movements (Fig. 5.10).

Further loss of dopamine is confirmed by reducing the release of dopamine, its receptor, decreasing the level of dopamine transporter and enzyme tyrosine hydroxylase that were reported via different research studies. Few studies target receptor-specific role of dopamine in AD that includes D1 and D2 receptors and their attributing role towards memory and cognition (Nyberg et al. 2016). Mostly receptor-specific studies from complex regulation of prefrontal cortex that shows an abundance of D1 receptors coordinate the executive and working memory. The D2 receptor is distributed in caudate, VTA, and hippocampus to regulate hippocampus

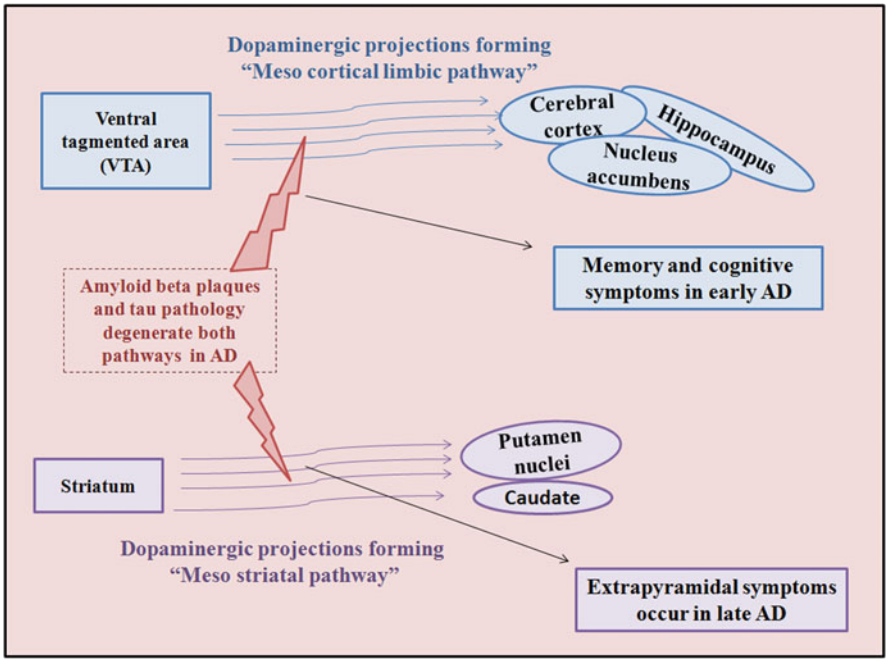

Fig. 5.10 Role of dopaminergic projections in AD

oriented cognitive functions. It also regulates episodic memory and executive functions in the brain. The decline in D2 and D1 receptors may be caused by excitotoxicity-mediated degeneration of dopaminergic neurons.

On this basis, different dopamine targeting drugs are going to be evaluated for therapeutic efficacy in AD. The neuroprotective approach like allopregnanolone (ALLO) has shown to increase dopaminergic neurons and improve cognitive deficits in AD studies. Another drug vindeburnol and L-DOPS promote catecholamine synthesis and found to enhance memory and concentration in preclinical AD studies (Feinstein et al. 2016). This way, there are a number of dopamine targeting drugs that exhibit good efficacy in AD studies (Table 5.6).

5.11 Role of Dopaminergic System in Schizophrenia and Enlisting Potential Therapeutic Drug Targets Under Research

Schizophrenia (SCZ) is a chronic heterogeneous mental disorder and is characterized by positive, negative, and cognitive symptoms that make the patient mentally impaired and socially distorted. The positive symptoms of SCZ include delusions, hallucinations, distorted thoughts, and abnormal behaviour, while negative symptoms include social withdrawal and reduced motivation. These symptoms

Table 5.6 Enlisting the compounds targeting dopamine for therapeutic efficacy in AD

Drug	Mechanism of action	Status in AD
Levodopa	DA precursor	Improves memory and decreases synaptic plasticity in hippocampus
Selegiline	MAO-B inhibitor	Improves memory and decreases synaptic plasticity in hippocampus
Allopregnanolone (ALLO)	Positive modulator of GABAB	Increases survival of dopaminergic neurons and improves cognitive deficits
Vindeburnol	Promotes catecholamine synthesis	Improves memory and concentration in AD studies
L-DOPS	Promotes catecholamine synthesis	Improves memory and concentration in AD studies
S33138, FP17141	D3 receptor antagonist	Reverse cognitive decline
SKF81297	Selective D1/D5 receptor agonist	Reduces long-term potentiation to prevent synaptic failure
PD168077	D4 receptor agonist	Enhances working memory and attention
Dimebon	Increases dopamine level in the brain	Effective in mild to moderate AD
Rimonabant	CB1 receptor antagonist Increases dopamine level	Improves social and working memory
Methylphenidate	Dopamine reuptake inhibitor	Improves neuropsychiatric symptoms
ITI-007	Dopamine receptor modulator	Improves neuropsychiatric symptoms
Rasagiline	Monoamine oxidase B inhibitor	Enhances cognition
Rotigotine	Dopamine agonist	Improves executive function in AD

proceed along with cognitive impairments like working memory and attention deficits (Robertson et al. 1990). The pathogenesis of SCZ strikes the neurochemical alterations occurring in patients with SCZ. It includes dopamine theory, glutamate theory, and other neurochemicals alterations like serotonin dysfunction (Laruelle 2014). As dopamine theory always remains a major contributor in pathogenesis, its role in SCZ does not remain controversial.

Initially proposed dopamine hypothesis postulates that hyperactivity of dopamine neurotransmission results in schizophrenic symptoms. This theory was put forward on the basis of evidence that the administration of dopamine releaser amphetamine and dopamine enhancer levodopa potentially exacerbate symptoms of schizophrenia (Brisch et al. 2014). On the other side, treatment drugs against enhanced dopamine levels decrease the activity of dopamine and improve therapeutic reliability on antipsychotic drugs including D2 antagonists. The mesostriatal dopamine system remains a key target of these drugs confirmed by blockage of high concentrations of D2 receptors that also contribute to extrapyramidal side effects. Later revisions to classical dopamine theory provided a new finding (da Silva Alves et al. 2008). This new evidence suggests that amphetamine does not exhibit all symptoms of schizophrenia but only exacerbates positive symptoms along with the improvement in

negative symptoms. Thus, it becomes confirmed that hypoactivity in the dopaminergic system contributes to negative symptoms of schizophrenia. This leads to the utilization of atypical antipsychotics including clozapine and typical antipsychotics like haloperidol that exhibit antipsychotic activity with no extrapyramidal side effects. This both typical and atypical antipsychotics exhibit selectivity for the mesolimbic dopamine system along with weak D2 blocking striatal activity (Brisch et al. 2014). Thus, DA hypothesis gets much more revised and postulates that hyperactivity of dopamine in the mesostriatal region contributes to positive symptoms whereas hypoactivity of dopamine in the prefrontal cortex results in negative symptoms of SCZ.

Moreover, subcortical dopamine dysfunction also remains a prominent pathogenic mechanism in schizophrenia. It includes dysfunction of cortical areas, functional abnormality in the frontal cortex, and structural changes in the prefrontal cortex, entorhinal cortex, hippocampus, amygdala frontal and temporal brain regions (Weiner and Joel 2002). Further, the degenerating changes in the mesolimbic pathway and targeting region of treatment drugs reveal nucleus accumbens that encounter glutaminergic projections from cortical areas whose dysfunction led to different symptoms of schizophrenia. It has been proposed that dysfunction of the frontotemporo limbic-mesolimbic DA pathway contributes to reduced cortical input to mesolimbic region (Weiner and Joel 2002).

On the basis of the above discussion, pharmacotherapy of schizophrenia includes second-generation atypical antipsychotics and first-generation typical antipsychotics (Patel et al. 2014). Here, the typical antipsychotics remain less preferable due to their extra pyramidal side effects but even atypical antipsychotics give metabolic adverse effects like obesity, hypercholesterolaemia, and diabetes mellitus. Further, in preclinical and clinical research, several dopamine modulating drugs are targeting schizophrenia (Table 5.7).

Table 5.7 Enlisting the compounds targeting dopamine for therapeutic efficacy in schizophrenia (SCZ)

Typical antipsychotics (first-generation antipsychotics)	Atypical antipsychotics (second-generation antipsychotics)	Drugs under preclinical and clinical trials
Chlorpromazine	Aripiprazole	ITI-007 (presynaptic partial D2 agonist and postsynaptic D2 antagonist)
Fluphenazine	Clozapine	RP5063 (partial D2, D3, and D4 agonist)
Haloperidol	Lurasidone	SKF-38393, SKF-83959, SPD-451 (partial D1 agonist)
Perphenazine	Olanzapine	DAR-0100A
Thioridazine	Paliperidone	SKF-81297
Thiothixene	Quetiapine	A-77636
	Risperidone	ABT-431

5.12 Role of Dopaminergic System in Anxiety and Enlisting Potential Therapeutic Drug Targets Under Research

Anxiety is considered to be the most prevalent psychiatric disorder today. It could be defined as an abnormal state of mind with excessive fear associated with sympathetic hyperactivity, apprehension, and hypervigilance that interfere with daily life. The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) classifies anxiety disorders that include panic disorder, phobias, generalized anxiety disorder (GAD), post-traumatic stress disorder (PTSD), and obsessive compulsive disorder (OCD) (Thibaut 2017). Often anxiety associates with mood disorder, severe neurodegenerative condition, and depression-like disorders. The contributing mechanism for anxiety in the mammalian brain includes the neurochemical and neuropeptide changes occurring at cortical and subcortical levels. Usually the regions affected in anxiety include the hippocampus, amygdala, septum, and prefrontal cortex. Altered neurochemistry remains a confirmed mechanism behind anxious state of mind, so different neurochemicals have been targeted for decades regarding their contribution to anxiety (Martin et al. 2009).

In recent years, altered dopamine levels in the brain emerge as potential therapeutics in anxiety disorders. Different studies have confirmed the role of dopamine in social behaviour, fear processing, and avoidance learning like phenomenon. The decrease in dopamine is considered as a possible cause of anxiety and depression as L-DOPA treatment improves its symptoms (Stansley and Yamamoto 2015). Furthermore, one study has confirmed that stress activates the mesolimbic pathway that causes increase in dopamine over synapse with reduced reuptake. Thus, dopamine results in the activation of dopamine receptors which contributes to stress. Dopaminergic neurons are abundant not only in the striatum but the ventral tagmental area also remains a major dopamine modulatory unit in the striatum. There are about 60% of dopamine neurons present in VTA. These neurons constitute D1 receptors in moderate to low density along with a high level of D2 receptors (Zarrindast and Khakpai 2015). Here, activation of D2 receptors contributes to stress and anxiety like condition by inhibiting dopamine release in VTA. On administering an antagonist of D2 receptor, D1 gets activated, thus favouring the release of dopamine to relieve anxiety. The contributory function of the amygdala in anxiety never remains controversial; it regulates anxiety, stress, fear conditioning, and emotional memory (Forster et al. 2012). The structural changes in the basolateral amygdala (BLA) including stress induced hypertrophy contributes to symptoms of anxiety. The BLA activity normally remains suppressed by PFC but on stress dopaminergic projections relieves inhibitory stimulus and triggers anxiety. Thus, mesolimbic dopaminergic projections highly contribute to fear-associated anxiety. Here, activation of D1 receptors of amygdala contributes to anxiety whereas D2 receptors associate with vestibular nuclei associated anxiety (de la Mora et al. 2010). Septum remains another region that lies in the basal forebrain that regulates fear, stress, anxiety, emotion, and aggression. Activation of septum generally contributes to anxiety as it gets dopaminergic projections from VTA that contributes to anxiety (Zarrindast and Khakpai

Table 5.8 Enlisting the compounds targeting dopamine for therapeutic efficacy in anxiety

Drugs	Mechanism of action	Effects observed (region specific)
Apomorphine	D1/D2 receptor agonist	Anxiolytic effects (amygdala), anxiogenic effects (hippocampus)
SCH23390	D1 antagonist	Anxiolytic effects (BLA, VTA, NAc), anxiogenic effects (hippocampus, amygdala)
Quinpirole	D2 agonist	Anxiolytic effects (VTA), anxiogenic effects (hippocampus, BLA)
Sulpiride	D2 antagonist	Anxiolytic effects (BLA), anxiogenic effects (amygdala)
Raclopride	D2 receptor antagonist	Anxiolytic effects (BLA), anxiogenic effects (CeA)
Eticlopride	D2 receptor antagonist	Anxiolytic effects (CeA)
SKF38393	D1 agonist	Anxiogenic effects (BLA, hippocampus)

2015). Thus, VTA remains a main unit in regulating anxiety and it is regulated by excitatory and inhibitory inputs.

Moreover, the hippocampus not only modulates learning and memory but also involved in fear and anxiety-like behavioural disorders. There are two different regions of the hippocampus including ventral hippocampus and dorsal hippocampus that differentially precede their functions. The ventral hippocampus involves fear and anxiety due to its projections to the prefrontal cortex whereas the dorsal just regulates memory and learning-like functions (Bannerman et al. 2004). This contribution is further confirmed by lesions of the ventral hippocampus that contributes to anxiety-like symptoms. The mesolimbic dopaminergic projections from ventral tagmental area and SNc when gets affected from plasticity also contribute to anxiety.

On this basis several agonists and antagonists are going to target the treatment of anxiety; some of them are enlisted in Table 5.8.

5.13 Role of Dopaminergic System in Epilepsy and Enlisting Potential Therapeutic Drug Targets Under Research

Epilepsy is the most widespread neurological disorder that initiates with uncontrollable excitatory neurotransmission. It affects 1–2% of the world's population and features recurrent epileptic seizures. Seizures may affect localized areas ("focal" or "partial" seizures) or spread throughout the whole cerebral hemisphere ("generalized" seizures). The predisposing factor behind epilepsy is the hyperactivity persisting in different brain regions that involve imbalance between excitatory and inhibitory impulses (Stafstrom and Carmant 2015). Currently most of the approved drugs in epilepsy target this imbalance by upregulating the GABAergic system in the brain. Whether, GABA/Glutamate remains a main target for the development of drug therapy of epilepsy but recently growing evidence strengthens the role of other neurotransmitters and neuropeptide in the epileptic brain.

Dopamine, acetylcholine, serotonin, and noradrenalin have gathered specific attention for their contribution to epilepsy pathogensis (Werner and Coveñas 2015).

Among this, dopamine seems to be a prominent neurotransmitter that could modulate GABA/glutamate ratio in epilepsy (Howes et al. 2015). Plenty of evidence has shown that dopamine crucially controls the activity of seizure. Even the epileptic brain suggested alterations that occur in the dopaminergic system reflected from altered release, synthesis, and metabolism of dopamine.

Moreover, different studies evaluated the role of dopamine receptors in seizure specifically in limbic epilepsy. The D1-like receptors are reported for their epileptogenic activity while D2-like receptors provides antiepileptic effects (Clinckers et al. 2004). The D1 receptors in limbic regions on activation stimulate glutamate release that contributes to seizure. The role of dopamine in epilepsy also depends upon the region involved in seizure generation. As the temporal lobe epilepsy is reported to be triggered from the hippocampus (Kandratavicius et al. 2012). Here, D2 receptors density is more than D1 receptors. So, dopamine provides inhibitory effects on seizure generation in the hippocampus. Similarly in the dentate gyrus, the rich distribution of D2 receptors offers positive effects for the control of epilepsy. The expression of dopamine receptors also gets varied in epilepsy as shown in animal and human studies. The decrease in D2/3 receptor binding is reported in the epileptic brain specifically in the temporal lobe, thalamus, and basal ganglia region (Paredes et al. 2015). Even the density of dopamine transporters gets reduced in the basal ganglia.

Compounds like apomorphine, amphetamine, L-DOPA, and anti-parkinson drugs including pergolide and bromocriptine (stimulate D2 receptor) provide antiepileptic activity in different studies. On the other side, antipsychotic drugs (D2-like antagonists) have demonstrated to decrease the seizure duration; however similar drugs are also reported to provoke seizures in previously unaffected individuals (Rezaei et al. 2017). The SKF38393 agonist of D1 receptor causes convulsions through G-protein signalling (Table 5.7). The D1-like receptor enhances the cAMP levels and activity of protein kinase A to mediate the adenylyl cyclase activation. Here, the downstream protein DARPP-32 (DA and cAMP-regulated phosphoprotein of 32 kDa) gets activated by PKA to regulate neuronal excitability. On the another side, D2 receptor provides antiepileptic activity due to its opposite action to D1. The D2 receptor activates Gi protein to decrease cAMP production and counteract the DARPP-32 signalling (Bozzi and Borrelli 2013). Even after a lot of positive results, dopaminergic drugs do not target therapeutic benefits in epilepsy as they exhibit a variety of side effects. The old findings suggested that the implication of bromocriptine D2 agonist provides antiepileptic action in animal studies (Table 5.9).

Table 5.9 Dopamine targeting drugs in epilepsy

Drugs	Mechanism of action	Therapeutic effects
Raloxifene	Selective oestrogen receptor modulator (SERM)	Antiepileptic effects
Fluoxetine	Selective serotonin reuptake inhibitor (SSRI)	Antiepileptic effects, antipsychotic effects
Bromocriptine	Dopamine agonist	Antiepileptic effects
Apomorphine	Dopamine agonist	Antiepileptic effects, antiparkinsonian effects
L-DOPA	Dopamine agonist	Antiepileptic effects
Pergolide	Dopamine agonist	Antiepileptic effects
Liraglutide	GLP-1 agonist	Antiepileptic effects
Haloperidol	DA antagonist	Decrease seizure threshold

5.14 Role of Dopaminergic System in Traumatic Brain Injury and Enlisting Potential Therapeutic Drug Targets Under Research

Traumatic brain injury (TBI) is a neurological disorder attributed by head injury. It could occur due to a violent blow, injury, or aggressive shakiness to head which produces functional and structural changes in the brain. The functional changes include behavioural cognition and motor abnormalities while structural changes include cerebral damage and neuron loss (Madikians and Giza 2006). The most common TBI-associated cases are prevalent in traffic accidents, military, sports, violence, construction, industrials sites, etc. These cases also occur in infants when sudden shake to babies body causes a violent impact on their heads. The common pathogenic features of TBI involve oxidative stress, mitochondrial dysfunction, excitotoxicity, and cerebral ischaemia (Quillinan et al. 2016). The damage to neurons results in neurotransmitter alterations in TBI. Among the different neurotransmitters, alterations in dopamine levels in TBI prominently contribute to abnormalities associated with posttraumatic brain injury. The breakdown of dopamine in the striatum and frontal cortex is reported in the TBI brain. Reduction in 25% of dopaminergic neurons of the basal ganglia is reported after cortical injury. The frontal cortex, striatum, and hippocampus are the major regions affected in TBI that reflect abnormalities in motor, behavioural, attention, execution, and memory. However, the dysregulation in the catecholaminergic system is strongly evidenced in TBI. Increased dopamine metabolism markers including DOPAC and increased expression of COMT are indicative of the dysregulation in the dopaminergic system (Chen et al. 2017). The initial increase in the release of dopamine after TBI may occur to combat excitotoxicity but when the disease gets severe, enhanced excitotoxicity and oxidative stress damage the cellular function of dopamine neurons that cause a deficit in its release in TBI brain.

Table 5.10 Enlisting the compounds targeting dopamine for therapeutic efficacy in TBI

Drugs	Mechanism of action	Pharmacological effects in TBI
Methylphenidate	Increases dopamine synthesis	Improves cognition, working memory, and attention
Bromocriptine	D2 receptor agonist	Improves cognition
Atomoxetine	Increases dopamine	Improves cognition and attention
Guanfacine	Increases dopamine	Improves cognition and attention
Levodopa	Dopamine agonist	Improves cognition and attention
Methamphetamine	Increases dopamine	Improves memory and cognition
Amantadine	Increases dopamine	Improves depression symptoms
Selegiline	Monoamine-oxidase-B (MAO-B) inhibitor	Prevents excitotoxicity
Rasagiline	Monoamine-oxidase-B (MAO-B) inhibitor	Prevents excitotoxicity
Pramipexole	Dopamine agonist	Prevents excitotoxicity
Ropinirole	Dopamine agonist	Prevents excitotoxicity
Buproprion	Increases dopamine	Reduces neuroinflammation

Different reports have reported the dysfunction in nigrostriatal and mesolimbic pathways after TBI. Either it could result from excitotoxicity or exacerbated neuroinflammation inside the TBI brain. The damage of dopaminergic neurons could also result from enhanced oxidative stress-mediated mitochondrial dysfunction in TBI. The alterations in glutamate release, sodium/potassium ATPase (Na/K ATPase) function, and enhancing the production of reactive oxidative species remain prominent pathogenic events in TBI. These further raise the metabolic needs of neuronal cell and cause depletion of adenosine triphosphate (ATP) that results in ischaemia/hypoxia-like conditions. Hypoxia increases phosphorylation of NMDA subunits including NR1 and NR2 that enhance phosphorylation of dopamine cAMP regulated phosphoprotein 32 kDa (DARPP-32) to alter downstream protein phosphatase activity (PP1). Further this PP1 causes transcription of nuclear cAMP response element binding protein (CREB) that further phosphorylates the Na/K ATPase. DARPP-32 functioning is tightly regulated by the release of dopamine, glutamate, and adenosine (Kochanek et al. 2015). Any abnormal alteration in their release hinders the neuron survival. Moreover, aggregation of α-synuclein protein and Lewy body formation are considered as the major pathological event of TBI (Irwin and Trojanowski 2013). The altered dopamine neurotransmission subsequently causes depression, anxiety, and substance abuse like common behavioural changes after TBI. Such evidence leads to utilize dopamine targeting drug for therapeutic benefits in TBI (Table 5.10). The low-dose methamphetamine improves memory and cognition after TBI evidenced by histopathological and neurochemical studies. The bromocriptine-like D2 agonists possess antioxidant properties that are proven to be protective against memory and cognition deficits (Jenkins et al. 2016). Another drug named as L-deprenyl enhanced dopamine and norepinephrine to restore cognition and memory deficits due to synaptic plasticity.

On the other side, dopamine antagonists including antipsychotics improve the agitation and psychotic symptoms occurring after injury. Whether haloperidol and risperidone provide positive symptoms over psychiatric illness, side effects like akinesia and pseudoparkinsonism limit their efficacy. But with the administration of olanzapine, with low D2 antagonism, no side effects appear. It has also been observed that haloperidol and risperidone recover motor symptoms but does not affect cognition and memory. Further, it is justified that D2 receptor inhibition is important as anti-inflammatory and immunological responses so may be the blockage of it gives negative effects. The D1 inhibition provides positive results in TBI and the utilization of dopamine agonist remains a beneficial neuroprotective approach.

5.15 Role of Dopaminergic System in Multiple Sclerosis and Enlisting Potential Therapeutic Drug Targets Under Research

Multiple sclerosis (MS) is a severe neurological disorder associated with autoimmune demyelination of the central nervous system. It affects the genetically susceptible younger adults ranging from 15 to 45 years old. Other factors like exposure to viruses like Epstein-Barr virus, smoking, and low serum vitamin D levels remain major environmental contributors in MS. Indeed, there is no exact cause and cure for the disease but the prevalence of MS is increasing day by day. Clinically, four forms of MS are there which are well known as relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS (PRMS) (Loma and Heyman 2011). The combined characteristic features of these forms of MS include impairments of functions in motor, visual, and sensory systems. Moreover, some undiagnosed features including cognitive dysfunction, fatigue, and mood disturbances contribute to cortical damage. The treatment therapy in MS includes interferons, monoclonal antibodies, and cytotoxic drugs which provide symptomatic relief but none of them are able to halt or cure the disease (Derwenskus 2011). The pathogenic mechanism behind these factors includes demyelination, axonal damage, dysfunction of glial cell, and inflammation like neurodegenerative features that are under research for therapeutic approach in MS (Loma and Heyman 2011).

The dysregulated dopamine in MS is reported as dopamine fatigue hypothesis postulated by Chaudhuri and Behan (2000). They suggested that abnormal dopamine release contributes to both mental and physical weakness and feebleness in MS patients. Chronic exhaustion that occurs in MS-affected individuals may result in abnormal functioning of neurons in the basal ganglia. Moreover, structural impairments in the brain due to white matter loss may damage dopaminergic projections including striatal and mesocorticolimbic pathway (Dobryakova et al. 2015).

The expression of D1 receptors gets reduced while D2 receptors increased to cause abnormal catecholamine release for inhibition in apoptosis. This way dopamine initiates abnormal autoimmune response that severe MS. The administration of dopaminergic agonists bromocriptine and methylphenidate to the mesocorticolimbic

Table 5.11 Enlisting the compounds targeting dopamine for therapeutic efficacy in MS

Drugs	Mechanism of action	Pharmacological effects in MS
Bromocriptine	Dopamine agonist	Improves chronic fatigue and other symptoms
Methylphenidate	Dopamine agonist	Improves chronic fatigue symptoms
Amantadine	Dopamine agonist	Improves chronic fatigue symptoms
Risperidone	D2 receptor like antagonist	Decrease spinal cord lesions and autoimmunity
Phenelzine	MAO-B inhibitor	Improves MS symptoms
Fenoldopam	D1-like receptor agonist	Decreases autoimmunity and improves MS symptoms
Dopamine	Dopamine agonist	Decreases autoimmunity and improves MS symptoms
L-DOPA	Dopamine precursor	Decreases autoimmunity
Pergolide	Dopamine agonist	Decreases autoimmunity
Haloperidol	D2 antagonist	Decreases autoimmunity
Pimozide	D2 antagonist	Decreases autoimmunity
Fluoxetine	D2 blocker	Decreases autoimmunity and neuroinflammation
Domperidone	D2 antagonist	Decreases autoimmunity and neuroinflammation

region improves chronic fatigue symptoms. The administration of modafinil and amantadine like dopaminergic agonists in MS patients effectively treats chronic fatigue. Thus, chronic fatigue occurring in MS seems to be a function of mesocorticolimbic dopaminergic projections and targeting it could provide therapeutic benefits in MS (Table 5.11).

Moreover, the abundance of dopamine not only distorts the dopaminergic neurotransmission but also damages the key players of the immune system like peripheral monocytes, lymphocytes T cells, B cells, and macrophages (Levite 2016). The stressful conditions induce dopamine release from immune cells in extracellular space that are further uptaken by lymphocytes to initiate phagocytosis and for activation of T cell and B cell lymphocytes to regulate adhesion, migration, survival, proliferation, and communication with other cells that express dopamine receptors. But in the case of MS, the dopamine receptors on immune cells get altered.

5.16 Clinical Trials and Investigational Drugs

The recent advancements in dopamine are fully contributed by eventual set of high pace discoveries occurred in the research field. The presence of dopamine in the brain was first reported in the 1960s but gets revolutionized when the mystery of parkinsonism got unlocked. Some Swedish works found it to be responsible for extrapyramidal symptoms of PD. Afterward scientific discoveries were mostly devoted to possible roles of catecholamine synthesis and dopamine until levodopa was introduced. The following years were remarkably well known for dopaminergic pathways and their contributory role in various disorders. In the 1980s, advancements in techniques and modern concepts of discovery highly evolved the

neurotransmitter studies through the introduction of dopamine receptors (D1 and D2). Later, a number of different substances were made to explore their specificity towards dopamine receptors including toxins for dopaminergic systems and different MAO and COMT inhibitors. A large number of molecules were evaluated for their therapeutic potential towards the dopaminergic system. Some of them are approved as a therapy for Parkinson's disease while few well known as typical and atypical antipsychotics which are utilized for psychosis and schizophrenia-like behavioural disorders. Recent outbreaks have suggested the role of dopamine in memory coordination and cognition. May the most reliable neurotransmitter dopamine also govern the mechanism to execute memory-oriented functions. Moreover, the localized administration of receptor-specific dopamine compounds to different regions of the brain has given unexpected results to control anxiety. Several new molecules like SCH23390, sulpiride, and eticlopride modulate the dopamine system to provide the anxiolytic action, while levodopa itself and other molecules like bromocriptine, sulpiride, and aripiprazole have improved the symptoms of chorea and rigidity in Huntington's disease. Other dopamine targeting drugs like BMY-14802, SCH23390, and piribedil have significantly reduced the motor complications of PD. Other recent outbreaks of dopamine in neurodegenerative disorders are enlisted in Table 5.12.

Currently, with the help of molecular cloning, two D1-like and three D2-like receptor genes have been successfully identified. The first dopamine receptor cloned was reported by Bunzow et al. (1988) and it was the D2 receptor that was cloned. Low stringency screening of a rat brain cDNA library helped in the isolation of this receptor. The rat D3 and D4 receptors, i.e. two additional members of this family, have been cloned by low stringency hybridization using probes derived from the D2 receptor. The first functional D1-like receptor (referred to herein as the D1A dopamine receptor) was cloned simultaneously in several laboratories (Dearry et al. 1990; Gerfen et al. 1990; Sunahara et al. 1990; Grandy et al. 1990). Similar strategies based on the sequences of the cloned D1A receptor were used for the cloning of the second member of the D1-like receptor family. This second D1-like receptor clone was isolated nearly simultaneously by several groups and has been referred to as the D5, the D1b, or the D1β receptor (Grandy et al. 1990; Sunahara et al. 1991; Tiberi et al. 1991; Gingrich and Caron 1993) (Table 5.12).

5.17 Conclusion

Dopamine regulates many functions through its receptor signalling which are complex and may also depend on cellular protein like kinases or other enzymes. The role of dopamine has been well studied extensively in Parkinson's, Alzheimer's, and Huntington's disease but its role is also widely expressed in various other neurological disorders like epilepsy, multiple sclerosis, schizophrenia, anxiety, and traumatic brain injury. In this chapter, various roles of dopamine and dopaminergic receptors have been brightly highlighted. Several disorders demand a lot of research on the dopaminergic system for various therapeutic approaches. Hence, the high pace

Table 5.12 Investigational drugs in clinical trials

Drug candidates	Therapeutic target	Clinical phase	Therapeutic intervention
Pridopidine	Stabilizes dopamine	Phase III	Improves motor symptoms of HD
Atypical antipsychotics	D2 receptor antagonist	Randomized controlled trials	Improve motor symptoms of HD
Bromocriptine	DRD2 agonist	Rodent studies	Enhances spatial memory and survival rate of hippocampal neuron in TBI
L-deprenyl	Enhances dopamine level	Rodent studies	Enhances cognition and improves neuroplasticity in TBI
Bromocriptine, lisuride, and methylphenidate	DA agonist	Observational studies and randomized trials	Improve positive and negative symptoms of SCZ
RP5063	Partial D2, D3, and D4 agonist	Phase III completed	Improves positive and negative symptoms of SCZ
ITI-007	Presynaptic partial D2 agonist and postsynaptic D2 antagonist	Phase III	Improves positive and negative symptoms of SCZ
Methylphenidate	Increases dopamine release	Randomized trials	Improves cognition and neuropsychiatric symptoms
Atomoxetine	Improves dopamine signalling	Observational study	Improves symptoms of TBI
Levodopa	Potentiates dopamine release	Observational study	Improves symptoms of TBI
Rasagiline	Potentiate dopamine	Phase II	Improves cognition and memory in AD
Piromelatine	DA agonist	Phase II	Improves cognition and memory in AD
Aripiprazole, brexpiprazole, and methylphenidate	DA agonist	Phase I	Improve cognition and memory in AD
Risperidone	D2 receptor antagonist	Accepted for human use	Improvement in psychiatric disturbances and stabilization of motor score
L-Dopa	Precursor of DA	Accepted for human use	Reduction in chorea in some HD patients
Zonisamide	Facilitates dopamine	Accepted for human use	Generalized epilepsy

of research along with newly developed advancements in the field of neuroscience and pharmacology will be useful to get more knowledge about dopamine receptor signalling in devastating disorders.

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Pharmacology of Serotonin and Its Receptors

6

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Abstract

Serotonin (5-hydroxytryptamine) is found in platelets, neuronal bodies, and with higher concentrations in GIT enterochromaffin cells and lesser amount in the brain. Serotonin is responsible for various secondary actions as it is one of the most important neurotransmitters in the CNS. It comprises seven families, namely 5-HT_1 to 5-$HT_{6,}$ which are further divided into different subfamilies. 5-HT is associated with the pathophysiology of many diseases including vomiting, IBS, anxiety, schizophrenia, depression, hypertension, migraine, obsessive-compulsive panic disorders, eating disorders, and carcinoid diarrhea. The present chapter gives emphasis on the action of serotonin on different physiological systems via the serotonin receptors along with their receptor pharmacology, including the agonists, antagonists, and SSRIs.

Keywords

Serotonin · 5-hydroxytryptamine (5HT) · Serotonin receptors · SSRIs

Abbreviations

5-CT	5-Carboxamidotryptamine
5-HIAA	5-Hydroxyindoleacetic acid

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5-HTT	5-Hydroxytryptophan transporter
5-HTTP	5-Hydroxytryptophan
8-OH DPAT	8-Hydroxy-di-N-propylamino tetralin
AC	Adenylyl cyclase
Ach	Acetylcholine
ACTH	Adrenocorticotrophic hormone
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CSF	Cerebrospinal fluid
DAT	Dopamine transporter
DOB	2,5-Dimethyl-4-bromoamphetamine
DOI	2,5-Dimethoxy-4-iodoamphetamine
GABA	Gamma-aminobutyric acid
GIT	Gastrointestinal tract
IBS	Irritable bowel syndrome
IL	Intracellular
IM	Intramuscular
IV	Intravenous
MAO	Monoamine oxidase
MAT	Monoamine transporter
MH	Malignant hyperthermia
NE	Norepinephrine
NET	Norepinephrine transporter
NO	Nitric oxide
PAH	Pulmonary arterial hypertension
PL_A	Phospholipase A
PL_C	Phospholipase C
PNS	Peripheral nervous system
PPH	Primary pulmonary arterial hypertension
SERT	Serotonin transporter
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressant
TM	Transmembrane
UTI	Urinary tract infection

6.1 Introduction

Serotonin is a chemical messenger that is available in each tissue of the human body as well as identified in plants and aerobic organisms like bacteria. Serotonin is a ubiquitous monoamine acting as a neurotransmitter and hormone (Mohammad-Zadeh et al. 2008), which is also recognized as 5-hydroxytryptamine (5-HT) (Shad 2017). Serotonin was first independently recognized in the late 1940s in the USA and Italy. At that time it was known as "Serotonin" in the USA and "Enteramine" in Italy. In 1950, it was confirmed that the structure of both the compounds were same. In the mid-1950s, serotonin was identified in the brain of animals (Glennon and

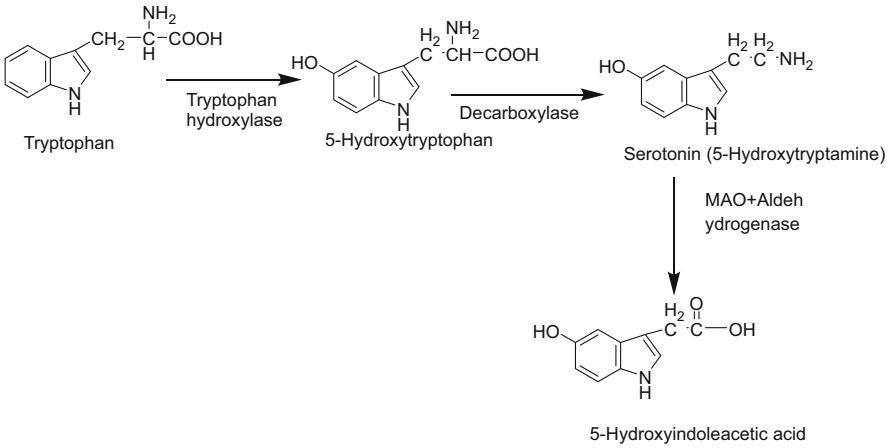

Fig. 6.1 Synthesis and degradation of serotonin (5-hydroxytryptamine) (Sources: Pytliak et al. 2011)

Dukat 2002). It is also found in the platelets, neurons, and GIT (enterochromaffin cells of the GIT mucosa). Over 90% (Shajib and Khan 2015) of the total amount in the body is present in the enterochromaffin cells of the gut and a lesser amount in the brain and retina (Pytliak et al. 2011; Peterlin and Rapoport 2007). In the brain, cell bodies of serotonergic neurons are mostly located in the brainstem midline with broad axonal ridge to all areas of the CNS (Peterlin and Rapoport 2007). Serotonin is associated with various diseases like schizophrenia, anxiety, depression, hypertension, migraine, carcinoid diarrhea, vomiting, irritable bowel syndrome, pulmonary hypertension, and eating disorders (Pauwels 2003; De Ponti 2004).

Serotonin secreted by the nuclei in the median raphe of CNS is transferred toward the spinal cord and other parts of the brain including the hypothalamus. Serotonin discharged from the enterochromaffin cells of GIT finds it own way out of the tissues into the blood, where it is taken up by the blood platelets (Zarrindast et al. 2014). Serotonin synthesis depends on two factors: (1) accessibility of tryptophan which is an amino acid obtained on or after nutritional ingestion, and (2) the action of rate-limiting enzymes tryptophan hydroxylase (Peterlin and Rapoport 2007). Serotonin synthesis starts through active uptake of tryptophan in neurons and enterochromaffin cell by use of amino acid transporters (Pytliak et al. 2011; Upadhyay 2003). Initially, in the presence of tryptophan hydroxylase, tryptophan is converted into 5-hydroxytryptophan, which is finally converted into 5-hydroxytryptamine (serotonin) with the help of aromatic L-amino acid decarboxylase (Fig. 6.1) (Berger et al. 2009). Serotonin is mainly degraded through oxidative deamination, catalyzed by monoamine oxidase A (MAO A), and followed by oxidation to 5-hydroxyindoleacetic acid (5-HIAA) (Ni and Watts 2006). In the brain, it is protected from enzymatic degradation by storing in synaptic vesicles of neurons. When 5-HT is released from the storage vesicles, it is either metabolized into 5-HIAA or reuptaken into the presynaptic

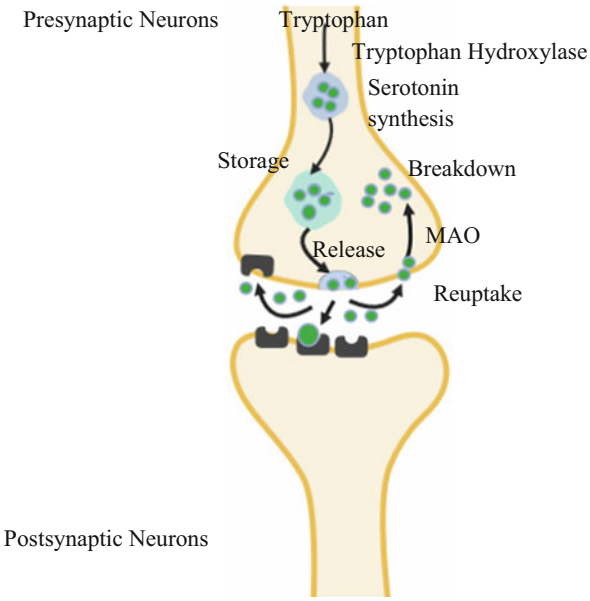

Fig. 6.2 Steps involved in synthesis and degradation of serotonin

neurons by the serotonin transporter (5-HTT or SERT) (Fig. 6.2) (Hamel and Currents 2007).

Serotonin possesses trophic factors in the early stages of pregnancy in humans. Serotonin secreted in mother's enterochromaffin cells of the GIT moves to the platelets in mother's blood. In the meantime from early stage of pregnancy, the fetus additionally begins its own 5-HT secretion process in the nuclei of the midbrain. After distribution of 5-HT by serotonergic neurons through the body and brain of the fetus, it expands division, relocation, and development of peripheral and central tissues (Shad 2017). Serotonin has vast numbers of physiological activity like modulation of platelet aggregation, contraction of vascular and nonvascular smooth muscle, regulation of appetite, mood, anxiety, controlling of body temperature, wakefulness, perception of sexual behavior, and hormone secretion (Upadhyay 2003; Kroeze et al. 2002). Serotonin and its receptors play a significant role in the functioning of the brain and therefore, dysregulation of serotonin system leads to various psychiatric and neurological disorders (Berger et al. 2009).

Serotonin, a notable neurotransmitter in the CNS, also plays a significant role in peripheral tissues as well as in immunity. The growing human body is the proof of recommending that a wide range of immune cells express the mechanism to create, store, react, and transport serotonin, including mast cells, T cells, macrophages, platelets, and dendritic cells. Moreover, there is rising evidence of a possible association between mood disorder, serotonin, and T cells. However, it is not clear how serotonin associates with immunity (Wu et al. 2019).

6.2 Action of Serotonin in Physiological Systems

6.2.1 Cardiovascular System

Acting on 5-HT_2 receptors, 5-HT causes narrowing of vascular smooth muscle. Although serotonin acts as a vasoconstrictor, in the heart and skeletal muscle it acts as a vasodilator. Serotonin releases adrenaline from the adrenal medulla, which affects ganglionic transmission and elicits cardiovascular reflexes. Serotonin has positive inotropic and chronotropic activities on the heart, which may be blunted by concurrent incitement of afferent nerves from baroreceptors and chemoreceptor. On vagus nerve endings, activation of 5-HT_3 receptors evokes the Bezold-Jarisch reflex which causes hypotension and bradycardia. On arterial blood vessels serotonin shows inhibitory action, which causes endothelial nitric oxide production, synthesis of prostaglandin, and obstruction of NE release from sympathetic nerves (Sibley et al. 2018; Tripathi 2013; Katzung 2018).

6.2.2 GIT

The direct action of serotonin on 5-HT_2 smooth muscle receptors of GIT causes enhancing tone and facilitates peristalsis movement. In the enteric nervous system, activation of 5-HT_4 receptors causes increased release of acetylcholine. Serotonin inhibits gastric secretion but increases mucous production. Overproduction of serotonin in carcinoid tumors causes severe diarrhea (Katzung 2018). Serotonin is also involved in digestion right after food enters the body. Actuation of taste bud cells on the tongue causes serotonin release onto sensory afferent nerves that move the taste information to the CNS. When food enters in the GIT, serotonin regulates the peristaltic movement and secretions. Modified 5-HT signaling has been concerned in bowel disorders like irritable bowel syndrome (IBS) (Gershon and Tack 2007) and is efficiently treated by the medication targeting both 5-HT_3 and 5-HT_4 receptors. Excessive serotonin secretion in GIT can also activate the 5-HT_3 receptors present in afferent vagal nerves that innervate vomiting center present in the brainstem, which also justifies why 5-HT_3 antagonists like ondansetron are valuable antiemetic (Berger et al. 2009).

6.2.3 Nervous System

Serotonin shows a significant feature in the development of the brain (Nordquist and Oreland 2010). In the CNS, serotonergic neurons secrete serotonin which influences various functions like temperature regulation, appetite, mood, hormone secretion, cognition, sensory perception, and motor activity (Shajib and Khan 2015). A decrease in serotonin levels leads to various psychiatric disorders like depression, suicidal tendency, and violence. Notably, females are more prone to depressive disorders than males, because the rate of serotonin synthesis is only about half of

that in males. Depressive disorders are prominent in aged person which may be because of less serotonin synthesis (Sibley et al. 2018).

6.2.4 Respiratory System

Serotonin has stimulant effect on smooth muscles of bronchiole through 5-HT_{2A} receptors. It enhances acetylcholine secretion from bronchial vagal nerve endings. Usually, serotonin possesses hyperventilation but excess doses cause transient apnea through coronary chemoreflex. Serotonin causes relaxation and constriction of bronchi and bronchioles (Katzung 2018). Augmenting the release of Ach or releasing Ach from activated cholinergic nerves causes bronchoconstriction (Cazzola and Matera 2000).

6.2.5 Platelets

In platelets, the synthesis of serotonin does not occur but expresses the mechanisms for serotonin uptake, storage, and exocytotic release. Serotonin promotes platelet aggregation by binding to the platelet $5HT_{2A}$ receptors. Serotonin released from adherent platelets causes vasodilation, when the endothelium is undamaged, aiding proper blood flow, whereas in damaged endothelium serotonin causes constriction and further impairs the blood flow. These impacts of platelet-derived 5-HT are believed to be significant in vascular disease (Sibley et al. 2018; Rang et al. 2016).

6.3 Role of Serotonin

The various roles of serotonin are highlighted in Fig. 6.3.

6.3.1 Neurotransmitter

Serotonin is a neurotransmitter present in different parts of the brain. Serotonin is concerned with the regulation of sleep, temperature, mood, behavior etc., and the imbalance of serotonin causes different diseases like schizophrenia, depression, and anxiety (Tripathi 2013).

6.3.2 Malignant Hyperthermia (MH)

The episodes of MH can be activated by ecological stress such as heating, exercise, excitement, and anxiety. Some investigations performed on pigs specify that the sympathetic nervous system is concerned in MH as a minor response. Stresses like environmental, exercise, and heating also increase serotonin release in the CNS and distribute serotonin levels in blood, which induces MH (Wappler et al. 2001).

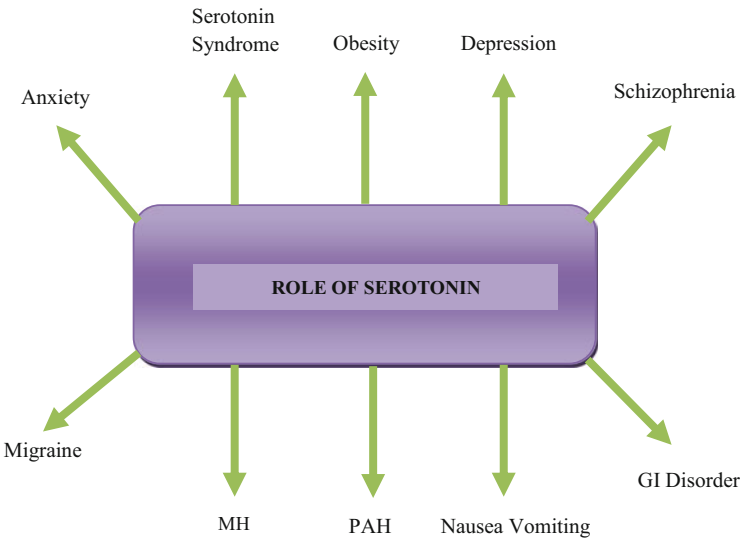

Fig. 6.3 Role of serotonin

6.3.3 Pulmonary Arterial Hypertension (PAH)

Serotonin increases pulmonary vascular smooth muscle cell proliferation, which causes pulmonary arterial vasoconstriction. Changes of serotonin yield cause increasing amount of free serotonin in the surrounding area of the pulmonary artery wall, which is an important pathophysiological process for the development of PAH. There are some conditions where changes in serotonin amount cause PAH such as increasing plasma concentration of free serotonin by lesser serotonin platelet storage, PPH patients with transplantation of the heart or lung, genetic defects in serotonin platelet storage, and low platelet count with PPH patients (Maclean et al. 2000).

6.3.4 Nausea and Vomiting

Serotonin plays an important role in nausea and vomiting induced by chemotherapeutic agents or antineoplastic agents. Vagal-dependent pathway is considered as the main mechanism to initiate vomiting after administration of antineoplastic agents (Hesketh 2008). Vomiting is activated when afferent impulses from the cerebral cortex to the vomiting center (located in medulla) travel through pharynx and vagal afferent nerves of the GIT. Efferent nerve impulses at that point make a journey from the vomiting center to the stomach muscles, salivary gland, cranial nerves, and respiratory system which cause vomiting. Chemotherapeutic agents cause vomiting by activation of neurotransmitters like serotonin, Ach, GABA situated in the chemoreceptor trigger zone, GIT, and vomiting center (Navari 2013).

6.3.5 Cancer

Recently it has been found that serotonin plays an important role in human tumor cells of different origins like glioma, carcinoid, and carcinomas. Serotonin is involved in the migration of cancer cells, tumor angiogenesis, and metastatic dissemination. Different studies show that the levels of serotonin play a major role in cancer cell growth (Sarrouilhe and Mesnil 2019).

6.3.6 Intestinal Motility and IBS

Serotonin has an important role in normal and dysfunctional GIT motility. Serotonin receptors especially 5-HT_3 and 5-HT_4 receptors are targeted to treat various GIT motility disorders. Different studies have been performed on rat to investigate the relevance of serotonin with colonic motility (Kendig and Grider 2015). Serotonin is a significant flagging particle in the gut particularly in enterocytes, smooth muscles, and enteric neurons. Most of the body serotonin is available in enterochromaffin cells. Serotonin promotes the activation of extrinsic and intrinsic afferent neurons to start the peristaltic and secretary reflexes and convey information to the CNS. Serotonin is reuptaken by the serotonin transporter (SERT) in the enterocytes or neurons. Exogenous serotonin application inspires such a large number of responses that it is hard to figure out which is physiologically relevant. This impact is to a great extent because of the nearness of multiple receptor subtypes, which seem, by all accounts, to be available on a few classes of myenteric neurons, on smooth muscle cells, and on epithelial cells. IBS is an unpredictable issue that is associated with altered gastrointestinal motility, discharge, and sensation. Changed serotonin signaling may prompt both intestinal and extraintestinal frameworks in IBS (Sikander et al. 2009).

6.3.7 Obesity

Serotonin receptors present in the CNS are related with the parameter of food ingestion leading to obesity (Thomsen et al. 2008). The capacity to store and prepare vitality is essential for physiologic capacity. Overabundance vitality is put away in fat tissue as triglycerides, which is discharged as free unsaturated fats when required, through cell forms firmly managed by insulin. Fat cell functioning is required to regulate the metabolism of lipid and body glucose level. Studies have been conducted to understand the effect of 5-HT on lipid digestion and glucose homeostasis and cytokine emission (Fex and Stenkula 2019). Obesity increases the risk of various diseases like diabetes mellitus, congestive heart failure, coronary heart diseases, stroke, hypertension, and osteoarthritis (Smith et al. 2009).

6.3.8 Migraine

Migraine pain is the most continuous neurological issue in the growing population around the world, influencing up to 12% of the all inclusive community and more common in women about 25%. It highly affects the general public because of its crippling nature and in that, decreased personal satisfaction and expanded absence from work. Cerebral pain is the essential clinical sign and it has been related with hereditary affectability of neurovascular responses to specific improvements or to cyclic changes in the central nervous system. Among the numerous synapses in the brain, the serotonergic system (5-HT) from the brainstem raphe core has been most convincingly ensnared in migraine pathophysiology. The changes in metabolism of serotonin and serotonin-mediated responses during the migraine pain recommend that migraine pain is a result of a central neurochemical imbalance that includes a low serotonergic character, although the correct flow between serotonergic neurotransmission to the sign and symptoms of migraine pain is still not completely understood (Hamel and Currents 2007).

6.4 Classification of Serotonin Receptors

Gaddum and Picarelli proposed the arrangement of 5-HT receptors in 1957, when it was exhibited that practical reactions of the guinea pig ileum could be mostly obstructed by morphine (M); at the same time the rest of the reaction can be obstructed by dibenzyline (D) and named them as M and D receptors, respectively (Göthert 2013).

Presently serotonin receptor families are classified into seven families (Table 6.1) naming 5-HT_1 to 5-HT_6. All classes are G-protein-coupled receptors (GPCRs)

Table 6.1 5-HT families and mechanisms

Family	Type	Mechanism of action	Signaling effect
5-HT_1	Gi/G_0-protein coupled	Decreasing intracellular concentration of cAMP	↓AC
5-HT_2	Gi/o-protein coupled	Increasing intracellular concentration of phospholipase A_2 and arachidonic acid	↑PL_C, PL_A
5-HT_3	Ligand-gated Na^+/K^+ channel	Depolarization of cell plasma membrane	Cations
5-HT_4	Gs-protein coupled	Increasing intracellular concentration of cAMP	↑AC
5-HT_5	Gi/G_0-protein coupled	Decreasing intracellular concentration of cAMP	↓AC
5-HT_6	Gs-protein coupled	Increasing intracellular concentration of cAMP	↑AC
5-HT_6	Gs-protein coupled	Increasing intracellular concentration of cAMP	↑AC

Sources: Pytliak et al. (2011), Leysen (2004), Sibley et al. (2018)

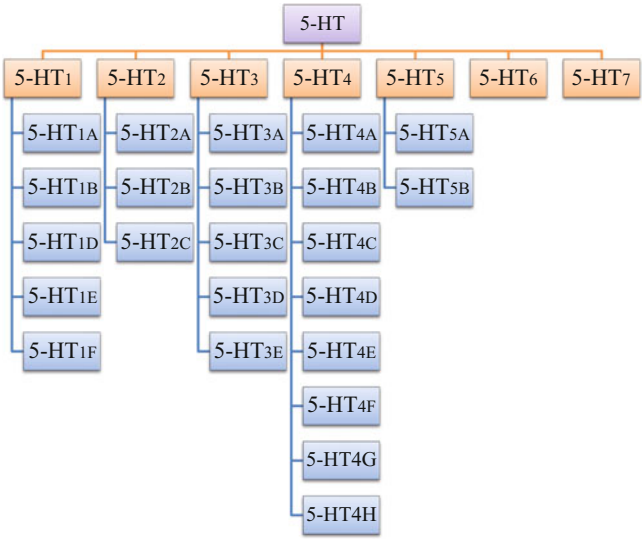

Fig. 6.4 Families and subfamilies of 5-HT

except 5-HT_3 receptor which is a ligand-gated ion channel (Kroeze et al. 2002). Based on sequence and pharmacological activity, all 5-HT receptors are further subdivided (Fig. 6.4) through alternative splicing, RNA editing, etc. (Barnes and Sharp 1999).

6.5 5-HT Receptors and Subfamilies

6.5.1 5-HT_1 Receptors Subfamily

The 5-HT_1 receptor is the largest class of 5-HT receptor, which has five subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} (Fig. 6.4). All the subfamilies of 5-HT_1 receptor show high affinity for the synthetic agonist, 5-carboxamidotryptamine (5-CT), except 5-HT_{1E} and 5-HT_{1F} receptors (Lanfumey and Hamon 2004). The mechanism of these receptors is to decrease adenylyl cyclase (AC) and cAMP levels by pairing to Gi/o protein. Additionally signal transduction mechanisms also have been described (Pytliak et al. 2011).

6.5.1.1 5-HT_{1A} Receptors

Mostly 5-HT_{1A} receptors are dispersed in the CNS, like in axon hillock of neurons, soma, dendrites, and the cell body. 5-HT_{1A} receptors control the production of adrenocorticotrophic hormone (ACTH) but they do not regulate prolactin discharge (Wang et al. 2009). In humans, 5-HT_{1A} receptor possesses a number of physiological and behavioral effects like aggression, anxiety, addiction, and appetite. They also regulate cardiovascular functions, heart rate, blood pressure, pupil dilation, and

Table 6.2 5-HT subtypes, their agonists and antagonists

Subtype	Localization	Agonists	Antagonists
5-HT_{1A}	Raphe nuclei, hippocampus	Buspirone, 8-OH-DPAT	WAY 100135, Spiperone
5-HT_{1B}	Subiculum, globus pallidus, substantia nigra, basal ganglia	Sumatriptan, Methysergide, Zolmitriptan	GR-55562
5-HT_{1D}	Cranial vessels,globus pallidus, substantia nigra	Sumatriptan, Eletriptan	SB714786
5-HT_{1E}	Cortex, striatum	-	-
5-HT_{1F}	Dorsal raphe, hippocampus, periphery	Eletriptan, Naratriptan	-
5-HT_{2A}	Platelets, smooth muscle, cerebral cortex	α – CH_3-5HT, DOI	Ketanserin, Cyproheptadine, Methysergide, LY53857
5-HT_{2B}	Stomach fundus	α – CH_3-5HT, DOI	LY53857
5-HT_{2C}	Choroid plexus, substantia nigra, basal ganglia	α-Methyl-5-HT, Aripiprazole, Ergonovine, Lorcaserin	LY53857, Cyproheptadine, Methysergide, Mesulergine
5-HT_3	Parasympathetic nerves, solitary tract, area postrema, GI tract	2-CH_3-5HT,Quipazine	Ondansetron, Tropisetron
5-HT_4	Hippocampus, striatum, GI tract	Cisapride, Tegaserod, Prucalopride, Renzapride, Metoclopramide	GR113808
5-HT_{5A}	Cortex, hippocampus	-	SB-699551
5-HT_6	Hippocampus, striatum, nucleus accumbens	WAY-181187	SB-271046
5-HT_6	Hypothalamus, hippocampus, GI tract	5-CT, LP-12	SB-269970, Clozapine

Source: Pytliak et al. (2011), Katzung (2018), Sibley et al. (2018)

vasoconstriction (Glennon and Dukat 2002). The quantity of 5-HT_{1A} receptors is high in hippocampus, limbic area, lateral septum, cortical area, and also in raphe nuclei. But in cerebellum and basal ganglia, binding sites are rarely found. Some selective agonists of 5-HT_{1A} are Gepirone, Dipropyl-5-CT, 8-OH-DPAT, etc. (Table 6.2) (Barnes and Sharp 1999).

6.5.1.2 5-HT_{1B} Receptors

The 5-HT_{1B} receptors are available on axon terminal of non-serotonergic and serotonergic neurons. They reduce the discharge of neurotransmitters, including gamma aminobutyric acid (GABA), serotonin, acetylcholine, glutamate, and noradrenaline. 5-HT_{1B} receptors are available in different parts of the brain but mostly found in the frontal cortex, basal ganglia, and striatum. 5-HT_{1B} receptors are also

found on cerebral arteries and additional vascular tissues mediate vasomotor properties of 5-HT (Lanfumey and Hamon 2004).

6.5.1.3 5-HT$_{1D}$ Receptors

By using the radioligand technique, 5-HT$_{1D}$ receptors were identified in the CNS and broadly dispersed in the brain. They inhibit AC and paired with G-protein. They are involved in locomotion and anxiety. However, clinical significance of 5-HT$_{1D}$ receptors remains still largely unknown (Glennon and Dukat 2002).

6.5.1.4 5-HT$_{1E}$ Receptors

The role of 5-HT$_{1E}$ receptor is still unidentified because of the deficiency of selective animal models, specific antibodies, and pharmacological tools. Hypothetically based on their distribution in the brain like olfactory bulb, frontal cortex, and hippocampus, 5-HT$_{1E}$ receptor regulates memory. The gene of 5-HT$_{1E}$ receptor is composed of 365 amino acids and located in chromosome position 6q14-q15. Higher amount of receptor distribution is found in cortex, claustrum, and caudate putamen but lesser amount is observed in amygdala and hippocampus (Barnes and Sharp 1999).

6.5.1.5 5-HT$_{1F}$ Receptors

The 5-HT$_{1F}$ receptors are mostly mRNA recognized in the cortex, dorsal raphe, hippocampus, hypothalamus, striatum, and thalamus of the human brain. From its distribution it suggests that it may act as a 5-HT autoreceptor (Hoyer et al. 2002). There were no selective ligands for the 5-HT$_{1F}$ receptors, but recently two agonists are found, i.e., LY344864 and LY334370. Depending on the anatomical location where the 5-HT$_{1F}$ receptors are present, they play an important role in cognitive and visual functions (Barnes and Sharp 1999).

6.5.2 The 5-HT$_2$ Receptor

The 5-HT$_2$ receptor is a G-protein-coupled receptor and shows a typical heptahelical structure (Leysen 2004). The 5-HT$_2$ receptors have three subtypes, i.e., 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors (Fig. 6.4); they are coupled to Gi/o. The three 5-HT$_2$ receptor subtypes trigger phospholipase A_2 and promote the discharge of arachidonic acid (Leysen 2004). The families of 5-HT$_2$ receptor have different regional distributions within the brain. Therefore each subtype may be capable of mediating definite physiological functions (Knight et al. 2004). The 5-HT$_2$ receptors have showed various roles in CNS disorders like eating patterns, and influence sleep, migraine, schizophrenia, depression, and anxiety (Knight et al. 2004).

6.5.2.1 5-HT$_{2A}$ Receptor

Both centrally and peripherally 5-HT$_{2A}$ receptors occur. They are found in postsynaptic nonserotonergic neurons in the claustrum, olfactory nuclei, basal ganglia, and neocortex of the CNS. They inhibit the release of neurotransmitter like dopamine, glutamate, noradrenaline, and acetylcholine (Naughton et al. 2000). They also

regulate the endocrine responses like secretion of ACTH, corticosterone, oxytocin, renin, and prolactin (Bortolozzi et al. 2005; Feng et al. 2001). 5-HT_{2A} receptors are peripherally found in bronchial, urinary, platelets, and vascular smooth muscle tissues. They cause platelet aggregation, bronchoconstriction, and vasoconstriction. 5-HT_{2A} receptors are concerned in various CNS diseases like migraine, anxiety, depression, schizophrenia, psychosis, and hypertension (Naughton et al. 2000).

6.5.2.2 5-HT_{2B} Receptors

The mRNA of 5-HT_{2B} receptors is found in the human brain. In the brain, 5-HT_{2B} receptors are observed in dorsal hypothalamus, lateral septum, cerebellum nuclei, medial amygdala, and also in the nucleus of dorsal raphe. Along with the brain, these receptors are also found in the intestine, stomach, myocardium, and pulmonary smooth muscles (Leysen 2004). On cerebral arteries of endothelial cells, stimulation of 5-HT_{2B} receptors leads to the secretion of nitric oxide, which causes vascular relaxation in arteries. These vascular relaxations of cerebral arteries lead to migraine headache (Schmuck et al. 1996). Activation of 5-HT_{2B} receptors produces pulmonary hypertension on pulmonary arteries (Launay et al. 2002). In GIT, 5-HT_{2B} receptors cause hypersensitivity induced by serotonin in colonic smooth muscle. These receptors also involved in the IBS, cardiac valvulopathy, and neuroendocrine malignancy (Leysen 2004).

6.5.2.3 5-HT_{2C} Receptors

The mRNA and protein of 5-HT_{2C} receptors are observed in striatum, thalamic nuclei, brain stem nuclei, spinal cord, hippocampal formation, septal nuclei, midbrain nuclei in high amount (Leysen 2004). 5-HT_{2C} is quite similar to 5-HT_{2A} receptors; they show high homologous series of amino acids and also coupled to phosphoinositol hydrolysis. Some studies reported that in migraine, 5-HT_{2C} receptors have greater role than 5-HT_{2A} receptors. 5-HT_{2C} receptors are applied in the treatment of schizophrenia, drug abuse, urinary incontinence, Parkinson's disease, obesity, anxiety, depression, etc. Lorcaserin and WAY-163909 are the examples of 5-HT_{2C} receptor agonists, which are used in the treatment of obesity (Glennon and Dukat 2002).

6.5.3 5-HT_3 Receptors

The 5-HT_3 receptor is only 5-HT receptor which is a part of ionotropic ligand-gated ion channel. The structure and functions of 5-HT_3 receptors resemble the members of the cys-loop ligand-gated ion channel family (Thompson and Lummis 2007). The receptors are recognized on neurons of both central and peripheral origin, where they produce quick depolarization because of a transient internal current, resulting in the opening of nonselective cation channels (Na^+, Ca^{++} influx, K^+ efflux). 5-HT_3 receptors cause vomiting and nausea during radiotherapy and chemotherapy process, which are treated with 5-HT_3 antagonist like ondansetron, granisetron, tropisetron, palonosetron, and dolasetron (Table 6.2) (Hoyer et al. 2002; De Ponti 2004). At

higher concentration 5-HT_3 receptors are present in the brainstem especially in nucleus tractus solitarius and postrema, which are involved in the process of vomiting. The 5-HT_3 receptors are available in postsynaptic and presynaptic neurons and activation can regulate the release of neurotransmitters like GABA, dopamine, acetylcholine, and substance P (Lummis 2012). These receptors also control GIT secretion, motility, and peristalsis in enteric nervous system and also are involved in information transfer in the GIT (Galligan 2002). 5-HT_3 receptors have five subunits, i.e., 5-HT_{3A} to 5-HT_{3E} (Fig. 6.4). The structure of subunits differ from each other like 5-HT_{3A} varies with 32 amino acids, 5-HT_{3B} varies with three translational variants, and 5-HT_{3E} with five isoforms. 5-HT_3 receptor antagonists are used not only to treat chemotherapy-induced nausea and vomiting but also useful in the treatment of IBS, ischemic colitis, bipolar disorder, anorexia, anxiety etc. (Lummis 2012).

6.5.4 5-HT_4 Receptors

In the CNS, 5-HT_4 receptors alter neurotransmitter (dopamine, acetylcholine, GABA, and serotonin) discharge and increase synaptic diffusion. They may likewise assume a role in memory upgrade; in any case, positive clinical investigations are still energetically anticipated (Hoyer et al. 2002). 5-HT_4 receptors are subdivided into seven subtypes, i.e., 5-HT_{4A}-5-HT_{4H} (Fig. 6.4), which are coupled to Gs to activate AC and increase cAMP production (Sibley et al. 2018), though all subtypes contain analogous pharmacological activity and connected with AC activity (Pauwels 2003). 5-HT_4 receptors are found in the septum, hippocampus, prefrontal cortex, and basal ganglia, which are related with cholinergic, glutamatergic, and GABAergic neuro-transmission (King et al. 2008). 5-HT_4 receptors are also available in the heart, bladder, enteric neurons, and smooth muscle cells of GIT. On activation of the receptor, acetylcholine releases from motor neurons and inter neurons; this increases the motility of GIT (Tack et al. 2012). Some examples of 5-HT_4 receptor agonists are cisapride, mosapride, renzapride, naropride, clebopride, and metoclopramide (Table 6.2) belonging to the benzamide groups. Besides benzamide group agonists, other 5-HT_4 agonists are tegaserod, velusetrag (TD-5108), prucalopride, etc. (Tack et al. 2012).

6.5.5 5-HT_5 Receptors

These receptors have two subfamilies, i.e., 5-HT_{5A} and 5-HT_{5B} (Fig. 6.4). They are coupled to Gi/o to inhibit AC. Humans only express functional 5-HT_{5A} receptors. From their regions, it has been theorized that they might be engaged with feeding, anxiety, motor control, depression, adaptive behavior, learning, memory consolidation, and brain expansion (Thomas 2006). Disturbance of 5-HT neuron-glial associations might be engaged with the advancement of certain CNS pathologies including Alzheimer's disease, Down's syndrome, and some drug-actuated

development deficits. After analyzing the location of chromosome, it was found that for human 5-HT_{5A} receptors: position 7q36 and chromosome 7; 5-HT_{5B} receptors: position 2q11–13, chromosome 2. 5-HT_{5A} consists of 357 amino acids but in 5-HT_{5B} receptor, end codons are present in the gene. Studies on 5-HT_{5A} and 5-HT_{5B} are very less among the other serotonin receptors (Nelson 2004).

6.5.6 5-HT_6 Receptors

It is the first receptor of 5-HT which is coupled to AC (Woolley et al. 2004) and increases cAMP intracellular level by pairing to Gs protein. 5-HT_6 receptors are located predominantly inside limbic and extrapyramidal cerebral zones in the CNS, which suggest that it is important for motor control and cognition (Sibley et al. 2018). The accurate scientific importance of 5-HT_6 receptors remains still indistinguishable. 5-HT_6 receptors have a role in learning and memory process. It also plays a role in obesity. Additionally, the 5-HT_6 receptors have been recommended to be concerned in psychotic disease such as epilepsy and anxiety (Kitson 2007). The 5-HT_6 receptor was first discovered in rats and humans in 1993 and 1996, respectively with the help of molecular biology. The 5-HT_6 receptor shows a unique pharmacological activity of high affinity toward antipsychotic molecules and tricyclic and atypical antidepressant molecule like clozapine, loxapine, amitriptyline, and mianserin. In 1998, first selective 5-HT_6 receptor antagonist was reported in humans, i.e., Ro04–6790; chemically it is 4-amino-*N*-(2,6-bis-methyl-amino-pyrimidin-4-yl)-benzene sulphonamide. Ro04–6790 has less penetration through BBB, but when 30 mg/kg dose intraperitoneal was given it showed 70% receptor occupancy from CSF levels (Woolley et al. 2004).

6.5.7 5-HT_7 Receptors

In human beings, 5-HT_7 receptors enhance the activation of AC by pairing to Gs protein. It is mainly distributed in the CNS (Sibley et al. 2018). 5-HT_7 receptors incite relaxation in human colonic smooth muscle and in the guinea pig ileum. 5-HT_7 receptors have a task in reducing the peristalsis by 5-HT. 5-HT_7 receptors are also expressed in extravascular GIT smooth muscles and CNS (De Ponti 2004). Recent studies have provided evidences on the modulation of 5-HT_7 receptors for the treatments of various CNS disorders like anxiety and depression. Also, the 5-HT_7 receptor agonist facilitates memory and has antiamnesic effects (Meneses 2015). AS-19, LP-44, and LP-211 are recently available as highly selective 5-HT_7 agonists (Ciranna 2006). The mRNA and protein of 5-HT_7 receptors, which are present in hypothalamic nuclei play a role in regulation of body temperature. 5-HT_7 receptors also regulate circadian rhythms and REM sleep (Thomas and Hagan 2004).

6.6 Clinical Pharmacology of Serotonin

6.6.1 5-HT Receptor Agonist

Azapirones like buspirone, gepirone, and ipsapirone are agonists of 5-HT_{1A} receptor which is partially active. These are used as anxiolytic drugs and may work on the autoreceptors to reduce serotonergic activity (Katzung 2018).

Dexfenfluramine (Fig. 6.5) is another agonist of 5-HT receptors, which is a selective agonist. It may be broadly used as a hunger suppresser but it was withdrawn due to cardiac valvulopathy (Katzung 2018).

Lorcaserin (Fig. 6.5) is approved for weight loss medication which is a 5-HT_{2C} agonist (Fig. 6.7) (Katzung 2018). The drug is thought to reduce food consumption and increase satiety by selectively activating $5HT_{2C}$ receptors on hypothalamus. Chemically lorcaserin is 1R-8-chloro-2,3,4,5-tetrahydro-1-methyl-1H-3-benzapine (Smith et al. 2009). In humans, lorcaserin shows 100-fold selectivity for 5-HT_{2C} versus the closely related 5-HT_{2B} receptor and 17-fold selectivity over the 5-HT_{2A} receptor (Thomsen et al. 2008; Smith et al. 2008). There is no useful action at additional 5-HT receptors when the concentration of lorcaserin is less than 1 micromol per liter. This leads to the minimal adverse effects than that of nonselective 5-HT receptor agonist (Smith et al. 2009). The clinical dose recommendation for 18 years and older patient of lorcaserin is 10 mg, two times in a day. On oral administration, lorcaserin absorbed rapidly greater than 90%. The C_{max} is about 1.5–2 h and $t_{1/2}$ is about 11 h. About 70% drugs are bound to plasma protein. Lorcaserin is metabolized in the liver and the main metabolite is lorcaserin sulfamate. It is excreted by the kidney through urine and the main metabolite is *N*-carbamoylglucuronides (Bai and Wang 2011). Common adverse effects are vomiting, nausea, diarrhea, constipation, fatigue, UTI, upper respiratory tract infections, rashes, headache, dizziness and back pain; memory and attention deficiency are also seen in 1.9% of patients (Brashier et al. 2014).

The 8-hydroxydipropylamino tetraline (8-OH DPAT) is an aminotetralin derivative and highly selective 5-HT_{1A} agonist. It is used as an experimental tool and not used therapeutically because of its low oral bioavailability (Tripathi 2013).

Sumatriptan and other triptans are selective 5-$HT_{1B/1D}$ agonist and effective to treat acute migraine pain attacks (Fig. 6.7). Migraine pain is a disease characterized by pulsating pain in the head lasting for 4–8 h and often associated with vomiting, nausea, sensitivity to sound and light, flashes of light, and other symptoms. Dilatation of certain cranial vessels causes migraine pain; selective 5-$HT_{1B/1D}$ agonists (triptans) constrict the dilated cerebral blood vessels. Triptans have three mechanisms of action which relieve the migraine pain. The mechanisms are by direct effect on vascular smooth cells which causes vasoconstriction of cranial vessels, reduction of nociceptive neurotransmission, and reduction of discharge of vasoactive neuropeptides (Tepper et al. 2002).

In 1984, Sumatriptan (Fig. 6.5) was discovered. It is the first selective 5-$HT_{1B/1D}$ agonist among the triptans. At first, it was introduced as injectable, followed by tablet, nasal spray, and suppositories (Dahlöf 2001). Sumatriptan demonstrates its

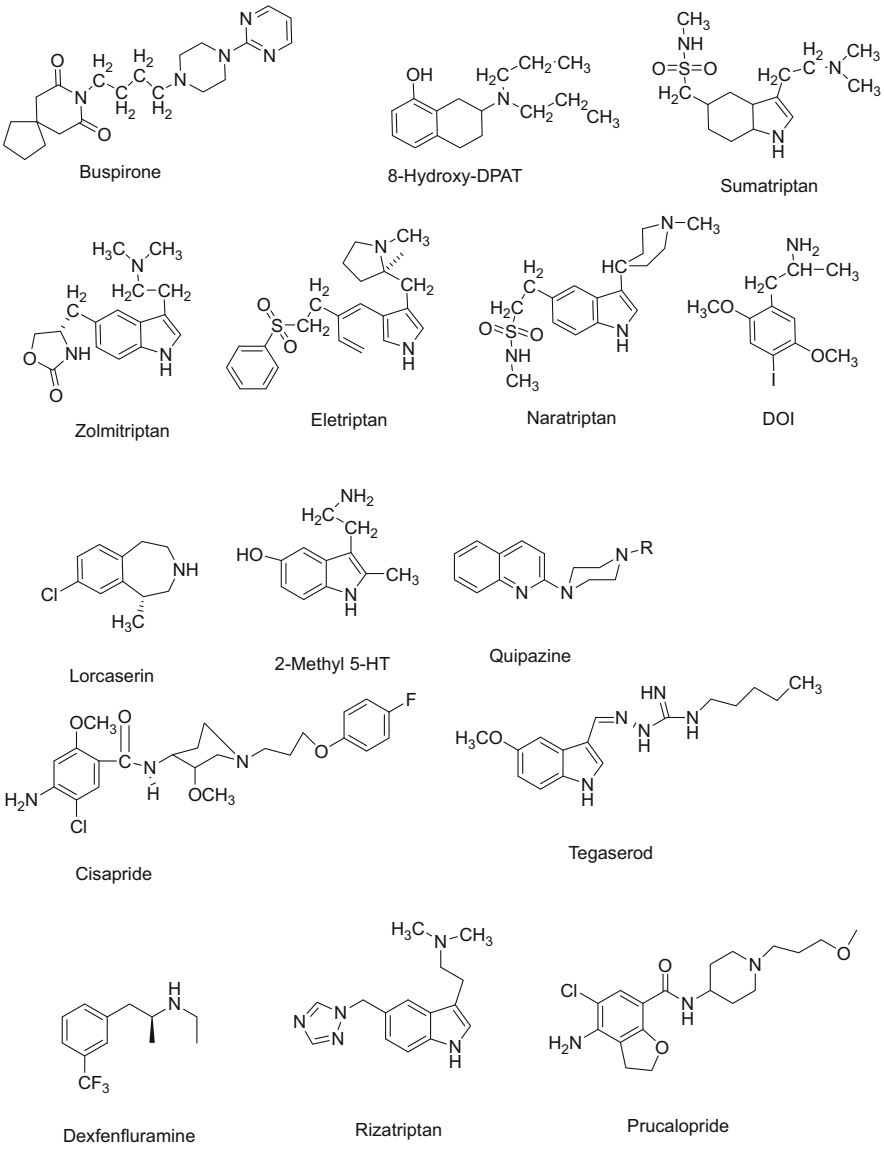

Fig. 6.5 5-HT receptor agonists (Sources: Glennon and Dukat 2002)

remedial impact by incitement of the $5HT_{1B}$ receptor on cranial vascular smooth muscle that causes vasoconstriction, which relieves the headache (Fuseau et al. 2002; Dahlöf 2001). Sumatriptan has definite limits like high headache reappearance, contraindications in patients with coronary artery disease, and low oral bioavailability (Villaln et al. 2003).

The bioavailability of sumatriptan is about 15% in intranasal, 14% after oral, and 96% after subcutaneous administrations. Incomplete absorption and presystemic metabolism cause low bioavailability. The therapeutic dose is 8–66 ng/ml. After subcutaneous administration C_{max} is 25 min and after oral or intranasal administration the C_{max} is 60–90 min. The $t_{1/2}$ is 1.5–2.6 h (Femenia-Font et al. 2005). Adverse effects of sumatriptan are difficulty and abnormal thinking, tiredness, dizziness, agitation, fatigue, tremor, vertigo, etc. (Dodick and Martin 2004).

Zolmitriptan (Fig. 6.5) is another Triptans which have higher oral bioavailability than sumatriptan, about 40% (Tepper et al. 2002). Chemically zolmitriptan is (*S*)4-[3-[2-dimethylamino-ethyl]-1*H*-indol-5-yl]methyl-2-oxazolidinone (Goads and Boes 2001). It is a selective 5-$HT_{1B/1D}$ receptor agonist. It is used effectively in the treatment of migraine and adolescent migraine (Lewis et al. 2007). It is also used in the treatment of cluster headache (Bahra et al. 2000). The $t_{1/2}$ is 3 h and T_{max} is 2 h. Zolmitriptan is metabolized in the liver and eliminated with cytochrome P_{450} pathways (Goads and Boes 2001).

Naratriptan (Fig. 6.5) has 60% more bioavailability than sumatriptan, greater lipophilicity, less readily metabolized, and better CNS penetration (Tepper et al. 2002). It belongs to triptans, a second-generation antimigraine drug. Naratriptan is 5-$HT_{1B/1D}$ receptor agonists. Chemically naratriptan is (*N*-methyl-3-1-methyl-4-piperidinyl)-1-H-indole-5-ethanesulphonamide hydrochloride. Among the orally administered triptans, naratriptans have highest bioavailability. The approximate dose is 35 microgram per Kg. The pharmacokinetics of naratriptan is that it has longest $t_{1/2}$ of about 5–6 h after oral administration. 70% of naratriptan eliminates unchanged; only a considerable part is subjected to P450 metabolism. Naratriptan has fewer side effects (Lambert 2005).

Rizatriptan (Fig. 6.5) has high bioavailability than sumatriptan, is more potent, and has a rapid onset of action (Tepper et al. 2002). Chemically rizatriptan is *N,N*-dimethyl-2-[5-(*1*H-1,2,4-triazol-1-ylmethyl)-1*H*-indol-3-yl]ethanamine. Metabolism of rizatriptan occurs in the liver by MAO-A. Rizatriptan-*N*-oxide and triazolomethyl indole-3-acetic acid are two major metabolites of rizatriptan; both are inactive. N-monodesmethyl-rizatriptan is a minor active metabolite of rizatriptan. After oral administration, bioavailability is 45%, C_{max} is 1–1.5 h, $t_{1/2}$ is about 2–3 h, and 14% bound to plasma protein. In 1998, the FDA approved rizatriptan and it is available as oral disintegrating tablets in a dose of 5 mg or 10 mg (Wellington and Jarvis 2002; Kacperski and O'brien 2012). Rizatriptan is used in the migraine with aura and migraine without aura treatment in adult patients (Wellington and Jarvis 2002).

Cisapride (Fig. 6.5) is a benzamide derivative (Crowell 2004) and it agonizes the 5-HT_4, which is used to treat GIT disorders like gastroesophageal motility and reflux (Fig. 6.7) (Katzung 2018). Cisapride speeds up the process of stomach emptying and intestinal passage but it has limitations on varying bowel functions. Some studies confirmed that it is used in chronic constipation. But nowadays it is available only for considerate use in the USA because of its toxicity like patient deaths and cardiac dysrhythmias (Crowell 2004).

Tegaserod (Fig. 6.5) is a partial agonist highly selective for the 5-HT_4 receptor (Camilleri 2001), which is used for IBS and constipation. It is an

indolecarbazimidamide (Tack et al. 2012), a new class of compound which shows GIT prokinetic effects (Crowell 2004). The chemical name of tegaserod is [3-(5-methoxy-1*H*-indole-3-ylmethylene)-N-pentyl-carboximidamide]hydrogen maleate (Müller-Lissner et al. 2001). After oral administration, tegaserod quickly absorbed and widely distributed in tissues. Presystemically, it is metabolized and excreted through bile as *N*-gluconides. From toxicity studies, it is confirmed that tegaserod is safe and there were no drug-drug interactions found clinically (Camilleri 2001). It is effective for the treatment of gastroesophageal reflux disease, lower bowel motility disorders, constipation, and predominant irritable bowel syndrome (Camilleri 2001).

Prucalopride (Fig. 6.5) is a benzofurancarboxamide (Tack et al. 2012), a newer 5-HT_4 agonist, stimulates peristalsis movement, and accelerates colonic transit (De Ponti 2004). It shows more potent laxative efficacy than tegaserod (Spiller 2002). Chemically prucalopride is (4-amino-5-chloro-2,3-dihydro-*N*-[1-(3-methoxypropyl)-4-piperidinyl]-7 benzofurancarboxamidemonohydrochloride (Briejer et al. 2001). Prucalopride is quickly absorbed from GIT after oral administration. It is excreted by urine about more than 60% and through feces about 6%. The dose recommendation for elderly person is started with 1 mg daily and it may be increased to 2 mg daily if required (Frampton 2009).

Velusetrag (TD-5108) is 5-HT_4 agonist which has high affinity and selectivity. Velusetrag is a derivative of dihydroquinoline carboxylic acid. It is an effective stimulant of GIT motility. It is more potent than other 5-HT_4 agonist in stimulating colonic transit (Tack et al. 2012).

6.6.2 5-HT Antagonist

These are a variety of compounds, which block serotonergic receptors and antagonize the action. Some newly developed antagonists are described below:

Cyproheptadine (Fig. 6.6) has potent 5-HT_2 blocking action. The major clinical uses include management of the smooth muscle manifestations, carcinoid tumor, and cold-induced urticaria. It is used in children for increasing appetite and poor eater for weight gaining. Cyproheptadine also reduces muscle spasms following spinal cord (Hoyer et al. 2002) injury, in which constitutive activity of 5-HT_{2C} receptors is connected with rising Ca^{2+} currents leading to spasms. It has some side effects like dry mouth, drowsiness, weight gain, and confusion ataxia (Tripathi 2013).

Methysergide (Fig. 6.6) antagonizes some action of 5-HT on smooth muscles including blood vessels. It is a 5-$HT_{2A/2C}$ antagonist. It is used in migraine prophylaxis and in the treatment of carcinoid and postgastrectomy dumping syndrome. Long-term use causes abdominal, pulmonary, and endocardial fibrosis (Tripathi 2013). Clinically, methysergide is available in tablet form of 1 mg. Long-term uses of methysergide has adverse effects like pleura pulmonary fibrosis, fibrotic thickening, and retroperitoneal fibrosis (Dahlöf and Maassen Van Den Brink 2012).

Ketanserin (Fig. 6.6) is a 5-HT_2 receptor antagonist. Among 5-HT_2 receptors, ketanserin shows stronger blockade in 5-HT_{2A} than 5-HT_{2C}. It antagonizes

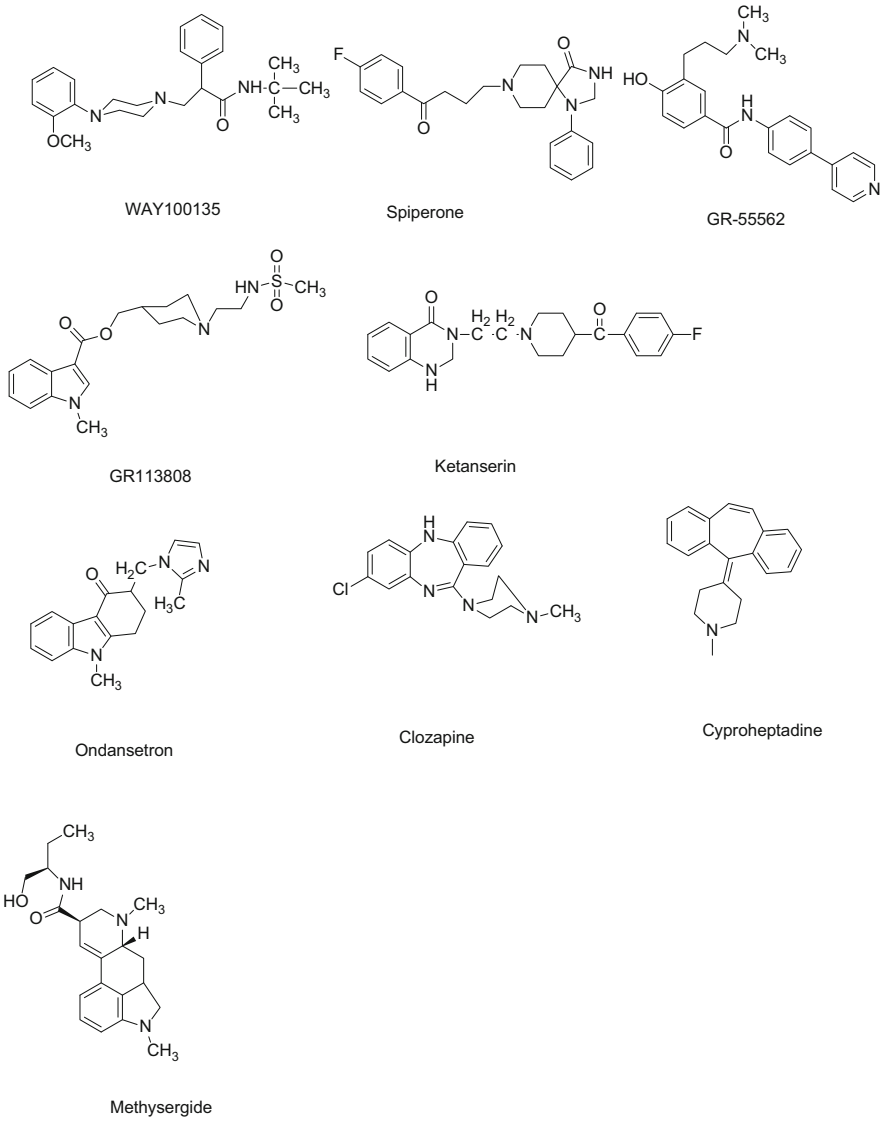

Fig. 6.6 5-HT receptor antagonists (Sources: Glennon and Dukat 2002)

vasoconstriction, platelet aggregation, and contraction of airway smooth muscles which are induced by serotonin. It is an effective antihypertensive drug (Katzung 2018). Another 5-HT_2 antagonist is ritanserin; it has no α-blocking action. It alters the bleeding time and reduces thromboxane formation (Katzung 2018).

Ondansetron (Fig. 6.6) is a newer selective 5-HT_3 antagonist, which has shown efficacy in controlling nausea and vomiting process of chemotherapy and radiotherapy of cancer (Fig. 6.7) (Katzung 2018). In the CNS region, ondansetron inhibits

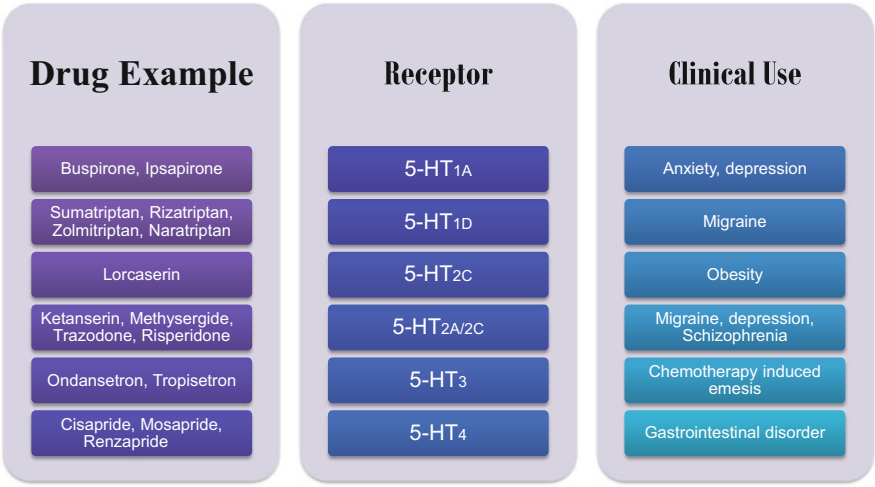

Fig. 6.7 Example of drugs with clinical uses

5-HT_3 receptors or antagonizes including the region of nucleus tractus solitarius, amygdala, postrema, and dorsal raphe nucleus. In the PNS, by antagonizing 5-HT_3 receptors, ondansetron blocks the myenteric neurons and depolarization of vagal afferent nerves, which cause a decrease of 5-HT_3 receptor-mediated nociceptive reaction. The bioavailability of oral ondansetron is low due to hepatic first-pass metabolism. The highest plasma concentration of ondansetron is about 1.5 h after oral administration (Ye et al. 2001). Plasma protein binding capacity of ondansetron is about 70–76%. It has 140 L volume of distribution after IV or IM or oral routes of administration. The $t_{1/2}$ is about 3–3.5 h. Metabolism occurs in the liver by cytochrome P450 enzyme pathway. Ondansetron is excreted about 5% through urine. Ondansetron is used to treat nausea and vomiting induced by postoperative, chemotherapy, radiation therapy and bone marrow transplantation etc. (Culy et al. 2001).

Palonosetron is another newer 5-HT_3 antagonist, which has been shown more efficacy than first-generation 5-HT_3 antagonist (ondansetron or granisetron) in clinical trials phase III to treat emesis during chemotherapy. Palonosetron has different chemical structure from the other drugs in its class. It is a compound tricyclic ring system attached to quinuclidine moiety. It shows longer activity (Rojas et al. 2008). Palonosetron has long $t_{1/2}$ which is about 40 h and slower elimination. Due to its long $t_{1/2}$, it shows better efficacy about 48 h after surgery (Srivastava et al. 2016).

6.7 Serotonin Transporter

Serotonin transporter (SERT or 5-HTT) is a member of monoamine transporter (MAT) (Xue et al. 2019). SERT terminates the actions of serotonin by various ways like enzymatic degradation, restriction of diffusion in synapse, and reuptake to presynaptic neuron from extracellular site (Glennon and Dukat 2002). The SERT is located in the cytoplasm of neuron; the protein consists of amino acids (in humans 630 amino acids) and 12 reversed topological transmembrane (TM) spanning helices with COOH and NH_2 group at end point (Fig. 6.8) (Beecher et al. 2019). Transmembrane domain has been interlined by 2 intracellular (IL1 and IL5) and 3 extracellular (EL2, EL3, and El5) hydrophilic loops (Beecher et al. 2019). SERT is 50% homologous to norepinephrine transporter (NET) and dopamine transporter (DAT) of MAT (Glennon and Dukat 2002). The SERT protein binds with sodium ion and chloride ion to form a complex for transportation of serotonin (Glennon and Dukat 2002; Barnes and Neumaier 2011). SERT has vital roles in homeostasis in the CNS, GIT, and blood platelets. SERT has in affective role in many disorders like stress, obsessive compulsive disease, poor mood, anxiety, sexual dysfunction, and depression (Beecher et al. 2019).

Some tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRIs) are used to block the SERT, which causes inhibition of uptake and increasing the amount of neurotransmitter in the synapse (Schloss and Williams 1998). Some examples of SERT inhibitors are fluoxetine, fluvoxamine, paroxetine, citalopram, sertraline, imipramine, and desipramine (Fig. 6.9) (Rudnick 2006; Glennon and Dukat 2002).

The bioavailability of fluoxetine is less than 90% due to hepatic first-pass metabolism after oral administration. It has long $t_{1/2}$ about 1–4 days. Fluoxetine is excreted through urine. More than 90% of fluvoxamine is absorbed after oral administration. About 100% of drug excreted through urine. Metabolism of fluvoxamine occurs in the liver. Clinically paroxetine is a potent chiral SSRI. It is

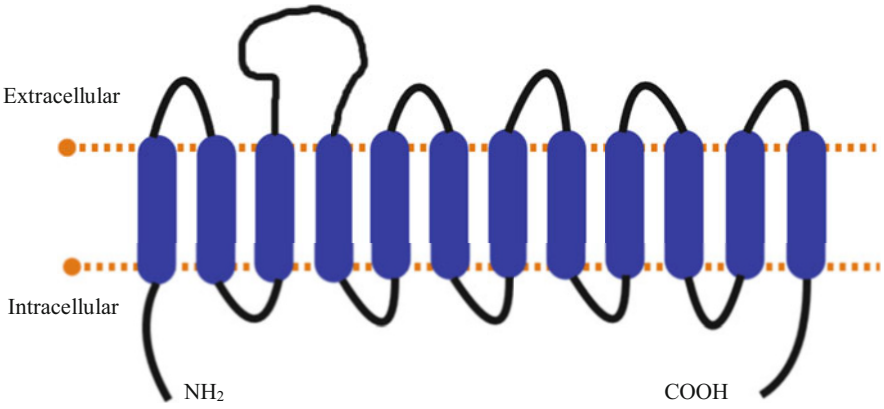

Fig. 6.8 Serotonin transporter

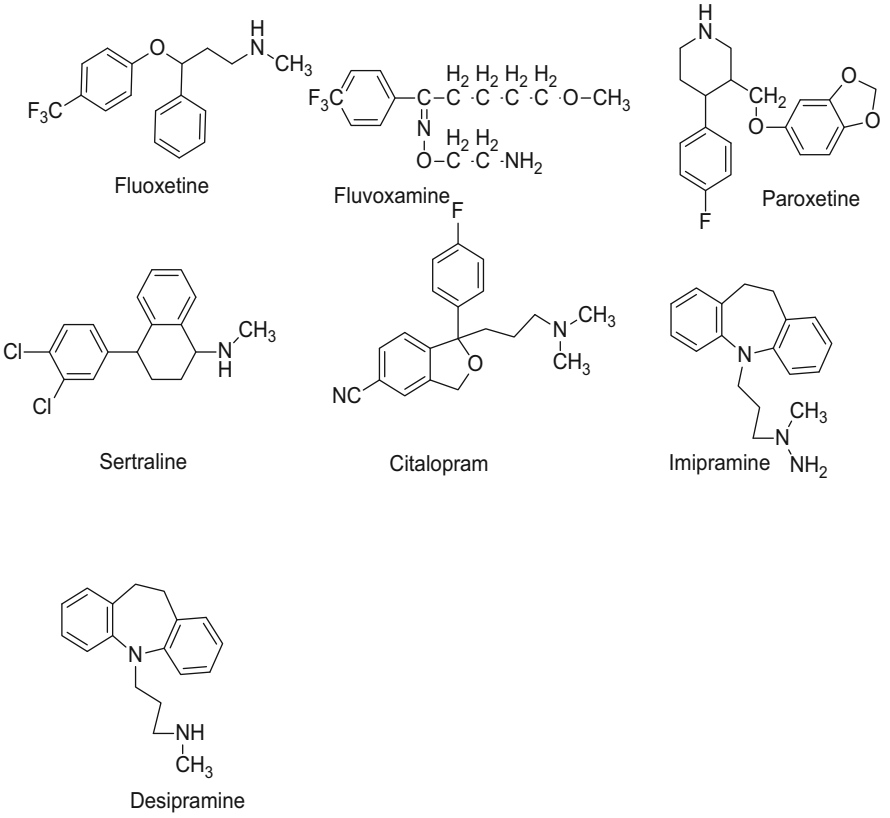

Fig. 6.9 Structure of some SERT inhibitors (Sources: Glennon and Dukat 2002)

absorbed from GIT and metabolized in the liver. Complete absorption takes place from the GIT in case of sertraline. The C_{max} of sertraline is 6–8 h (Hiemke and HÄrtter 2000).

6.8 Serotonin Toxicity or Serotonin Syndrome

Serotonin toxicity or syndrome is due to excess concentrations of 5-HT in the brain or CNS (Birmes et al. 2003). It is characterized by different dose-related adverse effects. Generally, it includes a group of symptoms like autonomic instability, neuromuscular excitability, and cognitive behavioral changes, called serotonin syndrome (Ener et al. 2003). Depending on the increased level of serotonin in the CNS, the toxic effect ranges from mild to severe. Overdose of drug and rising therapeutic doses cause moderate toxicity. The combination of two or more serotonergic drugs causes severe toxicity (Buckley et al. 2014). For analysis of serotonin syndrome, the patient's complete history is required including serotonergic drug uses, its signs and

symptoms, and the elimination of extra situation (Birmes et al. 2003). On clinical ground severe toxicity is diagnosed and characterized by a quickly rising temperature and inflexibility. Mild toxicity is difficult to recognize from numerous ailments or other unfriendly medication impacts (Buckley et al. 2014). Sternbach criteria are used for the analysis of serotonin syndrome, but it is still complicated in cases of benign symptoms or normal neurological test results (Birmes et al. 2003). Serotonin syndrome or toxicity is treated primarily by caring, charcoal lavage, consisting of external cooling with blankets, and dialysis in the case of lithium overdose (Ener et al. 2003). Generally, discontinuation of serotonergic medications is done to overcome from toxicity (Buckley et al. 2014). Benzodiazepines are used in some neurological symptoms, including serious myoclonus and hyperreflexia. In severe toxicity cases cyproheptadine is recommended (Frank 2008).

6.9 Clinical Studies on Serotonin and Its Receptors

Serotonin agonists and mood: A randomized phase I study was initiated in 40 participants by the University of Chicago to determine the efficacy of very low doses serotonergic agonist on a human volunteer with depression. The study will measure the changes from the baseline in the Profile of Mood States (POMS) in the subjects. This study was initiated in May 2018 and estimated to be completed by May 2020 (ClinicalTrials.gov Identifier: NCT03790358).

Serotonin 1_A agonists and cognition in schizophrenia: A phase III study was performed in 60 participants by Vanderbilt University to observe the cognitive functioning of schizophrenia patient with antipsychotic treatment. For 6 weeks patients were assigned to take their antipsychotic drugs with active medication buspar or placebo. Before and after treatment, memory and problem-solving abilities were evaluated. The study was initiated in January 2003 and completed in October 2004 (ClinicalTrials.gov Identifier: NCT00178971).

5-HT_4 agonist and chronic constipation: A phase II study was performed in 360 participants by Theravance Biopharma to compare the effectiveness and safety in chronic constipation of TD-5108 drug (Investigational drug) with a sugar pill (placebo). The study was performed to determine the efficacy and safety of a 5-HT_4 agonist in chronic constipation. The study was initiated in October 2006 and completed in May 2007 (ClinicalTrials.gov Identifier: NCT00391820).

Lorcaserin hydrochloride and smoking cessation: A randomized phase II study was performed in 603 participants by Arena Pharmaceuticals to evaluate the effect of lorcaserin hydrochloride in smokers for smoking cessation. The study was performed by giving treatment with lorcaserin and placebo. The effect was measured on the last 4 weeks of treatment. The study was initiated in March 2014 and completed in November 2014 (ClinicalTrials.gov Identifier: NCT02044874).

Sarpogrelate and coronary artery disease: A randomized phase IV study was performed in 40 participants by Seoul National University Bundang Hospital to determine the effect of sarpogrelate, a serotonin receptor antagonist, on coronary artery disease. The study was performed in Korean type 2 diabetic patients with

atherosclerosis disease and to review the comparisons of sarpogrelate with aspirin. The study was initiated in July 2015 and completed in April 2016 (ClinicalTrials.gov Identifier: NCT02607436).

Granisetron and sympathomimetics during cesarean section: An observational study was performed in 240 participants by Johann Wolfgang Goethe University Hospital to determine the effect of granisetron on usage of sympathomimetics during cesarean section. The study was performed in patient with cesarean section to quantify the usage and dose of sympathomimetics used. The study was initiated in October 2017 and completed in February 2016 (ClinicalTrials.gov Identifier: NCT03318536).

Ramosetron Plus DX, Dexamethasone, Granisetron plus DX, and vomiting and nausea: A randomized phase III study was performed in 287 participants by Astellas Pharma Inc. to compare Ramosetron Plus DX and Dexamethasone to Granisetron plus DX. The comparisons were measured on safety and efficiency parameter for the treatment of vomiting and nausea induced by chemotherapy. The study was initiated in January 2006 completed in March 2006 (ClinicalTrials.gov Identifier: NCT00272285).

5-HT_3 antagonists and cardiac safety: An observational study was performed in 250 participants by the University of British Columbia to evaluate cardiac safety of commonly used antiemetic drugs like ondansetron. The study was performed to determine the increased cardiac safety of ondansetron. The study was initiated in June 2014 and estimated to be completed by July 2020 (ClinicalTrials.gov Identifier: NCT02436798).

Emend and emetogenic chemotherapy: A non-randomized phase II study was performed in 22 participants by the University of Illinois at Chicago to review the effect of emend or aprepitant on chemotherapy-induced nausea and vomiting for multiple days. The drugs used in the study were aprepitant, dexamethasone, and ondansetron. The study was initiated in November 2005 and completed in January 2009 (ClinicalTrials.gov Identifier: NCT00711555).

5-HT_6 antagonist and bipolar disorder: A randomized study was initiated in 68 participants by King's College London to determine the efficacy of JNJ-18038683, a 5-HT_6 antagonist on cognitive impaired people with bipolar disorder and healthy volunteer using functional MRI. The study was also designed to validate 5-HT_6 antagonist for the treatment of bipolar disorders and effects of 5-HT_6 antagonist for brain functioning in healthy volunteers. The study was initiated in August 2018 and estimated to be completed by July 2020 (ClinicalTrials.gov Identifier: NCT03633357).

5-HT_3 antagonist and opioid withdrawal: A randomized study was performed in 133 participants by Stanford University to examine the uses of ondansetron on reducing the withdrawal symptoms of opioid and to prevent the sequence of physical dependence of opioid. The drugs used in the study were ondansetron, naloxone, and morphine. The study was initiated in April 2011 and completed in October 2016 (ClinicalTrials.gov Identifier: NCT01549652).

Ondansetron and lactulose-induced diarrhea: A randomized phase IV study was initiated in 16 participants by the University of Nottingham to determine the efficacy

of ondansetron (5-HT_3 antagonist) on the quantity of water in large and small bowel. The study will measure the mode of action of ondansetron in lactulose-induced diarrhea. The study was initiated in October 2018 and estimated to be completed by August 2019 (ClinicalTrials.gov Identifier: NCT03833999).

Aprepitant in preventing nausea and vomiting: A phase II study was performed in 22 participants by Wake Forest University Health Sciences to evaluate the effect of aprepitant in preventing nausea and vomiting in patients undergoing chemotherapy and radiation therapy for pancreatic cancer. The drugs used in the study were aprepitant, gemcitabine hydrochloride, capecitabine, and fluorouracil. The study was initiated in August 2006 and completed in August 2012 (ClinicalTrials.gov Identifier: NCT01534637).

Granisetron and myofascial pain: A randomized phase IV study was performed in 40 participants by Karolinska Institute to evaluate the effect of granisetron on myofascial pain in the orofacial muscles. The study was initiated in March 2007 and completed in July 2015 (ClinicalTrials.gov Identifier: NCT02230371).

ONZETRA® Xsail® and episodic migraine: A randomized phase III study was undertaken in 420 participants by Avanir pharmaceuticals to evaluate the safety and efficacy of ONZETRA® Xsail® (sumatriptan nasal powder) for the acute treatment of episodic migraine with or without aura in adolescents. The study was designed to examine the efficacy and safety of sumatriptan nasal powder for treating migraine in 12–17 years old patients. The study was initiated in November 2017 and completed by November 2019 (ClinicalTrials.gov Identifier: NCT03338920).

Ondansetron and CNS distribution: A non-randomized phase I study has been performed in 18 participants by Washington University School of Medicine to determine the time course of plasma and CSF concentrations of IV ondansetron in healthy subjects, with and without selective inhibition of Pgp efflux transporter. The study was initiated in May 2019 and estimated to be completed by June 2020 (ClinicalTrials.gov Identifier: NCT03809234).

Ondansetron and hypotension: A randomized phase IV study has been performed in 100 participants by Hospital de base to verify the hypothesis that ondansetron IV (5-HT3 receptor antagonist) decreases the occurrence of hypotension induced by spinal anesthesia. The study was initiated in March 2019 and estimated to be completed by March 2020 (ClinicalTrials.gov Identifier: NCT03973411).

6.10 Conclusion

Among the monoamines, serotonin or 5-hydroxytryptamine (5-HT) is unique and its effects are served by one ligand-gated ion channel and G-protein-coupled receptors. Seven families and different subfamilies of 5-HT receptor have been recognized. In recent two decades, a tremendous research work has been carried out to recognize the different 5-HT receptor families and subfamilies, and their uniqueness, revealing various connections between 5-hydroxytryptamine receptors and diseases. However, further studies would provide better insight about functions, effect of some receptors and their subtypes in health or disease.

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Pharmacology of Histamine, Its Receptors and Antagonists in the Modulation of Physiological Functions

7

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Abstract

Histamine is an important monoamine consisting of an imidazole ring, which is connected to the amino group by an ethylene group. It is biosynthesized through histidine decarboxylase-catalysed decarboxylation of the amino acid L-histidine. It acts through four different G-protein-coupled receptors in different locations of the body to exert its pharmacological response. There are different structural analogues that have been introduced to activate such receptors, whereas specific antagonists are also introduced to inhibit their effect. This chapter highlights the pharmacology of histamine along with structural biology, biological distribution and physiological role of the different histaminergic receptors within the biological system. Further, this chapter also includes different moderators to the specific histamine receptors and projection for their pharmacological response.

Keywords

Histamine · Histamine receptor · Pharmacology · Antagonists · Agonists · Biological role · Structural biology · Distribution

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Abbreviations

5-HT	5-hydroxy tryptamine
AC	Adenylyl cyclase
ADHD	Attention deficit hyperactivity disorder
BBB	Blood-brain barrier
BMMC	Bone marrow-derived mast cells
Ca^{2+}	Calcium
CCL	C-C motif chemokine ligand
cDNA	Complementary deoxyribonucleic acid
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
DNA	Deoxyribonucleic acid
GPCR	G-protein-coupled receptors
GSK	Glycogen synthase kinase
IgE	Immunoglobulin E
IP3	Inositol triphosphate
IPs	Inositol phosphates
kDa	Kilo Dalton
kg	Kilogramme
L	Litre
MAPK	Mitogen-activated protein kinases
MDR1	Multidrug resistance protein 1
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	Messenger ribonucleic acid
PI3K	Phosphatidylinositol 3-kinase
PLC	Phospholipase C
RA	Rheumatoid arthritis
REM	Rapid eye movement
TM	Transmembrane

7.1 Introduction

Histamine, a biologic amine, was isolated from a mould ergot by Sir Henry Dale and his group at the Wellcome Laboratories more than a century ago. Since then, this amine was studied enormously to explore associated physiological roles within the biological system (Parsons and Ganellin 2009). This amine is considered as the most imperative antique mediator within the biologic system, and it had been studied extensively among the chemical mediators, including catecholamine and others derived from amino acids for its role in the biological system (MacGlashan 2003). Officially histamine was synthesized and characterized as a potential biologic amine in 1907 and 1910, respectively (Barger and Dale 1910). During initial experiments with this amine, its capabilities of constricting guinea pig ileum and its convincing vasopressor potential had been reported. Further studies revealed the stimulatory

effect of this biologic amine on different smooth muscles of the respiratory or gastrointestinal tract, and injecting this amine into animal system prompted shock-like condition and cardiac contractility (Dale and Laidlaw 1910, 1919). In 1920, one of the scientist, Popielski, depicted the stimulatory role of histamine on secretion of gastric juice from the dog stomach. Later in 1924, Lewis demonstrated the "triple response" of histamine, which consisted of vasodilatation and altered vascular permeability leading to the formation of red spots, fluid extravasation and swelling effect. Together with incidence of cutaneous flare also results as a consequence of axon reflex (Lewis and Grant 1924). However, it took 17 years from its characterization to establish the existence of histamine in normal tissues as a natural constituent. In 1927, Best and his team had isolated this amine from lungs and liver samples (Best et al. 1927; Shahid et al. 2009). It was also rumoured that the presence of histamine in normal cells might be due to breakdown of histidine (Parsons and Ganellin 2009). Subsequently, the role of histamine in connection to anaphylactic reaction was established quickly in 1929 through analysis of histamine content within the lung prior and after the shock, and thereafter in 1932 this histamine was demonstrated as an important mediator of anaphylactic shock (Dale 1929; Shahid et al. 2009). However, the connection of histamine with mast cells was established in 1952 (Riley 1953), whereas linking with basophil was recognized in 1972 (Ishizaka et al. 1972).

7.2 Pharmacology of Histamine

To explain the biological role of histamine, it displays two basic and vital functionalities, viz. imidazole (pKa 9.4) and primary aliphatic amine (pKa 5.8). In aqueous environment, histamine establishes various acid-base equilibrium. Actually, the neutral histamine or the free-base is able to receive one or two protons to form the monocationic or dicationic fraction of histamine, respectively. The formation of cationic fraction of histamine depends on the pH of the media, where the major fraction (96%) of histamine forms the monocationic form at physiological pH (7.4), with 3% dicationic and a small remaining fraction of neutral form. Alternatively, the predominant form at less than pH 5 is the dicationic, and neutral at pH above 10 (Ramírez et al. 2003; Vianello and Mavri 2012).

Figure 7.1 represents the chemical structure of histamine with nomenclature of the positions. This nomenclature process of histamine represents its tautomeric forms, which is important to denote synthesis, storage, release, and metabolism of histamine, including derivatives of this biological amine.

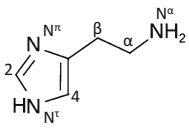

Fig. 7.1 Structure and nomenclature for histamine

7.2.1 Biosynthesis of Histamine

Histamine is a low molecular weight amine, which is synthesized from the amino acid L-histidine by the presence of histidine decarboxylase (Huang et al. 2018). Any other pathway has not been reported to achieve the transformation of histamine (Parsons and Ganellin 2009). Following purification of histidine decarboxylase protein from different cells of mouse and rat, a complementary DNA (cDNA) was cloned that encodes this protein. The gene responsible for histidine decarboxylase synthesis is *Hdc* gene, which prepares the proenzyme for the protein. The proenzyme consists no enzymatic activity encompassing a molecular mass of 74 kDa. Probably by Caspase-9, the proenzyme is cleaved to form two subunits, 20 kDa C-terminus and 53 kDa N-terminus subunits. This N-terminal subunit then forms a homodimer to form the active enzyme, which takes part in catalyse histamine synthesis (Furuta et al. 2007; Komori et al. 2012). The expression of histidine decarboxylase enzyme can be observed in several living cells in human body system, including mast cells, basophils, parietal cells, gastric mucosa, neurons, and in central nervous system (CNS). Therefore, this enzyme catalyses conversion of histamine from histidine in different cells and tissues to exert its physiological roles *via* acting on four different types of receptors (Oda et al. 2000; Akdis and Blaser 2003; MacGlashan 2003).

Recently, several lymphoid and myeloid cell types have been established with higher histidine decarboxylase response, which are also capable of synthesizing high quantities of histamine (Szeberényi et al. 2001). This type of amine is known as "neo-synthesized histamine", the presence of which has been located in haematopoietic progenitors, T cells, dendritic cells, platelets, macrophages, and neutrophils (Shiraishi et al. 2000; Tanaka et al. 2004; Dy and Schneider 2004; Shahid et al. 2009).

The preparation of histidine decarboxylase knockout mouse model generates a system devoid of histamine, where such models are more beneficial to establish the role of endogenous histamine in a broad range of diseased and normal living system. Due to lack of histamine, simultaneously a number of mast cells storage granules are found to decrease in such knockout system, which could be correlated with the action of histamine on production of mast cells granule proteins (Ohtsu et al. 2001). It had been shown that in the absence of specific antigens, immunoglobulin E (IgE) clones activate interleukin-3-dependent BMMC (bone marrow-derived mast cells), leading towards histamine production, cytokine secretion, adhesion, migration and improvement of survival time (Kawakami and Kitaura 2005). Simultaneous research by Tanaka and group added that the transient induction of histidine decarboxylase by the stimulation of IgE in the BMMC was approximately 200-fold higher than the production of histidine decarboxylase in the presence of any antigen (Tanaka et al. 2002). Therefore, such induction further increases in the quantity of stored histamine at the site.

7.2.2 Storage and Release of Histamine

It is evident from the earlier section that the production of histamine may occur in several tissues within the body. However, binding capabilities to different areas of the body and subsequent physiological roles are different because of the types of receptor where it binds to bring the conformational change and physiological response. Even though the heterogeneity of the histamine receptor had been established in the 1940s, much information was on histamine-related activities based on its release, storage and metabolism. Most of the related scientists of British pharmacology were in agreement, including West GB, Trendelenburg U, Schild HO, Riley JF, Perry WLM, Paton WDM, Mongar JL, Gaddum JH, Feldberg W and Blaschko H (Corcoran 1957). Much of the research related to histamine was compiled together and brought to the researchers worldwide jointly by the British Pharmacological Society and the Physiological Society in a CIBA Foundation symposium in honour of Sir Henry Dale (Schayer 1956). The mast cell as a storage location for histamine was recognized by Riley and West, and it had been established that certain components are responsible for inducing the release of histamine *via* disruption of the mast cells and thereby results in decrease in tissue histamine content (Riley and West 1952). Therefore, there was a strong positive correlation established between mast cell population and histamine content in a variety of tissues. Most of the studies performed initially to release histamine was compound 48/80, a polymer that induces histamine release. It was first described in 1951 by Paton (Feldberg and Mongar 1954). However, simultaneous comments by Riley and West made potential changes to the concept that some of the body tissues contained certain cells other than mast cells to store histamine to account their histamine content. Thus, subsequent research had brought forward towards those other cells that store histamine, including blood basophils and platelets in some species (Parsons and Ganellin 2006). Therefore, mast cells and basophils are the storage location of histamine in the haematopoietic system, where this histamine could be found within the specific granules. In mast cells, this amine is closely accompanying anionic proteoglycans heparin, whereas in basophils it is associated with chondroitin-4-sulphate. The release of a large quantity of histamine is potentiated during degranulation of the storage granules by the action of immunological response by IgE or cytokines, or by non-immunological response by calcium ionophore, compound 48/80, substance P, etc. (Dy and Schneider 2004).

The production of histamine within enterochromaffin-like cells also has been proven and its role in the secretion of gastric juice is established (Dy and Schneider 2004). Therefore, the presence of histamine can be found in all types of tissues within the biological system of mammalian species; however, the range of concentration of histamine may vary from 1 to over 100 μg/g. Moreover, major storage of histamine was found in the skin, lung, connective tissues and most of the gastrointestinal tract (Parsons and Ganellin 2006).

7.2.3 Metabolism of Histamine

So far, we have discussed the synthesis of histamine from L-histidine followed by storage within the granules over different tissues within the body. If the granules are stimulated to release histamine, it follows metabolic pathways to be excreted from the system. Small quantities of released histamine (2–3%) can be excreted from the system unchanged, whereas the larger fraction (>97%) follows major pathways of excretion. Two metabolizing enzymes control the major metabolic pathway for this biological amine, which includes histamine N^{τ} methyl-transferase (Fig. 7.1 to identify N^{τ} position) and diamine oxidase (Hill et al. 1997; Ogasawara et al. 2006). Among these two metabolizing enzymes, histamine N^{τ} methyl-transferase takes part in the metabolism of a major portion of released histamine (50–80%). Following methylation of histamine in this process, the product further undergoes oxidation by monoamine oxidase to form the primary urinary metabolite *M*-methylimidazole acetic acid. The other metabolizing enzyme, diamine oxidase, metabolizes around 15–30% of histamine to imidazole acetic acid (Akdis and Blaser 2003). Metabolic pathway of histamine involving histamine N^{τ} methyl-transferase can be prominently expedited through the application of a highly specific and potent inhibitor of diamine oxidase, aminoguanidine. Simultaneously, blockade histamine N^{τ} methyl-transferase using SKF 91488 has shown to result in *in vitro* and *in vivo* increase in bronchoconstriction potential of histamine, suggesting this an important enzyme in the metabolism of histamine, because inhibition of diamine oxidase did not affect any (Sekizawa et al. 1993). The location of histamine N^{τ} methyl-transferase was detected in the airway epithelial cells, and it has been hypothesized that the released histamine from the airway mast cells gets metabolized locally. Thereby, mechanical removal of the epithelium cells from the airway increases *in vitro* bronchoconstriction potential of histamine, which can be demonstrated by the removal of local metabolizing enzyme present on the epithelial cells (Barnes et al. 1985; Knight et al. 1990). Because of the immediate metabolism of histamine by two major enzymes, pharmacologically active doses of histamine has a very short half-life in experimental rats (10 s), whereas it is 20–30 s in dogs (Parsons and Ganellin 2006). It was also reported that the induction of infection by the virus reduces the activity of histamine N^{τ} methyl-transferase; thereby the responsiveness of inhaled histamine could be increased (Nakazawa et al. 1994).

7.3 Histamine Receptors

Being an important biological amine, histamine mediates several physiological effects *via* acting on specific receptors to the amine on different target cells. Four different types of histamine receptors have been identified so far, to produce histaminergic responses upon binding of histamine or any structural analogue to it. Based on the physiological role upon conformational change of the receptors, two distinct histamine receptors, H1 and H2, were identified in 1966 (Ash and Schild 1966). Further, it was reported that some of the histamine responses were inhibited by the use of low doses of

mepyramine, while other responses were indifferent. Just before two decades, another two types of histamine receptors, H3 and H4, were identified in consecutive years, 1999 and 2000, respectively (Oda et al. 2000; Nieto-Alamilla et al. 2016). The structural biology, biological distribution, physiological roles, and available antagonists are summarized in successive sections of this chapter.

7.3.1 Histamine H1 Receptor

The histamine H1 receptor belongs to rhodopsin-like G-protein-coupled receptors (GPCRs) family and is activated by histamine (biogenic amine) (Monczor and Fernandez 2016). The GPCR belongs to the largest family of membrane proteins and is being encoded by more than 800 genes in humans. As H1 receptor it is a GPCR (coupled to G_q); it works by activating phospholipase C and inositol triphosphate (IP3) signalling pathway (Church 2017). H1 receptors are found in smooth muscle, in the CNS and on vascular epithelial cells in the heart (Criado et al. 2010). Histamine release is mostly responsible for allergic reactions against various allergens (Church 2017). Histamine binding to the extracellular domain of H1 receptor induces conformational changes of the transmembrane section resulting in alterations in the C terminal area (Church 2017). The C terminal in turn, *via* its interaction with G proteins and activation of the Gq signalling pathway, elicits allergic reactions (Church 2017). Several antihistaminergic drugs are used against allergy, which bind to H1 receptor without causing activation of the receptor and thus preventing any response (Monczor and Fernandez 2016).

7.3.1.1 Structural Biology

The structure of H1 histamine receptor has been elucidated *via* binding of antihistamine, doxepin (Shimamura et al. 2011). Binding of doxepin in the transmembrane (TM) alpha helices of H1 receptor is stabilized by its interactions with amino acids. The binding domain consists of a conserved tryptophan residue, which is common among the GPCRs. The second-generation antihistamines have been shown to access an anion-binding site comprised of two lysine residues, and they interrelate with a phosphate (Shimamura et al. 2011).

Since histamine H1 receptor is a GPCR, it includes a conserved DRY (aspartate (D), arginine (R), tyrosine (Y)) motif adjacent to its cytosolic face, in its seven-helix TM surface. This motif is specialized for "ionic lock" interaction between an aspartate and arginine in some G protein receptors that stabilizes the inactive state. But the inactive state of the histamine H1 receptor is stabilized by the hydrogen bond between arginine 125 and glutamine 416 (Shimamura et al. 2011).

7.3.1.2 Biological Distribution

A number of research have been carried out to demonstrate the distribution of histamine H1 receptors in different mammalian tissues, using specific radio-ligands (Shahid et al. 2009). Back in 1997, the development of a selective radio-ligand, mepyramine, aided the identification of the H1 receptors in a wide array of tissues,

such as in the CNS, gastrointestinal tract, respiratory system and vascular smooth muscle, hepatocytes, endothelial cells, T and B lymphocytes, dendritic cells, chondrocytes, monocytes, neutrophils, cardiovascular system, genitourinary system and adrenal medulla (Chand and Eyre 1975; Hill et al. 1977; Matsuda et al. 2004; Sander et al. 2006; Shahid et al. 2009). A greater density of H1 receptors has been shown in the hippocampus, posterior hypothalamus, neocortex, nucleus accumbens and thalamus. These receptors are found in lower density in the cerebellum and basal ganglia (Shahid et al. 2009; Mahdy and Webster 2014).

7.3.1.3 Role in Biological System

Signalling Pathway

H1 receptors' established signalling mechanisms include Gq/11 protein activation along with subsequent activation of phospholipase C (PLC). This leads to increased intracellular inositol phosphates (IPs) and calcium levels, followed by activation of small G proteins, RhoA and Rac (Notcovich et al. 2010). Alternatively, in heterologous native H1 receptors expression systems, the pathway is mediated *via* Gi/o, phospholipase A2 activation and production of cyclic guanosine monophosphate (cGMP). This is followed by nitric oxide production to trigger inflammatory conditions (Monczor and Fernandez 2016). In certain tissues like the brain and adrenal glands in mammals, and ovary cells (as seen in Chinese hamster), H1 receptors may also lead to the activation of adenylyl cyclase (AC) followed by increased intracellular cAMP (3′,5′-cyclic adenosine monophosphate) production (Notcovich et al. 2010; Monczor and Fernandez 2016). Besides the signalling activations by ligand binding, H1 receptors have also been reported to show spontaneous receptor activities even when agonists do not bind them. They are able to activate both IP production and alter gene expression *via* nuclear factor-κβ (Fitzsimons et al. 2004; Notcovich et al. 2010; Monczor and Fernandez 2016).

Neurophysiology

Endogenous histamine from neurons that have their cell bodies in the hypothalamic tuberomammillary nucleus is able to activate the histamine H1 receptors (Haas et al. 2008). These neurons turn active during the "wakefulness" cycle (firing at about 2 Hz), while at the time of slow wave sleep the neurons firing rate reduces to as low as 0.5 Hz and finally ceases during REM sleep (Thakkar 2011). Thus, the tuberomammillary nucleus (histaminergic nucleus) plays the major role in regulating sleep-wakefulness cycle (Sherin et al. 1998). Therefore, these histamine H1 receptors are one of the most important receptors that mediate internal clock. As histamine acts upon these H1 receptors, it modulates the neurochemistry to trigger wakefulness and a state of alertness. H1-antihistamines that are able to breach the blood-brain barrier (BBB) are reported to inhibit H1 receptor activity on the histaminergic neurons arising from the tuberomammillary nucleus. This explains the effect of drowsiness associated with these drugs. In the cerebellum and hippocampus, plentiful histamine H1 receptors have been found in the Purkinje and pyramidal cells dendrites (Haas et al. 2008). Hippocampal activation of histamine H1 receptors

leads to inhibition of hyperpolarization in hippocampal neurons. This reflects upon intracellular calcium ions release (Hill 1990). However, neurons associated with cortex, thalamus, brainstem and supraoptic hypothalamus are excited by the histamine H1 receptor *via* blockade of potassium (K) conductance (Huang et al. 2006; Haas et al. 2008; Thakkar 2011; Sundvik et al. 2011).

Inflammation

Stimulation of H1 receptors (other than in the nervous system) attributes to allergic reactions such as motion sickness, bronchoconstriction, separation of the blood vessels cell-lining, vasodilatation, redness of skin, hives (skin rashes) and smooth muscle relaxation. Excessive stimulation of these receptors thus leads to aggravated allergic progression, such as hay fever and other seasonal allergies. H1 receptor induced inflammation is mostly mediated *via* expression of the transcription factor, NF-κβ. Histamine H1 receptors activation in vascular endothelial cells triggers the production and release of many neuromodulators, such as platelet-activating factor, prostacyclin and nitric oxide (Sharma 2004). H1 receptor activation can also cause vascular permeability alterations, specifically in the postcapillary venule by contraction of endothelial cells (Sharma 2004; Thurmond et al. 2008; Criado et al. 2010). H1-antihistamines have been reported to downregulate NF-κβ expression, thereby attenuating certain inflammatory processes (Church and Church 2011; Church 2017).

7.3.1.4 Antagonists of H1 Receptor

Treatment of allergic diseases mostly involves induction of antihistaminic action *via* competitive antagonism of histamine binding to cellular receptors, commonly to the H1 receptors, found on nerve endings, glandular cells and smooth muscles. Numerous *in vitro* and animal experiments suggest several pharmacological properties of the recognized H1 receptor antagonists. The first-generation H1-antihistamines are found to have local anaesthetic, anticholinergic, sedative and anti-5-HT effects that may mitigate symptoms of the allergic response, but are associated with various side effects (Church and Church 2011) (Fig. 7.2).

First-Generation H1-Antihistamines

The first-generation H1-antihistamines share similar chemical constituents with cholinergic muscarinic antagonists, antipsychotics, tranquilizers and antihypertensive drugs (Mahdy and Webster 2014). These similarities render them poorly selective leading to undesired cross-talks with other receptors and often causing anti-α-adrenergic, antimuscarinic, as well as antiserotonin effects. They are potent to cross the BBB and thereby can adversely affect histaminergic transmission (Church and Church 2011).

Physiologically, histamine release during the daytime leads to wakefulness or state of alert while the reduced levels at night account for passive decrease in arousal response. The first-generation H1-antihistamines during the day often result in daytime drowsiness and impaired concentration. At night, these drugs delay the onset of REM sleep and decrease its duration (Rojas-Zamorano et al. 2009). The lack

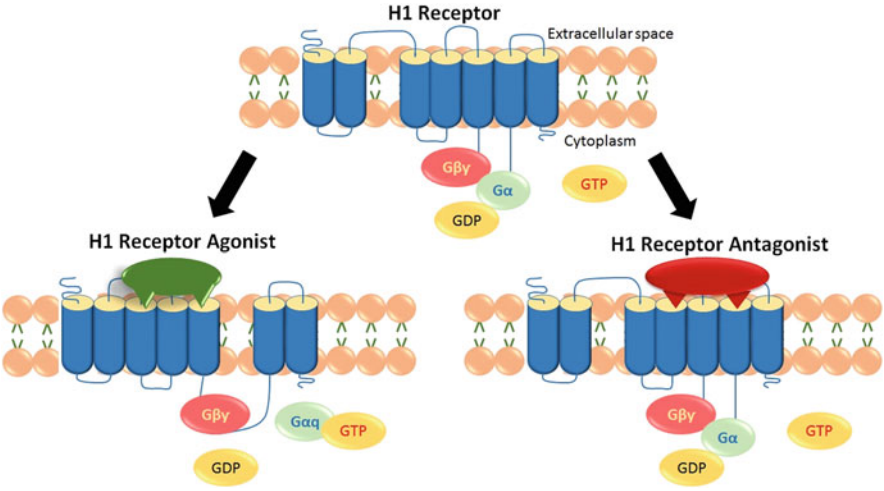

Fig. 7.2 Conformational changes in histamine H1 receptor on binding with agonists and antagonists

of sleep often impairs attention, working memory, as well as overall sensory-motor activities for prolonged duration (Church and Church 2011; Mahdy and Webster 2014). The first generation H1-antihistamines have been extensively studied for their detrimental effects upon the CNS, memory and learning, and in children, they may also affect the performance in examinations (Church and Church 2011). These reports have resulted in banning of these groups of drugs in several developed countries. For example, the Medicines and Healthcare products Regulatory Agency (MHRA), in the United Kingdom, in February 2009 (Anon 2009) has suggested stopping using of cough and cold remedial drugs, which contain particular components including the first-generation H1-antihistamines, while they are strictly prohibited for children younger than 6 years. The adverse effect of these drugs accounts much for their safety. According to published reports, more than 3000 people suffered from adverse effects of these drugs. Importantly, diphenhydramine and chlorpheniramine had accounted for 27 and 11 deaths, respectively (Church and Church 2011).

Second-Generation H1-Antihistamines

The 1980s had witnessed a major advance in antihistaminergic drug development with the introduction of the second-generation H1-antihistamines (Criado et al. 2010). These possess limited access across the BBB rendering them less sedating as compared to the first-generation H1-antihistamine. Moreover, they are more ligand specific and have no anticholinergic effects (Church and Church 2011).

Usually, two factors determine the net efficacy of H1-antihistaminer agents, one being its receptor affinity or absolute potency and the other is the drug concentration at the receptors site. It has been reported that desloratadine is the most effective antihistamine (Ki 0.4 nM), followed by levocetirizine (Ki 3 nM) and fexofenadine

(Ki 10 nM). These drugs attain higher potency in lower concentration. Physical conditions like temperature and pH may modulate the efficacy of these drugs in physiologic and pathologic conditions. For example, the H1 receptor affinity of fexofenadine and levocetirizine is increased even by fivefold in inflammatory conditions when tissue pH drops (Church and Church 2011; Mahdy and Webster 2014).

Several factors influence the free drug concentrations in the tissue compartments and extracellular fluids. In this regard, their absorption into the systemic circulation followed by ingestion of tablet or capsule or other oral dosage form containing the drug is vital. H1-antihistamines are properly absorbed, with the exception of fexofenadine which has variable absorption due to the effects of active transporter proteins (Devillier et al. 2008). Another important factor is the plasma binding of the drugs, which in case of this group is quite high (~65% with desloratadine, ~90% with levocetirizine) (Church and Church 2011). Finally, the volume of distribution of the drugs throughout the body influences its plasma levels after the distribution. For example, levocetirizine (0.4 L/kg) has low body distribution potency, while fexofenadine (5.4–5.8 L/kg) and desloratadine (~49 L/kg) are highly distributed throughout the body tissues (Molimard et al. 2004). Since H1-antihistamines concentrations in specific extracellular fluids are difficult to obtain, an indirect estimate of their efficacy can be calculated using the data on its receptor occupancy (absolute potency) and its peak plasma concentrations generally at ~4 h followed by one oral dose:

Receptor occupancy (%) = Bmax × L + Ki, where Bmax: maximal number of binding sites (set to 100%); L: plasma free drug concentration; Ki: equilibrium inhibition constant or absolute potency (Gillard et al. 2005).

The time required for onset of drug action correlates with its oral absorption rate, but this straight relation on speed of onset of antihistamine actions with its oral absorption rate is often breached by several confounding factors. In a study, it has been shown that plasma concentration of levocetirizine reached after 30 min from its injection in children with histamine-induced flare response. However, the drug had taken more than one and half-hour for its diffusion into the extravascular space for utmost clinical effect. Most of the H1-antihistamines take around 4 h to inhibit such flare responses to the maximal limits in adults (Tashiro et al. 2009).

Certain anti-H1-histamines, like levocetirizine and desloratadine, possess longer systemic action in mitigating histamine-induced flare responses than their predicted duration of action from their plasma concentrations (Tashiro et al. 2009). This presumably occurs owing to drug "trapping" by the H1 receptors by strong and enduring binding (Church and Church 2011). Alternatively, fexofenadine possesses comparatively shorter duration action. The primary reason behind this less prolonged action of fexofenadine is that it is actively secreted into the intestine and urine by P-glycoprotein (Tannergren et al. 2003).

A moderate quantity of research on H1-antihistamines has been centred on the early phase allergic responses of histamines, but recent studies focus upon the anti-inflammatory effects of these drugs (Boyle et al. 2006; Church and Church 2011). H1-antihistamines have been seen to attenuate inflammations owing to nasal

congestion and hyper-reactivity, while continued antihistamine therapy is needed for complete relief from such inflammatory diseases (Day et al. 2004).

The H1-antihistamines, cetirizine and levocetirizine, do not undergo any metabolism and are eliminated in the urine in unchanged condition (Simons et al. 2007). Desloratadine has been demonstrated to undergo metabolism in the liver but has no major drug interactions. Fexofenadine is minimally metabolized and follows faecal excretion in almost unchanged version (Mahdy and Webster 2014).

7.3.2 Histamine H2 Receptor

Ash and Schild proposed the existence of two classes of histamine receptors, H1 and H2, upon introduction of selective ligands for α- and β-adrenergic receptors and subsequently Black proved the concept following research outcomes with selective ligands for β-adrenergic receptors and later accepted upon synthesis of selective H2 blockers. The histamine H2 receptor is a Gs-coupled GPCRs and was firstly defined pharmacologically by Sir James (Church 2009; Timmerman and van der Goot 2009).

7.3.2.1 Structural Biology

The structural studies of histamine H2 receptor suggested that it has a molecular weight of 59 kDa in guinea pig striatum and hippocampus (Ruat et al. 1990). The presence of glycosylated native histamine H2 receptor with N-glycosylation sites in the N-terminus region in the guinea pig brain was evident with molecular weights 40.2–40.5 kDa of cloned H2 receptors (Fukushima et al. 1995; Shahid et al. 2009). However, N-glycosylation of the receptor is not vital for cell surface localization. For the first time, Gantz and colleagues cloned H2 receptor to strengthen a partial length H2 receptor sequence *via* polymerase chain reaction from canine gastric parietal cDNA and used it to identify a full-length H2 receptor clone following screening of a canine genomic library (Gantz et al. 1991). For human, canine and guinea pig the intronless gene (DNA) sequences encode 359 amino acids, whereas 358 amino acids for the rat H2 receptors. Histamine H2 receptor gene in humans was localized to human chromosome 5, which was evident in chromosomal mapping study (Traiffort et al. 1995). Birdsall demonstrated that an aspartate residue in TM domain 3 and an aspartate and threonine residue in TM domain 5 were accountable for histamine binding (Birdsall 1991). Pharmacological specificity of the H2 receptor is associated with specific key amino acid residues (Shahid et al. 2009).

7.3.2.2 Biological Distribution

Histamine H2 receptors are distributed on several morphologically diverse cell types and further it varies from species to species. It is distributed in rat uterus, gastric mucosa of rats, guinea pigs, dogs, humans, bronchi and bronchioles of sheep and mast cells of mice (Chand and Eyre 1975). Widespread distribution of H2 receptor mRNA expression was observed in layers III and V of cerebral cortex, granular cell layer and olfactory bulb of guinea pig brain. H2 receptors are highly distributed in the basal ganglia,

amygdala, hippocampus and cerebral cortex, whereas a low distribution is observed in cerebellum and thalamic and hypothalamic nuclei of human brain and guinea pig brain. The highest ligand binding with H2 receptors was evident with layers II of prefrontal cortex in the human brain. The H2 receptors expression is not only limited to neurons but also evident to be present in astrocytes and in cultured brain endothelial cells. The expression of H2 receptors is manifested in parietal cells of the rat gastric mucosa, mainly in the apical side as compared to basal parts (Panula et al. 2015).

7.3.2.3 Role in Biological System

Activation of H2 receptors is responsible for the production of key regulators responsible for neuronal physiology and plasticity. Therefore, the excitation of H2 receptor leads to increased production of protein kinase A, cAMP and the transcription factor cAMP response element-binding protein (Haas and Konnerth 1983; Pedarzani and Storm 1995; Atzori et al. 2000). In consequence, cognitive deficits and abnormalities in nociception were reported in mice deficient of H2 receptor functions (Panula et al. 2015). Investigation on the role of histamine in the CNS *via* H2 receptor had revealed inhibition of nerve cells; then again the most exciting action involves blockade of the long-lasting after-hyperpolarization along with accommodation of firing, which ultimately leads to the excitation of human and rodent brain (Haas and Konnerth 1983; Haas and Panula 2003). A slow excitation of the H2 receptor is also observed, as in oriens-alveus interneurons, where the fast spiking frequency is reduced by activation of Kv3.2-containing K-channel (Atzori et al. 2000; Haas et al. 2008). Further, long-lasting potentiation of synaptic transmission in the hippocampus is reported to be markedly increased or induced (Kostopoulos et al. 1988; Haas and Panula 2003). Further, depolarization of the thalamic relay neurons leads to opening up the doors of consciousness through an increase in hyperpolarization-activated inward current (Panula et al. 2015).

Furthermore, the activation of the H2 receptor is also found to inhibit phospholipase A2 along with arachidonic acid release, which interprets the contrast physiological role of stimulated H1 or H2 receptors in different other tissues (Traiffort et al. 1992). The prediction of the central role of histamine, including behavioural changes, through H2 receptor is quite difficult as the specific ligands of H2 receptors hardly cross the BBB biological barrier. However, H2 receptor deficit mice were found to display cognitive deficits, diminishing long-term potentiation of the hippocampal region, abnormalities in immune function, nociception and gastric function (Panula et al. 2015). The immune response suppression *via* H2 receptors is also evident in human antigen presenting cells, where stimulation of H2 receptors revealed downregulation of different cytokines (Glatzer et al. 2013).

7.3.2.4 Antagonists of H2 Receptor

H2 receptor antagonists are found to be useful in treating hyper acid-related disorders in the gastrointestinal system, such as gastroesophageal reflux disease and peptic ulcer (Parsons and Ganellin 2006; Rojas-Zamorano et al. 2009). Sir James Black won the Nobel Prize for his work on β-receptor antagonists and on H2 receptor antagonists (Parsons and Ganellin 2006).

N^{α}-guanylhistamine is a weak partial agonist to H2 receptors acting on the heart and on GI tract leading to gastric secretion. Around 1964 to 1972, burimamide was discovered, which is a highly specific and competitive H2 receptor antagonist with 100 times more potency than N^{α}-guanylhistamine (Black et al. 1972). However, further exploration of its clinical potential did not succeed due to the lack of oral efficacy of burimamide. Metiamide was the second potent H2 receptor antagonist, which showed good oral bioavailability with therapeutic efficacy against duodenal ulcer disease, however precluded commercialization due to the presence of thiourea group in the structural moiety, which caused toxicity. Afterwards, cimetidine, the first marketed H2 receptor antagonist, introduced by Sir James Black, was developed by replacing thiourea group with the highly polar cyanoguanidine group, effective in the treatment of peptic ulcer and gastro-oesophageal reflux disease (Black et al. 1972; Brimblecombe et al. 1975; Parsons and Ganellin 2006). Subsequently, H2 receptor antagonists became very popular medicines for the treatment of gastric and duodenal ulcers (Fig. 7.3) (Church 2009).

The development of H2 receptor antagonists located at the GI tract brought the lead in the physiological control of gastric acid secretion, which could inhibit histamine, gastrin and acetylcholine, and vagally stimulated acid secretion. Subsequently, various H2 receptor antagonists were developed and marketed; however, specially ranitidine become very popular among them due to the lack of drug-drug interaction potential, unlike cimetidine. Due to high attrition in drug development considering safety and efficacy in patients, there are only five H2 receptor antagonists (cimetidine and ranitidine, famotidine, roxatidine and nizatidine) marketed, although several candidates have undergone clinical study (Panula et al. 2015).

7.3.3 Histamine H3 Receptor

Histamine H3 receptor expressions are mostly predominant in the CNS rather than in the peripheral nervous system. These receptors function as autoreceptors in presynaptic histaminergic neurons (Nieto-Alamilla et al. 2016). H3 receptors also mediate histamine turnover *via* negative feedback to inhibit the synthesis and release of histamine. These also lead to feedback inhibition of several other neurotransmitters released by functioning as an inhibitory heteroreceptor. These neurotransmitters include gamma amino-butyric acid (GABA), dopamine, noradrenaline, acetylcholine, serotonin and histamine. The H3 receptors gene sequences possess as less as about 22% and 20% homology with H1 and H2 histamine receptors, respectively. The H3 receptor is a potential therapeutic target due to its regulatory functions in several neuronal mechanisms (Nieto-Alamilla et al. 2016).

7.3.3.1 Structural Biology

Histamine H3 receptors also belong to the GPCR group with the core seven-TM domain, an extracellular amino terminus (NT), an intracellular carboxyl terminus (CT), three extracellular (ECL) and three intracellular (ICL) loops (Nieto-Alamilla et al. 2016). The H3 histamine receptors consist of a DRF motif instead of common

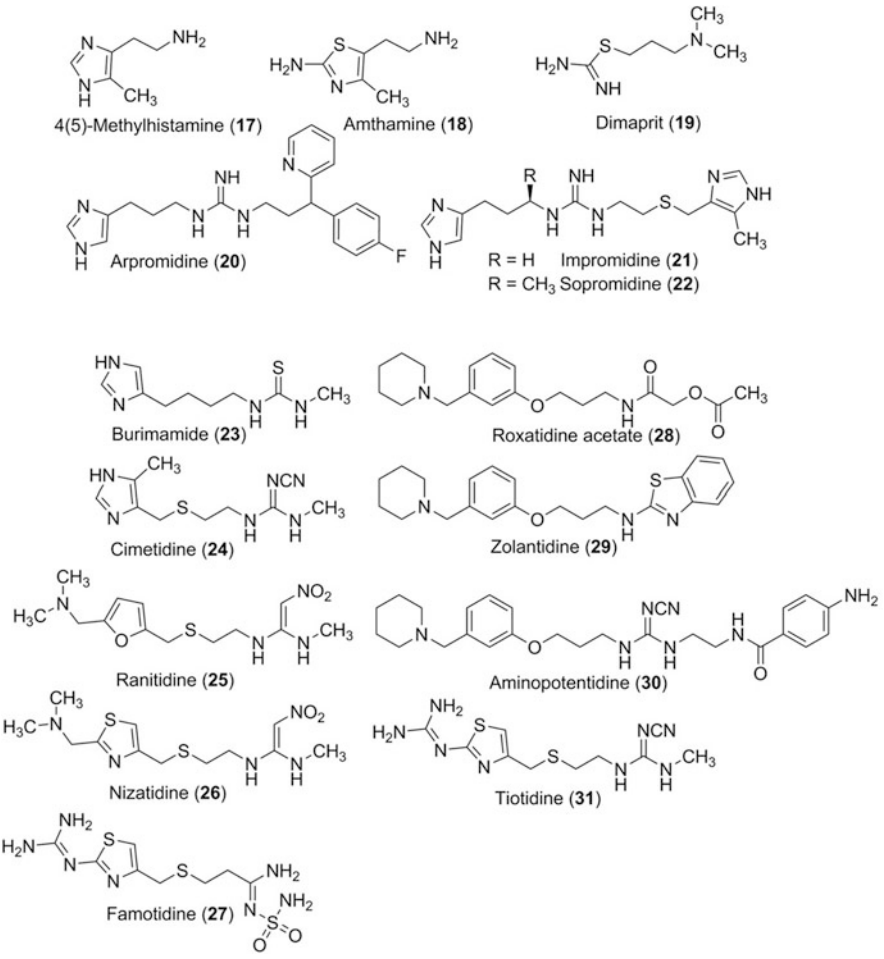

Fig. 7.3 Different ligands acting on H2 receptor (Panula et al. 2015)

GPCRs DRY sequence in the interface of TM domain and intracellular loops. Similar to NPXXY motif in all GPCRs, the histamine H3 receptor possesses a NPVLY motif in the TM domain (Bongers et al. 2007). The intracellular carboxy terminal of the receptor possesses a palmitoylation site, which aids the formation of helix 8. The Cys107 and Cys188 of the first and second extracellular loops form disulphide bonds. The receptor contains a short amino terminal of 39 amino acids, which have a glycosylation site on Asn11, while the third intracellular loop is long consisting of 142 amino acids. These sequences serve as loci for naturally occurring mutations that give rise to several isoforms of H3 histamine receptors (Nieto-Alamilla et al. 2016).

7.3.3.2 Biological Distribution

The histamine H3 receptor is a popular presynaptic autoreceptor acting upon histamine-containing neurones. These receptors are immensely distributed in the CNS, mostly in the cortex, thalamus, the caudate nucleus, hippocampus, hypothalamus and in the olfactory tubercle (Arrang et al. 1983, 1987). High expression of the histamine H3 receptors throughout the cortex suggests their significant role in the modulation of a wide range of neurotransmitters, such as GABA, acetylcholine, norepinephrine, dopamine and serotonin in the CNS and peripheral tissues. The distribution of histamine H3 receptors is quite low in the periphery including in the heart, small intestine, prostate and testis in humans, while their expressions in tissues like the lung and spleen are not reported (Lovenberg et al. 1999).

7.3.3.3 Role in Biological System

The histamine H3 receptors' potency in neurotransmitters modulation renders them a novel therapeutic target in the treatment of innumerable disorders. These include a number of inflammations and allergic reactions. In addition, these also account for various neurological disorders, leading to innumerable conditions. Obesity is caused by histamine cross-talks with orexinergic system, movement disorders owing to dopamine and GABA modulations by H3 receptor in the basal ganglia, schizophrenia and attention deficit hyperactivity disorder (ADHD) due to dopamine modulation, as well as irregular sleep-wakefulness cycle by alterations in noradrenaline, glutamate and histamine (Lovenberg et al. 1999; Morisset et al. 2000; Leurs et al. 2005, 2012). They also play a role in the regulation of satiety (Passani et al. 2011).

Histamine H3 receptors are coupled to G-protein Gi, inhibiting intracellular cAMP generation (Lovenberg et al. 1999). The interactions between the dissociated βγ and N-type voltage-gated calcium channels lead to decreased action potential-induced calcium influx. This ultimately results in reduced neurotransmitter release, thereby acting as presynaptic autoreceptors on histamine containing neurons (Arrang et al. 2007; Feuerstein 2008). The Gαi/o protein-dependent histamine H3 receptor signalling is usually mediated by both the Gα subunits and the Gβγ complexes, acting through the subsequent pathways that include one or more of the following: arachidonic acid, adenylyl cyclases; cAMP; Na^+/H^+ exchanger; mitogen-activated protein kinases; phosphatidylinositol 3-kinase; phospholipase A2; phospholipase C; protein kinase A (Fig. 7.4) (Morisset et al. 2000; Nieto-Alamilla et al. 2016).

Inhibition of Na+/H+ Exchanger

Histamine H3 receptors have been reported to inhibit Na+/H+ exchanger activities in sympathetic nerve terminals in myocardial ischemia, thereby inhibiting noradrenaline release. The mechanism may involve a direct interaction of Gαi/o subunit with the Na^+/H^+ exchanger (Bongers et al. 2007).

Inhibition of Voltage-Gated Calcium Channels

Histamine H3 receptors mediate inhibition of neurotransmitter through action potential-induced Ca^{2+} entry by Gβγ complexes binding to pore-forming α1-subunit of calcium channels (N- and P/Q-type voltage-gated) (Zamponi and Currie 2013).

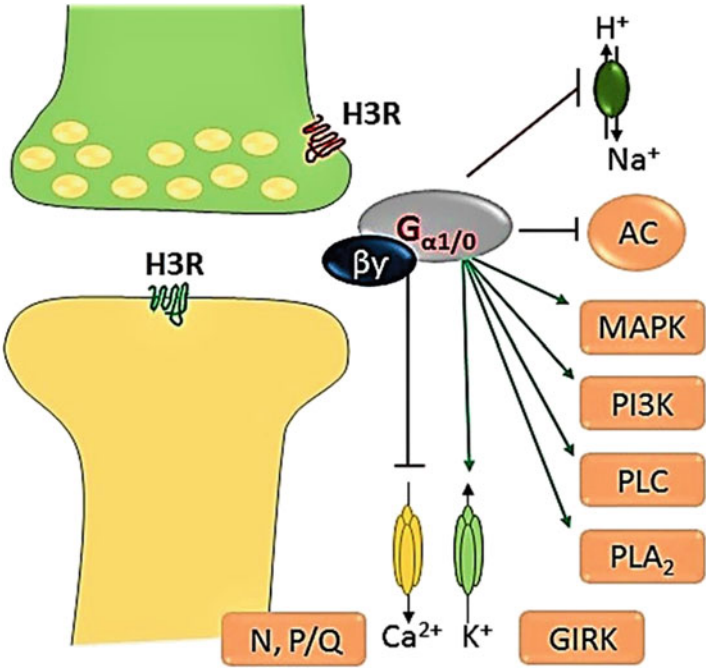

Fig. 7.4 Molecular signalling pathway of activated histamine H3 receptor (H3R) located in presynaptic and postsynaptic neuron to mediate biological functions. *AC* adenylyl cyclase; *MAPK* mitogen-activated protein kinases; *PI3K* phosphoinositide 3-kinase; *PLC* phospholipase C; *PLA*$_2$ phospholipase A$_2$; *GIRK* G-protein-coupled inwardly rectifying potassium channel

Accordingly, the histamine H3 receptor activation downregulates calcium influx in dissociated histaminergic neurons of hypothalamus, in the transfected human neuroblastoma cells, striatal synaptosomes, and transfected pheochromocytoma cells (Nieto-Alamilla et al. 2016).

Activation of G-Protein-Gated Potassium Channels

Gβγ subunits of the histamine H3 receptors bind and activate G-protein-gated inwardly rectifying potassium K^+ channels (GIRK) (Sahlholm et al. 2012), which can downregulate synaptic transmission (Meneses et al. 2015) and modulate neurotransmitter release. This occurs at the postsynaptic neurons secreting melanin-concentrating hormone (MCH) (Parks et al. 2014).

Activation of Phospholipase C

Histamine H3 receptors may activate phospholipase C (PLC), thereby leading to increased intracellular calcium ions concentration *via* IP3 pathway to mediate physiological functions (Bongers et al. 2007).

Activation of the MAPK Pathway

Histamine H3 receptors are able to mediate MAPK phosphorylation in heterologous systems as well as native tissues (Flores-Clemente et al. 2013). Gβγ complexes have been suggested to play a key role in this action (Lai et al. 2016).

Activation of the Phosphatidylinositol 3-Kinase (PI3K) Pathway

Histamine H3 receptor activation triggers PI3K pathway *via* Gβγ complexes and protein kinase B (Akt) phosphorylation, which by subsequent actions inhibits glycogen synthase kinase 3-β (GSK3β) activities (Bongers et al. 2007).

Stimulation of Phospholipase A2

Histamine H3 receptor-induced phospholipase A2 activation leads to the release of docosahexaenoic acid, arachidonic acid and lysophospholipids. These mediate physiological functions such as relaxation of bronchioles *via* endothelium-derived relaxing factor, which is an arachidonic acid metabolite (Nieto-Alamilla et al. 2016).

7.3.3.4 Antagonists of H3 Receptor

The histamine H3 receptor, as previously discussed, is an autoreceptor, which also regulates the release of several other neurotransmitters, including noradrenaline, dopamine and 5-HT acetylcholine. Thus, it serves as a popular therapeutic target for CNS disorders, as agents with multiple and complementary modes of action.

Early Pharmacophore

The initial focus of H3 receptor ligands development was upon the agonists with an imidazole ring within its structure (Celanire et al. 2005). The imidazole ring in these drugs leads to undesired inhibition of cytochrome P450 isoenzymes and this results in adverse drug interactions (Celanire et al. 2005). Moreover, they fail to cross the BBB and also proved to have a certain degree of toxicity (Schwartz 2011). Another problem with these drugs is less specificity and action upon other receptors such as on the histamine H4 receptor (Sadek et al. 2016).

Thioperamide represents the first imidazole-based histamine H3 antagonist. It was quite potent as well as selective in action but could cause hepatotoxicity. It was first designed in order to enhance cognition functions and wakefulness (Schwartz 2011). A potential study has reported the efficacy of thioperamide treatment in ameliorating circadian rhythm Parkinson patient (Masini et al. 2017).

New Pharmacophore

To mitigate the limitations of histamine H3 receptor imidazole based antagonists, non-imidazole H3 receptor antagonists emerged. These drugs can easily cross the BBB and reach the CNS. However, few problems also cropped up in the use of these drugs, for example phospholipidosis, its strong binding to potassium channel, and problems with P-glycoprotein also known as multidrug resistance protein 1 (MDR1) substrate (Gemkow et al. 2009). Pitolisant, a highly selective antagonist for the H3 receptor, was the first H3 receptor antagonist to proceed to clinical trials. Presently, it is the sole drug that is approved in the USA and Europe.

Histamine H3 receptor antagonists are immensely introduced in clinical trials for the treatment of cognitive impairments in Alzheimer's disease, narcolepsy, attention deficit hyperactivity disorder (ADHD), Parkinson's disease and schizophrenia (Benarroch 2010). A striking property of H3 receptors is their high degree of constitutive activity *in vivo* (Rouleau et al. 2002). This discovery is important for drug development, since the ability to compete with constitutively active H3 receptor states (inverse agonism) has important therapeutic implications. H3 antagonists are useful in treating sleep disorders (Lin et al. 2011). They are highly potent drugs for addressing cognition disorders as pharmacological blockade of H3 receptors has been reported to elicit procognitive outcomes in different preclinical models of Alzheimer's disease, ADHD and schizophrenia (Brioni et al. 2011). At present, the treatment modulation involves a single neurotransmitter system (e.g. cholinesterase inhibitors to treat Alzheimer's disease, dopaminergic stimulants for ADHD). However, multiple neural circuits and neurotransmitter systems are associated with such treatments as well. The histamine H3 receptor antagonism can increase the concentrations of noradrenaline, dopamine, acetylcholine, serotonin and histamine in the cortex resulting in alterations in cognitive processes (Esbenshade et al. 2008).

7.3.4 Histamine H4 Receptor

The gene of H4 receptor was discovered in 1999 from the Human Genome Project, which had a feature of class A GPCR. This receptor is found to have similarity with other histamine receptors in its homology; thus it became the fourth receptor in the histamine family. After the huge success of H1 and H2 targeting, H4 was also underwent investigation for its targetability and functions. A vast literature review revealed that some of the histaminic activities were not mediated by H1, H2 and H3 receptors. In this context, pruritus and asthma came up with the major area of investigation as histamine plays an important role in the pathogenesis of disease. However, H1 and H2 targeted histamines does not respond effectivity for these disease conditions. Later this gap was filled with the concept of H4 receptor mediation in inflammation and lung functions. Likewise, patients with atopic dermatitis were not responding to H1 receptor antagonists. Subsequently, the generation of H4 antagonist has shown tremendous effect on disease conditions (Leurs et al. 2012; Thurmond 2015; Kiss and Keserű 2016).

7.3.4.1 Structural Biology

Structural biology of a receptor is key to understanding the binding site of a receptor and to develop new ligands for receptors. After the discovery of the histamine H4 receptor in 1999, several studies were conducted to predict the structure; it was found that the organization of H4 is in homology with H3 receptors. In perspective to discover the binding site Shin et al. revealed the involvement of the Glu182$^{5.4.6}$ and Asp94$^{3.3.2}$ pockets in H4 receptors in the histamine binding site. It was reported that nitrogen atom of histamine imidazole ring interacts with Glu182$^{5.4.6}$ pocket; however, other identified pockets, such as Asn147 and Ser306, were involved in receptor

binding but play significant roles in receptor activation (Shin et al. 2002). Another study for homology model development confirmed Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$ as the major anchoring point for H4 receptor binding and has shown participation in GPCR ligand binding. Moreover, Asp94$^{3.3.2}$ is the major site for ligand interaction and situated closer to Glu182$^{5.4.6}$ (Evers and Klabunde 2005; Kiss et al. 2008a; Pontiki and Hadjipavlou-Litina 2017). The binding mode of antagonists and agonists was also investigated by using histamine as agonist and JNJ7777120 as antagonist. Experimental results revealed that antagonists interact with Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$, whereas agonists interact with Thr323$^{6.5.5}$, Asp94$^{3.3.2}$ and Glu182$^{5.4.6}$ which suggest the involvement of Thr323$^{6.5.5}$ in agonist binding and activation of receptors (Jablonowski et al. 2003; Kiss et al. 2008b; Jójárt et al. 2008).

7.3.4.2 Biological Distribution

H4 receptor is a pertussis-toxin-sensitive GPCR, mainly expressed on the cells of peripheral tissue (thymus, spleen, colon, bone marrow and blood leukocytes) and immune system (eosinophils, monocytes, T cells, dendritic cells and natural killer cells). The affinity of H4 receptor for inflammation *via* $G\alpha_{i/o}$ proteins is through activation of leukocyte chemotaxis and increases in intracellular Ca^{2+} concentration. Moreover, LEC/CCL16, which is a liver-expressed chemokine, is identified as a non-histamine endogenous H4 receptor agonist and involved in eosinophil trafficking (Nakayama et al. 2004).

7.3.4.3 Role in Biological System

Histamine H4 plays a significant role in immunomodulation due to high expression in several types of immune cells (Fig. 7.5). H4 receptors are reported to have eosinophil chemotaxis and control pro-inflammatory responses and leukocyte trafficking by induced activation of eosinophils (Raible et al. 1994; Barnard et al. 2008). The presence of H4 receptor on eosinophils came out after investigation on pharmacology of various histamine ligands. Researchers found that H1 and H2 receptor ligands are ineffective and the potency of H3 receptor ligands was also not appropriate in some disease. Therefore, they have evaluated the H4 receptor ligands and results revealed the presence of H4 receptor on eosinophils and showed effectiveness (Thurmond et al. 2009; Reher et al. 2012). Moreover, H4 receptor also governs paracrine histamine-induced processes and autocrine (Lippert et al. 2004).

The presence of histamine H4 receptor was noticed in mast cells, which is associated with high secretion of histamine. Activation of H4 receptor on mast cells causes intracellular Ca^{2+} mobilization and chemotaxis leading to chronic allergic inflammation (Hofstra et al. 2003). It was evident from the study on animal that absence of histamine induced calcium response in mast cell block by H4 receptor antagonist (Thurmond et al. 2004, Yu et al. 2010; Jemima et al. 2014). This receptor was also involved in zymosan-induced recruitment of neutrophils *via* regulation of leukotriene B_4 release from mast cells (Takeshita et al. 2003; Thurmond et al. 2004; Thurmond 2015). Additionally, cytokine and chemokine production was also controlled by H4 receptor on T cells. Immunomodulatory function of this receptor was also associated with the release of IL-16 from $CD8^+$ T lymphocytes (Gantner et al. 2002). It had been reported that H4 receptors

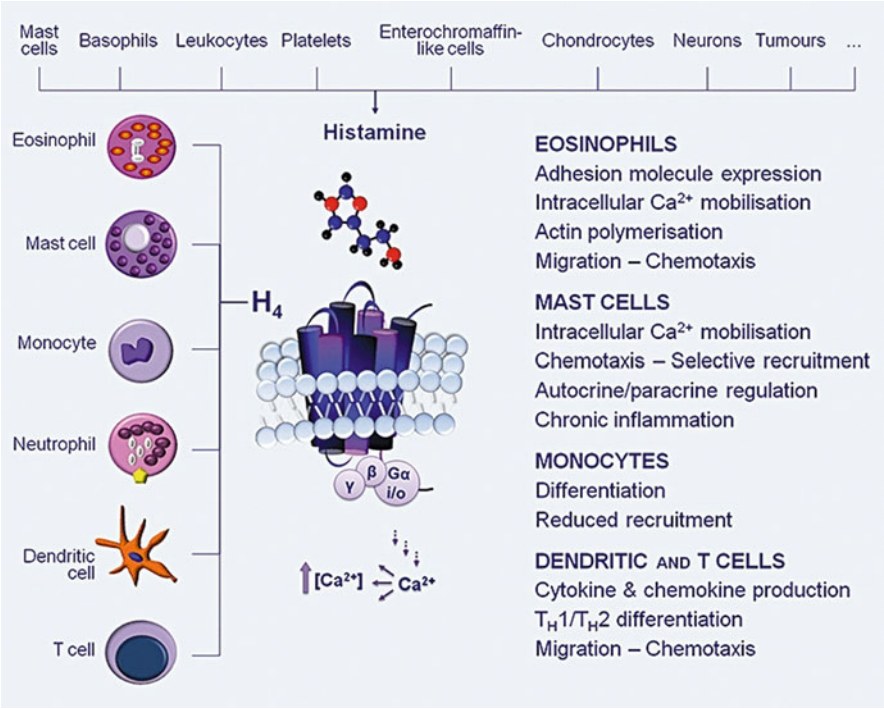

Fig. 7.5 Indicative immunomodulatory actions of histamine that are mediated through histamine H4 receptors (H4) predominately expressed in immune cells. Gαi/o, G-protein; TH, helper T cell (Zampeli and Tiligada 2009)

downregulate the production of ILs and that of CCL2 (C-C motif chemokine ligand 2) in human monocyte-derived inflammatory dendritic epidermal cells, the later leading to decreased monocyte migration (Dijkstra et al. 2007, 2008).

Diseases like asthma and atopic dermatitis are associated with mast cells and eosinophils. Histamine H4 receptors act as a mediator in lung disorders by controlling the migration of inflammatory cells, production of chemokine and cytokine. The expression of histamine H4 receptor in the lung is low; however, high expression in smooth cells and bronchial epithelial contributes to disease phenotype (Gantner et al. 2002). As discussed earlier, it also mediated the recruitment and distribution of mast cells in bronchial epithelium, thus leading to allergic inflammation of the airways (Thurmond et al. 2004). This was evident by a study on guinea pig model, where the H4 receptor antagonist JNJ7777120 showed reduction in inflammation, improved lung function and inflammation mediators synthesis in the lungs after allergen challenge (Somma et al. 2013). Histamine H4 receptor involvement is also recognized in autoimmune disease, such as rheumatoid arthritis (RA). Further, RA severity could be related to the expression of H4 receptor along with H1 and H2. This concept was supported by the presence of H4 receptor in vascular wall cells and synovial tissue of the patients suffering with RA and osteoarthritis (Ohki et al. 2007; Kiss et al. 2008a).

7.3.4.4 Antagonists of H4 Receptor

Thioperamide was developed as a H3 receptor antagonist and has affinity towards H4 receptor as well. However, it is not the ideal tool for proper understanding of H4 receptor. Jablonowski and team identified the first potent antagonist (JNJ 7777120) of H4 receptor, which was introduced to understand the physiological role of H4 receptors. Inflammatory properties of JNJ 7777120 was evident from the H4 receptor transfected SK-N-MC cell model and animal model (Jablonowski et al. 2003). Moreover, the reduction of neutrophil efflux in the mouse periodontal model was observed after pre-treatment with H4 antagonists (Varga et al. 2005). JNJ 7777120 and its analogue (JNJ 10191584) were found effective in the animal model of several diseases, such as colitis, asthma, pain and atopic dermatitis (Hsieh et al. 2010; Cowden et al. 2010). Potential issue was identified for JNJ 7777120 compound as it was reported that it had shown H4 receptor agonistic activity in some animal models and cell models due to arresting activation of receptor (Seifert et al. 2011). Additionally, short half-life and hypoadrenocorticism toxicity of JNJ 7777120 limit its clinical application. Thus another analogue of JNJ 7777120, JNJ 39758979, was discovered by Thurmond *et al.* with low toxicity and has shown effective anti-pruritic and anti-inflammatory activities in animals. However, this compound was withdrawn from study due to occurrence of drug-induced agranulocytosis. This leads to the development of toreforant, an antagonist of histamine H4 receptor, which did not possess any serious side effect and clinically tested on patients with RA, dermatitis and asthma (Thurmond 2015; Thurmond et al. 2016). Many histamine H4 antagonists have been developed, but only a few were investigated in humans for their effectiveness. In conclusion, still it is difficult to find the proper ligand for H4 receptor having human underscoring with all necessary properties.

7.4 Conclusion

Histamine is a well-preserved autacoid, found in most of the tissues in vertebrates. The histaminergic system is recognized by the action of histamine on four 7-TM GPCRs. These histamine H1, H2, H3 and H4 receptors showed molecular heterogeneity and constitutive activity. The physiological role of different modulators on these receptors varies largely, from antiallergic, sedating, antiulcer, wake promoting, anti-inflammatory actions to improvement of memory. Moreover, novel compounds for these receptors and exploration of their physiological role are still under investigation to bring new compounds in the treatment of complicated disorders.

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Pharmacology of GABA and Its Receptors

8

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Abstract

GABA is an important neurotransmitter in vertebrates where it acts at synapses of the CNS; in nematodes GABA acts primarily at neuromuscular synapses. Specifically, GABA acts to relax the body muscles during locomotion and foraging and to contract the enteric muscles during defecation. Following the recognition of GABA as an inhibitory neurotransmitter, the discovery of high-affinity GABA uptake and the characterization of GABA receptors have made great progress in developing GABA pharmacology. Tiagabine, the first marketed GABA uptake inhibitor, may be followed by new and more selective uptake inhibitors. This chapter centers on the discoveries made during more than six decades of neuroscience research on the role of GABA as a neurotransmitter. In doing so, special emphasis is placed on the significant involvement of GABA in the normal physiology of the human body such as sleep, reproductive system, heart, learning and memory. GABA dysregulation also categorizes various neurological disorders and enlisted their potential therapeutic drug targets under research that encompass Parkinson's disease, Alzheimer's disease, epileptic disorders, traumatic brain injury (TBI), Huntington's disease, anxiety, multiple

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sclerosis (MS), and schizophrenia. This chapter further highlights the recent and previously conducted clinical trials and future investigational targets of GABA. Now it is readily accepted as a vital spinal and supra-spinal inhibitory transmitter and we know many details regarding its molecular structure and trafficking around neurons. The chapter highlights the synthesis, reuptake, and metabolism of GABA. Thereafter, mechanisms of action of various GABA subtypes ($GABA_A$, $GABA_B$, $GABA_C$) have been discussed which make the prospects for further research very exciting.

Keywords

Basal ganglia · GABA · Hyperpolarization · Neurological disorders · Inhibition

Abbreviations

AC	Adenylyl cyclase
AD	Alzheimer's disease
AEDs	Antiepileptic drugs
BGT	Betaine-GABA transporter
BZD	Benzodiazepine
cAMP	Cyclic adenosine monophosphate
CB	Cannabinoid receptor
CNS	Central nervous system
D	Dopamine
DSM	Diagnostic and Statistical Manual of Mental Disorder
FSH	Follicle stimulating hormone
GABA	Gamma aminobutyric acid
GAD	Glutamic acid decarboxylase
GAD	Generalized anxiety disorder
GATs	GABA transporters
GDNF	Glial cell derived neurotrophic factor
GHB	Gamma-hydroxybutyrate
Gln	Glutamine
GnRH	Gonadotropin releasing hormone
GPCR	G-protein-coupled receptor
Gpe	Globus pallidus external
Gpi	Globus pallidus internal
HD	Huntington's disease
IN	Interneuron
IPSPs	Inhibitory postsynaptic potentials
mBDNF	mature Brain-derived neurotrophic factor
MnPO	Median preoptic nuclei
MNTB	Medial nucleus of the trapezoid body
MPN	Methylpyridoxine

MS	Multiple sclerosis
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
OCD	Obsessive compulsive disorder
PAG	Phosphate-activated glutaminase
PD	Panic disorder
PD	Parkinson's disease
PFC	Prefrontal cortex
PLTS	Persistent low-threshold spiking
PPMS	Primary progressive multiple sclerosis
PRMS	Progressive relapsing multiple sclerosis
PTSD	Post-traumatic stress disorder
RIMS	Rapid eye movement sleep
RRMS	Relapsing remitting multiple sclerosis
SACs	Starburst amacrine cells
SAD	Social anxiety disorder
SCZ	Schizophrenia
SNPc	Substantia nigra pars compacta
SPMS	Secondary progressive multiple sclerosis
SSADH	Succinate semialdehyde dehydrogenase
STN	Subthalamic nuclei
TBI	Traumatic brain injury
TGB	Tiagabine
THDOC	Tetrahydrodeoxycorticosterone
THIP	Tetrahydroisooxazolopyridinol
VGB	Vigabatrin
VLPO	Ventrolateral preoptic nuclei
VTA	Ventral tegmental area

8.1 Introduction

In the brain, neuronal excitation and inhibition is responsible for the basis of information transfer within the CNS. Amino acid neurotransmitters are the messengers which exert excitatory and inhibitory actions, which include glutamate and GABA, respectively. The balance between these two neurotransmitters ensures normal functioning of neurons showing rhythmic activity. GABA is the main inhibitory neurotransmitter in the brain and is made up of amino acid sequences. The primary action of GABA is exerted by binding to the postsynaptic receptor, which stimulates the Cl^- ion channels, opens Cl^- ions channels resulting in hyperpolarization of the cell, and thus exerts the inhibitory response, i.e., conductance of action potential is completely blocked. Clinically, the significance of GABA has not been estimated yet, but mainly it controls the transmission of neurotransmitters in the synapses and excitability of the neurons within the CNS. The ubiquitous distribution of GABA in the brain helps to exhibit a variety of physiological functions including

calmness, sleep, motor functions, cognition, and memory like behavioral phenomenon (Wu and Sun 2015). No doubt, it has an inhibitory control over mammalian brain but in neonatal brain it also exhibits some excitatory functions. Here, it participates in a variety of functions including neurogenesis, synapse formation, and synaptic plasticity. This indicates that GABA is well implicated in normal neural development and physiology of the brain. The inhibitory nature of GABA helps to execute over the excitatory neurons for maintaining the homeostasis between GABA and glutamate which is known as GABA/glutamate cycle. Any asymmetry in this balance creates the pathological situations associated with different disorders like epilepsy, schizophrenia, anxiety, dementia, and motor disorders like Parkinson's disease. Such pathological associations make GABAergic dysfunction as a major target for significant therapeutic approach in CNS disorders (Wu and Sun 2015). GABA signaling modulation or enhancing GABAergic inhibition is the way for the treatment of many pharmacologic drugs in various pathological situations.

GABA is present in different parts of the mammalian body, but its various roles are still unknown. Primarily it shows inhibitory responses in the CNS as well as in the spinal cord. Usually, the main function of the beta cells of the pancreas is to produce insulin but is also able to produce GABA. Thus GABA produced from the beta cell can stimulate the growth of beta cells, formation of beta cells from alpha cells, and inhibiting alpha cells. Within the body, the other parts contain low amounts of GABA, though the proper function and relevant clinical significance of this remain unknown, but it mainly counterbalances the excitatory responses.

Firing of the neuronal cell is too fast, in the absence of GABA. When GABA activity is significantly decreased in the CNS, various disorders like anxiety, convulsions, panic attacks, cognitive dysfunction, Parkinson's disease, drug addiction, etc. occur due to excitatory discharge. Transmission of nerve impulses is significantly hindered from one neuron to another by GABA, due to inhibitory responses; it has calming or quieting effect. In the brain, the activity of growth hormone, synthesis of protein, and plasma concentration are improved by GABA, but diminishes small airway-derived lung adenocarcinoma. Other responses include hypotensive, tranquilizing, diuretic, and antidiabetic effects.

Inhibitory neurotransmission is a complex process but it can be achieved through the activation of different types of GABA receptors. Historically only two types of GABA receptors (i.e., $GABA_A$ and $GABA_B$) were identified, but now GABA receptors are classified into three types ($GABA_A$, $GABA_B$, $GABA_C$) based on pharmacological and electrophysiological properties. Recently, $GABA_A$ and $GABA_C$ are classified as ligand-gated chloride channel receptors and $GABA_B$ receptors are known as metabotropic, i.e., G-protein-coupled receptors.

The therapeutic reliability of neurotransmitter GABA could be analyzed from the success of benzodiazepines (BZD). BZDs are a class of drugs which act by interacting with GABA receptors and utilized as the most commonly prescribed drugs clinically. BZDs are the positive allosteric modulator of $GABA_A$ receptor to potentiate the inhibitory neurotransmission derived calming effect in the brain (Griffin et al. 2013). The drugs under BZDs include diazepam, flurazepam, alprazolam, chlordiazepoxide, etc. which are commonly prescribed for anxiety, insomnia,

muscle relaxation, and epilepsy. Furthermore, several new possible indications of these drugs emerge under newly revealed mechanisms. New possibilities for classic medications working through GABA modulation may provide suitable therapeutic benefits in different neurodegenerative diseases. To study the possibilities for specific disorders, first it is essential to discuss the physiology of GABA inside the brain which includes synthesis, metabolism, and reuptake including the structure and localization of receptors mediating its actions.

8.2 History and Discovery of GABA; Agonists and Antagonists

In 1883, it was known only as a plant and microbial metabolic product. There are evidences which establish that in the mammalian CNS, γ-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter which was fully accepted at the end of the 1960s/early 1970s by its role. In 1910, it was shown to be present in biological tissues but at that time its presence in the mammalian CNS was not confirmed. Only after 1950, i.e., 40 years later, the presence of free amino acid was identified in the brain that arose the neurochemical significance of GABA. During the ensuing decade, how neuronal activity is affected by GABA and related compounds has been reported to define its role within the CNS.

Additionally, the role of excitatory and inhibitory activities of GABA in crustacean is defined by concurrent findings of Kuffler (1954) (Jijón-Lorenzo et al. 2018). The initial finding related to GABA was muted. After 5 years of discovery by Roberts and Frankel, only two articles related to GABA activity within the brain were reported in the *Journal of Biological Chemistry* and PubMed had only four articles related to GABA activity. Nevertheless, in those days due to the lack of methods that define its presence and function, research community remained silent. The inkling properties of GABA were not shown by anyone in the brain. In 1957, researchers in Canada confirmed the activity of GABA due to the inhibitory activity of an unknown compound on crayfish neuron. GABA transporters were studied by Baruch Kanner of Hebrew University, Hadassah Medical School in Israel, who is a member of the *Journal of Biological Chemistry* editorial board.

Kanner said, "GABA receptors have an inhibitory input which is the major inhibitory action within the brain." Roberts and Frankel formed the basis and provided evidence; then only it was clear about how neurotransmitters control the brain activity. Roberts analyzed various extracts and reported the existence of a free amine compound by migration of ninhydrin reactive compounds on chromatograms. This amino acid is accumulated in higher concentrations in the brain than in other tissues.

In 1987, Eric A Barnard in Cambridge (UK) who is a molecular biologist and Peter H Seeburg at Gene tech in San Francisco (CA, USA) succeeded to define $GABA_A$ receptor, which is then classified as ligand-gated ion channels. After a decade, airway epithelium containing GABAergic system which is excitatory was described in 2007. On exposure to allergens the system becomes activated and participates in the mechanisms of asthma. In the testis and eye lens GABAergic system is present as shown in Table 8.1.

Table 8.1 Historical aspects of GABA receptors

Year	Important event	Reference
1883	GABA synthesized, known as a product of microbial and plant metabolism	Cooper et al. (2003)
1950	GABA identified as a normal constituent of mammalian CNS	Roberts and Frankel (1950)
1959	GABA was extracted from mammalian brain	Florey and McLennan (1959)
1962	Baclofen, a lipophilic derivative of GABA, was synthesized	Keberle et al. (1968)
1967	It was recognized as an inhibitory neurotransmitter	Hall and Kravitz (1967)
1975	Benzodiazepines the important modulator of GABA was described	Haefely et al. (1975)
1977	Benzodiazepine binding site discovered in the brain	Tallman et al. (1977)
1978	The distribution of forming enzyme for GABA (GAD) in the mammalian spinal cord was described	Barber et al. (1978)
1981	$GABA_A$ and $GABA_B$ receptors were pharmacologically distinguished	Bowery et al. (1982)
1984	GABA receptors that were insensitive to both bicuculline and baclofen known as $GABA_C$ receptor	Chebib and Johnston (2000)
1987	$GABA_A$ receptors were cloned	Schofield et al. (1987)
1990	Molecular composition of $GABA_A$ receptors was elucidated	Martin and Olsen (2000)
1997	Structure of $GABA_B$ receptors was identified	Kaupmann et al. (1997)
1991	ρ1 subunit of $GABA_C$ receptors was cloned	Polenzani et al. (1991)
1992	ρ2 subunit was cloned	Cutting et al. (1992)
1996	ρ3 subunit was cloned	Shingai et al. (1997)
1997	$GABA_{B1a}$ $GABA_{B1b}$ cloned	Bettler et al. (2004)
1998	$GABA_{B2}$	Jones et al. (1998)
2001	Positive allosteric modulators of $GABA_B$ receptors were cloned	Martin et al. (2001)
2004	Glutamic acid decarboxylase activator, pregabalin	Silverman (2008)
2009	Malfunction in GABA and glutamate described as a pathway to cure depression	Sharpley (2009)
2010	Acute psychological stress on prefrontal GABA concentration plays an important role in the pathophysiology of anxiety disorders	Hasler et al. (2010)
2011	Modulation of GABA system by Passiflora	Appel et al. (2011)
2012	Role of GABA in primary insomnia	Plante et al. (2012)
2013	GABA exerts anti-inflammatory and immunosuppressor effects	Prud'homme et al. (2013)

(continued)

Table 8.1 (continued)

Year	Important event	Reference
2014	Inhibiting ARG1 or agonizing the $GABA_A$ receptor treated neuroblastoma	Hackett et al. (2014)
2015	GABA administration helps in improving action selection processes	Steenbergen et al. (2015)
2016	Obstructive sleep apnea is associated with low GABA and high glutamate in the insular cortex	Macey et al. (2016)
2017	Somatostatin-positive GABAergic interneuron: new targets for depression	Zhang et al. (2017)
2018	GABA modulating bacteria of the human gut microbiota	Strandwitz et al. (2019)
2019	$GABA_A$ receptor signaling mechanisms revealed by structural pharmacology	Masiulis et al. (2019)

8.3 Synthesis, Storage, and Release of GABA

The synthesis, storage, release, reuptake, and metabolism of GABA occur due to signaling of the GABA system. Both ionotropic and metabotropic receptors expressed in the plasma membranes of cells and GABA exert their action through these receptors (Olsen and Sieghart 2009). GABA is formed by various metabolic pathways, also called the GABA shunt, and operates via closed loop process. The purpose of this pathway is to produce and conserve the supply of GABA. Brain regions contain high amount of GABA and the amount is 1000 times greater than other monoamine neurotransmitters. The endogenous synthesis of GABA proceeds via α- decarboxylation of L-glutamate by action of the enzyme glutamic acid decarboxylase (GAD) as shown in Fig. 8.1. The functioning of GAD depends upon the presence of cofactor pyridoxal-5′ phosphate to catalyze the single-step irreversible reaction for production of GABA (Roth and Draguhn 2012). Here, GAD exists in two forms; GAD 65 (65 kDa) was found to reside in axons and synaptosomes along with plasma membrane whereas GAD67 (67 kDa) was found to be localized in the cytosol of neuronal cells. These both mediate the synthesis of GABA by vesicular mechanism and cytoplasmic production, respectively.

On production, GABA gets recruited to synaptic vesicles via vesicular GABA transporter (vGAT), which releases GABA in synapse on membrane depolarization of neurons. After the release, GABA interacts with its receptors for mediating its action. The small amount of GABA release is re-uptaken by membrane-bound GABA transporters (GATs), localized on neurons and astrocytes. The reuptake of GABA is temperature and ion dependent. The GABA transporters are usually $GABA/Na^+/Cl$ symporters that constitute four different types including GABA transporter 1 (GAT1), GABA transporter 2 (GAT2), GABA transporter 3 (GAT3), and the betaine-GABA transporter (BGT1). These transporters are engaged over synapses to clear GABA from the synaptic cleft (Roth and Draguhn 2012). Further,

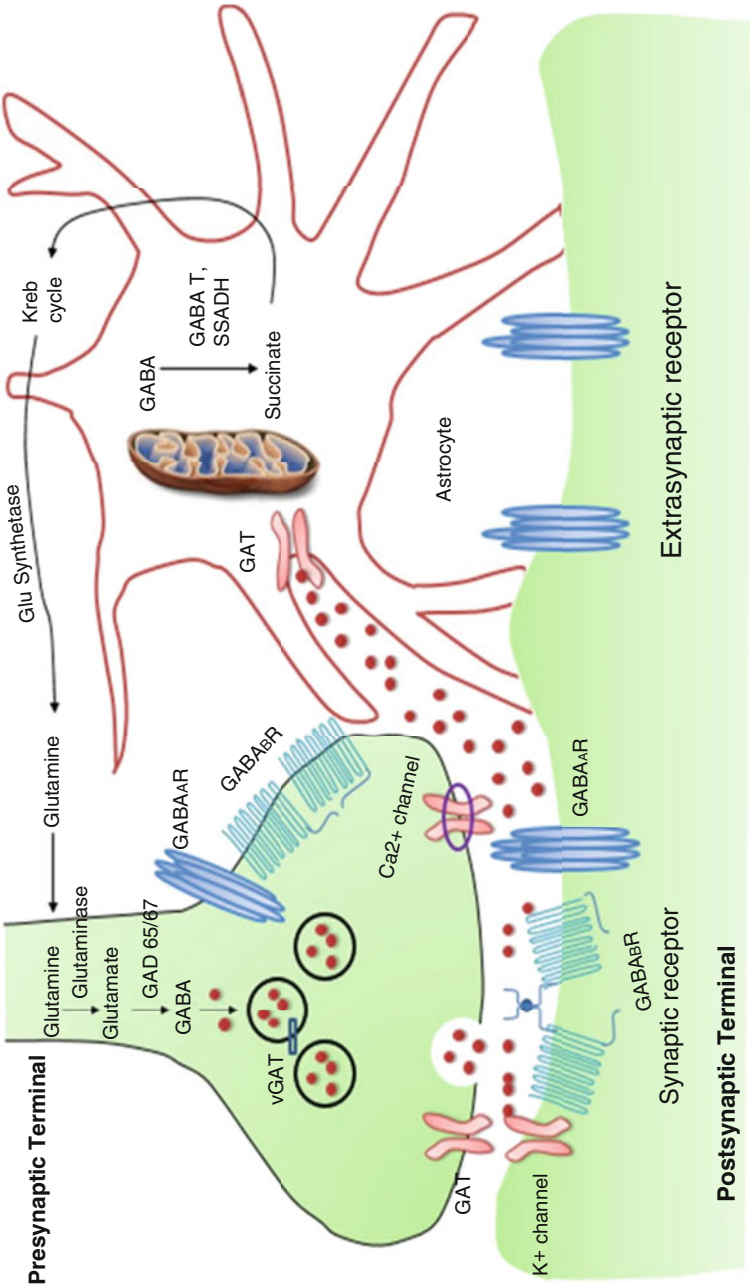

Fig. 8.1 Synthesis, storage, release, reuptake, and metabolism of GABA

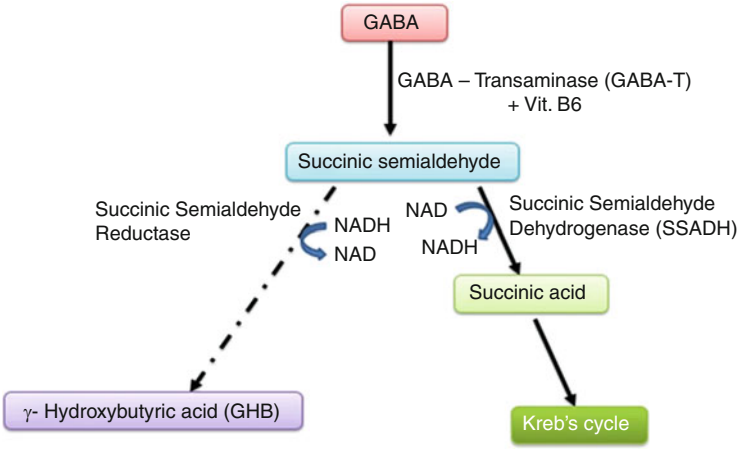

Fig. 8.2 The metabolic pathway of GABA

there is tight regulation over GABA metabolism maintained by astroglial processes through glutamate/GABA-glutamine cycle as shown in Fig. 8.2.

The astrocytes keep checking over GABA release in synapses and reuptake GABA, where it is further catabolized to succinate by mitochondrial enzymes, GABA transaminase (GABA-T), and succinate semialdehyde dehydrogenase (SSADH) in two steps reaction (Rowley et al. 2012). Succinate remains a sequential component of the Krebs cycle that gets frequently converted to glutamine (Gln) via the action of glutamine synthase. Estimation states that GABA metabolism constitutes 8–10% integral part of Krebs cycle and the produced glutamine is then delivered into neurons where it converts to glutamate (Glu) through neuron localized enzyme phosphate activated glutaminase (PAG). In this way, glutamate again gets readily available for the production of GABA.

8.4 Structure and Regulation of GABA

Neurotransmitter has the ability to affect the synaptic transmission by binding to the postsynaptic receptors. In the past, based on different structural and pharmacological classes, GABAergic receptors were divided into two distinct classes, i.e., $GABA_A$ and $GABA_B$. But now the third type of GABA is also recognized, i.e., $GABA_C$. At the end of 1980, the structure elucidation of the $GABA_A$ receptor was done after the clarification of the receptor subunits; it is the member of the superfamily to which various other types of receptors are included such as nicotinic acetylcholine and glycine. GABAρ receptor which was initially known as $GABA_C$ receptor is a subclass of $GABA_A$ receptor and is found in the retina (Johnston et al. 2003).

The $GABA_A$ receptor is the ligand-gated ion channel (anion selective) that induces inhibitory responses and is made up of different subunits of 8 subfamilies

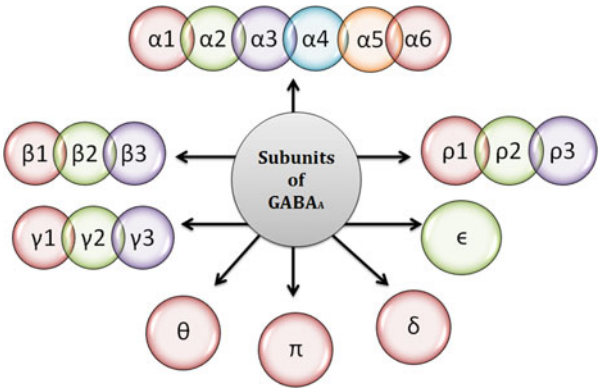

Fig. 8.3 Different subunits of $GABA_A$ receptor

α (1–6), β (1–4), γ (1–3), δ, ε, θ, π, and ρ (1–3) (Sigel and Steinmann 2012) as shown in Fig. 8.3.

Various combinations are possible when different 19 subunits are combined in the pentameric structure. However, the nerve cells contain around 25–30 $GABA_A$ receptor combinations (Birnir and Korpi 2007; Olsen and Sieghart 2009). Most common subunits are α, β, and γ which assemble to form a sequence of unique functionality, while δ, ε, and ρ remains less common and elusive for its functions (Sigel and Steinmann 2012). The most common ones are enlisted as α1β2, α3β3γ2, and α2β3γ2. The available research data provides evidence regarding the role and precise locations of GABA subunits. Immunocytochemistry studies reveal the wide distribution of α1, β1, β2, β3, and γ2 throughout the brain (Olsen and Sieghart 2009). The expression of β2 is specifically confined to the cerebellum, thalamus, hypothalamus, olfactory bulb, amygdala, and midbrain, whereas β3 is highly localized in cerebellum and midbrain regions like substantia nigra pars compacta and the ventral tegmental area. The expression of α2, α3, α4, α5, and α6 remains more region specific as can be studied from an example of α5 which is highly localized in olfactory bulb, hippocampus, and hypothalamus (Rudolph and Möhler 2014), and the α4 subunit which is highly localized in the thalamus, striatum, hippocampus, and outer cortex. Apart from specific regional findings, it is neuron specificity that reveals the presence of GABAergic subunits using immunofluorescence staining. The result of these studies postulates the pallidum neuron as α1 specific while brainstem as α1 and α3 specific. Moreover, their widespread distribution of serotoninergic neurons inside the raphe nuclei has α1 subunit of GABA while GABAergic neurons here express specificity for α1 and α3 subunits (Wu and Sun 2015). This way the widespread distribution of subunits and localized expression all over the CNS performs a number of various actions as shown in Table 8.2.

The GABA network is highly distributed in the CNS and plays a role in neuronal excitability. Different brain regions contain different expression of the receptor and distribution based on the function of the subunit as shown in Table 8.3.

Table 8.2 The role of $GABA_A$ receptor subunits in the CNS

Effect of benzodiazepines	α1	α2	α3	α5	γ2	β2	β3	δ
Sedation	+	−	−	−		+		
Anxiolysis	−	+	−/+	−				
Amnesia	+			+				
Myorelaxation	−		+					
Motor impairment	−	−	−					
Anticonvulsant	+	−	−	−				
Ethanol reinforcement	−			+				
Effects of anesthetics	−					+	+	+
Anxiety					+			
Learning/memory				+				+

Table 8.3 Different subunits in different regions with concentrations of $GABA_A$ receptors

Subunits	Concentration (%)	Regions
α1β2γ2	60	In most brain areas and it is localized in interneuron in hippocampus and cortex Purkinje cells
α2β2/3γ2	15–20	Forebrain especially hippocampus/striatum
α3βnγ2/γ3	10–15	Primarily in cortex
α2βnγ1	8	Bergman glia
α4βnδ	≤5	Thalamus and hippocampal dentate gyrus
α4βγ2	5	Thalamus and hippocampal dentate gyrus
α4βnγ	5	Hippocampal pyramidal cells, deep cortical layers, amygdala, olfactory bulb, hypothalamus, superior colliculus, spinal trigeminal nucleus
α5β3γ2/γ3	4	Hippocampus, cortex, and olfactory bulb
α5β2γ2	≤5	Hippocampus
α6β2/3γ2	≤5	Cerebellum and dorsal cochlear nucleus
α6βγ2	2	Cerebellar granule cell
α6βnδ	2–3	Cerebellar granule cell

Heteropentameric chloride channel formed from different five subunits is assembled to form functional $GABA_A$ receptor (Olsen and Sieghart 2009) and comprises a variety of different combinations of protein subunits. Here, the ionotropic receptor belongs to nicotinicoid superfamily which consists of a pentameric structure around a central pore for ion flow that mediates chloride ions influx. Different subunits of GABA have the same membrane topology, having four transmembrane α-helices (M1, M2, M3, & M4) consisting of long extracellular N-terminus and C-terminus regions with an intracellular loop between the M3 and M4 regions. The channel pore wall is formed from 5 subunits of the M2 domain. The functional properties such as

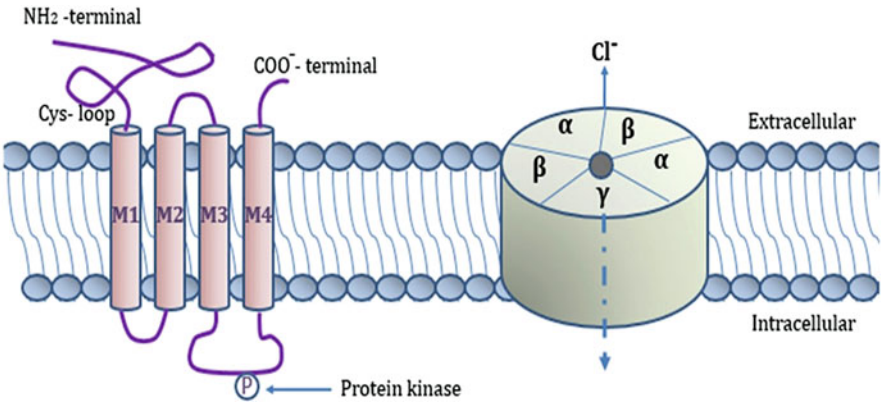

Fig. 8.4 Multiplicity of ionotropic $GABA_A$ receptors

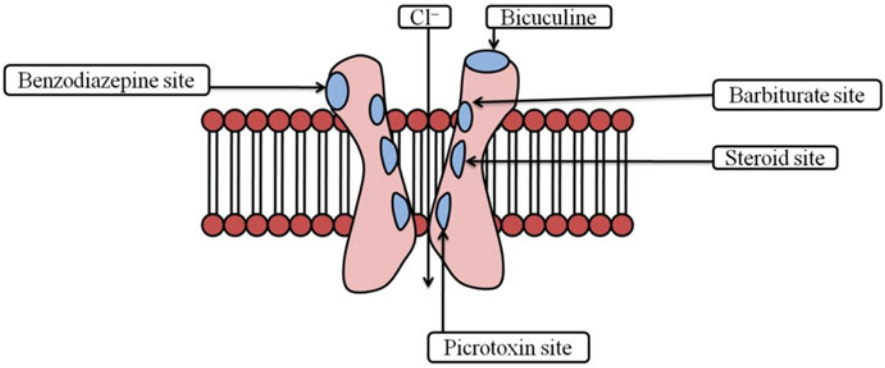

Fig. 8.5 Various binding sites at GABA

ion conductance of the receptor and fate of GABA are affected by intracellular loop because it contains the binding site of intracellular proteins and phosphorylation proteins (Olsen and Sieghart 2009) as shown in Fig. 8.4.

There are various binding sites located in the complex but the GABA binding site is between the interface of α and β subunits and other binding sites can modulate the action of GABA. Various drugs such as benzodiazepines, barbiturates, neurosteroids, and ethanol (Sieghart et al. 2012) positively modulate the $GABA_A$ receptors where as $GABA_A$ receptor blocked by bicuculline and picrotoxin (Semyanov 2002) as shown in Fig. 8.5. Different brain regions contain a combination of various subunits of $GABA_A$ receptor performing different functions at the same time. Each class of units has different pharmacological and electrical properties and performs specific functions.

On the other side, the metabotropic receptor ($GABA_B$) belongs to G-protein-coupled receptors (GPCRs) or metabotropic receptors for GABA and acts by coupling with G-proteins to mediate the intracellular signaling pathway. The

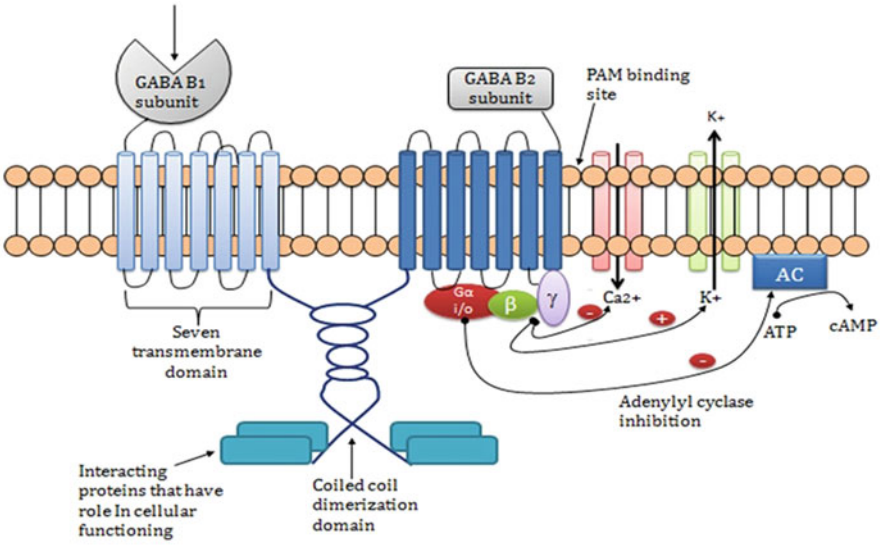

Fig. 8.6 Multiplicity of metabotropic $GABA_B$ receptors

$GABA_B$ receptors were determined by Norman Bowery and their team in 1981 when they are distributed in the CNS (Blackburn 2010). The $GABA_B$ receptors are activated by GABA which is the main inhibitory neurotransmitter present in the CNS. The $GABA_B$ receptors presynaptically inhibit voltage-activated Ca^{2+} channel to reduce the release of GABA and other neurotransmitters. On the other side, the $GABA_B$ receptor postsynaptically activates Kir3 channels and inhibits the neuronal activity by generating hyperpolarizing postsynaptic potentials, which overall minimize the excitatory neurotransmission of $GABA_B$ receptors by interacting with their effectors through Gα and Gβγ subunits of the G protein; the first effector of $GABA_B$ remains adenylate cyclase (AC) via activation of an inhibitory subunit (Gαis/Gα/Gβγ) that further inhibits voltage-dependent Ca^{2+} channels to limit the neurotransmitter release (Pin and Prézeau 2007). Here, $GABA_B$ receptors act as autoreceptors which therefore will inhibit the release of GABA and also the excitability of neurons. The $GABA_B$ receptors stimulate the opening of K^+ channels which brings the neuron closer to the equilibrium potential of K^+ thereby reducing the frequency of action potential and the release of neurotransmitters. Hence, $GABA_B$ receptors are inhibitory receptors. These receptors also reduce the activity of adenylyl cyclase and Ca^{2+} channels (Orts-Del'Immagine and Pugh 2018). Another important mechanistic regulation of $GABA_B$ involves activating the Kir3 channels which maintain slow inhibitory postsynaptic potentials (IPSPs) by inducing efflux of K^+ ions for hyperpolarization of the membrane (Pin and Prézeau 2007) as shown in Fig. 8.6.

Further, $GABA_B$ is of two subtypes: $GABA_{B1}$ which activates neurons and $GABA_{B2}$ that mediates specific sequential signaling. The $GABA_{B1}$ further consists

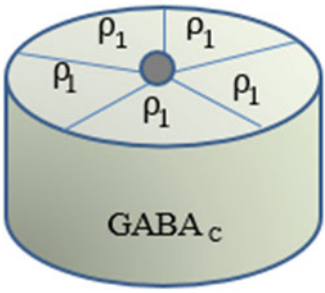

Fig. 8.7 Multiplicity of ionotropic $GABA_{\rho}$ receptors

Table 8.4 Difference between three types of GABA receptors

Receptor	$GABA_A$ receptor	$GABA_B$ receptor	$GABA_C$ receptor
Category	Ligand-gated channel	G-protein-coupled receptor	Ligand-gated channel
Subunits	α (1–6), β (1–4), γ (1–3), δ, ε, θ, π, and ρ (1–3)	GBR1, GBR2	ρ
Agonists	Muscimol, THIP	Baclofen	cis-4-aminocrotonic acid (CACA)
Antagonists	Bicuculline, Picrotoxin	Phaclofen	TPMPA, Picrotoxin
Desensitization	Yes	No	No
Modulator	Benzodiazepines barbiturates	Rac-BHFF	Zinc

of $GABA_{B1\alpha}$ and $GABA_{B1\beta}$ like subtypes; here the $GABA_{B1\alpha}$ carries a specific domain named "sushi," to control the physiological release of glutamate at presynaptic terminals. The $GABA_{B1\alpha}$ is encoded by the GABAR1 gene. But, $GABA_{B1\beta}$ receptors are located on postsynaptic terminals, encoded by GABBR2 gene and cause inhibition there. The $GABA_{B1\alpha}$ and $GABA_{B1\beta}$ are isoforms and combine with GABA to form a functional $GABA_{B(1a,2)}$ and $GABA_{B(1b,2)}$.

Another receptor for GABA includes $GABA_C$ which shows similarity to $GABA_A$ receptor. The $GABA_C$ receptors were first described in interneurons of the spinal cord. It remains ionotropic in nature and their pentameric chloride channels are composed only of ρ (1–3) subunits. This receptor is found to be expressed in the retina, observed by Miledi from bovine retina in Xenopus oocytes, and unlike $GABA_A$ it remains insensitive to bicuculline and baclofen like compounds (Johnston et al. 2003). GABA has high sensitivity for $GABA_C$ receptors. The pharmacological profile of $GABA_C$ receptors is different from $GABA_A$ and $GABA_B$ receptors. In rats, tiger salamander, and hybrid bass, $GABA_C$ receptors were found on bipolar cells (Du and Yang 2000). It is evidenced that $GABA_C$ receptors are composed of ρ subunits (ρ1,2 in humans; ρ1,2 in rat; ρ2,3 in rat; ρ1,2 in chicken) (Zhang et al. 2001) as shown in Fig. 8.7 and Table 8.4.

8.5 Agonists and Antagonists

GABA receptors exert inhibitory responses after binding to the neurotransmitter, i.e., gamma-aminobutyric acid (GABA).

$GABA_A$ receptors are ligand-gated ion channels which are pentameric proteins in nature and constitute 5 subunits belonging to different families (α1–6, β1–3, γ1–3, δ, π, ε, ρ, θ). They contain an integral chloride channel and hyperpolarize the receptor, and have modulatory sites for barbiturates, neurosteroids, ethanol, and benzodiazepines.

$GABA_B$ receptors are metabotropic in nature, i.e., G-protein-coupled receptors and exist as heterodimers of $GABA_{B1}$ and $GABA_{B2}$ subunits. The $GABA_{B1}$ subunit is able to bind with GABA, and the $GABA_{B2}$ subunit shows interactions as shown in Table 8.5.

8.6 Role of GABA in Normal Physiology

8.6.1 Sleep

Sleep in human beings and animals has been partitioned into rapid eye movement sleep (REMS) and non-REMS (NREMS). REMS keeps up the house-keeping function of the brain and its loss influences the vast majority of the psycho-physical physiological processes (Yadav et al. 2019). Another report proposes the sleep promoting neurons (SPNs) incorporate GABAergic neurons in the ventrolateral preoptic nuclei (VLPO)/intermediate nuclei, middle preoptic nuclei (MnPO), and brainstem parafacial zone (Ma et al. 2019; Anaclet and Fuller 2017).

Outstanding among other best-studied co-transmission system is the co-release of glutamate by primary GABAergic/glycinergic neuron terminals in the medial nucleus of the trapezoid body (MNTB). For instance, starburst amacrine cells (SACs) in the retina discharge GABA through vesicles in a Ca^{2+} dependent process (Lee et al. 2010). This enables few SACs to encode both movement and course sensitivities using GABA signaling, respectively. Glutamate is co-released from dopaminergic neurons in the ventral tegmental zone (VTA) (Chuhma et al. 2009). Dopamine acts upon a moderate timescale by binding to G-protein-coupled receptors, while glutamate acts upon a fast timescale when bound to ionotropic glutamate receptors and conveys temporarily exact signals. Glutamate co-release is helpful for exact prediction error signals, allowing the reward to be encoded in the firing rates of dopaminergic neurons and mediating dopamine-dependent behaviors (Mingote et al. 2017).

8.6.2 Female Reproductive System

GABA also appears to play a role in female reproductive functions, perhaps because hormonal cycles may synchronize with the body clock. GABA shows an increase in

Table 8.5 Selective agonist and antagonist of $GABA_A$, $GABA_B$, $GABA_C$ receptors

Name of compound	Description
$GABA_A$	
Muscimol	Competitive $GABA_A$ receptor agonist
GABA	Endogenous agonist
Isoguvacine hydrochloride	Selective $GABA_A$ agonist
L-838,417	$GABA_A$ partial agonist; displays subtype selectivity
MK 0343	$GABA_A$ partial agonist; displays subtype selectivity
Muscimol	Potent $GABA_A$ agonist; also $GABA_A$-ρ partial agonist
TACA	$GABA_A$ agonist; also GABA-T substrate and GABA uptake inhibitor
TCS 1205	$GABA_A$ agonist; displays subtype selectivity
THIP hydrochloride	$GABA_A$ agonist
ZAPA sulfate	"Low affinity" $GABA_A$ agonist; also $GABA_A$-ρ antagonist
SR 95531 hydrobromide (Gabazine)	Potent, selective, competitive $GABA_A$ receptor antagonist
(-)-Bicucullinemethiodide	Competitive $GABA_A$ receptor antagonist
Picrotoxin	Noncompetitive $GABA_A$ receptor antagonist/glycine receptor inhibitor
Flumazenil	$GABA_A$ receptor antagonist
MRK 016	α5-selective $GABA_A$ inverse agonist
TB 21007	α5-selective $GABA_A$ inverse agonist
Furosemide	$GABA_A$ antagonist; also $Na^+/2Cl^-/K^+$ cotransporter blocker
PHP 501 trifluoroacetate	Potent $GABA_A$ antagonist
Picrotoxin	$GABA_A$ antagonist
SCS	Potent $GABA_A$ antagonist; β1-subunit selective
SR 95531 hydrobromide	Competitive and selective $GABA_A$ antagonist
Suramin hexasodium salt	Competitive α1β2γ2 $GABA_A$ antagonist; also nonselective P2 antagonist
$GABA_B$	
Baclofen	Selective $GABA_B$ receptor agonist
SKF 97541	Potent $GABA_B$ receptor agonist
(*RS*)-Baclofen	Selective $GABA_B$ agonist
GABA	Endogenous agonist
SKF 97541	Highly potent $GABA_B$ agonist; also $GABA_{A\text{-}\rho}$ antagonist
CGP 55845	Potent, selective $GABA_B$ receptor antagonist
Saclofen	Selective, competitive $GABA_B$ receptor antagonist
SCH 50911	Selective, competitive $GABA_B$ receptor antagonist
CGP 35348	Selective $GABA_B$ antagonist; brain penetrant
CGP 36216 hydrochloride	$GABA_B$ antagonist; displays activity at presynaptic receptors
2-Hydroxysaclofen	Selective $GABA_B$ antagonist; more potent than Saclofen
Phaclofen	Selective $GABA_B$ antagonist
SCH 50911	Selective and competitive $GABA_B$ antagonist
$GABA_A$-ρ receptors/$GABA_C$	
GABA	Endogenous agonist

(continued)

Table 8.5 (continued)

Name of compound	Description
Muscimol	$GABA_A$-ρ partial agonist; also $GABA_A$ agonist
RuBi GABA trimethylphosphine	Caged GABA; inhibits neural activity
TACA	$GABA_A$-ρ agonist; also $GABA_A$ agonist, GABA-T substrate, and GABA uptake inhibitor
SKF 97541	$GABA_A$-ρ antagonist; also highly potent $GABA_B$ agonist
THIP hydrochloride	$GABA_A$-ρ antagonist; also $GABA_A$ agonist
TPMPA	Selective $GABA_A$-ρ antagonist
ZAPA sulfate	$GABA_A$-ρ antagonist; also $GABA_A$ agonist

the levels of luteinizing hormone, estrogen, and progesterone while decrease in the level of FSH. GnRH neurons express both GABA and GABA receptors and receive GABAergic input that expresses estrogen receptors; therefore GABA has been implicated as a major player in the regulation of GnRH neuron activity and secretion (Maffucci and Gore 2009).

8.6.3 Learning and Memory

Hippocampus is the region of the brain which is known to be involved in learning and memory functions and is prominent in GABA. It helps in reshaping the neural connections of the brain. GABA is reported to help and harm the formation of memories (Jay 2003). Ethanol stimulates transmission of GABA by enhancing its action. Therefore it relieves both anxiety and unwanted effects on memory and learning. Pentobarbital has strong memory inhibition in spatial discrimination activities in rats (Chapouthier and Venault 2002). Barbiturates show the positive modulation of GABAergic receptors that have been analyzed in various memory tasks. Kim and coworkers hypothesized that the enhancement of memory consolidation caused by blockade of $GABA_A$ receptors within a limited time window depends on the increase of BDNF levels, induced by $GABA_A$ receptor blockade. The results of their study suggest that the enhancement of the level of mBDNF and its function during a restricted time window after training are required for the enhancement of memory consolidation induced by $GABA_A$ receptor blockade. It is well known that hippocampal $GABA_A$ and NMDA receptors play an important role in spatial memory (Kim et al. 2012). Calcineurin signaling pathway involved in the interaction between $GABA_A$ and NMDA receptors, to justify this relationship Saito et al. 2010 explored the effects of cyclosporin A, a calcineurin inhibitor, along with muscimol, on the retention of a delayed SWSh task in a radial arm maze. The results of these studies indicated that hippocampal NMDA receptors regulate the effect of spatial working memory induced by muscimol. In addition, the calcineurin signaling pathway may be involved in muscimol-induced impairment of memory retention. The downregulation of GABA modulates memory storage by facilitating the release of norepinephrine, which, after binding to β-ARs, initiates an

intracellular cascade culminating in the synthesis of new proteins, which are utilized for the synaptic changes required to stabilize the new, reactivated, or inhibitory memory trace (Makkar et al. 2012).

8.6.4 Blood Pressure

In the mammalian central nervous system, GABA is the main inhibitory neurotransmitter. $GABA_A$ and $GABA_B$ receptors are activated by aminobutyric acid in neurocytes, which promote the dilation of blood vessels and also reduce blood pressure (Ma et al. 2015). GABA reduces the renal sympathetic nerve secretion in parallel with the fall in blood pressure. Several studies reveal that stimulation of the GABA receptor can centrally reduce sympathetic nerve activity, causing a drop in blood pressure. Although the physiological significance and location of these receptors are unknown, the pharmacological manipulations of these receptors can cause extreme functional changes and involve GABA as a possible central modulator of cardiovascular control.

8.6.5 Heart

GABA slows heart rate by activating GABA receptor. GABA inhibits the central norepinephrine neurotransmitter system and produces the tension booster effect and may increase the heart rate. Experimental study suggests that muscimol decreased heart rate in insulated heart experiments and may represent an effect on intrinsic cardiac activity (Bentzen and Grunnet 2011). Bicuculline, a GABA antagonist, shows a negative chronotropic effect which can be described by the excitatory actions of vagal neurons. Reports suggest that GABA has an excitatory effect in early development due to high chlorine intracellular concentrations. The change has inhibitory GABA phenotype due to the increased aspect of the cotransporter KCC2 chlorine output (Ben-Ari 2002). Lin et al. reported that there is a correlation between the reduction in HR and temperature and state that the temperature could influence ion channels, hence extending the duration of a cardiac tissue action potential, with a greater effect on atrial tissue (Lin et al. 2014). Rana et al. reported a decrease in HR with exposure to caffeine and caused cardiac arrest in some cases. The reports suggested that the negative chronotropic effects could be due to the presence of adenosine receptors, which modify the activity of potassium channels, sustain cardiomyocyte membrane hyperpolarization and therefore reduce heart rate (Rana et al. 2010).

8.7 Mechanism of Action of the Natural Neurotransmitter GABA and Benzodiazepines on Nerve Cells (Neurons) in the Brain

Benzodiazepines show their activity by increasing the activity of GABA. It is an agent that exchanges messages starting with one neuron then onto the next. The messages that GABA exchanges are inhibitory: they show neurons coming in contact, to slow down or stop firing. About 40% of the millions of neurons in the brain respond to GABA, which implies that GABA has a calming hypnotic effect in the brain. This regular activity of GABA starts with benzodiazepines, which thus exerts an additional extra (often excessive) inhibitory activity (Bowery and Smart 2006; Bateson 2004) as shown in Fig. 8.8. Its response with special sites (GABA-receptors) on the outside of the accepting neuron opens a channel, permitting negatively charged particles (chloride ions) to pass within the neuron. These negative ions "supercharge" the neuron making it less receptive to other neurotransmitters which would typically excite it. Benzodiazepines additionally react at their very own unique sites (benzodiazepine receptors), situated on the GABA-receptor. A combination of a benzodiazepine at this site acts as a booster to the activities of GABA, enabling more chloride ions to enter the neuron, making it considerably progressively resistant to excitation. Different subtypes of benzodiazepine receptors have slightly various activities. One subtype (alpha 1) is responsible for sedative effects, another (alpha 2) for anti-anxiety effects, and both alpha 1 and alpha 2, as well as alpha 5, for anticonvulsant effects. All benzodiazepines combine to a greater or lesser extent, with all these subtypes and all enhance GABA action in the brain (Rudolph et al. 2000). Enhancement of GABA's inhibitory activity caused by benzodiazepines, the brain's output of excitatory neurotransmitters, including norepinephrine (noradrenaline), serotonin, acetylcholine, and dopamine, is

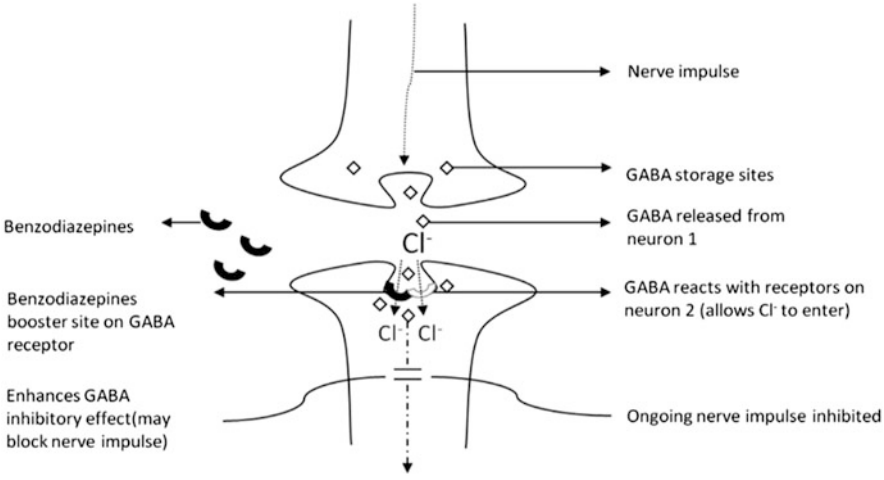

Fig. 8.8 Mechanism of action of GABA and BZD on nerve cells in the brain

diminished. Such excitatory neurotransmitters are important for normal alertness, memory, muscle tone and coordination, emotional responses, endocrine gland secretions, heart rate and blood pressure control, and a group of different functions, all of which may be impaired by benzodiazepines (Ciranna 2006).

8.7.1 Mechanism of Action of GABA Receptor as Shown in Fig. 8.9

Inhibitory synapses are the most abundant synapses in the CNS and include neurotransmitter GABA. Aspects of health, especially emotional and physical stability, are affected by GABA. During locomotion specifically, it relaxes the body muscles and foraging and helps in the process of defecation by contracting the enteric muscle. The important functions of this neurotransmitter are included in various natural processes and pathological processes as it is required for basic motor function. Various types of neurological and psychiatric disorders including anxiety, depression, insomnia, and epilepsy occurred due to the deficiency of GABA and also due to the obstruction of electrical impulses. GABA has been shown to affect many biological functions in the brain, e.g., cognition, learning, emotions, locomotion, circadian rhythms, and sleep. Furthermore, GABA also has roles in cellular events such as differentiation, proliferation, migration, axonal growth, synapse formation, and neuronal death (Kilb 2012; Birnir and Korpi 2007).

Pharmacological actions are responsible for the $GABA_A$ effects including excitability of the neuron (Carver and Reddy 2013), anxiety modulation (Nuss

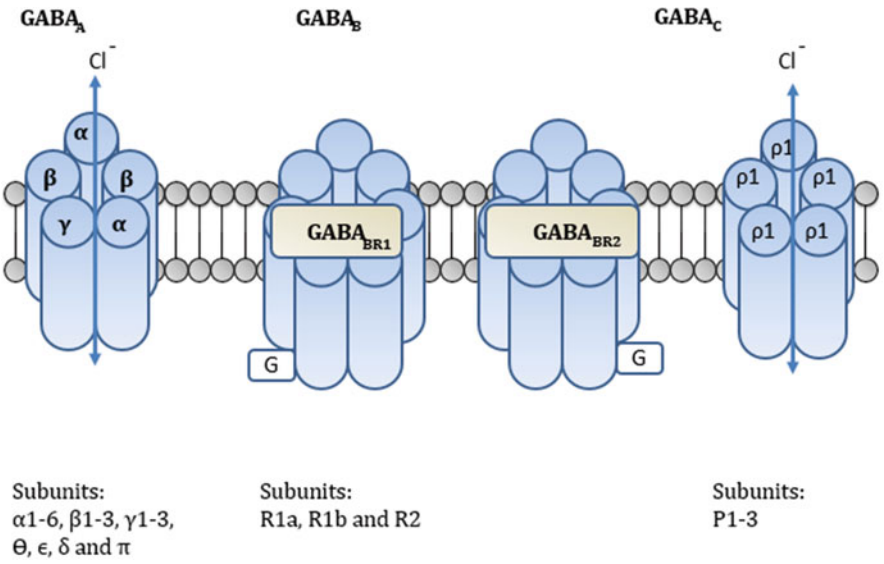

Fig. 8.9 Different subunits with different activity on receptor

2015), behavior (Caldji et al. 2004), circadian rhythms (Albus et al. 2005), and learning and memory abilities (Chapouthier and Venault 2002).

8.7.1.1 $GABA_A$ Receptor Action

Activation of $GABA_A$ receptor leads to the opening of the central pore to 0.7 nm, enough to allow passage of partially hydrated Cl^- ions. Due to this, hyperpolarization of neuronal membrane occurs. Hence, decreased occurrence of action potential results in inhibition of neurotransmission as shown in Fig. 8.10.

8.7.1.2 $GABA_B$ Receptor Action

Binding of GABA to the extracellular domain of B_1 leads to allosteric changes in B_2 subunit (coupled to G protein) which results in inhibition of adenylyl cyclase, activation of K^+ channels, and a decrease in calcium conductance and hence decrease in neurotransmitter release and action potential as shown in Fig. 8.11.

8.7.1.3 $GABA_C$ Receptor Action

GABA is more selective to $GABA_C$. Binding of GABA to the extracellular domain of C results in allosteric changes in the receptor structure. It leads to an influx of chloride ions, ultimately hyperpolarization which results in the inhibition of neurons as shown in Fig. 8.12.

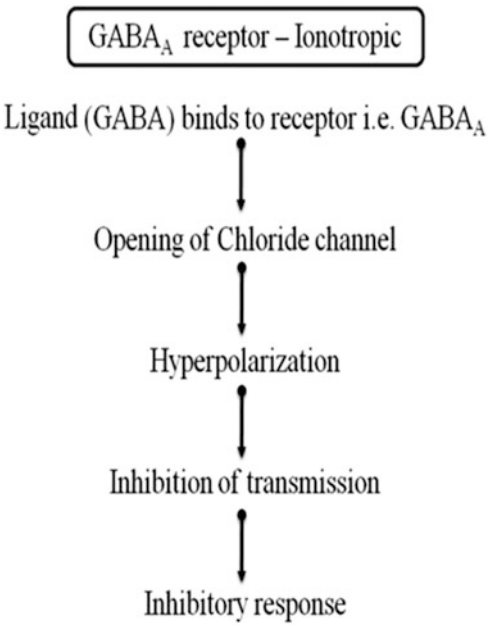

Fig. 8.10 $GABA_A$ receptor action

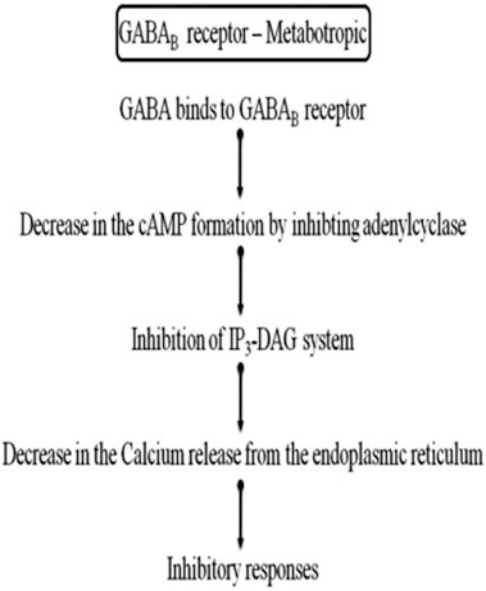

Fig. 8.11 $GABA_B$ receptor action

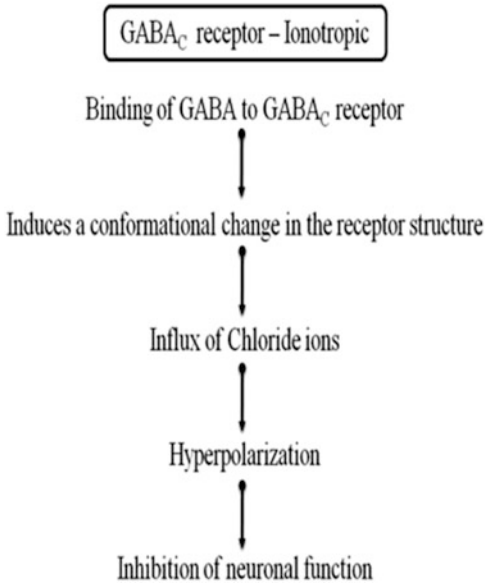

Fig. 8.12 $GABA_C$ receptor action

Pharmacology and Function of $GABA_A$ Receptor (Recent Update on Involvement of $GABA_A$ in Normal and Pathophysiological States)

$GABA_A$ trafficking and clustering are regulated by different intracellular and transmembrane proteins that form $GABA_A$ receptor (Birnir and Korpi 2007). The brain possesses rare expression of other subunits but α1, β2, γ2 subunits are highly

expressed according to their respective families (Bormann 2000). Hippocampus has a high expression of the α5 subunit and can combine with β and γ2 subunit in the other region of the brain mediating tonic inhibition by forming extrasynaptic channels (Korol et al. 2015). Similarly, forebrain and cerebellum contain α4 and α6 subunits mediating tonic inhibition by combining with β and δ subunit to form extrasynaptic channels (Farrant and Nusser 2005).

GABA acts via the ligand-gated ionotropic receptor and is a ligand for the receptor and N-terminus having a binding site between the interface of α and β subunits. Selective agonists such as muscimol and THIP activate the receptor and competitive antagonists such as bicuculline and gabazine (SR95531) block the receptor. At low concentrations steroids such as progesterone, pregnenolone, and their derivative neurosteroids such as allopregnanolone, THDOC, act as positive allosteric modulators of $GABA_A$ receptors but at a higher concentration alone they activate the $GABA_A$ channels (Uusi-Oukari and Kopri 2010).

The effects of diazepam that were mediated by respective receptors are lost and comparison does not occur. So it was demonstrated that various effects such as sedative, anterograde amnesic, and partly the anticonvulsant actions of diazepam (Crestani et al. 2000) are mediated by α1 subunit. $GABA_A$ receptor containing α5 subunits showed enhanced cognition properties without exerting convulsant, proconvulsant, or anxiogenic activity (Chambers et al. 2004). The function of β subunit of $GABA_A$ receptor is identified by a similar approach that was used to define α subunit. Different subunits such as α1, β1, β2, β3, and γ2 have different distribution throughout the brain suggested by immunocytochemistry data. Majorly, the $GABA_A$ receptors are formed from the high proportion of α1 and γ2 subunits and are widely expressed subunits. Other subunits such as α2, α3, α4, α5, and α6 have confined distribution.

Pharmacology and Function of $GABA_B$ Receptor (Recent Update on Involvement of $GABA_B$ in Normal and Pathophysiological States)

The highest concentration of $GABA_B$ is found in the molecular layer of the cerebellum, the frontal cortex, and certain thalamic nuclei (Billinton et al. 2000). $GABA_B$ receptors are generally divided into auto and hetero-receptors. $GABA_B$ receptors are composed of the two subunits $GABA_{B1}$ and $GABA_{B2}$, which convey particular capacities. $GABA_{B1}$ harbors the binding site for GABA to the large extracellular found in the N-terminal domain, though $GABA_{B2}$ is required for high-affinity agonist binding to $GABA_{B1}$. The clinical significance of $GABA_B$ receptors contributed to ongoing research, and their particular role in the overall regulation of gastric function is being discovered (Collares and Vinagre 2005). The receptor is coupled to adenylyl cyclase (AC) through Giα and Goα proteins which inhibit adenylyl cyclase types I, III, V, and VI, while Gβγ stimulates adenylyl cyclase types II, IV, and VII. $GABA_B$ receptors were shown to mediate presynaptic inhibition on some nerve endings and postsynaptic inhibition on some cell bodies or dendrites. The presynaptic $GABA_B$ receptors activation inhibits GABA and glutamate release through a decrease in Ca^{2+} conductance by inhibiting voltage-sensitive-P, N, and L-type Ca^{2+} channels, whereas postsynaptic initiation of $GABA_B$ receptors

resulting in an increase in membrane conductance through G-protein-coupled internally rectifying potassium channels (GIRK orKir3) and inhibition of adenylate cyclase (Emson 2007). Conversely, autoreceptors facilitate the LTP by limiting postsynaptic inhibition (Pinard et al. 2010). $GABA_B$ heteroreceptors mediate heterosynaptic depression of glutamate discharge at hippocampal neurotransmitters (Guetg et al. 2009). This was appeared to modulate the expression of LTP at neural connections in the hippocampus and the lateral amygdala (Shaban et al. 2006).

$GABA_B$ receptors are predominantly localized at extrasynaptic sites to control the excitability of neurons by inhibiting transmitter release as well as hyperpolarizing the neuronal membrane (Gassmann and Bettler 2012). The $GABA_B$ receptors are also responsible for most effects of the drug of abuse gamma-hydroxybutyrate (GHB) which acts as a $GABA_B$ partial agonist at high doses (Pin and Prézeau 2007; Drasbek et al. 2006). $GABA_B$ agonists also established several beneficial effects both in animals and in humans (Bowery and Smart 2006). $GABA_B$ receptors were pharmacologically recognized in the early 1980s and were selectively activated by baclofen (β-p-chlorophenyl-GABA) (McDonnell et al. 2007), the molecule that is clinically used for the treatment of spasticity in patients with multiple sclerosis, cerebral palsy, and spinal cord injury (Froestl 2010). Baclofen was synthesized by Heinrich Keberle in 1962, 30 years before a $GABA_B$ receptor was cloned (Froestl 2010). Baclofen is a lipophilic derivative of GABA and was designed to enhance the penetration of the blood-brain barrier. The R isomer of baclofen shows a three times greater affinity/efficacy for $GABA_B$ receptors than the racemate (Filip and Frankowska 2008). However the use of baclofen has been associated with serious side effects such as sedation, muscle relaxation, and marked hypothermia (Cryan et al. 2004).

Two recently discovered $GABA_B$ receptor positive allosteric modulators are COR627 and COR628 (Castelli et al. 2012). The advantage of these positive allosteric modulators (PAMs) is that they are free from side effects which are associated with $GABA_B$ receptor agonists such as sedation, muscle relaxation, and marked hypothermic potential (Cryan et al. 2004). Compounds showed high receptor selectivity but their low affinity and weak brain penetration after peripheral administration limited their use in pharmacological studies (Kerr and Ong 1996). More potent and selective $GABA_B$ receptor antagonists (phosphinic acid derivatives) like CGP36742 (also known as SGS742), CGP55845A and CGP56433A, CGP46381, CGP51176) (Froestl et al. 1995) and SCH50911 were developed. These $GABA_B$ receptor antagonists are used widely in research, and several compounds have shown promising results in preclinical behavioral models of depression and cognitive disorders (Nowak et al. 2006).

Pharmacology and Function of $GABA_C$ Receptor (Recent Updates on Involvement of $GABA_C$ in Normal and Pathophysiological States)

$GABA_C$ is a subtype and specially considered as part of $GABA_A$ receptor due to the presence of ρ subunit (Olsen and Sieghart 2009). Retina possess high expression of GABA receptor with ρ subunit which is insensitive to bicuculline- and baclofen-, showing pharmacology distinct features from the classical $GABA_A$ or $GABA_B$

receptors, known as $GABA_C$. Initially, the receptor comprised only three subunits (ρ1, ρ2, and ρ3), but later it was revealed that five (ρ4 and ρ5) subunits form the ionotropic $GABA_C$ receptors necessary to form a functional channel (Zhang et al. 2001). Also, the brain regions contain the expression of ρ subunits with function in the visual pathways (Lukasiewicz et al. 2004) as well as in the local GABA circuit of developing visual cortex (Morales et al. 2002), so expression is not only restricted to the retina and does not indicate a separate ionotropic receptor subfamily. It was confirmed that the human retina contains ρ2 subunits with significant abundance (Naffaa et al. 2017) and other regions of the brain such as hippocampus, cerebellum, and pituitary were detected with lower expression levels (López-Chávez et al. 2005).

The sensitivity of GABA to $GABA_C$ receptors is more as compared to $GABA_A$ and $GABA_B$ receptors. Ionotropic $GABA_C$ receptors desensitization and opening time of channel are also longer than in the other receptors. Excellent models are available to characterize GABAc receptors in the retina, i.e., rod-driven horizontal cells and availability of GABAc receptors on other types of retinal neurons.

The influence of $GABA_C$ receptors on sleep-waking processes is limited. $GABA_A$-modulatory drugs such as benzodiazepines, barbiturates, and neurosteroids do not affect $GABA_C$ receptors. $GABA_C$ receptors unlike the $GABA_A$ receptors show different electrophysiological responses and is additionally sensitive to the physiological agonist while the Hill slopes are steeper reflecting the presence of five ligands binding site whereas two sites appear to be present on the $GABA_A$ receptor. $GABA_A$ receptors (such as SR95531 and hydrastine) or $GABA_B$ receptors (such as phaclofen and saclofen) antagonists are not sensitive to $GABA_C$ receptors on retinal neurons. Detailed studies indicated that $GABA_C$ receptor binding preferences depend on different conformation of GABA molecule.

Recently, a novel target for analgesia is ρ2 receptors subunit implicated in pain perception found presynaptically in the spinal dorsal horn (Tadavarty et al. 2015). Sleep-waking behavior of rats demonstrated by behavioral pharmacological studies (Arnaud et al. 2001), learning and memory in chicks and rats (Chebib et al. 2009), the inhibitory modulation of the olfactory bulb (Chen et al. 2007) involves ρ1 receptors.

8.8 Therapeutic Potential and Recent Advances of GABA Receptor Agonist and Antagonist in Various Diseases

8.8.1 Role of GABAergic System in AD and Potential Therapeutic Targets Under Research

Alzheimer's disease is the most prevalent neurological disorder associated with the loss of neurons in the hippocampus, neocortex, and cerebral cortex. It is characterized by memory loss, cognition impairments, and behavioral abnormalities triggered by synaptic dysfunction (Kumar and Singh 2015). Whether acetylcholine remains a major neurotransmitter in memory formation and processing, the role of other neurotransmitters like GABA and glutamate becomes essential to discuss

due to their contribution to excitotoxicity-mediated cell death. Neuron loss in AD contributes to abnormal production and accumulation of protein misfolds over the synapses. Excitotoxicity, hypercholesterolemia, insulin resistance, oxidative stress, and genetic susceptibility remain major contributors in AD (Kumar and Singh 2015). Here, excitotoxicity-mediated neuron loss seems to be a predominant pathogenic mechanism in AD. GABAergic neurotransmission affected in excitotoxicity as the GABA and glutamate balance altered. Further, an in- depth analysis of GABAergic neurotransmission suggests the reliable therapeutics in AD. The amyloid beta formation upregulates the calcium release in hippocampal neuronal cultures which distorts neuronal function via overexcitability (Lazzari et al. 2015). Initially, NMDA gets activated and further GABA gets upregulated to counterbalance it. Abnormal elevation in GABAergic neurotransmission alters synapse function which results in long-term potentiation in dentate gyrus that is reversed by picrotoxin-like GABA antagonist. In hippocampal astrocytes, expression of bestrophin-1 (Best-1) has been reported to have decreased, which in turn confirms the altered GABA and glutamate in AD (Wu et al. 2014). It could be hypothesized that initial enhanced release of neurotransmitters to combat the enhanced excitotoxicity may damage the functionality of Best-1. Moreover, the enhanced astrocytic GABA resultant LTP deficits in the dentate gyrus have been proposed as the possible biomarker in AD. On the other side, the apoE-4 secreted from GABAergic neurons disrupts the functionality of GABAergic neurons which impairs cognitive function (Li et al. 2016).

It is fundamentally established that an adequate balance of GABA and glutamate is necessary for neuronal function and justifies the excitotoxicity-mediated inhibition of GABA expression in AD. Excitotoxicity-mediated overactivation of NMDA receptor causes degradation of subunits of $GABA_B$ in AD, as shown in Fig. 8.13.

The $GABA_B$ consists of $GABA_{B1}$ and $GABA_{B2}$ like subunits that get phosphorylated by calcium calmodulin complex on ser867 and ser783, respectively. This will mediate endocytosis of $GABA_B$ through lysosomal degradation and disrupt neural signaling via overexcitation of neurons (Kantamneni et al. 2008). Thus, a number of evidence strongly prosecute the deregulated GABAergic neurotransmission in AD. Many implicit the GABA agonists for neuroprotective action in AD-associated amyloid beta neurotoxicity. Etazolate is one of the GABAergic agonists that gave reliable effects in phase II clinical trials under AD research (Li et al. 2016). Other drugs like muscimol and propofol provide beneficial effects in AD studies. Further, there is more precise data available for the subunits of $GABA_A$. It has been reported that α5 subunit negatively regulates learning and spatial memory. The deletion of α5 enhances the hippocampus-dependent learning and spatial memory in mice. This led to the utilization of α5 subunit-specific inverse agonists in AD for beneficial effects over cognition.

Several inverse agonists like Ro-4938581 and Ro-4882224 are proven to be beneficial for cognition in clinical studies. Moreover, there are antagonists of $GABA_B$ which were also tested in AD studies for neuroprotection. It has been hypothesized that the astrocytic release of GABA may interfere with the synaptic activity of neurons which results in cognition impairment. On this basis, the compound SGS742 which is a novel $GABA_B$ antagonist provides beneficial effects in

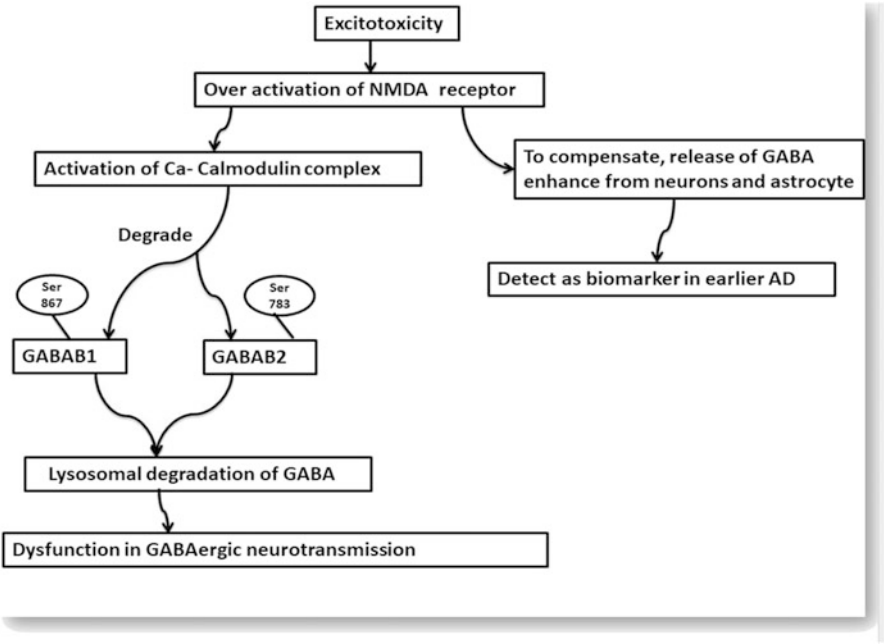

Fig. 8.13 GABAergic transmission and its receptor activation

clinical studies under AD research (Li et al. 2016). This way evidence-based studies over a number of pathogenic mechanisms made implicit different agonists. Inverse agonists and antagonists for GABA for therapeutic approach in AD are shown in Table 8.6.

8.8.2 Role of GABAergic System in Parkinson's Disease and Potential Therapeutic Targets Under Research

Parkinson's disease is the second most devastating neurodegenerative disorder affecting elderly people with motor and non-motor complications. The motor complications in PD include bradykinesia, rigidity, tremor, and postural abnormalities caused by damage in the dopaminergic neurons of substantia nigra pars compacta (SnPc), whereas the non-motor complications involve autonomic disturbances, olfactory dysfunction, and sleep problems which may originate due to loss of other neurons in specific regions of the brain (Maiti et al. 2016).

The voluntary control in the mammalian brain is coordinated by signaling of basal ganglia. The basal ganglia including the striatum, substantia nigra pars compacta (SNPc), subthalamic nuclei (STN), globus pallidus external and internal (GPe and GPi) like regions constitute to form a circuit to regulate voluntary movement control (Dézsi and Vécsei 2011). This circuit is incorporated by

Table 8.6 Drugs targeting GABA for therapeutic benefit in AD

Compound	Mechanism of action	Pharmacological action
Muscimol	$GABA_A$ receptor agonist	Protective against overproduction of amyloid beta
Propofol	$GABA_A$ receptor agonist	Protective against amyloid beta-induced neurotoxicity Improves cognition
MRK-016	Inverse agonists of $GABA_A$ at α5 subunit	Improved cognition in preclinical studies
CGS9896	Inverse agonists of $GABA_A$ at α5 subunit	Enhances memory in different tasks
Ro-4938581	Inverse agonists of $GABA_A$ at α5 subunit	Reverses memory deficit in different AD studies
Ro-4882224	Inverse agonists of $GABA_A$ α5 subunit	Reverses memory deficit in different AD studies
SGS742	$GABA_B$ receptor antagonist	Enhances memory and attention in human and animal studies
Etazolate	$GABA_A$ receptor agonist	Protects neurons against amyloid beta-induced neurotoxicity
CGP55845	$GABA_B$ receptor antagonist	Improves cognition in animal studies

GABAergic and glutaminergic neuronal projections that maintain inhibitory and excitatory control over movement through the availability of dopamine. Dopamine neurons are highly abundant in striatum along with D1 (excitatory) and D2 (inhibitory) receptors. In this circuit, the direct pathway includes D1 excitatory control over GABAergic inhibition of GPi that removes inhibitory control from thalamus which results in movement whereas the indirect pathway mediates D2-mediated inhibition over inhibitory control of GPe that further excites STN to mediate glutaminergic excitation of GPi to inhibit movement. This way the direct pathway acts as the inhibitory switch on GPi and the indirect pathway excites the GPi to control over thalamus which sends excitatory projections to the motor cortex which results in adequate movement. The loss of dopaminergic neurons (specifically D1) in PD results in interference to direct pathway, which causes hindrance in movement that results in hypokinesia, rigidity, and gait abnormalities as shown in Fig. 8.14.

The role of GABAergic system in motor complications is generally assessed on the basis of inhibitory GABAergic control over voluntary movements regulating brain regions. Dopamine loss in the striatum leads to excessive stimulation of GABAergic inhibitory control over movement which causes symptoms of PD (Alexander 2004). Another prospective target is the excitotoxicity-mediated loss of GABAergic neurons that results in a loss of adequate control over movement (Błaszczyk 2016). This way both the agonists and antagonists of GABA have made implicit for the treatment of PD. On one side progabide, a positive allosteric modulator of gamma-aminobutyric acid B, and Zolpidem like GABA facilitators improve the symptoms of PD, and on the other side antagonists for GABA also get evaluated for beneficial effects.

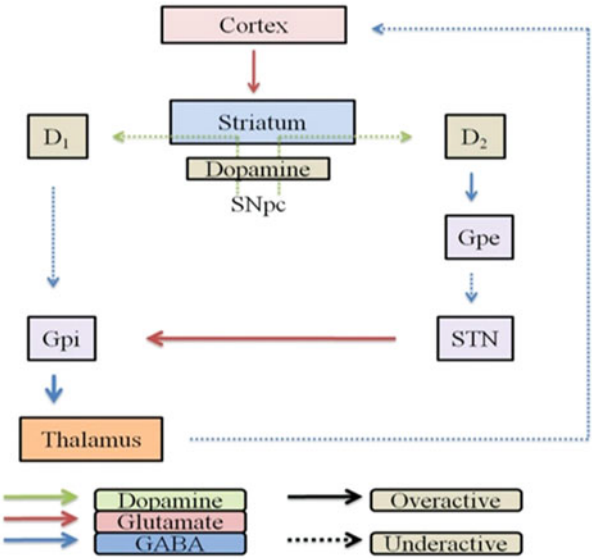

Fig. 8.14 Altered GABA and glutamate functioning in PD

Not only this, non-motor symptoms are believed to be brought out by GABAergic dysfunction. Here, the 80% of cases of PD reports for abnormal olfactory functions. The distorted olfactory function in PD is associated with the loss of dopaminergic neurons in olfactory bulbs and olfactory nuclei which depends upon glial cell-derived neurotrophic factors (GDNF) for survival. The availability of GDNF depends upon neuro-glial interactions effected by GABA/Ca^{2+} mechanism in both midbrain and olfactory nuclei. During normal conditions, the physiological release of GABA causes hyperpolarization of neurons for long-term inhibition of synaptic transmission. This hyperpolarization phase blocks the calcium channel and protects neurons from excitotoxicity (Błaszczyk 2016). The removal of calcium from mitochondria and cytoplasm requires a high amount of energy; therefore neuronal cells in the midbrain have energy requirements to cope with calcium overload. On the other side, calcium overloads lead to oxidative stress-mediated dysfunction of mitochondria. This way subsequent decline in ATP production, increase in oxidative stress, and calcium load result in mitochondrial dysfunction-mediated neuronal loss in SnPc in PD (Błaszczyk 2016). The excitotoxicity mediated initial release of GABA from astrocytic neurons but on exceeding the limits density of synaptic GABA receptors gets decreased which leads to an increase of GABA in synaptic cleft and initiate self-mediates apoptosis in neuronal cell. Furthermore, the defect in GABA-glutamate cycle (neuron-astrocyte interaction) for GABA recycling could also result in neuronal damage as shown in Table 8.7.

Table 8.7 Drugs targeting GABA for therapeutic benefit in PD

Compound	Mechanism of action	Pharmacological action
Tiagabine	Inhibitor of GABA transporter 1 Blocks microglia cell activation in PD	• Neuroprotective over dopaminergic neuron loss in PD • Protective against inflammation in PD
Muscimol	$GABA_A$ receptor agonist	Neuroprotective in PD
Baclofen	$GABA_B$ receptor agonist	Neuroprotective in PD
Flumazenil	GABA antagonist that binds to the benzodiazepine (BZ) binding site of the $GABA_A$ receptor	Treat hyper-GABAergic associated motor symptoms in PD
Zolpidem	• Facilitates GABAergic neurotransmission • Positive allosteric modulation of $GABA_A$ receptors	Improvement in motor symptoms of PD
Lycopene	• Enhances density of GABA receptors in SNPc • Antioxidant	Neuroprotective in PD

8.8.3 Role of GABAergic System in Huntington's Disease (HD) and Enlisting Potential Therapeutic Drugs Under Research

HD is the hereditary predisposed neurodegenerative disorder that is characterized by a progressive neuronal loss in the striatum that gradually spreads throughout the brain. Patients with HD suffer from motor, cognitive, and psychiatric disabilities (Rubinsztein 2006). The motor dysfunction-mediated loss over voluntary muscles remains major symptoms of the disease. The loss of striatal pathogenic neurons terminates their projections to different brain regions, which is assumed to be a major contributor in HD. The GABAergic projections are major abundant neurons in the striatum and their loss remains a predominant step in HD (Garret et al. 2018). The GABA neurons in indirect pathway loss first, that leads to overactivation of GPe and results in diminishing the activity of STN-mediated glutaminergic projections. The net result of this leads to an increase in thalamo-cortical activity that causes dance-like movements in HD. Simultaneously, the effects of disease on the direct pathway results in the activation of the subthalamic region as inhibitory tone is lost via deterioration of GABAergic neurons. This first effect SNr then proceeds to GPi that is seen via symptoms progression from dancing to akinesia. Here, the deterioration of GABAergic projections confirms that the brains of HD patients lose their capacity to synthesize and release the required amount of GABA. The remaining projections in the striatum are found to have increased GABA receptors whereas few studies report the loss of GABAergic neurons as an early event of disease. On this basis, different studies have implicit GABA replacement therapy and agonists of GABA for the treatment of HD disease (Mason and Barker 2009). The compounds like exogenous gangliosides, hesperidin, and quercetin ameliorate symptoms of HD by showing neuroprotective effect over alter neurochemistry as shown in Table 8.8.

Table 8.8 Drugs targeting GABA for therapeutic benefit in HD

Compound	Mechanism of action	Pharmacological action
Baclofen	GABA agonist	Neuroprotective in HD
Exogenous ganglioside GM1	Enhance synthesis of gangliosides Microglia protective	• Protective over alter neurochemistry • Decrease striatal atrophy and neuron loss • Protective over cognition and psychiatric symptoms
WIN55,212-2	Cannabinoid (CB1) agonist	• Protective over alter neurochemistry • Prevent excitotoxicity in HD
Spermidine	Enhances polyamines level in the brain Modulates NMDAR function	Protective over alter neurochemistry
Muscimol	GABAmimetic	• Protective over alter neurochemistry • Neuroprotective in HD

Among different receptors for GABA, the receptor $GABA_A$ remains a potential target for therapeutics in HD. The distribution of $GABA_A$ in striatum mediates tonic (α5 by α 1–3, δ or by γ 2 forming extrasynaptic receptor) and phasic inhibition (α1, α2, α3 in combination with β and γ2) via its different subunits (Waldvogel and Faull 2015). Here, α1 facilitates the fast inhibitory current and α2 subunit gets altered and hence confirms the decrease in $GABA_A$ in HD brain. The α1 participates in the co-release of GABA and dopamine in medium spiny neurons of the striatum and its decreased expression contributes to motor symptoms of HD.

Further, there is a spatial distribution of GABAergic interneurons (IN) in HD, different classes of IN including fast-spiking INs (FSIN) disposing parvalbumin, persistent low-threshold spiking INs (PLTS) expressing somatostatin or nNOS, and INs expressing calretinin. Specific degeneration of fast-spiking neurons specifically contributes to HD (Du et al. 2017). Moreover, the role of cholinergic IN remains essential to discuss as they are widely distributed in the striatum which bears localization of GABA receptors. The increase in α3 subunits of $GABA_A$ receptor on cholinergic IN mediates inhibition over acetylcholine release in HD. Decrease in Tonic inhibition also a prominent pathogenic mechanism in HD, which is confirmed by a decrease in tonic inhibition regulatory subunits like α5 and δ (Allen et al. 2009). Moreover, the reduced tonic current mediates the movement and psychiatric symptoms in patients affected with HD.

The GABAergic neurons remain major inhibitory control above overexcited GPe, so it is essential to discuss the subunits that are expressed there. The α1, β2, and γ2 subunits get reduced along with decreased expression of vesicular GABA transporter in GPe, which is also reported in HD. Moreover, the subunits like α2, α3, and γ1 remain expressed in GPe, which is reported to get altered in the HD brain (Du et al. 2017). So, in this way specific agonists and antagonists for different subunits could be more reliable towards therapeutics in HD.

8.8.4 Role of GABAergic System in Anxiety and Enlisting Potential Therapeutic Drug Targets Under Research

Anxiety is the most prevalent stress-associated behavioral disorder that brings an abnormal state of mind which is characterized by excessive anxiousness, fear, and nervousness. The neurobiological terms utilized to define anxiety include overactivation of the sympathetic nervous system, amygdala-linked fear response, and neurochemical alterations in associated brain regions (Shin and Liberzon 2010). Furthermore, anxiety brings about somatic, cognitive, and behavioral changes that impair the normal functioning of an individual. The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-4), and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), establish the diagnostic criteria for anxiety which states that anxiety should be treated properly when "the anxiety, worry, or physical symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning" (Andrews et al. 2010). The different classifications specified the five different subtypes of anxiety disorders which include generalized anxiety disorder (GAD), panic disorder (PD), obsessive compulsive disorder (OCD), social anxiety disorder (SAD), different phobias, and post-traumatic stress disorder (PTSD). The neurochemical alterations occurring in the brain evidence the involvement of GABA, serotonin, opioid peptides, endocannabinoids, neuropeptides-Y oxytocin, and corticotropin-releasing hormone in the pathogenesis of anxiety. These alterations provide significant therapeutic approaches for the treatment of anxiety.

About 50 years' time passage confirmed the successful clinical reliability of BZDs for the therapy of anxiety-associated disorders. The BZDs target the GABAergic neurotransmission to provide significant therapeutics in anxiety disorders (Möhler 2012). Moreover, the GABAergic neurotransmission in amygdala is shown to be a reliable therapeutic candidate for anxiety treatment. Preclinical studies show that the administration of GABA into amygdala relieves the fear associative anxiety symptoms whereas the antagonists of GABA give anxiogenic effect (Aroniadou-Anderjaska et al. 2007). Moreover, the human studies also give reliable results by the administration of BZDs which abolish the negative symptoms through amygdala activation. There are different subunits of $GABA_A$; the BZDs bind to the interface of α and γ subunits for inhibitory and calming effects in the brain but these drugs show sedative effects rather than calming. Therefore, this side effect of BZDs leads to the discovery of much more specific agonists of different subunits which are today under research as shown in Table 8.9.

Later the selective drugs for α1 given by compounds like zolpidem, zopiclone, (S)-zopiclone, and zaleplon show clinical reliability for sedation and hypnotic effects. The α2 selective compound with no other subunit binding might contribute to ideal anxiolytic properties. L-838417 which has partial agonist activity towards α2, α3, α5 subunits with α1 antagonistic activity shows good reliability for anxiolytic effect with no sedative side effects but its unfavorable pharmacokinetic restricts further possibilities (Nuss 2015). Another compound ocinaplon exhibits good nonsedative anti-anxiety profile even in humans but its hepatotoxicity limits its

Table 8.9 Drugs targeting GABA for therapeutic benefit in anxiety

Compound	Mechanism of action	Pharmacological effects
NS 11394	Partial agonist on α2 and α3 subunits and full agonist on α5 subunit ons $GABA_A$	Anxiolytic effect
L-838417	Partial agonist towards α2, α3, and α5 subunits but shows affinity even also towards α1 on $GABA_A$	Anxiolytic effect
Ocinaplon	Partial agonists towards α2, α3, and α5 subunits and full agonist for α1on $GABA_A$	Anxiolytic effect
SL 651498	Agonist for α2, α3 and partial agonist for α1 and α5 subunits on $GABA_A$	Anxiolytic effect
TPA023	Partial agonist on α2 and α3 subunits on $GABA_A$	Anxiolytic effect
TPA023B	Partial agonist on α2, α3, and α5 subunits on $GABA_A$	Anxiolytic effect
TPA123	Partial agonist on α1, α2, α3, and α5 subunits on $GABA_A$	Anxiolytic effect
TPA003	Agonist at α3 with high selectivity on $GABA_A$	Anxiolytic effect
ELB-139	Unknown selectivity	Anxiolytic effect
XBD173	Translocator protein (TSPO) ligand (TSPO is a benzodiazepine binding site protein that acts on $GABA_A$ receptor)	Anxiolytic effect with no sedation
CGP 56433A	Selective $GABA_B$ receptor antagonists	Anxiolytic effect Antidepressant effect
CGP 55845A	Selective $GABA_B$ receptor antagonists	Anxiolytic effect Antidepressant effect
CG 39783	$GABA_B$ receptor positive allosteric modulator	Anxiolytic effect Antidepressant effect
Compound	Mechanism of action	Pharmacological effects
Eszopiclone	Highly selective for α5, medium binding to α2, α3 but lower towards α1 on $GABA_A$	Anxiolytic effect Antidepressant effect
NS 11394	Partial agonist on α2, α3 subunits and full agonist on α5 subunit on $GABA_A$	Anxiolytic effect
L-838 417	Partial agonist towards α2, α3, and α5 subunits but show affinity even also towards α1 on $GABA_A$	Anxiolytic effect
Ocinaplon	Partial agonists towards α2, α3, and α5 subunits and full agonist for α1 on $GABA_A$	Anxiolytic effect
SL 651498	Agonist for α2, α3 and partial agonist for α1 and α5 subunits on $GABA_A$	Anxiolytic effect
TPA023	Partial agonist on α2 and α3 subunits on $GABA_A$	Anxiolytic effect
TPA023B	Partial agonist on α2, α3, and α5 subunits on $GABA_A$	Anxiolytic effect
TPA123	Partial agonist on α1, α2, α3, and α5 subunits on $GABA_A$	Anxiolytic effect
TPA003	Agonist at α3 with high selectivity on $GABA_A$	Anxiolytic effect
ELB-139	Unknown selectivity	Anxiolytic effect

(continued)

Table 8.9 (continued)

Compound	Mechanism of action	Pharmacological effects
XBD173	Translocator protein (TSPO) ligand (TSPO is a benzodiazepine binding site protein that acts on $GABA_A$ receptor)	Anxiolytic effect with no sedation
CGP 56433A	Selective $GABA_B$ receptor antagonists	Anxiolytic effect Antidepressant effect
CGP 55845A	Selective $GABA_B$ receptor antagonists	Anxiolytic effect Antidepressant effect
Eszopiclone	Highly selective for α5, medium binding to α2, α3 but lower towards α1 on $GABA_A$	Anxiolytic effect Antidepressant effect

further reliability. This way several different subunits-specific molecules are under investigation for lesser side effects with improved anxiolytic profile for therapeutic benefits.

Not only $GABA_A$ but $GABA_B$ also provides some remarkable therapeutic effects in anxiety. There are some endogenous steroids which also show specific binding towards the $GABA_A$ for anxiolytic activity with lesser side effects than BZDs. Animal studies revealed the anxiolytic effects of different neurosteroids including pregnenolone, dehydroepiandrosterone, and progesterone even at low concentrations (Nuss 2015). Etifoxine, which is a small structurally unrelated molecule to BZDs, promotes neurosteroids synthesis in the brain that shows anxiolytic effects in both animals and humans. The anxiolytic effects of etifoxine are contributed by positive allosteric modulating effect on $GABA_A$ by binding to β subunit specifically β2 and β3 sites. Therefore, GABAergic system potentially participates in the regulation of anxiety and could be a novel approach for future studies.

8.8.5 Role of GABAergic System in Multiple Sclerosis and Enlisting Potential Therapeutic Drug Targets Under Research

Multiple sclerosis (MS) is a severe neurological disorder characterized by an autoimmune demyelination of the CNS. It affects the genetically susceptible younger adults from 15 to 45 years old. Other factors like exposure to viruses like Epstein-Barr virus, smoking, and low serum vitamin D levels remain major environmental contributors in MS. Indeed, there is no exact cause and cure for the disease but the prevalence of MS is increasing day by day. Clinically, there are four forms of MS which are seen as relapsing remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and progressive relapsing MS (PRMS) (Loma and Heyman 2011). The combined characteristic features of these forms of

MS include impairments of functional symptoms in motor, visual, and sensory systems. Moreover, some undiagnosed features including cognitive dysfunction, fatigue, and mood disturbances contribute to the cortical damage. The treatment therapy in MS includes interferons, monoclonal antibodies, and cytotoxic drugs which provide symptomatic relief but none of them are able to halt or cure the disease (Derwenskus 2011). The pathogenic mechanism behind these factors includes demyelination, axonal damage, dysfunction of glial cell, and inflammation like neurodegenerative features which are under research for therapeutic approach in MS (Loma and Heyman 2011).

The role of GABAergic pathway in MS is uncertain but the structural and metabolic damage to neuronal and glial cells provides the evidence regarding the role of neurochemical alterations. It estimates the reduced level of GABA with a concomitant increase in the level of glutamate in the brain of MS patients. The adequate balance of glutamate and GABA is essential for motor performance and its alterations in MS cause sensorimotor dysfunction. Evidences remarkably report the deficits in GABA levels in the hippocampus, sensorimotor cortex, and prefrontal cortex that contribute to motor and cognitive dysfunction in MS (Cawley et al. 2015). These remarks get strengthened by excitotoxicity, inflammation, and demyelination like factors. This includes acute lesions enhancing the glutamate level implicit in cell death mechanisms for MS. The neuronal cell terminal resides in the $GABA_A$ receptors which could inhibit the calcium and glutamate release via depolarization. This way, the $GABA_A$ receptor could be a potential therapeutic target in MS. Further, the neurotransmitter GABA is also synthesized in astrocytes; hence it remain a major regulator of immunity. Here, it is important to notify that exogenous GABA could be deteriorating in MS-like conditions as it exacerbates the disease by worsening the immune system. It upregulates the cytokine release-mediated neuronal cell death (Mandolesi et al. 2015). Therefore, if drugs could enhance the endogenous GABA levels then it could be much more beneficial. For example, drugs like vigabatrin that inhibits GABA-transaminase give neuroprotective effect on systemic administration. Similar effects are gained from sodium valproate whereas diazepam and phenobarbitone sodium give mixed results.

Moreover, the presence of cannabinoid receptor CB1 over the GABAergic and glutaminergic neuronal terminals makes implicit endocannabinoids targeting drugs in MS (Scotter et al. 2010). Here, agonists of CB1 inhibit the activity of adenylyl cyclase that decreases the levels of cyclic adenosine monophosphate (cAMP) to decrease neurotransmitters release from synaptic vesicles as shown in Table 8.10.

It also inhibits the calcium channels by binding to Gi/o proteins that contribute to the inhibition of the neurotransmitter release. This mechanism could make possible successful implications of endocannabinoids targeting drugs like nabilone, HU210, URB597, and WIN 55,212-2 to protect GABAergic neurons from autoimmunity and excitotoxicity like factors in MS (Scotter et al. 2010).

Table 8.10 Drugs targeting GABA for therapeutic benefit in MS

Compound	Mechanism of action	Pharmacological effects
Phenobarbitone sodium	• GABA facilitatory as well as GABA mimetic effect • Blocks calcium channels to inhibit glutamate release	Neuroprotective
Sodium valproate	• Inhibitor of GABA transaminase and increases GABA levels • Inhibits glutamate-mediated excitotoxicity	Neuroprotective
Allopregnanolone	Agonist for GABA-A receptors on neurons and glial cells	Neuroprotective, Anti-inflammatory
Vigabatrin	Inhibitor of GABA transaminase and increases GABA levels	Reduces disease severity
Diazepam	GABA agonist	Reduces disease severity
Topiramate	Blocks sodium and calcium channel for enhancing action of GABA	Reduces disease severity
Exogenous GABA	Worsen immune system	Increases disease severity
Gabapentin	Inhibits calcium channel to enhance concentration of GABA over synapse	Improves disease symptoms
Baclofen	GABA agonist	Mixed effect
Ganaxolone	Synthetic allopregnanolone analogue, an agonist for $GABA_A$ receptors on neurons and glial cells	Improves disease symptoms
Etifoxine	TSPO agonists, enhance endogenous GABA	Improves disease symptoms
XBD-173	TSPO agonists, enhance endogenous GABA	Improves disease symptoms

8.8.6 Role of GABAergic System in Schizophrenia and Enlisting Potential Therapeutic Drug Targets Under Research

Schizophrenia (SCZ) is a heterogeneous disorder that markedly causes neuropsychiatric illness in patients and accompanies negative and positive symptoms along with cognitive abnormalities. The positive symptoms accompany hallucinations and delusions while negative symptoms bring out emotional blunting and decreased motivation. Although SCZ affects only 1% of the population of the world, it puts a huge economic and social burden on people that make this psychiatric illness much more severe (Rosenberg 2012). Moreover, the exact pathogenesis of SCZ is not clear and thought to be contributed by genetic and environmental factors. Initially the dopamine hypothesis was considered as a major lead for research of therapeutics in SCZ. No doubt, this dopamine hypothesis provided a number of therapeutics including typical and atypical antipsychotics. Growing evidences also suggested the role of GABA, glutamate, and serotonin in SCZ (Laruelle 2014). The abnormal activity of circuits of the prefrontal cortex (PFC), medial temporal lobe, and striatal region also gather attention in recent decades. Post-mortem studies of SCZ patients

evidenced the alterations in GABA levels to confirm its role in altered activity of the brain. Decreased activity of GABAergic interneuron observed in PFC along with reduced quantity of GAT1 and GAD67. The reduction in GABAergic signaling in PFC may contribute to cognitive, emotional, and enhanced dopaminergic function like abnormalities of SCZ patients (Nakazawa et al. 2012). The justification behind this associates with dysfunctioning of mesostriatal dopaminergic neurons in SCZ. Normally, the dopaminergic projections of mesostrial pathway give input to PFC GABAergic neurons, and this control becomes mature during younger age with rise in cortical activity. But any genetic or environmental risk may hinder this control by causing dysfunction in mesostriatal dopaminergic pathway which reduces GABA in SCZ (Selten et al. 2016). However, a decrease in dendritic spines and loss of pyramidal neurons also contribute to a decrease in GABA in SCZ.

Alteration in GABA levels contributes to diverse symptoms of SCZ including synaptic plasticity, memory, execution, and psychosis. The loss of parvalbumin GABAergic interneurons in the hippocampus and cortex may contribute to executive and cognitive symptoms of SCZ (Lewis et al. 2012). One report suggests that a decrease in GABAergic neurotransmission in the hippocampus contributes to deficits in working memory in SCZ (Gao and Penzes 2015). Moreover, psychosis is considered as the positive symptom of schizophrenia that triggers by dysfunction of the dopaminergic system in the schizophrenic brain (Kesby et al. 2018). The hyperexcitation of dopamine (D1) in the striatum is reported to crucially control cortical activity. As if the cortical interneuron gets inhibited, striatum dopamine neurons get overexcited that may result in schizophrenic symptoms. The debatable possible mechanism is that it may decrease in cortical GABAergic interneurons activity that inhibits ventral tegmental area to increase nucleus accumbens dopamine activity (Nguyen et al. 2014).

Accordingly, a number of drugs targeting dopamine are going to be evaluated for their beneficial role in SCZ. Direct infusion of GABA agonist muscimol in the hippocampus is shown to mitigate the memory deficits caused by phencyclidine (PCP) treatment (Riordan et al. 2017). The hormonal drug estradiol rescues memory in SCZ by upregulating the expression of GABA in the hippocampus. Moreover, a new compound named as imidazenil modulates GABAergic neurotransmission for beneficial effects in SCZ. It is highly potent partial GABA agonist with anticonvulsant and anxiolytic property without any side effect of sedation (Guidotti et al. 2005). Moreover, recent studies are also assessing the therapeutic efficacy of GABA targeting drugs via their subunit selectivity. The different subunits of $GABA_A$ including α5, α-2/3, and γ2 are ongoing major therapeutic targets in SCZ. The selective α5 inverse agonists and α-2/3 agonists are under preclinical studies for their therapeutic benefits over impaired cognitive and executive functions of SCZ (Charych et al. 2009). The γ2 subunit of $GABA_A$ is reported to participate in physical interaction with D5 receptor of dopamine which may initiate the symptoms of SCZ (Vinkers et al. 2010) as shown in Table 8.11.

Table 8.11 Drugs targeting GABA for therapeutic benefit in HD

Drugs targeting GABAergic system in SCZ		
MK-077	FG-7142	MRK-536
Tiagabine	NS11394	BZDs
Vigabatrin	RO4938581	Muscimol
Bretazenil	RO4882224	Imidazenil
Abecarnil	L-655	ELB-139
TPA023	L-708	Ocinapalon
MRK-409	MRK-016	PWZ-029

8.8.7 Role of GABAergic System in Traumatic Brain Injury and Enlisting Potential Therapeutic Drug Targets Under Research

Traumatic brain injury (TBI) is a neurologic disorder resulting from head injury that leads to temporary or permanent impairment of the structure and function of the brain. It could occur due to violent blow, injury, or aggressive shakiness to the head which produces rapid accelerated or decelerated impact on the brain that leads to structural damage, functional dysfunction, or neurological deficits leading to the death of patients. The most common TBI-associated cases are prevalent in traffic accidents, military, sports, violence, construction sites, industrials, etc. (Madikians and Giza 2006). These cases also occur in infants when sudden shake to babies cause a violent impact on their heads. The common pathogenic features of TBI involve the disturbance of the protective endogenous antioxidant system along with altered mitochondrial function, excitotoxic damage, and cerebral ischemia. Excitotoxicity is considered as the major pathological event occurring in TBI brain. It is associated with an imbalance in between the GABA/Glutamate ratio that raises susceptibility of neurons towards cell death (Yi and Hazell 2006). The glutamate is the major neurotransmitter present in pyramidal neurons localized in midbrain, hypothalamus, cortex, and hippocampus, while GABA interneurons are localized in cortical and thalamocortical regions to regulate motor functions, attention, and memory. The adequate balance of GABA/Glutamate is necessary for neuron survival; any shift to it results in cerebral ischemia, TBI, and other neurodegenerative disorders.

During focal TBI, penetrating injury causes local swelling and ischemia that may irreversibly damage localized tissue through excitotoxicity. One report suggested that posttraumatic epilepsy is caused by an imbalance in GABA and glutamate. The imbalance is reported to result from the loss of parvalbumin-positive GABA neurons (Guerriero et al. 2015). Moreover, brain studies of concussion-affected athletes reported that there is an increase in levels of $GABA_B$ after injury. It is believed to be proceeded to counteract the excitotoxicity-induced neuronal damage for survival. Further, synthesis of GABA is occurring from glutamic acid decarboxylase and its deficiency results in a decrease in GABA levels in the TBI brain. Some evidence has also contraindicatory reports that speculated that abnormal synaptic activity in the TBI brain results from a decrease in glutaminergic neurotransmission and

Table 8.12 Drugs targeting GABA for therapeutic benefit in TBI

Drug	Mechanism of action	Pharmacological effects in TBI
Levetiracetam	Stabilizes imbalance of GABA/glutamate by unknown mechanism	Improves posttraumatic epileptic symptoms
Diltiazem	$GABA_AR$ agonist	Improves posttraumatic symptoms
Diazepam	$GABA_AR$ agonist	Improves posttraumatic symptoms
MK-801	Stabilizes imbalance of GABA/glutamate	Improves posttraumatic symptoms
Zolpidem	Atypical GABA agonist	Insomnia, neurological complications, improves posttraumatic symptoms
Baclofen	$GABA_B$ receptor agonist	Improves posttraumatic symptoms
Tetrazepam	$GABA_A$ receptor agonist	Improves posttraumatic symptoms
Gabapentin	GABA analogue	Neuropathic pain Improves posttraumatic symptoms
Allopragnelone	GABA modulator	Improves posttraumatic symptoms
Modafinil	Decreases GABA and increases glutamate	Reduces daytime sleepiness

overactivation of GABAergic inhibitory impulses (Kochanek et al. 2015). This may be caused by modulation of $GABA_A$ receptor due to Ca^{2+} ions regulating NMDA receptors.

On this basis, different GABAergic drugs are evaluated for their neuroprotective efficacy in TBI including benzodiazepines (BZD) and other modulators of GABA. Few of these drugs provide successful results including MK-801, Diltiazem, and diazepam in animal and human studies (Gibson et al. 2010). So, targeting GABA for therapeutics in TBI could provide more successful results in future studies as shown in Table 8.12.

8.8.8 Role of GABAergic System in Epilepsy and Enlisting Potential Therapeutic Drug Targets Under Research

Epilepsy is a CNS disorder characterized by cerebral dysrhythmia. The brief episodes (seizures) of loss or disturbed consciousness with or without involvement of body movements are called convulsions. These episodes are unpredictable and have highly variable intensity depending on the site of origin in the brain. Earlier it was recognized as the "disease of lightening" (Browne and Holmes 2008).

8.8.8.1 Physiological Role of GABA in Epilepsy

GABA, the principal inhibitory neurotransmitter in the cerebral cortex, maintains the inhibitory tone and counterbalances neuronal excitation. When this balance is disturbed, seizures may occur. $GABA_A$ (ligand operated channels) receptor binding influences the early portion of GABA-mediated inhibitory postsynaptic potential, while $GABA_B$ (G-protein-coupled metabotropic receptor) influences the late portion (Badawy et al. 2009).

Most of the cases of epilepsy are idiopathic while some may be secondary due to trauma/intracranial tumor/cerebral ischemia. GABA is present relatively in high concentration in the mammalian brain which is further catalyzed by GABA aminotransferase into glutamate, i.e., an excitatory neurotransmitter responsible for the occurrence of epileptic seizures (Khazipov 2016; Rao and Zhang 2011), Alzheimer's disease (Solas et al. 2015), Huntington's disease (Byrne and Wild 2016), Parkinson's disease (Silverman 2012), tardive dyskinesia, anxiety, schizophrenia, and other behavioral disorders (Gajcy et al. 2010). The explanation for seizures is the inhibition of glutamate decarboxylase, a synthesizing enzyme of GABA by lowering brain GABA levels.

Experimental and clinical investigations demonstrate that GABA has a significant role in the mechanism and treatment of epilepsy: (1) Abnormalities of GABAergic function have been seen in genetic and acquired animal models of epilepsy; (2) Reductions of GABA-mediated inhibition, activity of glutamate decarboxylase, binding to $GABA_A$ and benzodiazepine sites, GABA in cerebrospinal liquid and brain tissue, and GABA detected during microdialysis studies have been accounted for investigations of human epileptic brain tissue; (3) GABA agonists suppress seizures, and GABA antagonist produces seizures; (4) Drugs that inhibit GABA synthesis cause seizures; and (5) Benzodiazepines and barbiturates work by enhancing GABA-mediated inhibition. Finally, drugs that increase synaptic GABA are potent anticonvulsants. Two recently developed antiepileptic drugs (AEDs), vigabatrin (VGB) and tiagabine (TGB), are examples of such agents. However, their mechanisms of action are quite different (VGB is an irreversible suicide inhibitor of GABA transaminase, though TGB blocks GABA reuptake into neurons and glia), which may represent observed differences in drug side effect profile (van Vliet et al. 2018; Löscher et al. 2013; Treiman 2001).

Hence to increase the level of GABA in the brain in order to prevent the occurrence of seizure disorders, either one of the following steps should be adopted:

1. Increasing the activity of the enzyme Glutamic Acid Decarboxylase (GAD) synthesizes GABA.
2. Selective irreversible/reversible inhibition of the GABA-catabolizing enzyme GABA-transferase.

8.8.8.2 Potentiation in Activity of GAD Activators as a Target for the Treatment of Epilepsy

Ginkgo biloba L. leaves extract containing various compounds such as flavonoids and terpenoids has clinical use in the treatment of certain cerebral malfunctioning and also exerts beneficial effects in the treatment of dementia of AD type and multi-infarct dementia. Sasaki demonstrated that bilobalide possessed dose-dependent anticonvulsant activity against many convulsions induced by isoniazid and 4-methylpyridoxine (MPN) in mice and causes elevation in hippocampal GABA levels through potentiation of GAD activity (Abdel-Wahab and Metwally 2011).

8.8.8.3 GABA Agonist and Antagonist Drugs in Epilepsy

GABA agonist drugs such as muscimol, tetrahydroisooxazolopyridinol (THIP), cetyl GABA, and progabide (PGB) are potential anticonvulsants in experimental animals, whereas GABA antagonist such as bicuculline and picrotoxin are proconvulsants. A number of GABA synthesis inhibitors can cause seizures, including 4-deoxypyridoxine, isoniazid, thiosemicarbazide, and L-allylglycine. Drugs that enhance GABA-mediated inhibition are also anticonvulsants including benzodiazepines which enhance binding to GABA receptor and increasing the frequency of chloride channel openings. The two recently developed and marketed antiepileptic drugs, VGB and TGB, are called "designer drugs" because they were specifically developed to increase synaptic GABA concentrations and thus inhibit seizure activity. VGB is an irreversible suicide inhibitor of GABA transaminase and thus inhibits degradation of GABA while TGB blocks GABA reuptake into neurons as well as glia. Hence the increased synaptic GABA concentration decreases the seizure frequency in patients with partial onset seizures (Lasoń et al. 2013; Besag and Patsalos 2012; Sałat and Kulig 2011).

8.8.8.4 Role of GABA Transporters in Epilepsy

There are 4 cloned isoforms of GABA transporters in rats and humans, termed GAT1, GAT2, GAT3, BGT-1. GAT1 is localized in presynaptic terminals and GAT2 in meninges, while BGT-1 in non-neural tissues and in glia. GABA transporters help in the GABA reuptake by clearing from extracellular space; blocking GABA transport induces an increase in the level of GABA and hence decreasing the occurrence of epileptic seizures (Lie et al. 2017).

8.8.8.5 Tonic $GABA_A$ Receptors in the Treatment of Temporal Lobe Epilepsy

Various studies showed that the potentiation of GAD activity causes the elevation in the level of hippocampal GABA that is contributed to the treatment of seizures and convulsions. Tonic $GABA_A$ receptors are new subpopulation of receptors that lead to prolonged inhibition and thereby control excitability. $GABA_A$ presynaptic receptors are repetitively activated which results in the increased level of GABA in the synaptic cleft, then diffuses to extracellular space where it activates postsynaptic receptors (Schipper et al. 2016) as shown in Fig. 8.15.

Secondly, $GABA_A$ receptors may be activated by the released GABA from non-vesicular sources. Extracellular GABA comes from the glial cells or dendrites by GAT reversal. Thirdly, the opening of GABA can occur spontaneously in the absence of synaptic activation and even in the absence of GABA. In case of excessive neuronal firing the GABA may spill into extracellular space. Neuromodulators can alter tonic GABA signaling. Various therapeutic agents act by a different facet of action such as GABA facilitatory, GABA mimetic, antiglutamate, Ca^{2+} entry reduction by inhibition of t-type Ca^{2+} current, prolongation of sodium channel inactivation, facilitation of GABA-mediated chloride channel opening, etc. Antiepileptic drugs suppress seizures, but do not cure

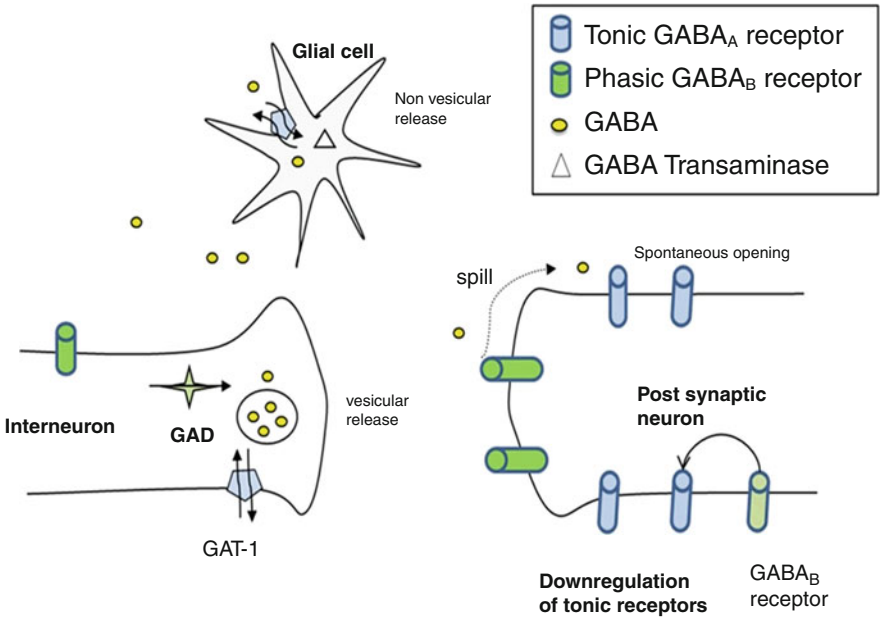

Fig. 8.15 Various mechanisms altering the activity of tonic $GABA_A$ receptors. Activated by four mechanisms: (1) spill of synaptically released GABA into extrasynaptic space due to insufficient clearance by GATs, (2) GAT reversal causes nonvesicular release, (3) spontaneous opening in absence of extracellular GABA, (4) $GABA_B$ receptor activation increases tonic $GABA_A$ signaling via an intracellular mechanism

the disease which may fadeout after years of successful control (Upadhyay et al. 2017) as shown in Table 8.13.

8.8.9 Recent Advances on GABA (Clinical Trials and Investigational Drugs)

Around 70 years ago, the discovery of neurotransmitter GABA was made and in this long passage of time, several findings including agonists and antagonists have proven it to be milestone in the scientific research field. Later, the identification of its receptors $GABA_A$ and $GABA_B$ made the elucidation of targets and therapeutic outcomes more significant. Today, the success of a number of molecules, specifically the clinical reliability of benzodiazepines and antiepileptic drugs, strongly attests to the remarked contribution of neurotransmitter GABA in neuro research. Initially, the benefits of GABA were assessed for only its calming, inhibitory, and impaired motor-oriented function but now also get diverted towards its role in neuron survival, memory, and other disease modifying functions. For example, nowadays the role of GABA receptor is getting explored for altered synapse function in AD. It is also expected to be a reliable biomarker for earlier diagnosis of disease in

Table 8.13 Drugs targeting GABA for therapeutic benefit in epilepsy

Compound	Mechanism of Action	Pharmacological action
Gabapentin	Gabapentin interacts with cortical neurons at auxiliary subunits of voltage-sensitive calcium channels. Gabapentin increases the synaptic concentration of GABA, enhances GABA responses at non-synaptic sites in neuronal tissues, and reduces the release of mono-amine neurotransmitters	Decreases release of glutamate from presynaptic terminals and has action in neuropathic pain
Lamotrigine	Inhibits excitatory amino acid release (glutamate & aspartate) by blockade of sodium channels	Add on therapy or monotherapy in partial seizures
Phenytoin	Membrane stabilization by blocking sodium & calcium influx into the neuronal axon	Prevents spread of seizure activity Abolishes tonic phase of GTC seizure
Topiramate	Topiramate blocks voltage-dependent sodium and calcium channels. It also inhibits the excitatory glutamate pathway while enhancing the inhibitory effect of GABA	Adjunctive therapy for refractory partial seizure
Benzodiazepine	Benzodiazepines increase the frequency of the chloride ion channel opening, thereby increasing the inhibitory effect of GABA on neuronal excitability	Reduction of anxiety Sedative and hypnotic action

dementia patients. Moreover, the usual side effects of BZDs also get eliminated as in-depth structural elucidation of receptors takes place. The knowledge regarding the different subunits of GABA and its functions raises more possibility with high accuracy and fewer side effects. Recently, the work is ongoing upon the different subunits of $GABA_A$ receptor for limiting side effects of anxiolytic drugs. Previously, the target of anxiolytic drug was the interface of α and γ subunits but it shows sedation rather than calmness. But now the specific agonists and partial agonists of α2, α3, and α5 subunits are getting evaluated for therapeutic efficacy. Apart from this, the role of these subunits is also getting explored for other therapeutic actions including memory, neurotoxicity, and neuron survival in multiple neurodegenerative disorders. The success of such findings and efforts could be analyzed from a plenty of molecules undergoing evaluation for GABA modifying properties under recent clinical trials (Schanzer et al. 2019; Zahn et al. 2019; Nikmaram et al. 2017) as shown in Table 8.14.

Table 8.14 Recent drug candidates targeting GABA in clinical phases

Drug candidate	Therapeutic target	Clinical trial	Therapeutic interventions
Allopregnanolone	$GABA_A$ receptor	Phase III	Super-refractory status epilepticus
AZD7325	$GABA_A$ receptor	Phase 2	Autism spectrum disorder
Allopregnanolone	$GABA_A$ receptor	Phase I	Increases neurogenesis and reduces amyloid beta formation in AD
Ganaxolone	$GABA_A$ receptor	Phase III	Refractory partial-onset seizures, female pediatric epilepsy
Vigabatrin	GABA potentiation	Completed	Complex partial seizures and infantile spasms
Clobazam	GABA potentiation	Phase III	Lennox–Gastaut syndrome
Muscimol	$GABA_A$ receptor	Phase I	Alzheimer's disease
Clarithromycin	Negative allosteric modulator of $GABA_A$ receptors	Phase I	Parkinson disease
YKP3089	Enhances GABA release	Phase III	Resistant partial onset seizures
SGS742	$GABA_B$ receptor antagonist	Phase II	Alzheimer's disease
Retigabine	GABA potentiation	Phase III	Epilepsy
L-830982	$GABA_A$ Alpha2/3 receptor agonist	Phase II	Cognitive disability in schizophrenia
Zolpidem	Positive allosteric modulator of $GABA_A$ receptors	Phase III	Improves neuropsychiatric symptoms (sleep disorders) in Alzheimer's disease
Flumazenil	$GABA_A$ receptor	Phase I	Parkinson disease

8.9 Conclusion

GABA is one of the major key players of inhibitory neurotransmission in the central nervous system and observed to be widely distributed throughout the mammalian brain. The $GABA_A$ receptor influences have been studied for decades and are beginning to be well understood; research on the $GABA_B$ and $GABA_C$ receptors are more recent and currently growing. The crucial regulatory functions of $GABA_B$ have become increasingly evident, whereas $GABA_C$ receptor studies are only at the beginning stage. GABA acts through their receptors and helps to control the excitability of cortical networks by modulating a network of cortical interneurons and useful in various CNS-related disorders including Parkinson's disease, epilepsy, anxiety, schizophrenia, and dementia. Numerous anxiolytic, sedative, and hypnotics show their molecular signaling through GABA receptors and dominantly emerge as powerful drug targets. $GABA_C$ receptors show a lower threshold of activation than $GABA_A$ and $GABA_B$ receptors, an essential property that is rich in promise for future clinical applications. Hence, exploring the more molecular mechanisms may result in a better understanding of their role in CNS disorder.

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Pharmacology of Melatonin and Its Receptors

9

Shamsher Singh, Arti Rana, Sunpreet Kaur, Jasdeep Singh, Vikrant Rahi, Hira Choudhury, and Puneet Kumar

Abstract

N-acetyl-5-methoxytryptamine or melatonin is a chronobiotic material, which proportionally acts as a rhythmic stabilizer of the body. This versatile moiety is secreted from a highly specialized gland called the pineal gland, located behind the third ventricle in the center of the brain. Not only the brain but some other parts are also responsible for the production of melatonin such as the gastrointestinal tract (GIT), skin, and lymphocytes. It is also called the hormone of darkness because of its versatile release pattern. Its production and release is controlled by light-dark cycle; the light inhibits the production whereas darkness stimulates it. Multiple evidence suggested the involvement of melatonin in biologic regulation of circadian rhythms, sleep, mood, and perhaps reproduction, tumor growth, and aging. For all these processes, receptors are the key element contributing to elicit all such responses. Melatonin receptors are generally classified into two subclasses, MT1 and MT2, expressed in different regions of the brain and MT3 receptors located in the liver and kidney. In this chapter, we discuss in detail the various aspects of melatonin with its key role in the body and how this versatile hormone and its receptors work in a synchronized way to alter the

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pathophysiological processes of the body according to the biological clock and circadian rhythm.

Keywords

Melatonin · Antioxidant · Circadian rhythm · Biological clock · Neurological disorders · Cancer · Cardiovascular diseases · Depression · Parkinson's disease · Alzheimer's disease

Abbreviation

4P-ADOT	4-Phenyl-2-acetamidotetralin
4P-PDOT	4-Phenyl-2-acetamidotetralin
5-HT	5-Hydroxytryptamine
5-MCA-NAT	5-Methoxycarbonylamino-N-acetyltryptamine
6-SMT	6-Sulphatoxymelatonin
A*b*	Amyloid β
AD	Alzheimer's disease
AFMK	*N*-acetyl-*N*-formyl-5-methoxy kynurenine
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATP	Adenosine triphosphate
Bcl-2	β-cell lymphoma 2
BDNF	Brain-derived neurotrophic factor
BKCa	Blocking of calcium-activated potassium channels
cAMP	Cyclic adenosine monophosphate
CCK-2	Cholecystokinin-2
cGMP	Cyclic guanosine monophosphate
CK1	Casein kinase 1
CK2	Casein kinase
CNS	Central nervous system
Cox-2	Cyclooxygenase-2
CREB	cAMP response element-binding protein
CVS	Melatonin actions on the cardiovascular system
DNA	Deoxyribonucleic acid
ERK	Extracellular signal-regulated kinase
ETC	Electron transport chain
FSH	Follicle-stimulating hormone
GABA	Gamma-aminobutyric acid
GDNF	Glial cell-derived neurotrophic factor
GDNF	Glial cell line-derived neurotrophic factor
GIT	Gastrointestinal tract
GPCR	G-protein-coupled receptor
GPx	Glutathione peroxidase
GRd	Glutathione reductase

GSK-3	Glycogen synthase kinase 3
HD	Huntington's disease
HIOMT	Hydroxyindole-O-methyl transferase
HIV	Human immunodeficiency virus
IL	Interleukin
IRI	Ischemic reperfusion injury
JNK	c-Jun N-terminal kinase
LH	Luteinizing hormone
MCF-7	Michigan Cancer Foundation
MI	Myocardial infarction
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	Messenger ribonucleic acid
MTNR1B	Melatonin receptor 1B
NADPH	Nicotinamide adenine dinucleotide phosphate
NAS	*N*-acetylserotonin
NAT	*N*-acetyl transferase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor
NMDA	*N*-methyl-D-aspartate receptor
NO	Nitrogen oxide
NREM	Non-rapid eye movement
OH	Hydroxyl radical
PD	Parkinson's disease
PKA	Protein kinase A
PKC	Protein kinase C
PTX	Pertussis toxin
QR2	Quinoreductase 2
REM	Rapid eye movement
RNS	Reactive nitrogen species
RORα	Related orphan receptors
ROS	Reactive oxygen species
SCN	Suprachiasmatic nucleus
SNc	Substantia nigra pars compacta
SNRIs	Serotonin norepinephrine reuptake inhibitors
SSRIs	Selective serotonin reuptake inhibitors
T2D	Type 2 diabetes
TLE	Temporal lobe epilepsy
TM	Transmembrane
TNF	Tumor necrosis factor

9.1 Introduction

Melatonin is a highly specific moiety, which serves in a versatile manner and expresses itself throughout the organs as well as tissues in the mammalian system through several mechanisms of action, whereas the sympathetic nervous system is directly involved in the control and coordination of melatonin synthesis as well as its production is in the pineal gland. Circadian rhythm originated from suprachiasmatic nucleus (SCN) in the hypothalamus and is also responsible for the control and coordination of melatonin. Not only the pineal gland but other organs like the retina, GIT, salivary gland, platelets, epithelial hair follicles, lymphocytes, and developing brain are also responsible for the production of melatonin (Singh and Jadhav 2014; Baltatu et al. 2017).

Chemically melatonin is *N*-acetyl-5-methoxytryptamine that was discovered by Aaron Lerner in 1958. Melatonin is a natural product of body and thus acts naturally in all living creatures at various stages of the daily cycle from algae to humans. It performs o'clock calendar functions in body, along with antioxidant action. Biologically melatonin makes modulations in sexual behavior, sleep, and circadian sleep; a low level of melatonin has been linked to PD, insomnia, AD, ischemic injury, epilepsy, and other neurodegenerative disorder (Singh and Jadhav 2014). In most organisms, these findings suggest its exclusive production and release during the night (Fig. 9.1) and it arbitrates information about the duration of darkness as well as the temporal position and therefore considered as a visceral indicator of **darkness** (Rocha et al. 2015).

In this chapter, we discuss the significance of melatonin as a hormone along with its synthesis, storage, and release and how it is involved in the control, coordination, and regulation of important biological features of mammalian systems such as circadian rhythm, adolescent growth, and seasonal variation. Furthermore, it is interesting to know about its essential antioxidant action; in association with

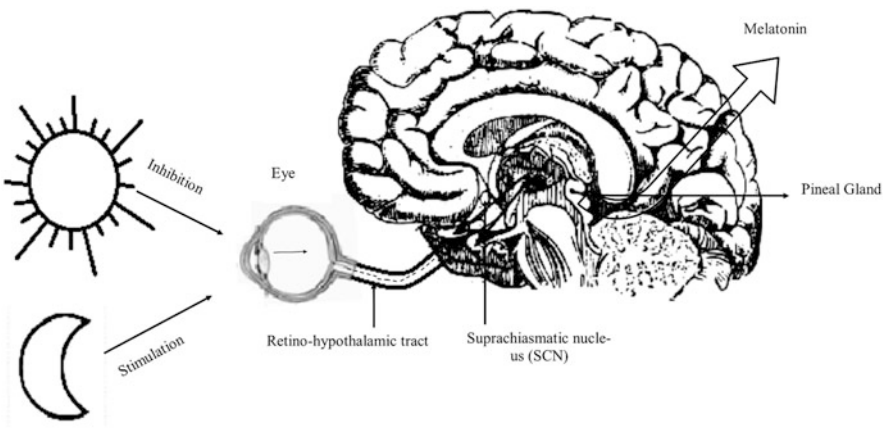

Fig. 9.1 Melatonin stimulation and inhibition

membrane receptors melatonin elicits various biological responses via involving integral proteins and MT1 and MT2 receptors (Dawoodi et al. 2012). It consists of MT1 and MT2 melatonin receptors which belong to G-protein-coupled receptors (GPCRs) family present in different regions of the CNS such as ganglion cells, retinal horizontal cells as well as distant body parts like ovary, prostate, and gastrointestinal tract. Quantitative changes in melatonin receptors occur day by day. The correlation between receptor alteration and alteration in endogenous melatonin creation is clearly proved in day-to-day rhythm sleep syndromes. Recent evidence supports the statement of its active role in animal ovary and to furthermore improve its abilities; it is important to research and investigate the clinical features, effects, efficacy and safety of melatonin along with its ligand involved in various diseases.

9.1.1 History

Before the discovery of melatonin, researchers used the injection of pineal gland extract to find out the role of pineal gland in different biological processes of the body. In some studies the researchers used pineal gland extract in tadpoles, frogs, toads, and fish to reveal the skin lightening effect of pineal gland biochemicals (Whitlock et al. 1954). In 1958, Aaron B. Lerner revealed the role of a neurochemical which reverses the darkening effect of the melanocyte stimulating hormone and named it "Melatonin" (Lerner et al. 1958) (Table 9.1).

9.2 Synthesis, Storage, Release, and Metabolism

The synthesis of melatonin is a multistep process, which includes the transformation of circulatory tryptophan to serotonin that further gets converted to melatonin. These conversational processes depend upon the action of enzymes, and serotonin-N-acetyl transferase (NAT) and hydroxyindole-O-methyl transferase (HIOMT) are the enzymes involved in the conversion of serotonin to melatonin as shown in Fig. 9.2 (Ganguly et al. 2002). Acute tryptophan reduces in melatonin synthesis; therefore melatonin synthesis is dependent on tryptophan availability. Other nutritional factors that influence melatonin synthesis are folate status and vitamin B_6, a coenzyme in tryptophan decarboxylation that possesses the ability to stimulate melatonin production in prepubertal children but not in adults.

Once synthesized, it expeditiously moves in blood and becomes a part of circulation. Therefore, the plasma concentration delivers the actual picture of pineal secretion. In order to measure melatonin concentration, body fluids like saliva and urine can be measured. It is estimated that 10–80 μg of nocturnal melatonin produced endogenously at night and in comparison to the day time, production is significantly less (Peuhkuri et al. 2012).

Environmental light and endogenous circadian clock are the major factors responsible for controlling the release of melatonin. Light is considered as the major environmental stimulatory factor of melatonin release (Fig.9.3). Elevation in the

Table 9.1 Historical development in the discovery of melatonin and its receptors

Name	Year	Discoveries	Reference
Herophilos	325–280 BC	First time discovering the pineal organ in man	Kappers (1979)
Aaron Lerner	1958	Discovered the chemical structure of melatonin	Lerner et al. (1958)
Bubenik	1993	Found melatonin in human intestine and the retina of many nonmammalian vertebrates	Filadelfi and Castrucci (1996)
Miles, Stankov and Reiter, Stankov Kennaway and Hugel, Almeida	1989; 1990; 1991; 1992; 1951	The highly specific ligand 2 ['25I]-iodomelatonin allowed the expansion of receptor investigation to peripheral tissues, such as retina, spleen, gastrointestinal tract, uterus, ovary, liver, and cultured normal and tumor cells	Filadelfi and Castrucci (1996)
Dubocovich	1998, 1981 1988, 1995 1990,1994	Melatonin receptors were first classified according to classic pharmacological criteria	Dubocovich et al. (2010)
Nosjean	2000	Identification and characterization of a third membrane-bound melatonin binding site MT3 receptor	Nosjean et al. (2000)
Ebisawa	1994	The MT1 receptor (Mel1c) was first cloned in frogs	Ebisawa et al. (1994)
Reppert	1995	The MT2 receptor was cloned from the brain, retina, and human pituitary gland	Reppert et al. (1995)
Ayoub	2004	Both MT1 and MT2 can form homo and heterodimers	Ayoub et al. (2004)
Takeda Pharmaceutical Company	2005	Development of melatonin receptor agonist TAK-375, now known as ramelteon	Neubauer (2008)
Christian de Bodinat	2010	Agomelatine, the first melatonergic antidepressant	De Bodinat et al. (2010)
Daniel P. Cardinali	2013	Role of melatonin and its analogs in insomnia and depression	Cardinali et al. (2012)
Gabriella Gobbi	2019	Differential function of melatonin MT1 and MT2 receptors in REM and NREM sleep	Gobbi and Comai (2019)

level of pineal melatonin is being observed late in the evening which reaches maximum up to 4:00 a.m. (2:00 and 4:00 a.m. is a maximum observed range) and then gets back down to the normal daytime levels. It is very difficult to detect the daytime level of melatonin. In contrast to sunlight, artificial lights are sufficient to block the nocturnal release of melatonin (Peuhkuri et al. 2012).

Melatonin possesses rapid metabolism with a half-life that varies between 10 and 60 min in humans following exogenous administration. It is catabolized mostly by the liver and gets excreted in the urine. Metabolite like 6-sulphatoxymelatonin

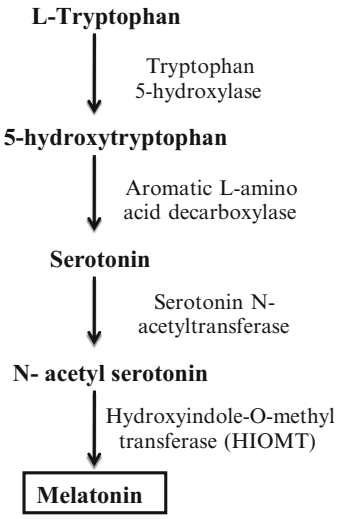

Fig. 9.2 Synthesis of melatonin

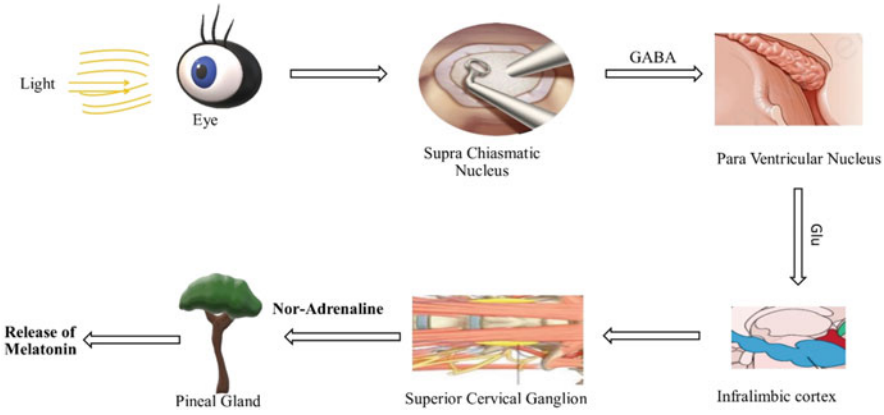

Fig. 9.3 Release of melatonin and suppression with light

(6-SMT) in urine indicates that melatonin status can be evaluated melatonin in the overnight urine sample (Peuhkuri et al. 2012).

9.3 Melatonin Receptors

Two distinct classes of melatonin receptors are found in different regions of the body, which are expressed in humans and have been reported so far, MT1 and MT2, formerly designated as Mel1a and Mel1b, respectively (Dubocovich et al. 2003). The majority of the melatonin receptors are found in the following regions of the

body such as cardiovascular system, coronary or cerebral arteries, cardiac ventricular wall, brain, liver and gallbladder, retina, exocrine pancreas, skin, parotid gland, colon, duodenal enterocytes, platelets, white and brown adipocytes, kidney, cells of the immune system, ovary/granulosa cells, placenta, myometrium, and fetal kidney. In other systems like GIT, these receptors are abundant in colonic mucosa and jejunal. Melatonin is involved in modulating functions of FSH and LH via affecting the expression of their receptors (Table 9.2).

Adenylyl cyclase activity gets inhibited due to the coupling of MT1 and MT2 melatonin receptors with higher affinity to pertussis toxin-sensitive G proteins. They are unique molecules possessing individual chromosomal localization with 60% amino acid identity. These moieties possess a long chain of amino acids (about 350 and 362) having calculated molecular weight about 39–40 kDa. In N-terminus MT1 and MT2 receptors contain one or two potential glycosylation sites and protein kinase A (PKA), protein kinase C (PKC), casein kinase 2 (CK2), and casein kinase 1 (CK1) which promote and regulate the receptor functioning. In the superfamily of GPCR, melatonin receptors are present as distinct groups due to the presence of NRY motif (letter amino acid code) which is a DRY (or ERY) variant present G protein-coupled receptors intracellularly in loop II. This is the particular region having higher involvement in the process of G protein signal transduction. The essential melatonin biding sites are Ser 8 (TM III), His 7 (TM IV), Gly 20 (TM VI), Ser 12 (TM III), Val 4 (TM IV), and Gly 20 (TM VI) in MT1 receptor, whereas two residues Cys 113 (in EL1) and Cys 190 (in EL2) are present in MT2 melatonin receptor which are conserved in most GPCRs and estimated to make disulfide bond which is important for melatonin binding with good affinity (Cajochen et al. 2002; Pala et al. 2013). Leucine zippers are involved in protein-protein interactions; melatonin receptors contain 6 leucines in the MT2 and 7 leucines in the MT1 in TM IV and possess protein-protein interactions.

MT1 and MT2 receptors generally show their effect after adhesion to the cell surface by G-protein. Forskolin inhibits the protein kinase A (PKA) and cAMP formation, followed by the activation of MT1 receptor. Similarly, cAMP formation is inhibited in MT2 receptors stimulated by forskolin; they also inhibit cGMP formation. In the central nervous system, membrane receptors are present in the brain as well as in the periphery RZR/RORα is located. Membrane receptors with their agonists are associated with circadian rhythm, whereas RZR/RORα is responsible for cellular growth, differentiation of bone, and immunomodulation in the periphery. To form melatonin effectively, protein kinase C-α activation is a crucial step.

9.3.1 MT1 Receptors

MT1 receptors are high-affinity receptors that fall into the GPCR superfamily and the binding of melatonin to these receptors inhibits the activity of adenylate cyclase activity in target cells (Pandi-Perumal et al. 2008). The two subgroups of the MT1 receptors are MT1a receptors and MT1b receptors. Cardiac vessels constriction and circadian rhythms are modulated by MT1 melatonin receptor generally found in

Table 9.2 Functions of melatonin receptors based on location in the different regions of the body

Location	Receptor type	Function	Reference
CNS			
Hippocampus	MT_1, MT_2	Inhibition of neuronal activity and excitatory responses in memory Modification in Alzheimer disease Enhancement in seizure threshold via suppression in $GABA_A$-receptor function	Savaskan et al. (2005, 2002)
Cerebellum	MT_1, MT_2	Involvement and interaction with glutamatergic synapse	Al-Ghoul et al. (1998)
Various retinal cell	MT_1, MT_2	Dopamine release evoked by stimulation gets inhibited Adaptation to low light intensity Modification in photoreceptor functions rod phototransduction pathways	Reppert et al. (1995)
SCN	MT_1	Initiation and improvisation in sleep Modification of circadian rhythm (blind people, phase shift worker, jet lag)	
Central dopaminergic system	MT_1	Modulation of dopamine synthesis and release Increased sensitivity and activation of dopamine receptors	Uz et al. (2005)
CVS			
Cerebral arteries	MT_1	Unknown effects	Savaskan et al. (2001)
Aorta	MT_1, MT_2	Vasodilation may occur	Ekmekcioglu et al. (2003)
Cardiac ventricular wall	MT_1, MT_2	Modulation of beta-adrenergic receptor-mediated cAMP signaling processes may be initiated Increased stimulation of voltage-activated calcium current Negative inotropic effects may occur	Ekmekcioglu et al. (2003, 2001)
Coronary arteries	MT_1, MT_2	MT_2 receptors-mediated vasodilation MT_1 receptors-mediated vasoconstriction	Ekmekcioglu (2006); Ekmekcioglu et al. (2003, 2001)
GIT			
Duodenal enterocytes	MT_2	Stimulation of HCO_3–secretion via neural stimulation	Sjöblom et al. (2001); Sjöblom and Flemström (2003)
Pancreatic cancer cell lines	MT_1	Acid/base homeostasis regulation (stimulation of HCO_3–secretion)	Ekmekcioglu (2006)
Gallbladder epithelia	MT_1	Gallbladder contraction may occur	Aust et al. (2004)

SCN and cardiac vessels. Not only in these regions but these sets of receptors are also found in peripheral tissues as well as other parts of the brain.

9.3.1.1 Receptor Signaling Mechanism of Melatonin MT1 Receptors

Pertussis toxin (PTX) sensitive (Gi2 and Gi3) and PTX-insensitive (Gq/11) G proteins mediate multiple cellular responses through activation of MT1 receptors; they generate signal transduction as well as physiological response of melatonin. According to the recent studies, the activation of MT1 receptors is exposed to different signal transduction pathways in mammalian cell line. According to these transduction pathways, MT1 receptor activation is responsible for inhibition of the activity of PKA, cAMP formation followed by forskolin and phosphorylation of that is cAMP-responsive element binding protein (CREB) (Fig. 9.4).

Further, the activated MT1 receptor also increases the phosphorylation of mitogen-activated protein kinase or extracellular signal-regulated kinase, kinase 1 and 2 (MEK1 and MEK2), and extracellular signal-regulated kinases 1 and 2-(ERK1/2). Thus, these signaling pathways possibly mediate the induction of filamentous structures in non-neuronal cells. Potentiation of prostaglandin F2α (PGF2α) and adenosine triphosphate (ATP)-induced phosphoinositide proceeds through the activation of βγ subunit of PTX-sensitive G proteins.

Ion channel regulation is responsible for the numerous functional responses of melatonin such as activation of PTX-insensitive G proteins via an increase in

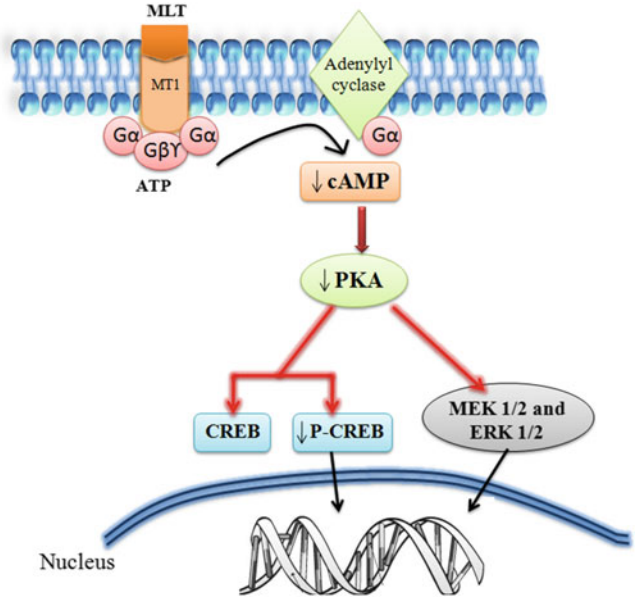

Fig. 9.4 MT1 receptor-mediated signaling mechanism

intracellular calcium by melatonin in ovine pars tuberalis cells expressing MT1 receptors, but obstructs the influx of calcium at AtT20 cells by PTX-sensitive G proteins and in pituitary cells of neonatal rats (Dubocovich et al. 2003). Due to MT1 receptors activation, the potentiation of adrenergic vasoconstriction is mediated by melatonin. In smooth muscles, due to this activation, vasoconstriction is potentiated by blocking of calcium-activated potassium channels (BKCa), and this blockage may be due to BKCa channel phosphorylation by PKA and cAMP reduction. Through inhibition of BKCa channel, melatonin vasoconstricts cerebral artery. Multiple mechanisms lead to the regulation of the inward rectifier potassium channel by melatonin in the SCN region. At T20 cells or Xenopus oocytes contain MT1 receptor that activate G protein along with activated inward rectifier potassium channel, (GIRK) Kir3 through a PTX-sensitive mechanism in which βγ subunits of the Gi protein are involved. In SCN neuronal firing is inhibited with the elevation in potassium conductance mediated by melatonin as a result of activation of Kir3 channel. This phenomenon (inhibited neuronal firing) was absent in knockout mice with MT1 melatonin receptor, which further makes conformation of MT1 receptor involvement for effect. The MT1 receptor may mediate membrane potential hyperpolarization in neonatal pituitary cells. There are various tissue-dependent signaling responses elicited by MT1 receptor activation mediated by PTX-insensitive G or PTX-sensitive proteins.

9.3.2 MT2 Receptors

The MT2 receptors are low-affinity receptors that are coupled to phosphoinositol hydrolysis. MT2 receptors are involved in retinal physiology, modulating circadian rhythms, dilating cardiac vessels, and affecting inflammatory responses in the microcirculation (Pandi-Perumal et al. 2008). Unlike the MT1, the restriction is higher with regard to their localization; the involving regions are SCN of the hypothalamus, cerebellum, cardiac vessels, kidney, ovary, retina, and other multiple cell lines. The protein reflecting similar binding affinities to MT2 receptor is now denoted as MT3 and is affinity purified from the Syrian hamster kidney (Nosjean et al. 2000). It is found that an enzyme involved in detoxification named quinone reductase 2 shares 95% homology to this enzyme. Lowering of intraocular pressure and leukocyte adhesion induced by leukotriene B4 is inhibited by MT3 receptors activation.

There are only one nuclear receptor and three membrane receptors.

9.3.2.1 Type 1a Receptors: Mel 1a, ML1a, ML1, MT1, MTNR1A

It consists of 351 amino acid chain encoded in chromosome number 4 of humans. Adenylate cyclase is inhibited when MT1 receptors bind to multiple G-proteins. MT1 receptors are mainly found in the human skin (Emet et al. 2016). Findings suggest age-dependent decrease in the expression of the MT1 receptor, especially in cortex SCN and Alzheimer's disease (AD). The decline in prolactin secretion and neuronal discharge in SCN is recorded due to MT1 receptor.

9.3.2.2 Type 1b Receptors: Mel 1b, ML1b, MT2, MTNR1B

It consist of 363 amino acid chain encoded in chromosome number 11 of humans. Adenylate cyclase is inhibited when MT1 receptors bind to multiple G-proteins (Emet et al. 2016). It leads to the inhibition of soluble guanylyl cyclase pathway. Through activation of melatonin receptor, adenylate cyclase inhibition occurs and reduces the production of cyclic AMP (cAMP).

Eccrine sweat glands and malign melanocytes are the specific parts of the skin whereas MT2 receptors are present. In hippocampus region of rats, functions associated with GABA-$_A$ are inhibited. It is found that these receptors are responsible for antidepressant activity and the expression of MT2 receptors is reduced in (AD). In the pharmacology and pathophysiology of disorders like AD, anxiety, sleep disorders, depression, and pain, MT2 receptors play a vital role. For the development of hypnotic agents MT2 receptors may rise as a new target. For the anxiolytic effects of melatonin, these types of receptors are responsible.

9.3.2.3 Receptor Signaling Mechanism of Melatonin MT2 Receptors

Physiological response as well as signal transduction mechanism of melatonin by MT1 activation can be modulated by the levels of both guanosine 3′-5′ monophosphate (cGMP) and cAMP. Similarly, melatonin inhibits the forskolin-stimulated cAMP formation and stimulates JNK through the MT2 receptor activation (Hardeland 2009). Further, cGMP formation is inhibited by the activation of MT2 receptor. In ***SCN***, the activity of PKC is increased by MT2 receptor activation and that response was blocked by the selective MT2 receptor antagonist 4P-PDOT. It is also reported that the diacylglycerol (DAG) and phospholipase C pathway can be stimulated by MT2 receptors (Fig. 9.5).

During the activation of the MT2, several physiological responses to melatonin were identified. These include:

1. Change in the fashion of release of dopamine from the retina of rabbit.
2. Activation of PKC due to phase advance of circadian rhythms in the isolated SCN.
3. Increase in humoral and cell-mediated immunity.
4. In microvasculature inhibition of rolling leukocyte.
5. Delay of the G_1 to S cell cycle transition leads to the inhibition of proliferation of human choriocarcinoma JAr cells.
6. Activation of MT2 receptors includes the following events: decline expression of Glut4 (glucose transporter), decrease glucose uptake in human brown adipocytes, mediates vasodilatation in arterial beds, and modulates neuronal activity in the hippocampus.

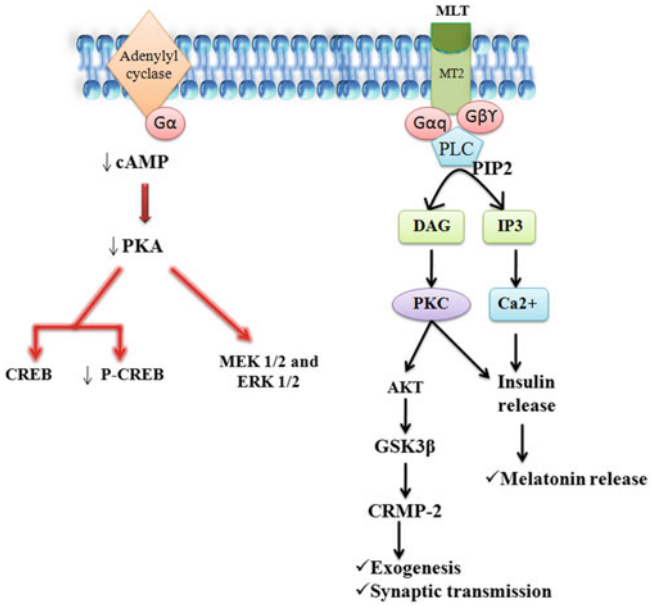

Fig. 9.5 MT2 receptor-mediated signaling mechanism

9.4 Physiological Role of Melatonin

9.4.1 Direct Antioxidant Actions of Melatonin

Melatonin uses multiple mechanisms to decrease the oxidative stress. According to previous experimental results, it directly acts as a free radical scavenger and indirectly act as antioxidant through stimulating glutathione synthesis (antioxidant), stimulating antioxidant enzymes, its ability to prevent antioxidative enzymes from oxidative damage, its potency to elevate the efficiency of, i.e., electron transport chain (ETC), and hence reducing the generation of free radicle by decreasing leakage of electrons. As discussed melatonin proved as effective agents in preventing molecular damage associated with elevated stress conditions. All these above-mentioned factors which possess the ability to restrain the resulting molecular mutilation have an unknown mechanism which is to be explored.

9.4.1.1 Direct Scavenger Action of Melatonin at Oxygen Based Free Radicals and Related Specie\s

Scavenging Action of Melatonin on Hydroxyl Radical (OH)

- 2 OH radicals are scavenger by each melatonin molecule.
- A free radical scavenging action shown by cyclic 3-hydroxy melatonin acts as a detectable marker which appears in urine.

Scavenging Action of Melatonin on Superoxide (O_2)

- Xanthine/hypoxanthine O_2 producing system is also called the pure chemical system in which O_2 is scavenged by melatonin.

Melatonin Scavenger of H_2O_2

- Melatonin scavenger H_2O_2 in a pure chemical system.
- A mechanism of the oxidation of melatonin by H_2O_2 was suggested based on two major resulting metabolite, i.e., N-acetyl-N-Formyl-5-Methoxy kynurenine (AFMK).
- Cyclic 3-hydroxyl melatonin and AFMK function as a scavenger of toxic reactants as like as parent molecule, i.e., melatonin.

9.4.1.2 Scavenger Action of Melatonin on Nitrogen-Based Free Radicals and Related Species

- Only in the presence of molecule oxygen interaction between NO and melatonin takes place.
- The chief product of melatonin/NO reaction is N-nitromelatonin.

9.4.2 Indirect Antioxidant Actions of Melatonin

Melatonin can scavenge nitrogen and oxygen-based reactants directly and along with reducing the oxidative stress by multiple pathways. The relative importance of the direct and indirect antioxidative processes of melatonin in vivo remains unknown.

9.4.2.1 Melatonin Stimulation of Glutathione Synthesis

GSH is a well-known antioxidant and free radical scavenger that possess high abundance intracellularly. According to a single reported result, an increase in GSH concentrations is observed intracellularly due to glutamylcysteine synthase, a rate-limiting enzyme stimulated by melatonin. Unlike other functions of melatonin, there are specific receptors that mediate direct free radical scavenging function of the indoleamine. Melatonin-mediated GSH synthesis is the major antioxidative action of melatonin.

9.4.2.2 Synergistic Actions of Melatonin with Classic Antioxidants

The important actions of melatonin at the level of mitochondria are as follows:

- It is an efficient action of ROS/RNS which are abundant in mitochondria than any other portions of cell and melatonin concentration is higher than serum.
- GPx and GRd are the efficient enzymes stimulated by melatonin useful for GSH cycling, because mitochondria is not capable of synthesizing GSH by itself.
- Antiapoptotic effects of melatonin have been reported along with mitochondria originated apoptotic signals.
- Higher concentrations of melatonin may be present in the mitochondria than any other portions of cell and melatonin concentration is higher than serum.

- Moreover, melatonin was shown to inhibit NADPH-dependent lipid peroxidation in human placental mitochondria to protect the fetal rat brain against oxidant-mediated mitochondrial damage and stimulate mitochondrial respiration in the brain.

9.4.2.3 Action of Melatonin in Immune System

- When melatonin acts on receptor with higher affinity, i.e., MT_1 and MT_2 it elicit immunological effects.
- Melatonin may enhance the cytokines production (IL-6, IL-1β, and TNF-α) and the main function of humoral response is to destroy extracellular microorganisms and preventing the spread of action.
- In order to fight against infectious diseases such as bacterial infections, HIV, cancer, etc. useful role of melatonin should be found.

Two GPCRs mediate the physiological actions of melatonin, MT1 and MT2. In sleep regulation, the role of MT1 and MT2 with high affinity in SCN is observed. The neuronal firing rate is suppressed by MT1 receptor activation in the SCN, and on the other hand circadian rhythm phase shifts are induced by MT2 receptors. In peripheral organs both MT1 and MT2 receptors are present and make their contribution to manage physiological functions. MT1 receptors activation pursue the following events which include vasoconstriction of cerebral and peripheral arteries, regulating the expression of Per1 gene in the anterior pituitary, and inhibiting the secretion of prolactin from the pars tuberalis (Doghramji 2007). Various studies support the involvement of MT2 receptors in the inhibition of dopamine release in the retina, vasodilation, in retinal physiology, and also increasing splenocyte proliferation. MT3 binding sites are widely distributed in the brain, liver, heart, kidneys, and lungs. Several studies performed in vivo suggest its possible role in the regulation of intraocular pressure and inflammatory responses inside the microvasculature.

9.5 Pharmacology

In order to identify and characterize the melatonin receptor in their heterozygous or native system, a good knowledge of the pharmacological property of radioligands is required in a receptor system. To characterize the potential therapeutic system, this knowledge plays an important role in defining ligand selectivity and specificity, affinity, efficacy, and potency which are further used as an example to demonstrate the characterization and use in the discovery of functional receptor in native tissue.

9.5.1 General Compounds Acting on Melatoninergic System

Several compounds have revealed the binding efficacy towards melatonin receptors for significant therapeutic actions. Any compound having 100-fold or more binding potential for specific receptor is called a ligand. Both the endogenous and exogenous

melatonin proved to be potent agonists for MT1 and MT2 receptors providing a variety of physiological responses (Dawoodi et al. 2012). Another compound named as *N*-butanoyl-2-(2-methoxy-6H-isoindolo [2, 1-a] indol-11-yl)-ethanamine (IIK7) that emerged as a selective MT2 melatonin receptor agonist proved to have higher affinity towards MT2 in comparison to MT1. On the contrary, luzindole acts as a selective antagonist for MT2 receptor as it shows a higher affinity towards MT2 in comparison to MT1 receptor. It provides significant results in circadian rhythm disorders and depression-associated studies. Besides this, other compounds such as 4-phenyl-2-propionamidotetralin (4P-PDOT) and 4-phenyl-2- acetamidotetralin (4P-ADOT) have also revealed significant antagonist properties for MT2 receptor (Cecon et al. 2018). Research studies have shown that the success of selective ligands for MT1 is less as compared to MT2 receptors as their efficacy and binding affinity are low. To tackle this, a dimer of agomelatine is formed as ligand S26131 which shows 200-fold higher affinities for MT1 receptor in cell line studies (Liu et al. 2016). Identical to these compounds, a number of different ligands have been recognized for their specificity towards each melatonin receptor which is enlisted in Table 9.3.

Moreover, the formation of MT1 or MT2 receptors with 5-HT_{2C} provides successful result in higher efficacy in various disorders. Such drugs include agomelatine and TIK-301 which provide reliable results in depression studies (Liu et al. 2016). Further on the basis of research data available, several melatonin targeting drugs are successfully running for their therapeutic efficacy in different clinical conditions (Dawoodi et al. 2012). Such compounds include ramelteon, agomelatine, TIK-301, and tasimelteon that have gained clinical reliability for their efficacy in circadian rhythms-associated disorders (Table 9.4).

Table 9.3 Ligands for melatonin receptors

Receptor	MT1	MT2	MT3
Agonists	Melatonin, 2-Iodomelatonin, N-propionyl melatonin, N-butanoyl melatonin, 6-Chloromelatonin, 2-Methyl-6,7-dichloromelatonin, S20098, GR 196429, 8M-PDOT, S26131	Melatonin, 2-Iodomelatonin, N-propionyl melatonin, N-butanoyl melatonin, 6-Chloromelatonin, 2-Methyl-6,7-dichloromelatonin, S20098, GR 196429, 8 M-PDOT, IIK7	2-Iodomelatonin, 6-Chloromelatonin, Melatonin (M5250), N-Acetylserotonin, 5-MCA-NAT
Partial agonists	5-Methoxyluzindole, N-acetyltryptamine	5-Methoxyluzindole, N-acetyltryptamine	NA
Antagonists	Luzindole, S20928	Luzindole, S20928, 4P-PDOT, 4P-ADOT, K185	Luzindole, Prazosin, Prazosin

Table 9.4 Clinically approved compounds

Clinically approved compounds	Mechanism of action—drug target	Therapeutic uses
Melatonin controlled release tablets (prolong release melatonin)	MT1 and MT2 receptor agonism	Treatment of insomnia in amyotrophic lateral sclerosis and other disorders
Ramelteon	MT1 and MT2 receptor agonism	Treatment of insomnia
Agomelatine	MT1 and MT2 receptor agonism Serotonin antagonist (exhibit little affinity for $5HT_{2C}$)	Anxiolytic action Antidepressive action Increase daytime alertness
TIK-301	Melatoninergic agonist Serotonin antagonist (exhibit higher affinity for $5HT_{2C}$)	Antidepressive action
Tasimelteon	MT1 and MT2 receptor agonism	Sleep-promoting action Antidepressive action

9.6 Physiological Responses Mediated by Activation of Specific Melatonin Receptors (MT1, MT2, and MT3)

Melatonin plays a very important role in ensuring that the organism is adjusted to seasonal and environmental changes. Endogenous and exogenous melatonin play a very important role in the control and coordination process. The release of endogenous melatonin takes place in seasonal and circadian fashion. On the other hand, behavioral and physiological responses are regulated by exogenous melatonin. In this segment multiple mechanisms through which melatonin controls and coordinates functions are to be discussed such as cardiovascular responses, endocrine functions, circadian rhythms, and immune system.

9.6.1 Pharmacology and Function of M1 Receptor (Recent Update on Involvement of M1 in Normal and Pathophysiological States)

9.6.1.1 Sleep, Addiction, and Behavioral Associated Disorders

The unique characteristic features of MT1 receptor make it a reliable target in various disorders like insomnia, circadian sleep disorders, major depression, and cancer. Regulation of sleep is a major therapeutic action which MT1 receptor contributes (Liu et al. 2016). It makes melatonin efficacious in insomnia and other sleep-associated disorders. Here, treatment with melatonin activates the MT1 receptor in the SCN and other limbic regions to decrease neuronal firing for sleep induction. The region basis localized distribution of MT1 receptor in the brain has

been explored for its in-depth analysis. The thalamic reticular nucleus is the major region to coordinate sleep. Here, the localized MT1 receptor serves to modulate the REM sleep whereas the localized MT2 receptor takes part in NREM sleep (Ng et al. 2017). The presence and activation of MT1 receptor in the retina regulates the neurotransmitter release. Once melatonin get secreted from photoreceptors, it immediately binds to melatonin receptors on amacrine cells and affects the subsequent release of dopamine and GABA. Melatonin and dopamine act as a mutual antagonists of one another. So, if melatonin binds on dopaminergic amacrine cells then the subsequent release of dopamine decreases but if itself dopamine interacts with D2 receptor then it leads to inhibition of melatonin release (Ng et al. 2017). Normally, the binding of melatonin to MT1 receptor directly inhibits dopamine release to facilitate dark adaptation.

9.6.1.2 Circadian Rhythm and Melatonin

Melatonin in relation to circadian rhythm has direct effects on sleep-inducing thermoregulatory mechanisms via loss of body temperature and sleep-inducing tendency. The disturbed circadian rhythms and abnormal sleep are major symptoms of depression and other mood-related disorders which make melatonin therapeutically relevant among them (Kasper and Hamon 2009). Sleep and circadian rhythms are most commonly affected by melatonin. At pharmacological and physiological dose, melatonin decreases alertness and core body temperature at different times of the day. Melatonin stimulates both phases, delays and phase advances of the circadian system, in humans when timed correctly. A decrease in alertness and temperature is observed in time zone travelers and shift workers, when timed to advance (Arendt and Deacon 1997).

9.6.1.3 Manipulating Rhythms with Melatonin

Shift work: Sleep disturbance, fatigue, and gastrointestinal problems are primary health compliance among shift workers. Poor adaptation of the circadian system is considered as a major cause of all these problems. Circadian state clearly depends upon core body temperature, and temperature and melatonin rhythms are closely coupled. The potency of the temperature rhythm is inversely correlated with the quality of sleep (Arendt and Deacon 1997). Night shift workers tend to sleep in the morning for short period when there is a rising phase of core temperature rhythms started, and hence, task performance abilities decrease along with declining core temperature and imbalanced melatonin. Melatonin improvises the shift worker/jet-lag conditions. It improvise sleep, performance, alertness, and disturbed homeostasis (body temperature). Melatonin can be used in conditions where night shift workers are exposed to morning bright light on the way to their home and it induce natural sleep.

Jet Lag: The condition of jet lag and shift workers is almost similar. The only differences between these situations are that time zone travelers can predict the time to avoid themselves to natural light with the help of natural zeitgebers they can adapt to situations. They are almost similar to shift workers, subject to other factors such as meals at inappropriate times, flight times of arrival, stress, difficulty in sleeping on

the aircraft, dehydration, etc. (Arendt and Deacon 1997). Therefore, melatonin is again considered as a good approach to treat time zone travelers; it improvises sleep pattern without hindering daily performances and tasks.

Other effects: Other actions like the antidepressant action of melatonin is confirmed after the discovery of agomelatine which acts synergistically via the melatonin receptors and 5HT-2C receptors (Kasper and Hamon 2009). The drug agomelatine has been proven to be better and well tolerated than SSRIs and SNRIs in clinical studies. This drug not only mitigates stress, depression, neurochemical abnormalities, and neuronal atrophy but also normalizes distorted sleep and circadian rhythm. The presence of the MT1 receptor in the dorsal raphe nucleus suggests their involvement in the pathogenesis of depression (Ng et al. 2017). The knockout model of MT1 receptor resembles the behavioral changes of depression in experimental animal studies.

The role of melatonin was also evaluated for drug addiction, abuse, and reward. It is observed via locomotor sensitization, a phenomenon involving repetitive injections of psychostimulants to induce locomotion in rats during drug free period. Genetic deletion of MT1 receptor enhances locomotor sensitization in rodents against methamphetamine (Hutchinson et al. 2012). Hence, the MT1 receptor could be a novel target to treat the addiction behavior induced by psychostimulants.

9.6.1.4 Neurological Disorders

Various studies confirmed that chronic administration of melatonin not only provides antidepressant action but also enhances hippocampal neurogenesis, neuronal differentiation, and neuronal survival via the activation of melatonin receptors: both M1 and M2 (Ramírez-Rodríguez et al. 2009). The expression of MT1 receptor is predominant in the hippocampus to regulate inherent circadian time keeping capacity, which remains essential to perform basic functions like memory, learning, neurogenesis, and long-term potentiation. This physiological participation makes melatonin an important target in hippocampus-dependent disorders such as epilepsy, dementia, and addiction (Tchekalarova et al. 2015).

The therapeutic efficacy of melatonin is also under evaluation for its role in excitotoxicity-dependent neuronal cell death. Excitotoxicity is the pathophysiological event which involves a variety of neurological disorders. It depends either upon ionotropic receptors (NMDA or AMPA) or metabotropic receptor (G-protein-coupled receptor). Among these receptors, the NMDA receptor excitotoxicity gets major concern due to its diverse role in synaptic plasticity, neuroendocrine regulation, and neuronal injury. Several studies have evaluated the role of melatonin against excitotoxicity-associated damage to neuronal cells (Escames et al. 2004). It has been confirmed that melatonin administration significantly ameliorates excitotoxicity via its anti-inflammatory effect through the MT1 receptor. Further, few studies have evaluated the specific role of melatonin receptor for the mechanism behind this action. For example, Das et al. have demonstrated that MT1 and MT2 receptors nonselectively participate in the neuroprotection against glutamate-associated excitotoxicity (Das et al. 2013). The study also reports that overexpression of melatonin receptor increases the expression of calcium-binding proteins

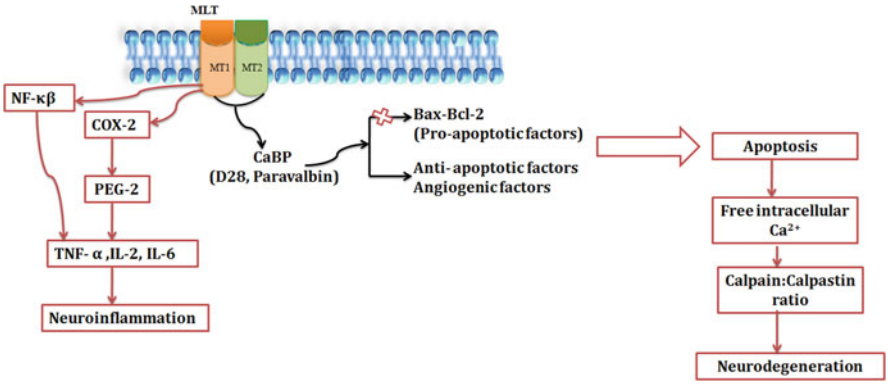

Fig. 9.6 Role of melatonin receptors in neuroinflammation and neurodegeneration

(Calbindin D28K and parvalbumin) which in turn decreases the calcium-dependent mediators of cell death including protease, calpains, and pro-apoptotic (Fig. 9.6).

Besides, the mediators of proinflammatory cytokines are expected to decrease as the expression of their upstream regulators (NF-κβ and COX-2) gets diminished on melatonin treatment. This anti-inflammatory effect probably gets maintained via the MT1 receptor. Consequently, neuroinflammation and intracellular calcium-mediated cell death associated neurodegeneration decrease via the upregulation of melatonin receptors. The efficacy of MT1 receptor is also proved in striatal disorders like Huntington's disease (HD) and Parkinson's disease (PD). Mutant striatal cell model evaluates the efficacy of melatonin against mitochondrial dysfunction in HD (Wang et al. 2011). The efficacy of melatonin in this model is confirmed to be mediated by the activation of MT1 receptor as mitochondria get reversed by overexpression of similar receptor. On the other hand in PD, the role of melatonin is under research for its contribution to motor symptoms and non-motor symptoms like depression, anxiety, and insomnia. The maintenance of dopaminergic neurons survival is a primary strategy to treat PD-associated neurodegeneration and symptoms, and the glial cell-derived neurotrophic factor (GDNF) remains an essential component to maintain the survival of dopaminergic neurons (Mack et al. 2016). The intrastriatal melatonin treatment upregulates the GDNF expression in striatum against MPTP-induced PD. Further, the neural stem cell studies prove it to be upregulated by the activation of MT1 receptor.

Melatonin has a neuroprotective effect in many other disorders, such as AD, epilepsy, PD, ischemic, amyotrophic lateral sclerosis, injury, and head injury. Such disorders concerned with degradation, loss of neurons, and mitochondrial dysfunction initiating glutamate and free radical overactivity may lead to disease progression.

Epilepsy

Various studies on the role of epilepsy have revealed that epileptic seizures are improvised with the help of melatonin but the effect of melatonin as a single

therapeutic moiety is still to be investigated. Due to its ability to cross the blood–brain barrier it may be used in the treatment of seizures. For example, temporal lobe epilepsy (TLE) involves progressive development of complex partial seizures that originate from the temporal lobe (hippocampus) and its various symptoms including seizures, cognition, and behavioral abnormalities are caused by localized regional damage in the hippocampus. It includes a gradual decrease of neurogenesis, loss of GABAergic interneurons, synaptic plasticity, and chronic inflammation which occur in a bidirectional manner with irregular circadian rhythms. This interaction has been validated via observing the decreased expression of MT1 receptor in the hippocampus which usually provides an inhibitory effect on the CNS. In this way, agonists of melatonin receptors could provide an anticonvulsant effect in epilepsy. Both melatonin and agomelatine have been reported to have anticonvulsant action in acute and chronic preclinical epilepsy. Even the protein and mRNA expression of the binding site for melatonin named RORα gets decreased in pilocarpine model of epilepsy (de Alencar Rocha et al. 2017). It has been estimated that RORα may contribute to temporal epileptic seizures identical to its participation in circadian rhythm, anti-inflammatory action, and antioxidant properties of melatonin.

Insomnia

High levels of melatonin is observed in adolescence, which declines slowly after the age of 20. Thus, it affects the age group of 20 more efficiently. Soporific and hypothermic effects are observed after administration of melatonin during the day in the young age group. Improvisation in sleep patterns is observed with increased nocturnal melatonin level via oral administration. Therefore, melatonin therapy is considered good to treat insomniac conditions.

Depression

It is the property of ideal antidepressant to reduce sleep onset difficulties, without hindering freshness and daytime alertness. Melatonin does not possess any abuse potential and adverse effects like "hangover": it has the ability to improvise patterns of sleep in patients with insomnia associated with depression.

Alzheimer's Disease

Accumulation of amyloid β (Aβ) protein and neurofibrillary tangles in the brain is the main cause of AD. Degeneration takes place as a result of hyper-phosphorylation of nerve fibers due to age-dependent decline in melatonin. Melatonin plays an important role in glycogen synthase kinase 3 (GSK-3) modulation; it helps to prevent neurodegeneration by the influence of AD by the interaction of GSK-3 with presenilin-1, a cofactor for G secretase. It is found that due to its antioxidant potential, melatonin has direct action in the inhibition of Aβ accumulation and improving sleep disturbances caused by AD.

Parkinson's Disease

Oxidative stress is the major factor responsible for the progression of PD; dopamine metabolism and mitochondrial impairment are the leading causes to generate

oxidative stress; these are the major factors responsible for the progression of PD. Positive therapeutic results are observed when melatonin is used against rotenone-induced dopamine loss. Due to excessive stimulation of glutamate receptors, neuronal damage may occur and melatonin has been shown to have a neuroprotective action against glutamate-induced excitotoxicity. Oxidative stress and mitochondrial dysfunction in the brains of patients with PD showed elevated oxidative damage to DNA, decreased levels of glutathione, and increased monoamine oxidase activity. Reduced antioxidant defense mechanisms in PD brains are observed due to a reduction in catalase activity and reduced glutathione (GSH) (Singh and Jadhav 2014).

9.6.1.5 Cancer Studies

Several cancer studies revealed the remarkable success of melatonin treatment in lung, gastrointestinal, neck, breast, and head cancer. On one side, melatonin treatment counteracts side effects associated with chemotherapy such as anxiety, depression, and toxicity but on the other side, it provides some significant results in both in vitro and in vivo cancer studies. The role of MT1 receptor is specifically confirmed in breast cancer cell line studies where molecular estimation is performed on mRNA level of receptor in human specimens. It has been estimated that melatonin receptors (MT1) are expressed over the MCF-7 and MDA-MB-231 (human breast cancer cell lines) (Hill et al. 2015). The exogenous administration of melatonin and estradiol in MCF-7 cells reduces the expression of MT-1 receptor as they control one another receptor binding. In this way, interaction takes place in between MT1 receptors and estrogen receptors results in antiproliferative effects of melatonin. Moreover, melatonin treatment enhances the caspase-independent apoptotic response via upregulation of Bcl-2/BAX ratio. Overall, melatonin therapy in cancer cells documented to regulate estrogen receptor binding stimulates the immune system and mediates apoptosis for anticancer action. It continuously varies in different cancer cell lines and modulates by melatonin treatment. Overexpression of MT1 receptor antagonizes the proliferation of MCF-7 cells which confirm its active participation in antiproliferative action against rapidly growing cancer cells. It enhances the reliability of selective agonist of MT1 receptor for therapeutic efficacy in research studies.

9.6.1.6 Cardiac Disorders

Peripheral as well as central interventions are regulated by melatonin; hence cardiovascular system is considered as the most important site of action for melatonin. The presence of melatonin receptors over the ventricular walls, aorta, peripheral and coronary arteries makes it highly recommended in cardiac disorders. It has been reported to possess antihypertensive, anti-atherosclerotic, and myocardium protective properties. The cardioprotective effect of melatonin is thought to be contributed by its antioxidant and anti-inflammatory potential. In recent years, our changed lifestyle has encountered late night shifts, indoor work life, and multiple jet lags which disrupt circadian rhythm leading to melatonin dysfunctional disorders.

A number of epidemiological studies have recognized the circadian rhythm as a contributory factor to enhance the incidence rate of diabetes, premature aging, and cardiac disorders (Zhong and Liu 2018). Additionally, the decreased level of melatonin in blood circulation has been reported in such disorders. For example, melatonin has been proven to be a potent antiadrenergic molecule in myocardial infarction. This cardioprotective effect is contributed by the activation of MT1 and MT2 receptors in coronary arteries as it gets inhibited via luzindole which is a nonselective blocker of melatonin (Favero et al. 2017). There is one explanation behind the therapeutic action of melatonin which strikes on the role of adhesion molecules in the pathophysiology of MI. It has been observed that patients with impaired circadian rhythm have upregulated endogenous vascular cell adhesion molecule-1, which is a marker of MI as it rarely occurred in the normal person. Adhesion molecules become upregulated along with endothelial and platelet activation after myocardial ischemia. Therefore, the melatonin treatment could effectively reduce these adhesion molecules to reduce the migration and edema of endothelium for prevention of heart attacks.

Ischemic perfusion injury remains the most serious complication of ischemic heart disease. It occurs after hypoxia and plaque removal, when blood starts to reflow. It frequently damages the cardiac cells through excessive myocardium contracture, low ventricular pressure, and ventricular fibrillation to enhance the irreversible damage over the heart. One study report suggests that damage caused by ischemic reperfusion injury gets enhanced by pinealectomy and significantly reduced by administration of exogenous melatonin (Nduhirabandi et al. 2011). Another preclinical study has implicated in the ligation of the coronary artery in rats which later increases the melatonin receptor expression (MT1 and MT2) to protect the heart (Lochner et al. 2013). It ensures the cardioprotective role of melatonin in ischemic reperfusion injury via both endogenous and exogenous manner.

9.6.1.7 Melatonin and Blood Pressure (BP)

Melatonin actions on the cardiovascular system (CVS) are well known. Since the classical work of Zanoboni group and Holmes and Sugden, hypertension is observed in rats after removing circulatory melatonin which gets reversed upon melatonin therapy is given. BP is regulated by melatonin, either by increasing the parasympathetic or reducing the sympathetic tone, acting centrally in the posttrauma area or peripherally, acting in the heart, kidney, and directly in the blood vessels, mediating vasoconstriction and vasodilation. Reduction in diastolic and systolic blood pressure during the night is the active part of the daily rhythm of blood pressure, and in humans these rhythms are directly controlled by melatonin. Moreover, as an important genetic factor, melatonin participates in programming of adult blood pressure in neonatal and fetal regulation (Baltatu et al. 2017).

9.6.2 Pharmacology and Function of M2 Receptor (Recent Update on Involvement of M2 in Normal and Pathophysiological States)

As already discussed, MT2 receptors remain low-affinity receptors which participate in the regulation of circadian rhythms, inflammation processes, and retinal and cardiac pharmacology.

9.6.2.1 Neurological Disorders

Recent data implicates the role of MT2 receptor in memory deterioration, neuroinflammation, and some other neurodegenerative disorders. Hippocampal expression of MT2 receptor is proved to decrease in AD while treatment drug ameliorates the memory dysfunction in CA1, CA2, CA3, and dentate gyrus region by upregulating the MT2 expression (Bahna et al. 2014). Hence, the melatonin system plays a significant role in the pathophysiology of AD. Besides the hippocampus, expression of MT2 receptor is reported to decrease in the pineal gland and occipital cortex of AD patients. AD involves mitochondrial dysfunction, neuroinflammation, and cerebral ischemia like abnormalities which accelerate oxidative stress to damage the neuronal function. Meanwhile, melatonin is well known for its antioxidant potential which serves via the activation of MT2 receptor. No doubt, the MT1 receptor is also present in the hippocampus but the comparatively high expression of MT2 receptor makes it more significant in AD pathology. Other major functions of MT2 receptor in the hippocampus are neurogenesis, neuronal differentiation, and neuronal survival (Ramírez-Rodríguez et al. 2009), and melatonin treatment proves to be neuroprotective over hippocampal neurons via upregulating the expression of MT2 receptor.

Several studies have assessed the neuroprotective potential of melatonin in Parkinson's disease (PD). Various models of PD such as MPTP, rotenone, and maneb have induced the PD through a similar mechanism of mitochondrial dysfunction, which is triggered by oxidative stress and apoptotic pathways. The antioxidant property of melatonin reported for therapeutic efficacy in PD-associated neurodegeneration. Even the neuroinflammation that occurs in PD gets significantly attenuated by melatonin treatment. These neuroprotective effects of melatonin contribute by the modulation of redox state in neuronal cells during disease conditions. Besides this, neurotrophic factors including nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF) are vital key components for neuronal survival in PD (Mack et al. 2016). Different PD studies have demonstrated that expression of BDNF gets reduced in SNc of PD patients whereas melatonin treatment using MT1/MT2 agonist agomelatine enhances the BDNF expression for neuroprotection in PD.

9.6.2.2 Metabolic Disorders

The crucial role of melatonin in metabolic disorders such as diabetes and obesity has also gathered specific attention of the researchers. Few studies have shown the

stimulatory action of melatonin on insulin secretion while some indicate it to inhibitory over the insulin release. An epidemiological study has suggested that the disruption of circadian rhythm in late night workers raises the chances of type-2 diabetes (T2D) in their life. Moreover, the presence of melatonin receptors in adipose tissues, liver, and muscles confirms their participation in glucose homeostasis. The gene encoding for MT2 receptor named MTNR1B is revealed to have a specific polymorphism that associates with T2D (Karamitri et al. 2018). The exact mechanism behind this is unclear but it may associate with the dysregulation of GPCR-dependent downstream signaling of MT2 receptor. Another study has also confirmed the protective role of melatonin against smoking-induced distortion of glucose homeostasis via activation of MT2 receptors (Filadelfi and Castrucci 1996; Cecon et al. 2018). The protective effect is also reported to potentiate via anti-inflammatory action which is receptor independent action.

In the case of obesity, the disease models of hibernating animals raise queries regarding the role of melatonin in weight gain. Before going to hibernation, these animals have more body weight as they take more food as energy reserve while the hibernation period involves a reduction in weight as food intake decreases with the reduction of energy consumption. Few animals of such models are reported to have variation in melatonin release with weight gain due to abnormal circadian rhythm. The evaluation of the model reveals the modulation of the sympathetic nervous system through melatonin receptors present on adipose tissue. Further genetic studies evaluate the role of melatonin on the regulation of body weight, lipid metabolism, and obesity (Karamitri and Jockers 2018). The polymorphism in the gene encoding for MT2 receptor in MTNR1B may associate with an increase in the body mass index and obesity.

9.6.2.3 Sleep, Addiction, and Behavioral Associated Disorders

The role of melatonin in the regulation of sleep disorders is not controversial today. Several drugs including melatonin and its agonist have gained reliability to treat insomnia and other sleep-related disorders. Novel compounds including melatonin, tasimelteon (nonselective MT1/MT2 agonist), and ramelteon (nonselective MT1/MT2 agonist) have raised the reliability of melatonin in clinical trials. Further in-depth analysis allows researchers to investigate the role of selective receptor compounds in circadian rhythm regulation. The presence of MT2 receptor upon GABAergic neurons enhances its consideration in research studies. As GABA, produce inhibitory and calming effect and the localized distribution of MT2 receptor on GABA neurons expected to increase the latency and maintenance of sleep (Comai and Gobbi 2014). The partial receptor agonist *N*-{2-[(3-methoxyphenyl) phenylamino] ethyl} acetamide (UCM765) has provided significant result in the preclinical model of NREM sleep. These positive results are achieved on reticular thalamic GABAergic neurons via the preclinical studies (Fig. 9.7).

Moreover, research studies also evaluate the role of melatonin in depression, anxiety, and pain. Firstly, the administration of melatonin gives antidepressant effects in depression-associated behavioral studies. A similar study has suggested that the significant result obtained by melatonin may mediate via the activation of

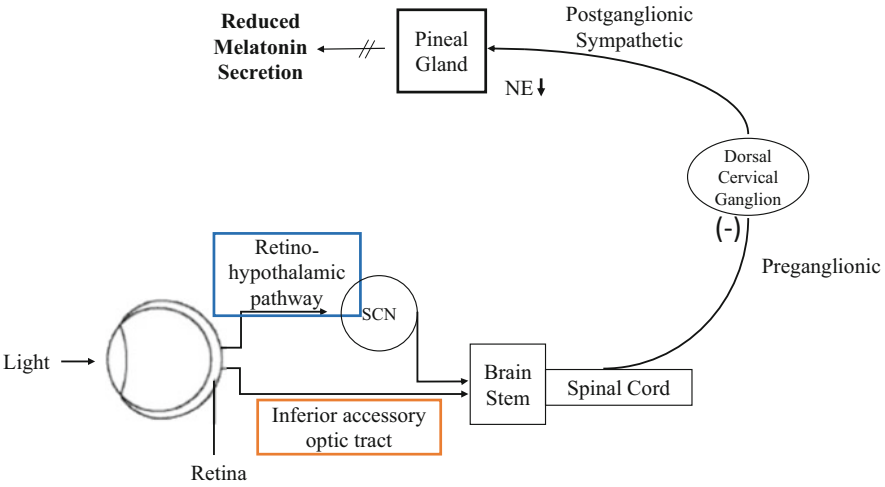

Fig. 9.7 Retinal pathway

MT2 receptor. Secondly, the identical receptor has been shown to have analgesic properties in hot water tail flick test (Yu et al. 2000). The positive result obtained from the administration of melatonin gets blocked by luzindole and 4P-PDOT which demonstrate the nociceptive action of MT2 receptor. Besides this, oral administration of melatonin decreases the flinching behavior associated with tactile allodynia in diabetic rats (Ambriz-Tututi and Granados-Soto 2007), and this analgesic effect is reported to get reversed by MT2 receptor antagonist (K-185).

9.6.2.4 Cardiovascular Diseases

The major source of melatonin is the pineal gland, but the presence of numerate extrapineal sources (mast cell, platelets, leukocytes, and endothelial cells) makes it possible that melatonin play important role in cardiac disorders. The functions like maintenance of blood vessel diameter regulate arterial blood pressure and blood flow in tissues and arteries. Experimental studies proposed the melatonin receptor MT1 and MT2 for their specific role in vasculature such as vasoconstriction and vasodilation respectively. A research study performed in porcine coronary arteries has accessed the inhibitory role of MT2 receptor in nitric oxide-induced relaxation (Tunstall et al. 2011). The positive significant effect of melatonin gets decreased via the MT2 receptor antagonist (4P-PDOT), which confirms it to be a novel candidate for coronary artery disorders.

Disorders such as ischemic reperfusion injury (IRI), myocardial infarction, and coronary heart disorders are usually mediated by cardiomyocyte damage that enhances the infarct size via microvascular dysfunction. The administration of melatonin has provided significant results in such disorders via receptor dependent or independent pathway. For example, the administration of luzindole like

nonselective antagonist of melatonin confirms it to be the receptor-dependent action of melatonin against IRI (Singhanat et al. 2018).

9.6.2.5 Cancer Studies

Cancer studies are associated with uncontrollable proliferation and activation of specific cell including gastric cell, skin cell, brain cell, etc. Numerous research studies have evaluated the efficacy of melatonin against a variety of cancer types including pancreatic cancer, skin cancer, liver cancer, breast cancer, and oral cancer via its protective action on cell function. The efficacy of melatonin has been assessed against skin cancer as MT1 and MT2 receptors are present on skin cells. MT1 receptor is specifically present on keratinocytes and epidermis layer while MT2 receptor is found to be localized on malignant and normal melanocytes (Pourhanifeh et al. 2019). Melatonin has been evaluated to have anticancer activities against UV rays and X-ray while the inhibition of cancerous growth in infected cells is reported to be mediated via the MT2 receptor. Moreover, the administration of melatonin also provides relief against glucocorticoid-induced suppression caused by neoplastic infections (Singh et al. 2017). Here, the immunomodulatory action in the spleen is attained via the activation of the immune response through overexpression of the MT2 receptor.

Besides this, melatonin acts as a physiological antagonist of serotonin in the gut via the activation of CCK-2, 5-HT3, and MT2 receptors which make it implement in gastric cancer (Asghari et al. 2017). The secretion of melatonin increases in the intestine during fasting conditions. It also initiates the secretion of mucosal bicarbonate by releasing calcium in enterochromaffin cell. Moreover, the activation of MT2 receptor causes the pancreatic secretion of amylase and cholecystokinin along with free radical scavenging action to heal the ulcerative conditions of the gastrointestinal tract.

9.6.3 Pharmacology and Function of M3 Receptor (Recent Update on Involvement of M1 in Normal and Pathophysiological States)

MT3 receptors are abundantly distributed in the peripheral tissue as well as the brain of hamster. It is believed that phosphoinositide hydrolysis is stimulated by these receptors. Both melatonin and its precursor (N-acetylserotonin) possess the ability to activate MT3 receptor, and they also possess pharmacological profile arranged according to affinities: the sequence includes 2-iodomelatonin > N-acetyl-serotonin > melatonin and the sequence is slightly similar to the human MT1/MT2 receptors (2-iodomelatonin > melatonin > N-acetyl-serotonin). Specific ligands for the MT3 melatonin receptor are N-acetyltryptamine and prazosin (Oxenkrug 2005). According to suggested hypothesis, mammalian MT3 site radioligand 2-[125I]-MCA-NAT binds to an enzyme quinone reductase 2 in the kidney membrane of hamster. The cloning of this enzyme is done followed by its purification from hamster kidney membranes. Decreasing intraocular pressure and inhibiting

leukocyte adhesion to vascular endothelial cells were reported when 5-MCA-NAT activates MT3 receptor (Doghramji 2007). Further investigation is needed to understand the binding affinities of MT3 and furthermore evaluating that MT3 melatonin binding proteins to represent a binding site for quinone reductase 2 or receptor to G-protein-coupled receptor. Structurally, this receptor is different from MT1 and MT2 receptors and revealed to be a classic low affinity melatonin membrane receptor (Tan et al. 2007). The temperature and binding rate based kinetics studies indicate it to be an enzyme rather than that of a receptor. And, mass spectroscopy and enzymatic studies confirmed it to be identical to quinoreductase 2 (QR2) which is a detoxifying agent. This property of MT3 receptor makes it relevantly applicable for therapeutic applications in different diseases. Currently, several studies are evaluating the therapeutic potential of MT3 receptor whether the signaling mechanism of MT3 receptor has not been discovered yet.

9.6.3.1 Cancer Studies

The MT3 receptor is reported to present in the liver, kidney, oocytes, brain, and ovaries. The MT3 receptor proves to be cytotoxic and proapoptotic in cancer studies which are performed on HT-29 cells (Nair et al. 2018). The anticancer potential of this receptor gets justified on the basis of its antioxidant efficacy. The MT3 receptor carries QR2 as binding site in its cleft, which makes it act as a detoxifying and antioxidant enzyme. An activation of QR2 converts the quinone reductase into more highly reactive species which exacerbates the cellular damage while the knockdown model of QR2 in K562 enhances the expression of antioxidant and detoxification enzymes to reduce proliferation rates in cancer studies. Hence, it is the inhibition of QR2 which regulates the antioxidant profile of the MT3 receptor.

9.6.3.2 Depression

Melatonin and its immediate precursor N-acetylserotonin (NAS) have been reported to possess antidepressant action in preclinical and clinical studies. Further different studies have evaluated the role of selective agonist and antagonist of MT3 receptor in depression studies which include 5-methoxycarbonylamino-*N*-acetyltryptamine (5MCA-NAT) and prazosin, respectively (Oxenkrug et al. 2010). The antidepressant activity of 5-MCA-NAT has been reported to provide significant results in tail suspension through QR2/MT3 receptor binding site which reverses via the administration MT3 receptor antagonist, prazosin. Another drug like resveratrol is also proven to be potent antagonists of QR2 binding site of MT3 receptor which may potentiate via the inhibition of indoleamine 2, 3-dioxygenase, a rate limiting step in tryptophan metabolism and kynurenine pathway. In other compounds like melatonin and 5-MCA-NAT, this effect may be achieved through competitive inhibition of tryptophan 2, 3-dioxygenase. In this way, the protective effect of melatonin via MT3 receptor may provide more reliable results in future preclinical and clinical studies.

9.7 Conclusion

Melatonin is a highly significant moiety, which is not only responsible for inducing sleep but also contributes in a versatile manner to control and coordinate the different biological processes of the body which includes maintenance of homeostasis, secretion of essential hormones, bowel movement, biological clock, circadian rhythm, and many more. MT1 and MT2 are the two important receptor types, and its subtypes are located at different regions of the body such as skin, parotid gland, colon, duodenal enterocytes, platelets, white and brown adipocytes, kidney, cells of the immune system, ovary/granulosa cells, placenta, and myometrium. These receptors are generally GPCR type, playing a major role at ground level to achieve various biological goals. Therefore, this luminary molecule is a crucial component which directs the smooth biological functioning of the human body.

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Pharmacology of Adenosine Receptors

10

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Abstract

Adenosine is an endogenous nucleoside molecule, regulating a myriad of physiological and pathological effects in almost all the organs systems including central nervous system (CNS), cardiovascular system (CVS), respiratory system, renal system, and immune system. Biological functions of adenosine are mediated by its interactions with four subtypes of G-protein-coupled receptors (GPCRs), namely A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ARs) which are ubiquitously present throughout the body. However, ubiquitous distribution of ARs in both healthy and diseased tissues imposed a great challenge to the researchers in the discovery and development of ligands targeting a particular AR subtype in a specific tissue, devoid of undesirable side effects. This chapter

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provides an overview of the synthesis, metabolism, and cellular transport of adenosine, with particular emphasis on the distribution and signaling mechanisms of ARs, including specific examples of agonists/partial agonists, antagonists, and allosteric modulators of ARs as potential therapeutic agents.

Keywords

Adenosine · A_1, A_{2A}, A_{2B} and A_3 adenosine receptors · G-protein-coupled receptors (GPCRs) · Adenosine receptors signaling

Abbreviations

AC	Adenylyl cyclase
ADA	Adenosine deaminase
AK	Adenosine kinase
AMP	Adenosine monophosphate
AR	Adenosine receptor
ARNO	ADP ribosylation factor nucleotide site opener
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
CADD	Computer-aided drug design
cAMP	Cyclic adenosine monophosphate
CNT	Concentrative nucleoside transporter
COPD	Chronic obstructive pulmonary disease
CREB	c-AMP-responsive element binding protein
DAG	Diacylglycerol
ENT	Equilibrative nucleoside transporter
ERK	Extracellular signal-regulated kinase
GPCR	G-protein-coupled receptor
GSK-3β	Glycogen synthase kinase-3β
HFpEF	Heart failure with preserved ejection fraction
iNKT cells	Invariant natural killer T cells
iNOS	Inducible nitric oxide synthase
IP_3	Inositol 1,4,5-triphosphate
IR	Ischemia-reperfusion
JNK	c-Jun N-terminal kinase
LBDD	Ligand-based drug design
MAPK	Mitogen-activated protein kinase
MPI	Myocardial perfusion imaging
OHT	Orthotopic heart transplantation
PAM	Positive allosteric modulator
PD	Parkinson's disease
PDEs	Phosphodiesterases
PKA	Protein kinase A
PKC	Protein kinase C

PLC	Phospholipase C
PLD	Phospholipase D
SAHH	*S*-adenosyl-homocysteine hydrolase
SAMe	*S*-adenosylmethionine
SBDD	Structure-based drug design
SPECT	Single photon emission computed tomography
TNFα	Tumor necrosis factor-alpha
TRAX	Translin-associated protein X
US FDA	United States Food and Drug Administration
USP4	Ubiquitin-specific protease

10.1 Introduction

Adenosine is an endogenous nucleoside molecule, regulating various physiopathological functions by interacting with four subtypes of G-protein-coupled receptors (GPCRs): A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ARs). The primary mechanism of signal transduction of A_1 and A_3 ARs involves the inhibition of adenylyl cyclase (AC), thereby reducing the cyclic adenosine monophosphate (cAMP), whereas the activation of A_{2A} and A_{2B} ARs results in the stimulation of AC and consequent increase in cAMP levels (Fredholm et al. 2001, 2011). However, adenosine shows varying affinity for ARs. In particular, A_1, A_{2A}, and A_3 ARs show moderate to high affinities towards adenosine, requiring only 10 nM to 1 μM concentration for their activation, whereas A_{2B} AR is comparatively a low affinity receptor which requires a higher concentration of adenosine (10 μM) for its activation (Borea et al. 2018a, b; Fredholm 2014). Table 10.1 provides the molecular characteristics and mechanism of action of adenosine receptors. All the ARs are ubiquitously present throughout the body, influencing various physiological and pathological processes of almost all the

Table 10.1 Molecular characteristics and mechanism of action of adenosine receptors

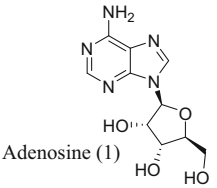
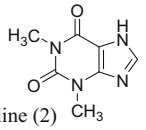
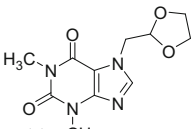
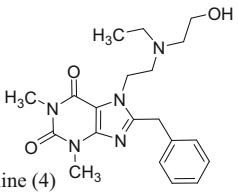
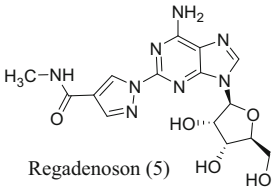
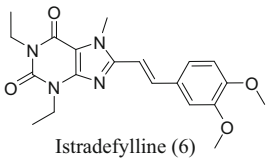
	A_1 AR	A_{2A} AR	A_{2B} AR	A_3 AR
Amino acid residues	326	410	328	318
Amino acid sequence similarity (%) vs hA_1AR		38.3	44.0	46.5
Amino acid sequence similarity (%) vs $hA_{2A}AR$			46.6	31
Amino acid sequence similarity (%) vs $hA_{2B}AR$				35.7
Affinity for adenosine (nM)	1–10	30	1000	100
G-protein coupling	$G_{i/o}$	G_s	$G_sG_{q/11}$	$G_sG_{q/11}$
Signaling system	↓AC, ↑PLC ↑PI3 kinase ↑MAPK, ↑K^+, Ca^{2+}	↓AC, ↑MAPK	↓AC, ↑PLC, ↑MAPK	↓AC, ↑PLC, ↑PI3 kinase, ↑MAPK

organ systems including central nervous system (CNS), cardiovascular system (CVS), respiratory system, renal system, and immune system among others. Thus, ARs represent potential drug targets for various therapeutic interventions (Borea et al. 2018a, b, 2016). Various agonists/partial agonists, antagonists, and allosteric modulators of A_1, A_{2A}, A_{2B}, and A_3 ARs have been discovered, patented, and are currently being investigated in clinical trials (Al-attraqchi et al. 2019; Borah et al. 2019; Chandrasekaran et al. 2019; Deb 2019a, b; Deb et al. 2019a, b; Mailavaram et al. 2019). But only few molecules could successfully reach the market either due to their poor pharmacokinetic profiles or because of the ubiquitous distribution of the ARs both in normal and diseased tissues imposing nonspecific actions or undesirable side effects of the drugs (Borea et al. 2018a, b; Chandrasekaran et al. 2019; Shaik et al. 2019). Istradefylline, the selective A_{2A} AR antagonist, was initially marketed in Japan (2013) for the treatment of Parkinson's disease (PD), but recently (2019) it has got approval from the US FDA as an add-on treatment to levodopa/carbidopa for PD (Hoffman 2019; Voelker 2019). Table 10.2 provides a list of clinically approved drugs and their therapeutic applications targeting ARs. Furthermore, growing advancement in the computer-aided drug design (CADD) software tools and algorithms has been significantly facilitating both the ligand-based and structure-based drug design (LBDD and SBDD) strategies for the discovery and development of novel drugs targeting ARs (Agrawal et al. 2019; Al-Shar'i Nizar and Al-Balas 2019; Deb 2019c; Deb et al. 2018a, b; Deb et al. 2019a, b; Kishore et al. 2011; N et al. 2019; Samanta et al. 2019). In particular, the recent discovery of the 3D crystal structure of A_1 AR (Cheng et al. 2017; Glukhova et al. 2017) along with the previously identified 3D structure of A_{2A} AR (Jaakola et al. 2008) has augmented the understanding of the molecular structures of ARs as well as physicochemical requirements of ligands for selective binding with ARs. This chapter highlights the synthesis, metabolism, and cellular transport of adenosine, with particular emphasis on the body distribution and signaling mechanisms of ARs in various physiological and pathological conditions. Important examples of agonists/partial agonists, antagonists, and allosteric modulators of ARs and their pathophysiological roles are also briefly discussed.

10.2 Synthesis, Metabolism, and Cellular Transport of Adenosine

Adenosine metabolism plays an important role in regulating various pathophysiological functions of the body. In physiological conditions, adenosine is available in low concentration (20–300 nM). However, under metabolic stressful conditions including pain, inflammation, and various disease states, extracellular adenosine concentration increases up to 30 μM due to ATP catabolism, where adenosine exhibits a helper/protective role by restoring the imbalance between energy demand and availability of working cells like neurons and cardiomyocytes by adapting some of their activities such as reducing heart inotropic effect, increasing oxygen and nutrition supply through vasodilation, thereby reducing the ATP requirement (Borea

Table 10.2 Therapeutic applications of clinically approved drugs targeting ARs

Name and structure of drugs	Mechanism of actions	Therapeutic applications
 Adenosine (1)	A_1 AR agonist	Paroxysmal supraventricular tachycardia (PSVT)
	A_{2A} AR agonist	Myocardial perfusion imaging
 Theophylline (2)	A_1 AR antagonist	Treatment of asthma
 Doxofylline (3)	A_1 AR antagonist	Treatment of asthma
 Bamifylline (4)	A_1 AR antagonist	Treatment of asthma
 Regadenoson (5)	A_{2A} AR agonist	Myocardial perfusion imaging
 Istradefylline (6)	A_{2A} AR antagonist	Adjuvant therapy of Parkinson's disease

et al. 2016, a, 2018b). Because of these protective roles, adenosine is considered as a "retaliatory metabolite" rather than a secondary metabolite of cAMP pathway (Newby 1984). Adenosine facilitates tissue protection from ischemic damage via preconditioning cell as well as exerting anti-inflammatory response and promoting angiogenesis (Linden 2005).

In physiological conditions, adenosine is synthesized intracellularly from AMP and *S*-adenosyl-homocysteine (SAH) hydrolysis by endo-5′-nucleotidase and *S*-adenosyl-homocysteine hydrolase (SAHH), respectively (Chen et al. 2013). It should be noted that the SAH hydrolysis leading to the formation of adenosine and homocysteine is a reversible process. The formation of SAH from adenosine and homocysteine is mainly favored under thermodynamic equilibrium conditions, consequently inhibiting the *S*-adenosylmethionine (SAMe) transmethylation due to increased levels of SAH. Thus, an effective decrease in adenosine levels mainly by adenosine kinase (AK) triggers the transmethylation process. Therefore, SAHH can facilitate both the synthesis and removal of adenosine (Bjursell et al. 2011; Finkelstein 1998; Moffatt et al. 2002). Extracellularly, adenosine is mainly produced under stressful conditions in high concentrations from the ATP, ADP, and AMP dephosphorylation with the help of two hydrolyzing enzymes, namely ectonucleosidase triphosphate diphosphohydrolase (CD39) and ecto-5′-nucleotidase (CD73), respectively (Zimmermann 2000). Additionally, extracellular conversion of cAMP to AMP with the help of ecto-phosphodiesterase (ecto-PDE) can further trigger the formation of adenosine via CD73 (Godinho et al. 2015; Pleli et al. 2018; Sassi et al. 2014).

Adenosine, once generated, travels across the cell membrane with the help of concentrative nucleoside transporters (CNTs) and equilibrative nucleoside transporters (ENTs). There are three isoforms of energy-dependent cation-linked (Na^+) CNTs (1–4) facilitating adenosine influx and four energy-independent isoforms of ENTs (1–3) which can assist in influx or efflux based on the concentration of adenosine. In general, adenosine influx takes place from extracellular to intracellular region, whereas the reverse condition is evident in hypoxia (Bading et al. 1993; Deussen 2000; Deussen et al. 1999).

Biotransformation of adenosine inside the cell takes place by hydrolysis to SAH, phosphorylation to AMP, and deamination to inosine with the help of SAHH, adenosine kinase (AK), and adenosine deaminase (ADA), respectively. Under physiological conditions, AK is mainly responsible for adenosine metabolism, whereas under pathological conditions, ADA preferentially facilitates adenosine clearance. Extracellular adenosine clearance occurs through ecto-ADA and influx through ENTs (Boison 2018; Boison et al. 2013; Gracia et al. 2012; Pacheco et al. 2005). Figure 10.1 represents the synthesis, metabolism, and cellular transportation of adenosine.

10.3 Molecular Structure of Adenosine Receptors (ARs)

All the four subtypes of ARs present common molecular structure arrangement, composed of seven transmembrane helices (TMs 1–7) which are connected to each other through three intracellular loops (ILs 1–3) and three extracellular loops (ELs 1–3) of varying lengths and functions. These three ELs play important roles in mediating receptor functions, where cysteine residues connect these ELs by forming disulfide bonds. The N-terminal containing glycosylation site is present on the

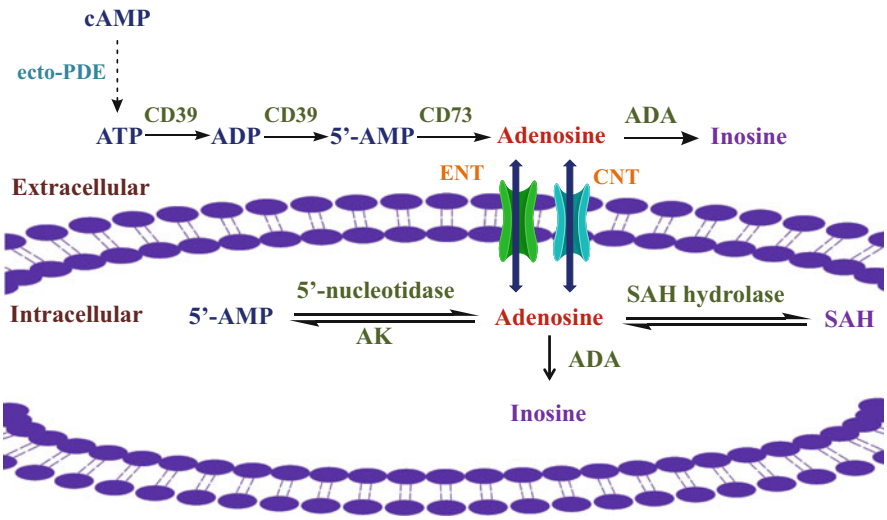


Fig. 10.1 Synthesis, biotransformation, and cellular transportation of adenosine

extracellular region, while the intracellular C-terminal possesses phosphorylation and palmitoylation sites that are responsible for desensitization and internalization of the receptor. The A_{2A} AR possesses longer C-terminal (122 amino acid residues) as compared to A_1, A_{2B}, and A_3 ARs (30–40 amino acid residues). Adenosine receptors present 41–58% amino acid sequence similarity among human species (Table 10.1) (Fredholm et al. 2001, 2011, 2000). Among all the subtypes, only the crystal structures of A_1 AR (Cheng et al. 2017; Glukhova et al. 2017) and A_{2A} AR (Jaakola et al. 2008) have been resolved, based on which several homology models of A_{2B} and A_3 ARs have been constructed to gain insight into their binding interactions with both agonists and antagonists ligands as well as to facilitate the structure-based drug design (Deb et al. 2018a, 2018b; Gutiérrez-de-Terán et al. 2017). ARs also exist in the form of homomer, heteromer, and oligomers, such as A_1 AR-A_{2A} AR, A_1 AR-A_3 AR, A_{2A} AR-D_2 dopamine receptor. In particular, A_{2A} AR-D_2 dopamine receptor complex that is present in striatum is considered as a significant therapeutic target for the treatment of Parkinson's disease (Brugarolas et al. 2014; Ferre et al. 2010; Navarro et al. 2016).

10.4 Distribution of Adenosine Receptors

Adenosine receptors are distributed throughout the cardiovascular, nervous, gastrointestinal, respiratory, urogenital, as well as immune systems. ARs were also detected in bones, eyes, joints, and skin (Peleli et al. 2017). Each subtype has a distinctive cell and tissue distribution, signaling transductors, and hence unique physiological effects (Fredholm et al. 2001).

10.4.1 Distribution of A_1 AR

A_1AR has shown a high abundance in the brain as well as other organs and tissues. This receptor subtype has been demonstrated by radioligand-receptor binding studies and imaging (Elmenhorst et al. 2012; Hayashi et al. 2017), along with RNA expression, Western blot, as well as functional characterization. Therefore, the wide distribution of this receptor has suggested its important physiological roles including spanning neurotransmitter release, neuronal excitability dampening, sleep/wakefulness control, reduction of pain, along with the sedative, anxiolytic, anticonvulsant, as well as locomotor depressant effects (Gessi et al. 2011; Sawynok 2016). In the central nervous system (CNS), A_1AR is mainly expressed in the brain cortex, hippocampus, cerebellum, spinal cord, autonomic nerve terminals, and glial cells (Ballesteros-yáñez et al. 2018; Chen et al. 2013). In the heart, the expression of A_1AR has been shown to be higher in atria and much less in the ventricular myocardium (Stenberg et al. 2003; Varani et al. 2017). At the vascular level, A_1ARs are found on the coronary smooth muscle arteries as well as endothelial cells (Headrick et al. 2013). Moreover, A_1ARs have been detected in the endothelial cells of the lung, in the airway's smooth muscles, in the alveolar epithelial cells, and in immune cells such as macrophages, neutrophils, eosinophils, and monocytes (Boros et al. 2016; Sachdeva and Gupta 2013; Sun et al. 2005), where they essentially promote some proinflammatory effects (Ponnoth et al. 2010). A_1AR is also found in the kidney, adipose tissue, and pancreas, where it causes induction of negative chronotropic, inotropic, as well as dromotropic effects, reduction in the renal blood flow and renin release, and inhibition of lipolysis and insulin secretion, respectively (Dhalla et al. 2009; Prystowsky et al. 2003; Rabadi and Lee 2015; Sun et al. 2001; Vallon and Mu 2006; Vincenzi et al. 2012). In the kidney, A_1ARs mostly present in the papilla's collecting ducts, inner medulla, in addition to the cells of the juxtaglomerular apparatus. A_1ARs have been also detected in the retina, skeletal muscle, intestine, and vascular cells of skeletal muscle (Soni et al. 2017; Varani et al. 2017).

10.4.2 Distribution of A_{2A} and A_{2B} ARs

The A_{2A} AR is present centrally and peripherally, where it serves a number of functions that are related to excitotoxicity, the release of spanning neuronal glutamate, glial reactivity, the permeability of the blood-brain barrier (BBB), as well as the migration of the peripheral immune cells (Koupenova et al. 2012; Merighi et al. 2015; Pedata et al. 2016), and greatly expressed in the striatum, the olfactory tubercle, as well as the immune system. However, lower levels are present in the cerebral cortex, heart, hippocampus, lung, and blood vessels. In the peripheral immune system, A_{2A} AR has been shown to have a great expression particularly in leukocytes, platelets, as well as the vasculature, in which it mediates numerous anti-inflammatory, antiaggregatory, as well as vasodilatory effects, respectively (Ruiz et al. 2014). A_{2A} ARs are found in the bowel, lung, bladder, vas deferens, as

well as in other different cell types such as fibroblasts, smooth muscles, alveolar epithelial, chromaffin, and taste cells, platelets, myocardial cells, and retinal, intestinal, endothelial and pulmonary epithelial cells (Aherne et al. 2011).

It has been shown in recent development of A_{2B}AR-knockout/lacZ-knocking mice (Yang et al. 2006) that A_{2B} AR has a wide distribution in numerous tissues and organs, and this includes the aortic vascular smooth muscle, vasculature, cecum, brain, large intestine, and urinary bladder (Wang and Huxley 2006; Yaar et al. 2005). Moreover, A_{2B} AR was found to be highly expressed in various cell types, including several immune cells such as mast cells (Hua et al. 2007; Yang et al. 2006), neutrophils (Ryzhov et al. 2008), dendritic cells (Addi et al. 2008), macrophages (Novitskiy et al. 2008), as well as lymphocytes (Yang et al. 2006), in addition to other cell types that include the type II alveolar epithelial cells (Eckle et al. 2008), endothelial cells (Cagnina et al. 2009), chromaffin cells (Yang et al. 2006), astrocytes (Peakman and Hill 1994), neurons (Christofi et al. 2001), and taste cells (Stein et al. 2001).

10.4.3 Distribution of A_3 AR

The identification of the A_3 AR distribution has been made possible after the generation of cDNA for this receptor (Nishida et al. 2014). The A_3 AR subtype was found to have wide expression in various primary cells, tissues, as well as cell lines. In the brain, A_3AR has been reported in low levels, where it is expressed particularly in the hypothalamus, thalamus, hippocampus, cortex, as well as retinal ganglion cells, and motor nerve terminals, in addition to the pial and intercerebral arteries (Burnett et al. 2010; Janes et al. 2014). Studies have also shown that the expression of A_3 ARs is also reported in microglia and astrocytes; thus inhibiting the neuro-inflammatory response in these particular cells was shown to be associated with the analgesic effect they induce (Borea et al. 2016). Despite the cardioprotective effects that have been related to the A_3 AR, as well as the great expression of this receptor subtype in the coronary and carotid artery, its precise location in the heart is not yet reported. At the periphery, A_3 AR was found to be expressed in enteric neurons, epithelial cells, lung parenchyma, colonic mucosa, and bronchi. Moreover, a broad distribution of A_3 AR subtype has been reported in inflammatory cells (Janes et al. 2014) including mast cells, eosinophils, monocytes, neutrophils, macrophages, dendritic cells, foam cells, lymphocytes, bone marrow cells, splenocytes, lymph nodes, chondrocytes, synoviocytes, as well as osteoblasts, where it is responsible for mediating various anti-inflammatory effects (Borea et al. 2015). It is worth mentioning that A_3 AR subtype is overexpressed in some cancer cells and tissues, which therefore shows the important antitumoral role of this receptor subtype (Borea et al. 2016). At cellular level, A_3 ARs have shown wide expression in motor nerve terminals, astrocytes, microglia, cortex, as well as retinal ganglion cells (Borea et al. 2015; Gessi et al. 2013).

10.5 Signal Transduction Pathways of Adenosine Receptors

Numerous signal transduction pathways are triggered by all the four G-protein-coupled ARs based on the activation of a particular type of cell (Fredholm et al. 2001, 2011).

10.5.1 Molecular Signaling of A_1 AR

The activation of the Gi-protein-coupled A_1 AR causes inhibition of adenylyl cyclase (AC), leading to the reduction of cyclic adenosine monophosphate (cAMP) production (Fredholm et al. 2000), resulting in the reduction of cAMP-dependent protein kinase A (PKA) and cAMP-responsive element-binding protein 1 (CREB-1) phosphorylation (Ellis et al. 1995). A_1 AR can stimulate the phospholipase C (PLC)-β, increasing diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3) levels, thus enhancing calcium (Ca^{2+}) concentrations inside the cell, stimulating the activation of Ca^{2+}-dependent protein kinase C (PKC) and/or other binding proteins (Basheer et al. 2002; Biber et al. 1997; Borea et al. 2018a, b; Nalli et al. 2014). Activation of A_1 AR also results in the opening of potassium (K^+) channels in neurons and cardiac tissue, while inhibiting Q, P, and N-type Ca^{2+} channels (Kirsch et al. 1990; Kunduri et al. 2013; Schulte and Fredholm 2003, 2000). Additionally, A_1 AR activation is also linked to the phosphorylation of mitogen-activated protein kinases (MAPK) like p38, ERK1/2, and JNK (Schulte and Fredholm 2003, 2000). The signal transduction pathway of A_1 AR is depicted in Fig. 10.2.

10.5.2 Molecular Signaling of A_{2A} AR

The activation of Gs-protein-coupled A_{2A} AR triggers AC activity and increases the cAMP levels, thereby stimulating PKA which causes phosphorylation and further activation of several proteins including receptors, PDEs, CREB, and dopamine- and c-AMP-regulated phosphoprotein (DARPP-32) (Preti et al. 2015). Additionally, A_{2A} ARs inside the brain can stimulate neuron-specific Gs-protein called G_{olf} that is also connected to c-AMP (Kull et al. 2000). Moreover, in the brain, adenosine level increases following ischemia-reperfusion injury leading to the stimulation of A_{2A} AR resulting in the potentiation of neuronal damage by increasing ERK and consequent stimulation of microglial activation, glial TNFα, glutamate, iNOS, and apoptosis (Mohamed et al. 2016). In the artery of rat tail, it has been observed that A_{2A} AR can also regulate the release of norepinephrine through the stimulation of both PKC and PKA (Fresco et al., 2004). A_{2A} AR is also found to bind with the help of its C-terminus with various other proteins such as dopamine D_2 receptor, α-actinin, ARNO, USP4, and TRAX (Baraldi et al. 2008). Importantly, A_{2A} AR can also modulate the signaling of MAPK (Baraldi et al. 2008; Chen et al. 2013). A_{2A} AR activation also plays an important role in cancer cells by stimulating

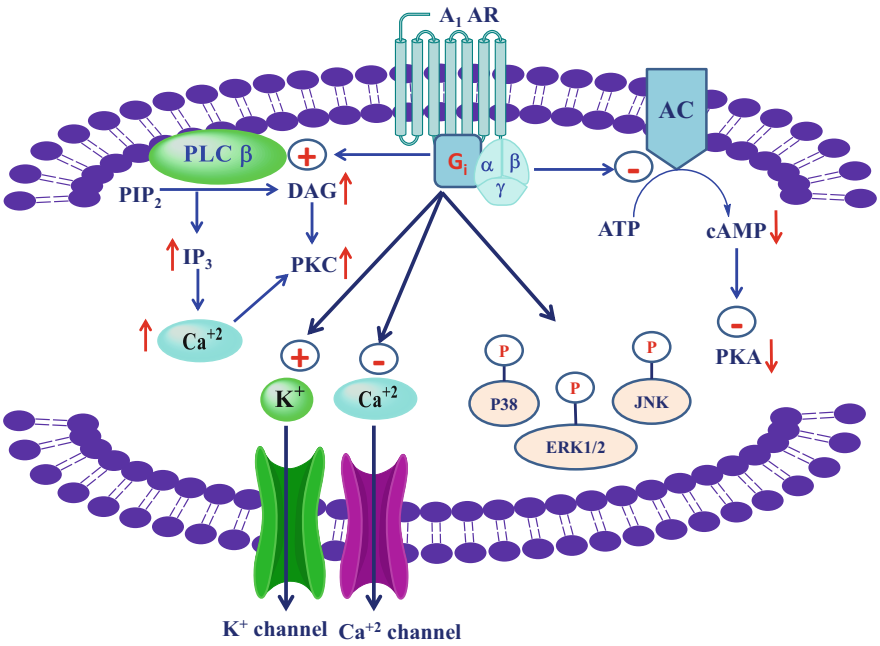


Fig. 10.2 Molecular signal transduction pathways of A_1 AR

proliferation PLC, PKC-δ, ERK, JNK, and AKT (Gessi et al. 2017). Signal transduction pathway of A_{2A} AR is depicted in Fig. 10.3.

10.5.3 Molecular Signaling of A_{2B} AR

Similar to the A_{2A} AR subtype, the A_{2B} AR is also coupled to Gs protein, triggering the AC activity and thereby increasing the cAMP levels, PKA phosphorylation, and cAMP-dependent recruitment of different effectors like exchange proteins (Epac) (Fredholm et al. 2011). A_{2B} AR-stimulated activation of Epac was also found to affect the proliferation of umbilical vascular endothelial cells and induce early gene expression reducing the proliferation of smooth muscle cells of coronary artery in humans (Fang and Olah 2007; Mayer et al. 2011). Unlike A_{2A} AR, the A_{2B} AR is also coupled to Gq protein, stimulating PLC leading to Ca^{2+} mobilization, while regulating the ion channels through the recruitment of γ subunits. A_{2B} AR can regulate various pathophysiological functions in the central and peripheral system through the activation of MAPK and AKT (Sun and Huang 2016). Additionally, A_{2B} AR responses can be influenced by its various binding partners like netrin-1, E3KARP-EZRIN-PKA, SNARE, NF-κB1/P105, and α-actinin-1. In particular, the neuronal guidance protein netrin-1 can bind and activate A_{2B} AR during hypoxia, reducing the migration of neutrophils and consequent inflammation (Rosenberger

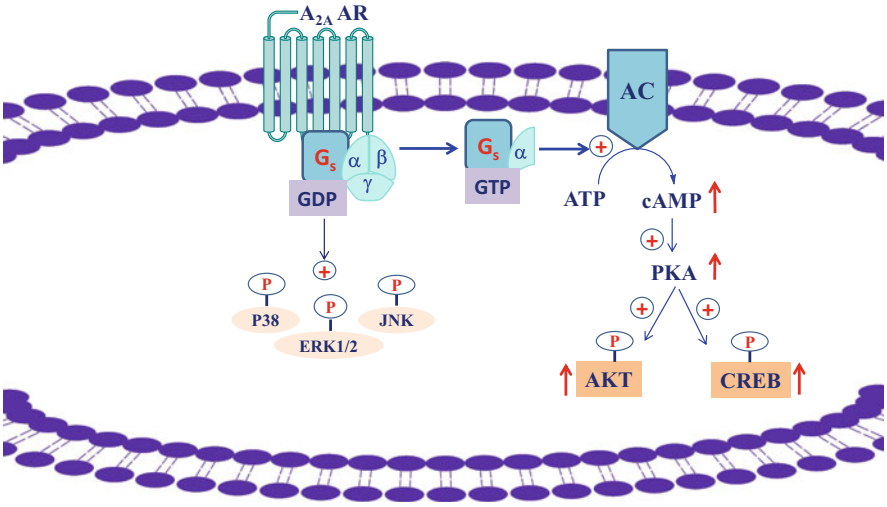


Fig. 10.3 Molecular signal transduction pathways of A_{2A} AR

et al. 2009). SNARE protein can bind and translocate the A_{2B} AR from the cytoplasm to the plasma membrane following agonist binding (Wang et al. 2004) and consequently, a multiprotein complex with E3KARP (NHERF2) and ezrin enables the fixation/stabilization of the A_{2B} AR at the cell surface (Sitaraman et al. 2002). Interestingly, α-actinin-1 can promote the dimerization of A_{2A} and A_{2B} ARs, inducing the cell surface expression of the later (Moriyama and Sitkovsky 2010). Furthermore, interaction of P105 with A_{2B} AR has shown to reduce the inflammatory effects of NF-κB (Sun et al. 2012). Recently, it has been reported that the stimulation of A_{2B} AR reduces ERK1/2, p38, and NF-κB induced by RANKL, thereby reducing osteoclastogenesis in bone (Kim et al. 2017). Several reports also indicate the role of A_{2B} AR signaling in neuroinflammation (Koscsó et al. 2012; Merighi et al. 2017), inflammatory bowel disease (Chin et al. 2012; Dammen et al. 2013), cardiac ischemic preconditioning (Yang et al. 2011), atherosclerosis development (Gessi et al. 2010a), and reduction of cardiac fibrosis (Phosri et al. 2018, 2017). The signal transduction pathway of A_{2B} AR is depicted in Fig. 10.4.

10.5.4 Molecular Signaling of A_3 AR

The A_3 AR subtype is coupled to Gi protein and inhibits AC with consequent reduction of the cAMP levels, while at high concentrations of agonist, A_3 AR couples to Gq protein, thereby stimulating PLC and increasing the Ca^{2+} release from the intracellular storage (Borea et al. 2018a, b). A decrease in cAMP level further causes inhibition of PKA leading to increase in glycogen synthase kinase-3β (GSK-3β); decrease in β-catenin, cyclin D1, and c-Myc; and reduction of NF-kB

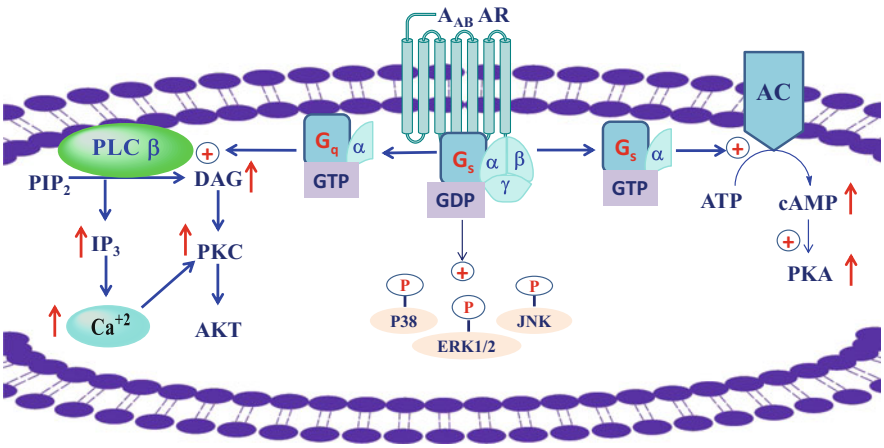


Fig. 10.4 Molecular signal transduction pathways of A_{2B} AR

DNA binding capability (Fishman et al. 2012, 2004, 2002; Stemmer et al. 2008). A_3 AR facilitated neuro- and cardio-protection is regulated via different signaling pathways including G-protein RhoA and phospholipase D (PLD) (Borea et al. 2018a, b). A_3 AR-mediated anti-inflammatory effects are regulated through MAPK, PI3/Akt, and NF-kB transduction pathways (Ochaion et al. 2008). A_3 AR is also found to induce ERK1/2 and proliferation of cells in human fetal astrocytes, microglia, glioblastoma, and melanoma among others (Hammarberg et al. 2003; Merighi et al. 2007; Neary et al. 1998; Soares et al. 2014). Interestingly, reduced ERK activation was also evident in melanoma, prostate cancer, and glioma cells, decreasing the proliferation of cells and release of TNF-α (Hyun et al. 2012; Martin et al. 2006). Activation of A_3 AR also modulates p38 and JNK in various cell types including cancer cells like colon carcinoma (Gessi et al. 2010b). The signal transduction pathway of A_3 AR is depicted in Fig. 10.5.

Readers are also encouraged to read the valuable chapter written by Merigi et al., highlighting various research findings showcasing the involvement of AR signaling in diverse pathophysiological conditions (Merighi et al. 2018).

10.6 Agonists, Partial Agonists, Antagonists, and Allosteric Modulators of Adenosine Receptors

10.6.1 Agonists of Adenosine Receptors

Important agonists of adenosine receptors are presented in Fig. 10.6.

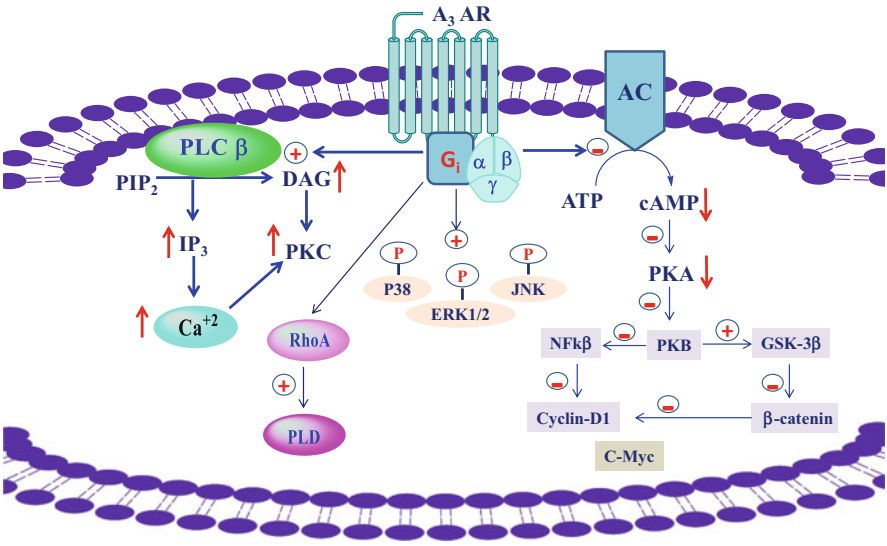


Fig. 10.5 Molecular signal transduction pathways of A_3 AR

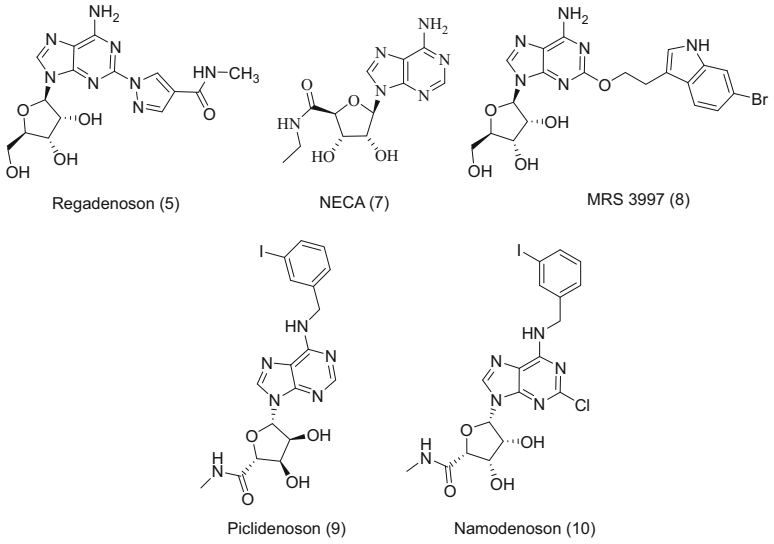


Fig. 10.6 Important agonists of ARs

10.6.1.1 Regadenoson

Regadenoson (5), a selective A_{2A} adenosine receptor agonist, was approved by the FDA (Food and Drug Administration) in 2008 in the injection form as a pharmacologic stress agent for patients unable to perform adequate exercise in order to increase blood flow in coronary arteries for myocardial perfusion imaging (MPI)

test (Thompson 2008). Regadenoson produces coronary arteries vasodilation by selectively activating A_{2A} AR; however it shows a very weak agonist activity on A_1 receptors and a negligible affinity for A_3 and A_{2B} adenosine receptors. Regadenoson has longer half-life than adenosine (Vij et al. 2018).

Following the approval of the FDA for regadenoson, many diverse clinical trials have been performed for the diagnosis and treatment of cardiovascular conditions. For instance, a phase IIIb study (NCT01618669) sponsored by Astellas Pharma Inc. on 1147 participants was conducted to compare between administration of regadenoson after inadequate exercise and administration of regadenoson without exercise for MPI by using single photon emission computed tomography (SPECT). Results have shown that the administration of regadenoson after 3 min of inadequate exercise is well tolerated with careful monitoring in patients without signs and symptoms of ischemia during exercise or after (Thomas et al. 2017). A study on 123 patients to determine the safety of regadenoson stress testing after orthotopic heart transplantation (OHT) has shown that dyspnea was the most common side effect with 66.7% of patients. However, there were no serious adverse effects such as hemodynamic changes and life-threatening arrhythmias which supports its safety and tolerability in OHT patients (Lazarus et al. 2018). Several studies have shown that dyspnea (the most common side effect) is not caused by bronchoconstriction, which makes regadenoson administration safe for patients with mild to moderate COPD and mild to moderate asthma (Golzar and Doukky 2014; Raines et al. 2019).

Agonists of A_{2A} AR have shown to decrease hypoxia/reoxygenation-induced tissue inflammation in mice with SCD (sickle cell disease). A_{2A} agonists reduced invariant natural killer T (iNKT) cells activation, which is higher than normal in patients with SCD. A phase II randomized trial (NCT01788631) on patients with SCD was conducted to test whether regadenoson can reduce iNKT cells activation and vaso-occlusive crises. After 48-h infusion of regadenoson (1.4 mg/kg/h) during vaso-occlusive crises the patients did not show significant decrease in iNKT cells activation as compared to placebo patients which indicates that regadenoson infusion in low doses is not sufficient to induce a significant reduction in iNKT cells activity (Field et al. 2019). The iNKT cells are also activated after lung transplantation due to activation of NOX2 (NADPH oxidase 2) causing ischemia-reperfusion (IR) injury following lung transplantation, and the activation of iNKT cells and NOX2 increases the production of interleukin-17 (IL-17). An *in vivo* study showed that A_{2A} receptor agonists attenuate the production of IL-17 and reduce IR injury in murine and human iNKT cells which indicates that A_{2A} AR agonists offer a possible therapeutic strategy to prevent IR injury and graft dysfunction (Sharma et al. 2016).

Regadenoson has also shown to cause BBB disruption in healthy rodents, which presents a potential solution for the limitations caused by the BBB in preventing many therapeutic agents including chemotherapy to reach the brain in higher concentrations. In a study on healthy rodents, regadenoson increased the concentration of temozolomide (a chemotherapeutic agent used in the treatment of glioblastoma) (Jackson et al. 2016). However, a clinical trial (NCT02389738) by Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins included six patients with

recurrent glioblastoma that received regadenoson with temozolomide. Results showed no increase in temozolomide concentration in brain unlike previous studies on rodents indicating that further studies and trials with different doses are needed for determining the optimum regadenoson dose to induce the desired BBB disruption and increase chemotherapeutic agent concentration in CNS. Another phase I trial (NCT03971734) is estimated to start in March 2020 by the same cancer center to determine regadenoson dose that can alter BBB integrity in patients with high-grade gliomas (Jackson et al. 2018).

Approximately 2–8% of patients experienced gastrointestinal side effects including abdominal discomfort, diarrhea, and nausea after receiving regadenoson with higher side effects frequency in patients with advanced renal disease. However, in 2017, there has been 11 cases of partial seizures and seizure-like adverse effects and 55 cases of convolutions reported to the FDA, which resulted in The American Society of Nuclear Cardiology (ASNC) guidelines to consider seizure disorders a relative contraindication with regadenoson administration (Andrikopoulou and Hage 2018; Henzlova et al. 2016).

10.6.1.2 NECA

In recent years, adenosine receptors have shown to be possible pharmacological targets to alter BBB integrity. A study included intravenous administration of NECA (5′-N-ethylcarboxamide adenosine) (6), a nonselective ARs agonist, has resulted in increasing brain concentration of dextrans (both low molecular weight and high molecular weight). However, NECA pharmacological effect was dose-specific, producing highest effect at 0.08 mg/kg; lower or higher doses showed less effect. It was interpreted that doses higher than 0.08 mg/kg of NECA showed less effect due to adenosine receptors desensitization. The fact that adenosine receptor agonists can be found in the market and are clinically approved makes these findings even more valuable presenting a possible less invasive method for BBB disruption (Carman et al. 2011; Cheng et al. 2016; Malpass 2011).

NECA intraperitoneal administration has shown to increase fasting serum glucose level. Further investigation showed that NECA administration has elevated glucose 6-phosphatase (G6Pase) enzyme mRNA leading to an increase in the liver G6Pase enzyme and gluconeogenesis, which is thought to be the cause for serum glucose elevation (Matsuda et al. 2014). NECA has also been studied for reducing intestinal IR injury in rats. Results showed that NECA reduced leukocyte activation and caused a significant improvement in capillary perfusion, thus reducing intestinal IR injury (Zhou et al. 2015).

10.6.1.3 MRS 3997

MRS 3997 (7) is a potent adenosine receptor agonist that activates mainly A_{2A} and A_{2B} AR and acts as a weak agonist for A_1 and A_3 ARs (Adachi et al. 2007; Gao et al. 2014).

10.6.1.4 Piclidenoson, CF101

Piclidenoson or CF101 (8) is a highly specific A_3 AR agonist that has proven to have an anti-inflammatory effect in many preclinical studies for conditions such as uveitis, rheumatoid arthritis, colitis, and osteoarthritis. Piclidenoson mechanism of action is mainly through the downregulation of NF-κB signaling pathway which causes an inhibition in TNF-α. Phase II clinical studies of piclidenoson on patients with plaque psoriasis have shown its efficacy in reducing signs and symptoms (Cohen et al. 2018).

A phase IIb clinical study (NCT01034306) utilizing piclidenoson as a monotherapy drug was conducted on 79 patients with rheumatoid arthritis sponsored by Can-Fite BioPharma. After 12 weeks of twice daily administration of 1 mg of piclidenoson or placebo, the patients treated with piclidenoson showed a significant improvement compared to placebo and reduction in rheumatoid arthritis symptoms, supporting previous clinical studies (Fishman and Cohen 2016; Stoilov et al. 2014). The same company is currently developing piclidenoson in an oral form as a first-line treatment for patients with moderate to severe plaque psoriasis (Fellner 2016).

10.6.1.5 Namodenoson, CF102

Namodenoson (CF102) (9) is a potent and selective A_3 AR agonist that is considered safe and tolerable after phase I and II (NCT00790218) clinical trials for hepatocellular carcinoma in combination with sorafenib. In those trials namodenoson has caused an increase in the median overall survival by approximately 7 months (Stemmer et al. 2013). Namodenoson has been tested in a phase II trial (NCT02128958) as a second-line treatment of Child-Pugh B (CPB) advanced hepatocellular carcinoma (HCC). Despite the fact that the primary end point has not been met in this trial, the median overall survival of CPB patients increased. Namodenoson was well tolerated by patients and considered safe for further phase III trials. Adverse effects that were observed in almost $>10\%$ of the patients were nausea, fatigue, anemia, asthenia, peripheral edema, and abdominal pain (Stemmer et al. 2019).

10.6.2 Partial Agonists of Adenosine Receptors

Important partial agonists of adenosine receptors are presented in Fig. 10.7.

10.6.2.1 CVT 2759

CVT-2759 (10) is a partial A_1 AR agonist that has shown to have the ability to selectively inhibit AV conduction in a moderate rate without causing an AV block despite application of high concentrations. It means that CVT-2759 has the ability to cause a predictable moderate inhibition on the AV nodal conduction while avoiding the risk of AV blockage. It has been observed in these studies that CVT-2759 has a minimum effect on the sinoatrial rate or on action potential durations (ventricular and atrial) (Szentmiklósi et al. 2015; Wu et al. 2001). Accordingly, CVT-2759 administration does not induce flutter of atrial fibrillation. Most importantly, A_1

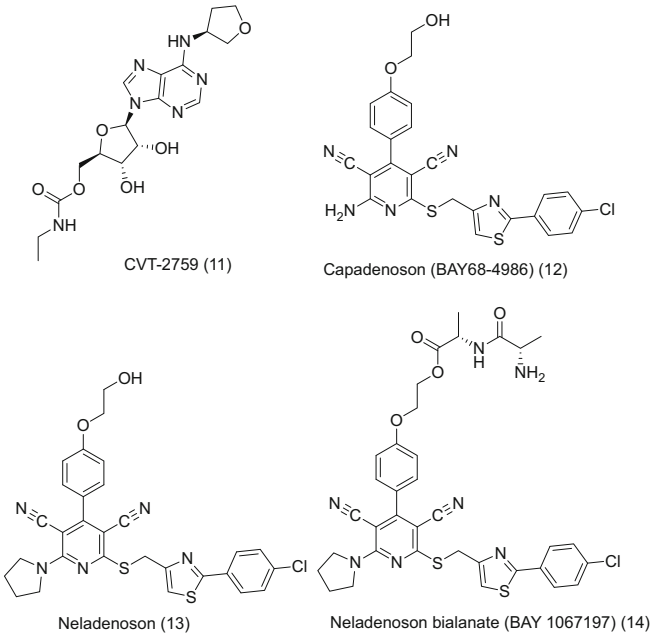


Fig. 10.7 Important partial agonists of ARs

AR partial agonists induce desensitization and downregulation of the receptor much less than full agonists, which makes these compounds a great option in treating certain cardiac arrhythmias while avoiding nonspecific adverse effects that are seen with adenosine administration.

10.6.2.2 Capadenoson (BAY68-4986)

Capadenoson (11) is a non-nucleoside A_1 AR partial agonist that has reached clinical trials, two phase II trials, one for patients with atrial fibrillation and the other for patients with stable angina (Albrecht-küpper et al. 2012; Szentmiklósi et al. 2015). Capadenoson was also investigated for advanced heart failure in animal models and has shown to reduce cardiac remodeling. Neladenoson bialanate is a capadenoson derivative that has entered clinical trials to treat patients with chronic heart failure. Mainly the therapeutic action of capadenoson is due to the partial activation of A_1 adenosine receptors, but it is important to note that capadenoson can also stimulate A_{2B} adenosine receptors. A study was conducted to investigate the effect of capadenoson on A_{2B} AR in cardiomyocytes, cardiac fibroblasts (physiologically relevant cells). Results have shown a significant effect on A_{2B} AR by capadenoson, suggesting that capadenoson should be reclassified from an A_1 AR partial agonist into a dual $A_1AR/A_{2B}AR$ agonist (Baltos et al. 2017). A phase II clinical trial (NCT00518921) of capadenoson was also conducted to evaluate the efficacy and safety in patients with stable angina with 1–4 mg doses; however, the trial was later withdrawn (Jacobson et al. 2019).

10.6.2.3 Neladenoson

Neladenoson, an A_1 AR partial agonist (12), currently is being tested clinically on patients with chronic heart failure in the form of dipeptide prodrug. Neladenoson shows higher selectivity to A_1 AR as compared to capadenoson. Many promising effects caused by Neladenoson have been observed including improvement in cardiac function without causing undesired effects on blood pressure, atrioventricular blocks, or bradycardia. The preference of using a partial agonist instead of full agonist of A_1 AR is due to the fact that partial agonist can activate the receptors without producing severe adverse effects as compared to full agonists. A multiple dose phase II study (NCT02040233) of Neladenoson has been also conducted to investigate tolerability, pharmacokinetics, and safety in patients with chronic heart failure (ParSiFAL study) (Jacobson et al. 2019; Voors et al. 2017).

10.6.2.4 Neladenoson Bialanate

Neladenoson bialanate (13), also referred to as BAY-1067197, is a prodrug of Neladenoson, an A_1AR partial agonist with high potency and selectivity. The need to develop a partial A_1 AR agonist comes from the fact that a full agonist produces extra-cardiac adverse effects including neurological (e.g., sedation) and anti-diuretic effects due to the vasoconstriction of renal afferent arterioles caused by the activation A_1 AR (Dinh et al. 2017; Greene et al. 2016).

Preclinical studies of Neladenoson bialanate have shown promising results including anti-ischemic cardio-protective properties, improved mitochondrial function, and preventing ventricular remodeling, which further supported this compound for phase II clinical trials such as PANACHE (NCT03098979) and PANTHEON (NCT02992288) trials. PANACHE trial was to evaluate Neladenoson in patients with chronic heart failure with preserved ejection fraction (HFpEF) while PAN THEON trial was for evaluating it on patients with chronic heart failure with reduced ejection fraction (HFrEF). Both trials were conducted to evaluate the safety and efficacy of the compound and both of these trials were sponsored by Bayer (Voors et al. 2018). In PANACHE trial, no significant dose to response relationship has been detected after 20 weeks of neladenoson administration, which indicates the need for further investigation and development required for Neladenoson to treat conditions such as HFpEF (Shah et al. 2019).

10.6.3 Antagonists of Adenosine Receptors

Important antagonists of adenosine receptors are presented in Fig. 10.8.

10.6.3.1 Caffeine and Theophylline

Caffeine (14) (3,7-trimethylpurine-2,6-dione) is a nonselective natural methylamine that acts as an A_{2A} and A_1 AR antagonist. Caffeine can be found in common beverages such as tea, coffee, products containing cocoa, soft drinks, dietary sources, and some medications. In the United States, the daily intake of a caffeine consumer is approximately 280 mg. The main purpose of caffeine consumption is to

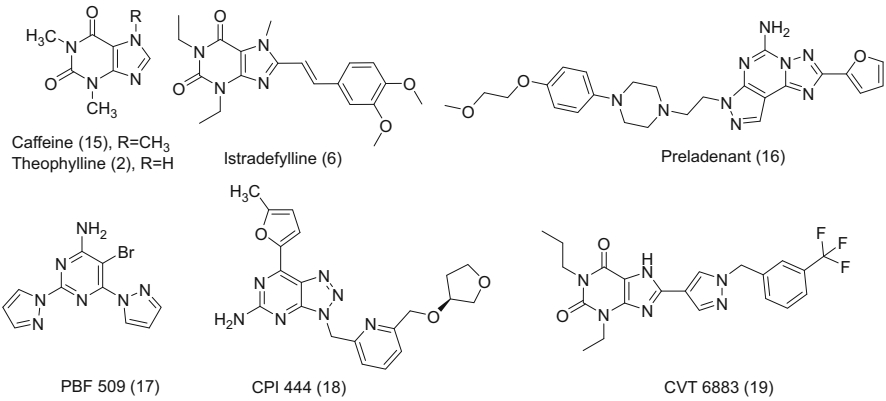


Fig. 10.8 Important antagonists of ARs

increase energy, alertness, and arousal. In normal population, caffeine consumption has been associated with mood and cognitive performance changes with more observed enhancement in performance in fatigued individuals compared to well-rested ones (López-cruz et al. 2018). Caffeine's antagonist effect on A_{2A} adenosine receptors has shown its potentials in treating PD. *In vitro* and *in vivo* studies have both shown that caffeine reduces parkinsonian motor symptoms. Also, drug tolerance associated with current PD drugs has been found to be reduced when co-administered with caffeine (Chen et al. 2010; Roshan et al. 2016). Currently, caffeine is used as an adjuvant treatment for migraine headache. Clinical trials have shown that caffeine can reduce postdural puncture headache (PDPH). In addition, caffeine was reported to produce an effective result in the treatment of hypnic headache; however, further clinical trials are still needed to prove its efficacy as a first-line treatment choice (Baratloo et al. 2016, 2015).

Theophylline (dimethylxanthine) (2) is a nonselective A_1 and A_2 AR antagonist. It has been used for over 80 years to treat airway diseases. Originally it was used as a bronchodilator; however the doses that were required were relatively high, which caused frequent occurrence of adverse effects that lead to the decline of its use and it was more widely used in inhaled form. Recent studies have shown that theophylline possess an anti-inflammatory effect in chronic obstructive pulmonary disease (COPD) and asthma at lower concentrations. Currently, theophylline is used in patients with asthma as an add-on therapy to inhaled corticosteroids. Theophylline is also given to patients with severe COPD when symptoms cannot be controlled by bronchodilators. Side effects of theophylline are related to the plasma concentration of the drug; most common side effects are headaches, vomiting, and nausea that are caused by phosphodiesterase (PDE) isoenzymes inhibition. At high concentrations the inhibition of A_1 receptors caused by theophylline induces seizures and cardiac arrhythmias (Barnes 2013).

10.6.3.2 PBF-680

The PBF-680 is an A_1 AR potent antagonist (structure not disclosed) that is currently in clinical trials for the treatment of asthma. An ongoing phase II trial (NCT02635945) aimed to evaluate the efficacy of PBF-680 in patients with mild to moderate asthma. In this study, 10 mg of PBF-680 was administered orally for 5 days; the efficacy was evaluated by the amount to attenuation of late asthmatic responses that occurs due to allergen broncho-provocation. Previous studies have shown that the activation of adenosine A_1 receptors has a pro-inflammatory role in certain immune cells and also broncho-constrictory effect in pulmonary tissue. Adenosine on the other hand has shown to provoke bronchoconstriction in asthmatic patients, while an adenosine receptor antagonist such as theophylline is an effective drug for asthma treatment. Selective A_1 receptor antagonists may offer a promising therapeutic option for asthmatic patients in the future (Gao and Jacobson 2017).

10.6.3.3 Istradefylline

Istradefylline (15) was the first selective A_{2A} AR antagonist; initially it was available only in Japan for treating the wearing-off phenomenon in Parkinson's disease patients receiving levodopa-containing treatment (Saki et al. 2013).

A recent clinical trial of Istradefylline on 31 patients with Parkinson's disease has proven its effect in decreasing gait disorders including slow walking speed, short steps, forward-bent posture, toe dragging, and reduced arm swing which improved the quality of life of those patients without a serious adverse effect detected (Iijima et al. 2019). Istradefylline has also been investigated in clinical trials for improving mood disorders in PD patients. Doses between 20 and 40 mg of Istradefylline were administered for 12 weeks. Results have shown an improvement in overall mood disorders. However, further trials are needed to confirm the effectiveness of istradefylline due to the fact that this trial recruited only 30 patients with dropout rate of 17% and it was an open-label trial which indicates the possibility of placebo effect in patients (Nagayama et al. 2019). Recently, it has got the US FDA approval (2019) and available in the market as an add-on to levodopa/carbidopa for the treatment of PD (Hoffman 2019; Voelker 2019).

10.6.3.4 Preladenant

Preladenant (16) is an A_{2A} AR antagonist; mainly it was developed to treat patients with PD. However, clinical trials have not been successful and got discontinued. The development of preladenant was discontinued in 2013 after two phase III clinical trials to test its efficacy in treating fluctuating motor disturbances in patients. Results indicated that preladenant had no significant effect as compared to placebo (Pinna et al. 2018).

A preladenant phase I study (NCT03099161) in combination with pembrolizumab was conducted to treat neoplasm. Solid tumors that do not respond to conventional therapy were targeted in the trial. The study was to assess the efficacy and safety of preladenant as a treatment and to set the recommended dose for further clinical trials. However, the study was terminated because the data did not support the study end point (Congreve et al. 2018).

10.6.3.5 PBF-509

PBF-509 (17) is a non-xanthine potent A_{2A} AR antagonist that has been tested for the treatment of PD on rodent models. Studies have shown its efficacy in reducing pilocarpine-induced tremulous jaw movements, haloperidol-mediated catalepsy, and L-DOPA-induced dyskinesia, which indicates that PBF-509 is an anti-dyskinetic agent along with reversing parkinsonian motor impairments making it a potential treatment option for PD in the future (Núñez et al. 2018).

10.6.3.6 CPI-444

CPI-444 (18) is a selective and highly potent A_{2A} AR antagonist for oral administration. The adenosine A_{2A} receptors expressed on immune cells have a suppressive effect on antitumor activity. Blockage of this receptor with a compound such as CPI-444 has shown to restore IL2 and IFNγ production and T-cell signaling in *in vitro* studies. Preclinical studies of CPI-444 on mice have proven its efficacy in producing antitumor response when anti–PD-L1 immunotherapy failed to produce the required therapeutic response. The mechanism that explains how blocking of A_{2A} receptors can overcome the resistance of anti–PD-L1 treatment is still under investigation (Willingham et al. 2018).

A clinical phase I trial (NCT02655822) is currently ongoing (by Corvus Pharmaceuticals, Inc.) for dose selection, tolerability, and safety of CPI-444 as a single antitumor agent or in combination with atezolizumab. Adenosine has shown to suppress antitumor activity in immune cells (T-cells) (Mobasher et al. 2019).

10.6.3.7 CVT 6883 (GS-6201)

CVT-6883 (19) is a selective and potent A_{2B} AR antagonist. Preclinical studies have shown that CVT-6883 has an inhibitory effect on pulmonary injury and inflammation in bleomycin-induced fibrosis models and adenosine deaminase-deficient mice. CVT-6883 has also shown to reduce airway reactivity induced by allergen or NECA in sensitized mice. However, CVT-6883 was discontinued from phase I clinical trials (Basu et al. 2016).

CVT-6883 has also shown to significantly reduce lung fibrosis mediators in multi-walled carbon nanotube (MWCNT) treated mice. CVT-6883 has also decreased inflammatory and cytotoxicity in animal models, which indicates that a selective A_{2B} AR antagonist might offer a possible treatment option for MWCNT-induced lung fibrosis in humans and requires further investigation and development (Liu et al. 2019).

10.6.4 Allosteric Modulators of ARs

Important allosteric modulators of adenosine receptors are presented in Fig. 10.9.

10.6.4.1 T-62 and LUF 5484

T62 (20) is a positive allosteric modulator (PAM) of A_1 AR. T62 preclinical studies have shown that oral administration caused a reduction in hypersensitivity

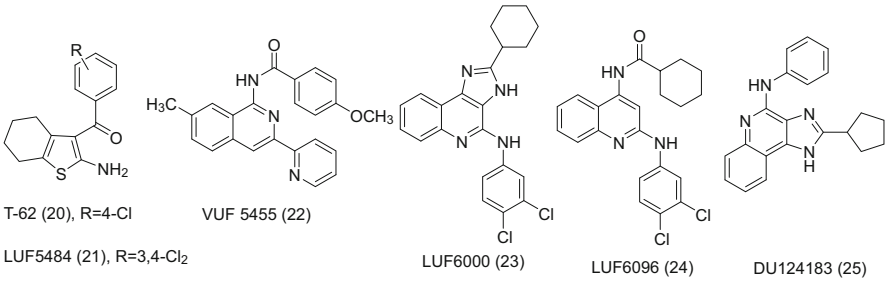


Fig. 10.9 Important allosteric modulators of ARs

neuropathic pain and inflammatory models. It was also noticed to induce sedation after the initial dosing; 5 days after daily administration tolerance has occurred due to downregulation of the A_1 AR. T62 has progressed into clinical trials, a phase II trial (NCT00809679) to evaluate the safety and efficacy of this compound as an analgesic for patients with postherpetic neuralgia. However, some patients experienced transient elevations in liver enzymes (transaminases) which terminated the study (Romagnoli et al. 2015; Sawynok 2016). LUF 5484 (2-amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(3,4-dichlorophenyl)methanone (21) is an A_1 adenosine receptor allosteric modulator (Bueters et al. 2002).

10.6.4.2 VUF5455

VUF5455 (22) is a 3-(2-pyridinyl) isoquinoline derivative, the first selective PAM of A_3 AR. VUF5455 enhances the binding of A_3 receptor agonists and increases the dissociation rate of antagonist (Briddon et al. 2018; Soudijn et al. 2006).

10.6.4.3 LUF6000

LUF6000 (2-Cyclohexyl-*N*-(3,4-dichlorophenyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine) (23), an A_3 AR PAM, increases the activity of orthosteric agonists. The maximal effect of the native ligand increases by 45% when an allosteric enhancer binds to the receptor. LUF6000 has been studied on animal models including mice and rats, and results have shown that LUF6000 induces anti-inflammatory effect by slightly stimulating neutrophils and normal white blood cells (Cohen et al. 2014).

10.6.4.4 LUF6096

LUF6096 (*N*-{2-[(3,4-dichlorophenyl)amino]quinolin-4-yl}cyclohexanecarboxamide) (24) is a positive A_3 AR allosteric modulator; it was developed by the scission of the imidazole ring of LUF6000. LUF6096 has been through preclinical studies on animal models and human cell membranes to evaluate its efficacy in reducing myocardial ischemia/reperfusion injury. Results have shown that LUF6096 is well tolerated and effective in deceasing the myocardial ischemia/reperfusion injury on dog models (Du et al. 2018, 2012).

10.6.4.5 DU124183

DU124183 (2-cyclopentyl-4-phenylamino-1*H*-imidazo[4,5-*c*]quinoline) (25) is a selective allosteric modulator that enhances agonist binding and function of A_3 AR (Göblyös and Ijzerman 2009). DU124183 causes a decrease in agonist potency meanwhile enhancing its maximum effect (Emax) (Gao et al. 2008).

10.7 Conclusions

Adenosine and its four receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3 ARs) are widely distributed throughout the body, modulating the physiological and pathological conditions of almost every organs and tissues. The ubiquitous distribution of ARs not only signifies their potential drug targets but also imposed a great challenge in the process of discovery and development of drugs selectively targeting a particular subtype of AR in disease-specific tissues, while culminating in undesirable side effects. In the last three decades, extensive research efforts from academia and pharmaceutical industries resulted in the discovery of various potential ligands targeting ARs, but only few of them could sustain the clinical trials to successfully reach the market. Istradefylline, an A_{2A} selective antagonist, is the most recently US FDA approved (2019) drug available in the market as an add-on to levodopa/carbidopa for the treatment of PD. Moreover, the recent discovery of the 3D crystal structure of A_1 AR and the previously identified 3D structure of A_{2A} AR have not only enhanced the understanding of the binding site topology of these receptors but also facilitated the development of improved homology models of other two AR subtypes as well as computer-aided structure-based strategies to design and discover novel AR-specific ligands. In this regard, the future discovery of the 3D crystal structures of remaining A_{2B} and A_3 ARs would further provide a clear insight into all the four subtypes of ARs, thus boost up the rational drug discovery process and development of novel clinical candidates, selectively targeting a particular AR subtype relevant to the therapeutic intervention of specific pathological disorders.

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Pharmacology of Angiotensin and Its Receptors

11

Satyajeet Biswal, Rajat Ghosh, and Pratap Chandra Acharya

Abstract

Angiotensin is a peptide hormone produced by the proteolytic cascade initiated by the enzyme renin. The physiological effects of angiotensin are articulated by a particular receptor subtype, and it allows the cells to respond to extracellular signals. In earlier days, receptors were used to be identified using in vitro radioimmuno assay methods similar to the method used to identify receptor-binding properties of antibodies. However, nowadays the validation of receptors is done by doing the molecular or gene grafting into an unresponsive cell and then by observing the changes in chemical messengers. These innovative methods of identifying receptors have led to the discovery of two major angiotensin receptors, angiotensin type 1 receptor (AT_1 receptor) and type 2 receptor (AT_2 receptor), which produce cellular signals. Angiotensin has various physiological functions in different places such as juxtaglomerular cells, aldosterone, heart and kidney. The pharmacological intervention of renin–angiotensin system can be done by using beta blockers which create the inhibitory effect on renin secretion from juxtaglomerular (JG) cells. There is another method which involves the use of the renin inhibitory peptide. However, this method is not yet proved to be a successful approach for controlling the renin–angiotensin system. By far the most appropriate method of controlling the renin–angiotensin system is by using orally active angiotensin-converting enzyme (ACE) inhibitors, which interrupt the whole system. However, due to the associated adverse effects of ACE inhibitors, angiotensin receptor blockers (ARBs) are chosen over them. This chapter describes the history and origin of angiotensin, its biosynthesis, its mechanism of action and its physiological role. Further, the chapter also narrates the role of

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angiotensin as drug target and the use of ARBs for the pharmacotherapeutic intervention of hypertension.

Keywords

Angiotensin · Angiotensin receptor blockers · Renin–angiotensin systems · *Ang II* · AT_1 · AT_2 · Sartans · Hypertension

Abbreviations

ACE	Angiotensin-converting enzyme
Ang II	Angiotensin II
ARBs	Angiotensin receptor blockers
AT_1	Angiotensin type 1 receptor
AT_2	Angiotensin type 2 receptor
AT_3	Angiotensin type 3 receptor
AT_4	Angiotensin type 4 receptor
CHF	Congestive heart failure
CKD	Chronic kidney disease
GPCR	G-Protein-coupled receptor
JG Cells	Juxtaglomerular cells
mRNA	Messenger RNA
NC-IUPHAR	International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification
RAS	Renin–angiotensin system
US FDA	United States Food and Drug Administration
WHO	World Health Organization

11.1 Introduction

11.1.1 History

Angiotensin is a peptide hormone produced by the proteolytic cascade which is initiated by the enzyme renin. The physiological effects of angiotensin are expressed by a particular receptor subtype, and it allows the cells to respond to extracellular signals. Primarily, the response to angiotensin signal is mediated by the peptide hormone after binding to their specific receptors (Goodfriend 2000). The angiotensin receptors were earlier identified by in vitro radioimmuno assays similar to the method used to identify receptor-binding properties of antibodies (Goodfriend et al. 1968; Goodfriend and Lin 1970). It has been observed that angiotensin targets are distributed in blood vessels and the adrenal glomerulosa by tracking the radioactive angiotensin infused into rats (Bumpus et al. 1964). However, every hormone-binding site cannot be considered as receptors. In the 1960s, the metabolic enzymes

used to be particularly confusing with angiotensin receptors, because they do not transduce the presence of hormone into a cellular response. Nowadays, the identification of receptors is done by doing molecular or gene grafting into an unresponsive cell and then by observing the changes in chemical messengers (Sasaki et al. 1991; Mukoyama et al. 1993).

11.1.1.1 Discovery of Angiotensin Receptor Subtypes: Insights from Angiotensin Receptor Binding

The identification of angiotensin receptors was done by performing simpler binding assays on tissue homogenates. The binding sites for angiotensin II were unknown until 1970 and were found in tissue homogenates (Lin and Goodfriend 1970; Goodfriend and Lin 1970). Angiotensin-binding sites have been found outside the blood vessels in a large number at unpredicted places with the help of autoradiography (Mendelsohn et al. 1984; Saavedra et al. 1986). The angiotensin hormone binds with the cells of the central nervous system, pituitary–reproductive tract, fetus, bladder, gut and blood vessels (Goodfriend et al. 1996). It has been suggested that the renin–angiotensin system affects embryogenesis, reproduction and natural defences against infection. These effects probably have evolutionary significance on the cardiovascular functions of angiotensin. All other biological functions may have therapeutic implications; however, the potential uses or side effects of angiotensin receptor blockers (ARBs) are yet to be explored (Goodfriend 2000).

The presence of abundant recepto-like binding sites in the kidney of calf fetus has been observed (Lin and Goodfriend 1970). However, there are vast differences in the binding properties of angiotensin between foetal and adult sites. This result suggested the availability of different types or subtypes of receptors for angiotensin (Simpson et al. 1980). The presence of receptor subtypes was further established by using specific nonpeptide antagonists leading to the discovery of two receptor subtypes labelled as AT_1 (angiotensin II receptor type 1) and AT_2 (angiotensin II receptor type 2) (Timmermans et al. 1993; De Gasparo et al. 1995).

One of the major challenges in the discovery of angiotensin receptors has been the cellular response of angiotensin in surprising locations. Sometimes, 'receptor-like proteins' with unknown ligand known as 'orphan receptors' or receptors with known ligand and location but unknown function, called as 'bureaucrat receptors', have been found. Although the working principle of these receptors is known, it is difficult to predict their physiological functions (Goodfriend 2000).

Angiotensin has been found to bind bovine heart mitochondria leading to inconsistent oxidative phosphorylation. This result suggested that the peptide hormone affects the energy metabolism in myocardial muscle (Goodfriend et al. 1971). This was further supported by the positive inotropic effect of angiotensin III on cat papillary muscle, which was exclusively resistant to hypoxia (Kent et al. 1972).

Angiotensin receptors have been located on human platelets (Moore and Williams 1982). Moreover, aged platelets have more angiotensin receptors compared with the younger ones (Siebers and Goodfriend 1986). Further, animal experiments suggest that angiotensin can speed up the clotting sequence through its receptors (Chabielska et al. 1998).

11.1.1.2 Regulation of the Receptor Binding

Inhibition of hormone-receptor binding is one of the lucrative approaches for drug development. The same approach is also utilized by the human body for physiological regulation. The angiotensin receptors are known to interact with intracellular G-proteins and can affect the angiotensin binding to their receptors (Glossmann et al. 1974).

The angiotensin receptors have been found in the lipid layer of the cell membrane. It is further known that the dietary lipids also influence the tertiary structure and binding kinetics of the angiotensin receptors. For example, the diets containing γ-linolenic acid can reduce the angiotensin binding by adrenal cells. The endogenous steroids and eicosanoids also have similar yet stronger effects. These modulators also affect the balance between angiotensin hormone and receptor antagonists. Because of the difference in the diet-induced microenvironments, some patients respond more to a new drug. The binding of agonist and antagonist at the same place on the receptors is also not possible precisely due to diet-induced microenvironment (Simpson et al. 1980; Campanile and Goodfriend 1981; Carroll et al. 1983; Engler et al. 1998; Carroll and Goodfriend 1984).

11.1.1.3 Effect of Receptor Density

The intensity of the effects of drug, hormone or autacoid depends on the number of its receptors, assuming that a fixed proportion of the receptor is involved in the signal transduction process. However, in the case of angiotensin, this generalization may not be applicable. For example, response of the angiotensin peptide is unimpressive and delayed in adrenal glomerulosa cells although they have a large number of angiotensin receptors, whereas vascular smooth muscle cells contain less number of receptors but mediate a rapid and dramatic response. The number of receptors at the cell surface is regulated by nuclear events to synthesize new receptors, whereas internalization of receptors from cell membrane to cytosol is due to either degradation or recycling. The internalization process is generally accelerated by the binding of agonists to the receptor, not by the binding of antagonists (Hunyady et al. 1994). Angiotensin receptors of vascular smooth muscle stick to this scheme, and hence the hormone downregulates the receptors in the vasculature. However, on the surface of adrenal glomerulosa cells, angiotensin upregulates its receptors (Conlin et al. 1993).

11.1.1.4 Clinical Relevance of Angiotensin Receptors

Angiotensin plays a vital role in the dysregulation of blood pressure and extracellular fluid volume due to its effects on blood vessels and aldosterone secretion. Further studies have revealed that angiotensin contributes to the pathogenesis of a wide range of diseases, such as vascular fibrosis, cardiac hypertrophy, atherosclerosis and diabetic nephropathy (Re 1993; Kabour et al. 1994; Weber et al. 1995a; Gansevoort et al. 1994b). Receptor abnormality is also induced due to the structural defects or changes in their surroundings. However, factors like tissue distribution, genetic sequences regulating receptor number, signal transduction coupling and the membrane components can also affect receptor function (Bonnardeaux et al. 1994; Kainulainen et al. 1999).

11.1.2 Discovery

The pressor principle 'renin' was extracted from the kidney by Tigerstedt and Bergman in 1897 and provided the first insight into the regulation of blood pressure. This revolutionary work further directed the discovery of reno-vascular hypertension in mammals (Goldblatt et al. 1934). When Goldblatt isolated the vasoconstrictor substance renin from the renal venous blood of a hypertensive dog, nobody believed it till 1940 (Braun-Menendez et al. 1940). A similar discovery was made simultaneously and independently by Page and Helmer in 1940, which showed that the so-called renin activator, later proved to be angiotensinogen, can be isolated from the intact animal after injecting it with renin. In Argentina, the pressor substance was known to be 'hypertension' whereas in the United States of America it was called angiotonin and was later shown to be an octapeptide (Skeggs Jr. et al. 1956; Bumpus et al. 1957; Elliott and Peart 1956). There were differences between laboratories concerning the nomenclature, but it was later established that both hypertensin and angiotonin were the same octapeptide, and the hybrid term 'angiotensin' was unanimously accepted by Braun-Mene'ndez and Page.

Components of angiotensin II-forming cascade such as angiotensinogen, angiotensin-converting enzyme (ACE), and angiotensins I, II and III were further characterized. In 1987, a committee was formed comprising members from the 'International Society for Hypertension', 'The American Heart Association' and the 'World Health Organization' which proposed to abbreviate angiotensin to *Ang* using the decapeptide angiotensin I as the reference for numbering the amino acid sequence of all angiotensin peptides (Dzau et al. 1987). *Ang II* regulates vascular resistance and blood volume and has been found in tissues such as kidney, adrenals, brain, sympathetic nervous system, pituitary gland and vascular smooth muscle. *Ang II* is also suggested to function as a paracrine and autocrine hormone in the regulation of cellular growth, proliferation and extracellular matrix formation (Dzau and Gibbons 1987; Grady et al. 1991; Weber et al. 1995b; Weber et al. 1995c). Metabolites of angiotensin such as angiotensin 2–8 (*Ang III*), angiotensin 1–7 or angiotensin 3–8 (*Ang IV*) have also been shown to exhibit biological activities (Peach 1977; Schiavone et al. 1990; Chappell et al. 1991; Iyer et al. 1998; Wright and Harding 1995).

At the end of the 1980s, it was demonstrated that at least two receptor types exist for *Ang II* in many tissues. The conventional peptide analogue such as saralasin and nonpeptide antagonist such as losartan were found to have a high affinity for both the receptors but without any selectivity (Chiu et al. 1989; Whitebread et al. 1989; Speth and Kim 1990). The initial nomenclature of the receptor subtypes was confusing, for example the receptor sensitive to losartan was called *1, B* or *a,* whereas the receptor with no affinity for losartan was termed *2*, *A* or *b*. The 'High Blood Pressure Research Council' in 1990 and the 'International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification' (NC-IUPHAR) appointed a subcommittee to address the problem, and a classification was proposed in 1991 and updated in 1995 (Bumpus et al. 1991; De Gasparo et al. 1995). The details of the receptor subtype classification are described in Sect. 11.3.

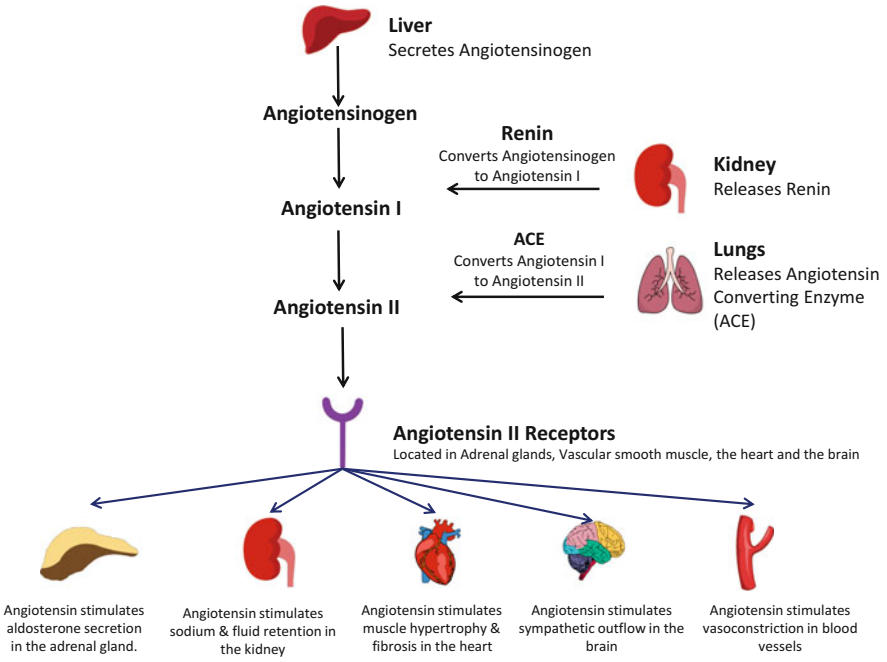

Fig. 11.1 Regulation of renin–angiotensin–aldosterone system

11.2 Angiotensin

11.2.1 Synthesis, Storage, Release and Removal

The enzyme renin and its greater precursor, prorenin, are produced by the zona glomerulosa cells present in renal afferent arterioles of the kidney (Fig. 11.1). The regulation of the renin–angiotensin–aldosterone system is dependent on several enzymes with the end product being *Ang II*. The physiological action of *Ang II* is further mediated by the interaction of the peptide with the specific cell surface receptors namely AT_1 and AT_2 (Guthrie Jr. 1995).

11.2.2 Physiological Role and Recent Advancement

During the circulation, *Ang II* contracts vascular smooth muscle by which elevation of blood pressure happens due to the resistance developed in the arterioles. The renal and mesenteric beds are known to be most sensitive to the *Ang II* action. Arteriolar smooth muscle hypertrophy and increased peripheral resistance are also contributed by *Ang II* by acting as a growth factor for blood vessels. This action is particularly important in the case of vascular injury (Naftilan 1992).

Ang II is known to play a major role in the fluid and electrolyte balance as well as cardiovascular functions. However, the circulating *Ang II* can activate the angiotensinergic sympatho-excitatory pathway in the brain leading to the development and progression of hypertension. Evidences indicate that central sympathetic nerve activity can trigger pathogenicity of essential hypertension. The sympatho-adrenomedullary system is known to have an adaptive response to stress. However, excessive or sustained stress can contribute to the state of hypertension (Mcdougall et al. 2005; Esler 2009).

Ang II, the peptide hormone, sustains blood pressure and vascular volume through several actions such as vasoconstriction, stimulation of aldosterone secretion, increased renal tubular absorption of sodium, activation of the sympathetic nervous system and increased cardiac contractility. However, these actions are impaired in the pathophysiologic states of hypertension and congestive heart failure (CHF). Increased release of renin and *Ang II* has been found in renal diseases like renal artery stenosis, renin-secreting tumours or kidney injury leading to hypertension (Guthrie Jr. 1995).

Therefore, drugs such as ACE inhibitors and ARBs are able to antagonize the actions of the renin–angiotensin axis leading to effective treatment of hypertension and heart failure.

11.3 Angiotensin Receptor

11.3.1 Types, Subtypes and Localization

11.3.1.1 The Type 1 (AT_1) Angiotensin Receptor

The AT_1 receptor is better known for its versatile physiological actions of *Ang II* like blood pressure regulation in arteries, balancing of water and electrolyte, dehydration, hormone secretion and renal function in the target cells present in cardiovascular, endocrine, neuronal, renal and hepatic tissues. The AT_1 receptor belongs to the G-protein-coupled receptor (GPCR) superfamily, and the cellular responses to AT_1 receptor signalling include smooth muscle contraction, neuronal activation, neurosecretion, ion transport, adrenal steroidogenesis, aldosterone secretion, cell growth and proliferation. The AT_1 receptor is coupled to the Gq-mediated calcium and protein kinase C signalling pathways as well as the intracellular signalling cascades extending to the nucleus. These signalling pathways regulate gene transcription and protein expressions to control cell proliferation in several *Ang II* target tissues (De Gasparo et al. 2000).

11.3.1.2 The Type 2 (AT_2) Angiotensin Receptor

The differential stability of dithiothreitol towards *Ang II* binding site suggested the presence of more than one form of the receptor (Chang et al. 1982; Gunther 1984; Chiu et al. 1989; Speth et al. 1991; Whitebread et al. 1989; Chang and Lotti 1990). The cloning and expression of the receptor types AT_1 and AT_2 provided clear evidence regarding the presence of the second isoform of *Ang II* receptor (Murphy

et al. 1991; Sasaki et al. 1991; Kambayashi et al. 1993; Mukoyama et al. 1993). However, the biochemical and physiological functions of the AT_2 receptor are still a matter of intense research.

11.3.1.3 AT_3 Receptor

The existence of AT_3 receptor subtype has been reported with its characteristic pharmacology. However, the distinct gene for this receptor in humans has not been established to date (Chaki and Inagami 1992; Inagami et al. 1993).

11.3.1.4 AT_4 Receptor

High-affinity binding sites of the *Ang IV* peptide were found to be concentrated predominantly in the brain and to some extent in heart, kidney, adrenals and blood vessels, which were termed as AT_4 receptors in 1995 (Harding et al. 1992). The AT_4 receptor does not bind the peptide analogues of *Ang II* as well as the non-peptide inhibitors of AT_1 and AT_2 receptors such as losartan, CGD42112A and PD123177 (Karnik et al. 2015).

11.3.2 Regulatory Pathways and Functions

11.3.2.1 Function of AT_1 Receptors in Juxtaglomerular Cells

The overexpression of AT_1 receptor on juxtaglomerular (JG) cells may hinder the release and production of renin through short-loop feedback mechanism (Kakinuma et al. 1993; Matsusaka et al. 1996). Moreover, losartan shows promising results by inducing mRNA when applying into in vitro culture of freshly isolated renin-releasing cells (Tufro-Mcreddie et al. 1994). When *Ang II* is administered in subpressor doses, the renal renin mRNA gradually decreases and can be stimulated by the treatment with enalapril. The mechanism behind this release and production of renin is not well understood because the regulation is primarily done by macula densa and baroreceptor. The inhibitory effect of *Ang II* receptor on renin-producing cells cannot be confirmed from systemic effects like low blood pressure. Thus the production of renin by overexpression of AT_1 receptor on JG cells remains unclear (Matsusaka et al. 1996).

11.3.2.2 Effect of Angiotensin on Aldosterone

Being the primary regulator of aldosterone synthesis, the overexpression of AT_1 receptor in the adrenal gland stimulates aldosterone secretion. The expression of AT_{1A} and AT_{1B} in the rat adrenal by appropriate hybridization has been explored (Gasc et al. 1994). The overexpression of AT_{1B} mRNA by zona glomerulosa is higher when compared with AT_{1A}. The stimulation of aldosterone secretion by *Ang II* is done in three different ways: (1) stimulation of the proliferation of adrenocortical cells, (2) induction of enzymes that are required for aldosterone synthesis and (3) induction of AT_1 receptors.

11.3.2.3 Effect of Angiotensin and Its Production in the Heart

In the year 1979, captopril showed a promising result in the case of myocardial hypertrophy by inhibiting the ventricular myocardial mass of spontaneous

hypertensive rats (SHR), which cannot be done by the vasodilator drug hydralazine (Antonaccio et al. 1979). This advanced research resulted in a number of in vitro studies showing the positive effect of Ang II on cardiomyocytes and vascular smooth muscle cells and was identified as a promising cardiovascular growth factor.

11.3.2.4 Stimulatory Effect of Potassium on Aldosterone via AT_1 Receptor

Potassium has long been thought to be a key mediator for the JG cells in response to sodium depletion (Boyd et al. 1971). It is also well known that extracellular potassium concentration carries a vital role in the modulation of aldosterone synthesis. Yet the presence of *Ang II* is mandatory for the steroidogenic effect of potassium. Moreover, when potassium supplement downgrades the plasma renin activity, simultaneously it upgrades the adrenal renin (Nakamaru et al. 1985).

11.3.2.5 Renal AT_1 Receptor Regulation

Recent studies show that not renin but angiotensinogen and ACE are rate-limiting factors, and the involvement of *Ang II* receptors in the activity of renin–angiotensin system (RAS) is not yet clear.

11.4 Role of Angiotensin Receptors as Drug Target

11.4.1 Angiotensin II Receptors

Interaction of *Ang II* with specific cell-surface receptors triggers the secondary signalling pathways, and the confirmation of the types and subtypes of angiotensin receptors has been done by the binding studies (Griendling et al. 1994). With the development of receptor-specific ligands, the different angiotensin receptors are confirmed as AT_1 and AT_2 subtypes. The characteristics of AT_1 and AT_2 receptors are summarized in Table 11.1.

11.4.1.1 Pharmacological Inhibition of the Renin–Angiotensin System

There are several ways to interrupt the renin–angiotensin system like the inhibition of renin secretion from the JG cells by using beta blockers. Another method is by using the peptide which inhibits renin itself; some of them are orally active, yet none of them is clinically proven. The most appropriate method for interrupting the system is by using the orally active ACE inhibitors. These ACE inhibitors show promising results by inhibiting the *Ang II* production. There are many ACE inhibitors available in the market which are safe and effective, but due to some of the side effects, *Ang II* receptor antagonists are being used (Guthrie Jr. 1995).

Table 11.1 List of characteristics of AT_1 and AT_2 receptors

	AT_1 receptor	AT_2 receptor
Selective antagonists	Losartan Valsartan Irbesartan Telmisartan TCV-116	CGP 42112A PD 123319
Signalling pathways	(+) Phospholipase C (+) Phospholipase D (−) Adenylate cyclase	(−) Guanylate cyclase
Tissue distribution	Vascular smooth muscle Heart Liver Brain (pressor nuclei) Kidney Adrenal cortex Pituitary	Brain (sensory nuclei) Uterus Ovary Adrenal medulla Neointima
Function	All major angiotensin II responses	(?) Ovulation (?) Neural tissue (?) Growth, wound healing

Key: (+) = Stimulates, (−) = inhibits, (?) = unclear

11.4.2 Advantages of the Newer 'Sartans'

Single dose a day, negligible adverse effect, patient tolerance to side effect and most importantly the lower cost make these sartans the first-line choice as antihypertensive drugs. These ARBs have a good tolerance profile as compared to the ACE inhibitors in both long term and short term due to which the patient compliance is much higher than other antihypertensive drugs. In the initial phase, hypertension is asymptomatic, and long-term treatment is necessary to control the blood pressure. Patient compliance is very much essential, and there should not be any interaction with food. Sartans come into this category and make oral administration very easy (Guthrie Jr. 1995).

11.4.2.1 Combination Therapy

Patients having poor control over blood pressure are usually prescribed a combination therapy of calcium channel blocker and angiotensin receptor blocker. The main strategy for using combination therapy is to provide both inhibitory action and treatment to the heart diseases. On the one hand, ARBs give renal protection and stroke protection without causing any metabolic adverse effects, and on the other hand calcium channel blockers help to treat angina and cardiac ischaemia. During a randomized double-blind clinical trial of 8- to 16-week weeks duration, it was observed that the combination therapy of once daily dose of amlodipine and valsartan showed promising results in lowering and maintaining the blood pressure level for approximately 1 year. So it is well known that a combination therapy would give much more benefit to the patient for reducing the blood pressure instead of using amlodipine or valsartan monotherapy (Sabbah et al. 2013).

11.4.2.2 ARBs and Chronic Kidney Disease (CKD)

The overexpression of renin–angiotensin–aldosterone system leads to the development of CKD despite the initial nephropathy. To preserve the renal function, it is important to block the renin–angiotensin system. Therefore, the use of ARBs has been extensively recommended as preferred antihypertensive agents in patients with pre-existing CKD (Sabbah et al. 2013).

11.4.2.3 Angiotensin II Receptor Antagonists: Effective Antihypertensive Agents

A numbers of pre-clinical and clinical studies have been done with all *Ang II* antagonists to validate their antihypertensive activity. During placebo-controlled clinical studies, losartan and valsartan showed to be the most effective drugs for treating hypertension; however, irbesartan and candesartan also showed promising hypotensive effect in double-blind placebo-controlled trials. But the effect of irbesartan and candesartan is clearly dose dependent as compared to losartan and valsartan. The dose-dependent pattern of both irbesartan and candesartan was 75 and 300 mg and 4 and 16 mg, respectively (Sabbah et al. 2013).

11.4.2.4 Angiotensin II Receptor Antagonists: An Excellent Tolerability Profile

Due to the long-term treatment regimen of ARBs, it is very important to observe the adverse event profile and tolerability of new therapeutic agents. Studies have shown that ARBs have an excellent tolerability similar to placebo. Losartan and valsartan showed a clear and confirmed result in contrast to ACE inhibitors in that these drugs don't induce cough (Benz et al. 1997; Lacourciere et al. 1994). The problem with ACE inhibitors is their lack of specificity, which causes dry cough. Among all angiotensin II antagonists, losartan is the only drug which has the ability to lower the plasma uric level by increasing uric acid excretion. However, this uricosuric effect of losartan, whether its advantage or disadvantage, remains unclear.

11.4.2.5 Renal Effects of Angiotensin II Antagonists: Comparable to Those of ACE Inhibitors

Ang II receptor antagonists do not interfere with any of the effects caused by kininase II inhibition. So, the importance of *Ang II* antagonists on the dynamics of renal blood flow and the renoprotective effect remains unclear. However, the laboratory experiments as well as clinical studies postulate that the effects of ACE inhibitors on kidney are somehow similar to that of ARBs (Burnier et al. 1993; Burnier et al. 1995; Gansevoort et al. 1994a). In contrast to the above facts, a few studies done on both the normotensive subjects and hypertensive patients suggest that *Ang II* antagonists don't affect the glomerular filtration and increased renal blood flow; however, it increases the urinary sodium excretion. According to a previous study, it has been suggested that natriuresis caused by ACE inhibitor is due to the inhibition of prostaglandin metabolism. Studies on the effects of indomethacin on renal haemodynamics showed that it acts as an antinatriuretic in both conditions (Burnier et al. 1993; Minghelli et al. 1998; Burnier et al. 1995). Hence it is proved that

non-steroidal antiinflammatory drugs (NSAIDS) are not class specific, and clinically it may interfere with the antihypertensive effect of ARBs.

11.5 Clinical Status of Various Angiotensin-II Antagonists

The clinical status of various angiotensin-II antagonists or ARBs has been compiled in Table 11.2 and described in the following section.

Azilsartan

Azilsartan (Fig. 11.2) is the latest *Ang II* receptor blocker, developed by Takeda Pharmaceuticals in the brand name Edarbi, and has been approved by the United States Food and Drug Administration (US FDA) on 25 February 2011. Azilsartan is well accepted in the market because of its sustained blood pressure control, which lasts for 24 h. It is a potent and highly selective *Ang II* receptor blocker having a bioavailability of 60% and an elimination half-life of 11 h. The prodrug azilsartan medoxomil gets hydrolysed to azilsartan very quickly. The dose range of 40 mg or 80 mg once daily shows promising results in systolic and diastolic blood pressure by reducing 12–15 mm Hg and 7–8 mm Hg respectively. Till now azilsartan is prescribed only for hypertension, and there are no sufficient human data supporting the use of azilsartan for the improvement of cardiovascular outcomes.

Candesartan

In the year 1993, candesartan (Fig. 11.3) was first examined by Japanese scientists, and they published the effectiveness of the compound as an angiotensin receptor blocker (Mizuno et al. 1992; Ogihara et al. 1993). Candesartan is available in the market as its prodrug candesartan cilexetil (cyclohexyl 1-hydroxyethyl carbonate), which is an ester and gets completely metabolized to its active molecule candesartan. Candesartan has much lower bioavailability when compared with other ARBs. It has an absolute bioavailability of 15–40% and an elimination half-life of 9 h. Candesartan can be given in a dose range of 4–16 mg/day for better therapeutic effectiveness. Candesartan is proved to be lethal if it is taken by pregnant women during the second or third trimester. Patients with renal artery stenosis are very prone to face high risk because of the reduction in renal glomerular filtration rate.

Irbesartan

In the year 1990, irbesartan (Fig. 11.4) was patented for use in the treatment of high blood pressure and heart failure.

Irbesartan is available in the market under the brand name Avapro. This drug is chosen as an initial treatment for high blood pressure. The dose range of this drug is 150–300 mg. Bioavailability is 60–80% and has an elimination half-life of 11–15 h.

Losartan

Losartan (Fig. 11.5) is the first discovered *Ang II* receptor antagonist, which was patented in the year 1986 and got approved by US FDA in 1995. Because of its effectiveness and less adverse effect, it got placed on the WHO (World Health Organization) List of Essential Medicines. The primary function of the drug is to lower the blood pressure, but it shows promising results in the case of renal disease reduction in patients with type-2 diabetes, hypertension and microalbuminuria or

Table 11.2 Pharmacological characteristics of the angiotensin II receptor antagonists available on the market

Drug name	Trade name	Prodrug	Bioavailability %	Dose recommended (mg/day)	Half-life (h)	Protein binding %
Azilsartan	Edarbi	Azilsartan medoxomil	60	40–80	11	99
Candesartan	Atacand	Candesartan cilexetil	42	4–16	9	>99
Irbesartan	Avapro	–	60–80	150–300	11–15	90
Losartan	Cozaar	–	25–35	50–100	1.5–2	99.7
Olmesartan	Benicar	Olmesartan medoxomil	26	5–40	13	99
Telmisartan	Micardis	–	42–100	20–80	24	99.5
Valsartan	Diovan	–	25	80–160	6	95

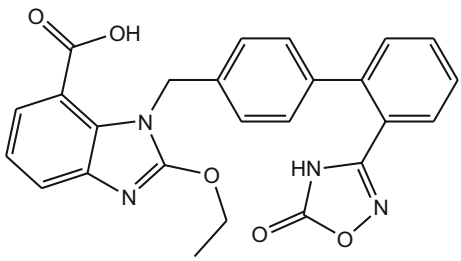

Fig. 11.2 Chemical structure of azilsartan

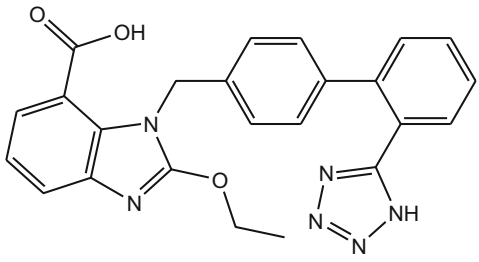

Fig. 11.3 Chemical structure of candesartan

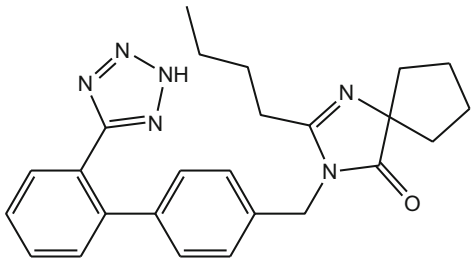

Fig. 11.4 Chemical structure of irbesartan

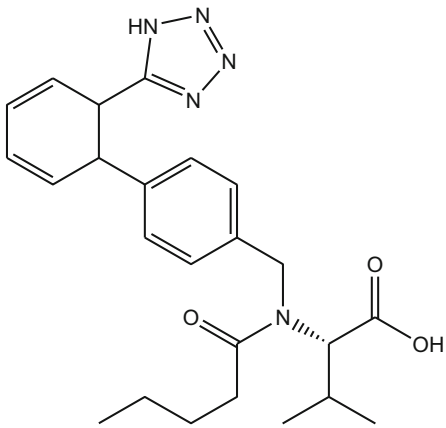

Fig. 11.5 Chemical structure of losartan

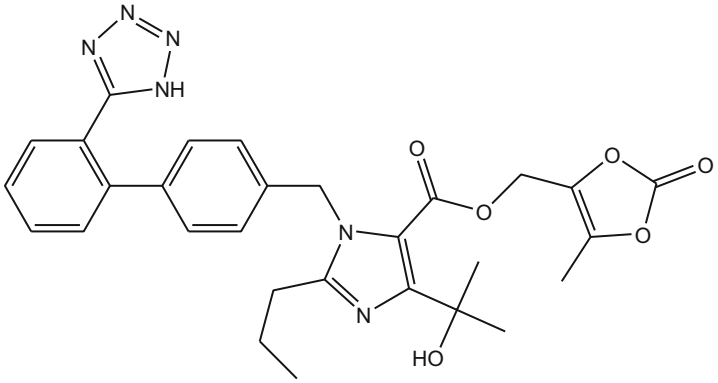

Fig. 11.6 Chemical structure of olmesartan

proteinuria. However, calcium channel blockers and thiazide diuretics are prescribed to most patients, but patients who have intolerance to ACE inhibitors are prescribed with losartan. The bioavailability of losartan is around 25–35% and the elimination half-life is 1.5–2 h.

Olmesartan

The drug olmesartan (Fig. 11.6) got approved by the FDA in April 2002 for the treatment of hypertension. Olmesartan medoxomil is a prodrug and gets completely metabolized to its active metabolite olmesartan rapidly. The bioavailability of olmesartan is around 26%, and it reaches its peak plasma concentration in 1 or 2 h. The oral dose of olmesartan is 20–40 mg once in a day. Sometimes a diuretic can be given with olmesartan to the patient when blood pressure cannot be controlled by only olmesartan.

Telmisartan

Telmisartan (Fig. 11.7) is a nonpeptide *Ang II* receptor antagonist, which got FDA approval in the year 1998 for use in the treatment of hypertension.

After oral administration, the peak plasma level is obtained in 0.5–1 h. The bioavailability of telmisartan is around 42–100% and protein binding is high at more than 99.5%. The elimination half-life is 24 h. Due to the biliary secretion of the

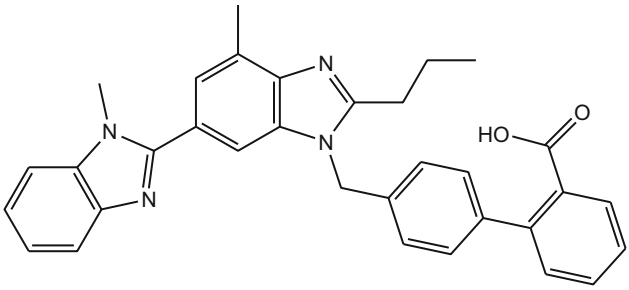

Fig. 11.7 Chemical structure of telmisartan

intact drug, telmisartan is cleared completely from circulation. The recommended dose of telmisartan is 40–80 mg once daily. Combined administration of telmisartan and digoxin increases the peak and trough plasma concentrations of digoxin by 49% and 20% respectively.

11.6 Conclusions

The physiological effect of the peptide hormone angiotensin was unknown till the discovery of two major angiotensin receptors, AT_1 and AT_2. Research on the physiological role of angiotensin has led to the finding that *Ang II* is the primary culprit in the development of hypertension and related cardiac diseases. However, *Ang II* is also known to maintain the fluid and electrolyte balance in the body. Therefore, the effective treatment of hypertension and heart failure can be achieved by blocking the angiotensin receptors using ARBs or angiotensin antagonists. The ARBs have been proven to be a better therapeutic intervention for the management of hypertension than the ACE inhibitor, due to its fewer adverse effects, although they both share the same biochemical pathway for the regulation of blood pressure.

In the recent years, the ARBs, also known as 'saratans', have been the cornerstone in the management of hypertension and heart failure. The sartans are typically the AT_1 receptor blockers without any effect on AT_2 receptor. It has been more than three decades since the identification of the AT_2 receptor. However, the biochemical and physiological functions of the AT_2 receptor are yet to be established. It is still a matter of debate whether the AT_2 receptor is involved in ovulation, neural tissue regeneration and wound healing process. Therefore, at this juncture when the role of AT_1 receptor has been fully understood, the exploration of physiological properties of AT_2 receptor is warranted. This could possibly lead to the development of selective AT_2 receptor modulators for the management of diseases other than of cardiovascular system.

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Pharmacology of Endogenous Opioids, Opiates and Their Receptors

12

Mohammed Noorladeen Al–Qattan, Nirupam Das, and Rati Kailash Prasad Tripathi

Abstract

The search for analgesia is the main drive for the discovery of opioid receptors and their endogenous ligand peptides. Although opioid peptides are very similar in their *N*-terminal sequence, they are classified into different types according to their precursor proteins. The peptides activate opioid receptors by binding to orthosteric-binding sites to mediate intracellular second messengers. Biased signaling and allosteric modulation is a new approach to obtain receptor subtype selectivity and separates the desirable from a myriad of unwanted pharmacological effects. This chapter describes endogenous opioids and opiates with an emphasis on structure, origin and processing, receptors, physiological roles, and potential involvement in therapeutic interventions. Further, it also provides a brief discussion on the effect of opioids on various ion channels and recent developments of established and investigational opioid molecules.

Keywords

Opioids receptors · Endorphin · Enkephalin · Dynorphin · GPCR · Neurological disorders · Ion channels

Abbreviations

ACTH Adrenocorticotropin
BACE1 Beta-site APP cleaving enzyme 1

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CLIP	Corticotropin-like intermediate peptide
DOR	Delta opioid receptor
GPCR	G protein-coupled receptor
KOR	Kappa opioid receptor
MOR	Mu opioid receptor
MSH	Melanotropins or melanocyte-stimulating hormone
N/OFQ	Nociceptin/orphanin FQ
OP	Opioid peptide
OR	Opioid receptor
ORL-1	Opioid receptor-like-1
POMC	Proopiomelanocortin

12.1 Introduction

The inscription of the ancient Sumerian clay tablet insinuates the use of the natural extract of poppy plant (*Papaver somniferum)* as analgesia and post-surgical pain (Norn et al. 2005; Brownstein 1993). The active ingredient of the extract is morphine, which necessitates the presence of receptors for binding inside the human body. The search for endogenous ligands acting on those opioid receptors (ORs) started in the 1960s and succeeded in a few years by using radiolabeled ligands (Pert and Snyder 1973). On the search for ligands for those ORs, several peptides were isolated. The early peptides were enkephalins (Hughes et al. 1975) which showed higher potency than morphine on opioid receptors (Kosterlitz and Hughes 1975). Soon the untriakontapeptide (endorphin) was discovered (Li and Chung 1976), then dynorphins (Cox et al. 1975) and endomorphins (Zadina et al. 1997).

Although opioid peptides (OPs) share almost similar *N*-terminal amino acid sequence, their precursor proteins are different. The processing of precursor proteins differs from one tissue to another as well as variation of the half-life of opioid peptides. Some endogenous opioid peptides like endomorphins act as a selective agonist for μ-opioid receptor (MOR) and thus have a good potential for clinical use. However, the low oral bioavailability of endomorphins restricts its use unless given as glycosylated derivatives (Varamini et al. 2012). Currently, three types of opioid receptors are known, namely Mu, Delta, and Kappa opioid receptors (MORs, DORs and KORs, respectively). The receptors can be activated by orthosteric ligands and cooperated with allosteric ligands to produce modulation of orthosteric affinity/efficacy as well as biasing the intracellular signaling.

12.2 Opioid Peptides

12.2.1 Classification, Synthesis, Storage, Release, and Removal

Endogenous opioid peptides are classified into three categories, namely endorphins, enkephalins, and dynorphins. The peptides are endogenously produced by peptidases digestion of larger precursor proteins, namely proopiomelanocortin,

proenkephalin and prodynorphin, respectively (Pleuvry 1991). Endomorphins is regarded as the fourth category of OPs; however, the precursor for endomorphins is not precisely known from the human genome (Terskiy et al. 2007) and may involve an oxidative transformation of guanine nucleotide from suspected gene leading to G → T transversion in the produced mRNA (messenger ribonucleic acid) (Matsushima et al. 2019). The proopiomelanocortin (POMC) precursor is processed to give rise to adrenocorticotropin (ACTH), melanotropins (MSH) and β-endorphin (Castro and Morrison 1997). The processing of precursor protein depends on a particular cell type. At anterior pituitary, POMC is processed in corticotrophs to generate mainly ACTH and β-lipotropin. When processed in melanotrophs, it produces MSH primarily (De Wied 1999). Both ACTH and β-lipotropin (1–91 aa) have no analgesic activity. The further processing of β-lipotropin gives rise to β-endorphin (61–91 aa), which in turn may yield [Met]-enkephalin (61–65) (Cox et al. 1976; Takeuchi 2001). In melanotrophs of pars intermediate of the pituitary, POMC is processed further to produce α-MSH (from *N*-terminal of POMC) which is attributed to different levels of specific peptidases in different regions of the pituitary (Day 2009). In addition to the pituitary, POMC is also detected in the arcuate nucleus of the hypothalamus, solitary tract of the medulla and several peripheral tissues (Papadimitriou and Priftis 2009).

Proenkephalin peptide contains six copies of [Met]-enkephalin and one copy of [Leu]-enkephalin, which are separated from each other by dibasic peptides as digestive site (Takahashi 2016). Although proenkephalin is produced by both the brain and adrenal gland, however, it is processed differently in each tissue (Geracioti et al. 2009). Proenkephalin-producing neurons are more widespread in the brain than POMC neurons (Geracioti et al. 2009). Prodynorphin is also called proenkephalin-B since it contains three copies of [Leu]-enkephalins in addition to dynorphin A, dynorphin B and α-neoendorphin (Fig. 12.1 and Table 12.1).

In neuron soma, precursor neuropeptides are synthesized in the endoplasmic reticulum, and through Golgi apparatus, the peptides are processed by proteases and undergo functional group modification and then transported to axons as vesicles. At the nerve terminal, the vesicles may contain both precursor and opioid peptides (Hökfelt et al. 2000). The processing of precursor peptide is activated by potassium-induced depolarization of the neuron (Yakovleva et al. 2006). Moreover, the acidic pH of secretory vacuoles promotes digestion of precursor peptides such as POMC (Cawley et al. 2016). The opioid peptides vesicles appeared distinct from amino acid neurotransmitter vesicles, and the former appear denser under a microscope. The dense vesicles are distributed over neuronal cells but especially abundant in dendrites, cell body and axon varicosities and can be released from either site with machinery different from neurotransmitters (Russo 2017; Gu et al. 2017).

Post-translational modifications may take place on opioid peptides such as glycosylation, acetylation, methylation and phosphorylation which alter their biological activities (Froehlich 1997). While glycosylation improves the blood-brain barrier (BBB) penetration (Egleton et al. 2000), acetylation attenuates the activity of the peptide. Glycosylation may occur even on precursor proteins and thus controls the degree of digestion to active opioid peptides and protects against non-specific digestion (Hughes et al. 1980; Loh and Gainer 1978).

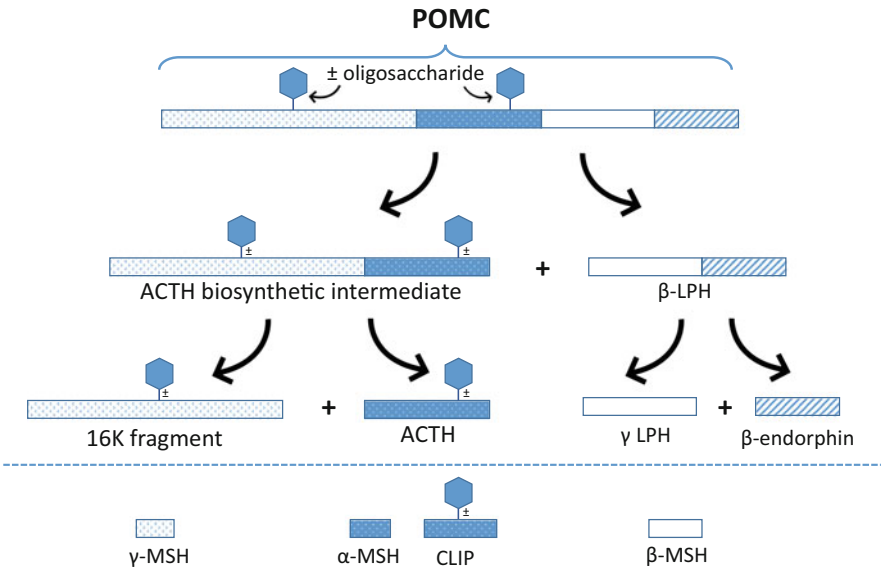


Fig. 12.1 The processing of POMC (Retrieved from Mains and Eipper 1981)

The neuropeptides (including opioids) may diffuse to distal sites (up to millimeters), unlike other neurotransmitters, which remain within the synaptic space. They exert their effect by interacting with opioid and other receptors and may have longer extracellular half-lives (van den Pol 2012; Russo 2017). Long-distance signaling within the brain through no synapses is called volume transmission (Agnati et al. 1995; Veening et al. 2012). Volume transmission is common with G protein-coupled receptors (GPCRs) that are more sensitive to neuropeptides where the nanomolar concentration of opioid peptides is sufficient to activate the receptors. This is unlike the ionotropic receptors like γ-aminobutyric acid (GABA), where micromolar concentration is needed (Ludwig and Leng 2006).

Another difference between opioid peptides and neurotransmitters is that opioid peptides lack reuptake mechanism by releasing neurons. Therefore, the inactivation mechanism is limited to digestion by peptidases extracellularly or after cellular internalization. Many peptidases degrade opioid peptides. The major peptidases involved in the degradation of opioid peptides are endopeptidases (those acting on *N*-terminal of peptides, the pharmacophoric domain). However, other peptidases also contribute such as an insulin-degrading enzyme, angiotensin-converting enzyme, neprilysin, serine peptidases and other dipeptidyl peptidases (Asvadi et al. 2014a; Malfroy et al. 1978; Turner 2004; Hersh and Rodgers 2008). Since the activity of these peptidases is affected by the pH of the tissue, the half-life of these peptidases increases in inflamed tissues that have acidic pH; therefore, opioid peptides are degraded more rapidly and differently (Asvadi et al. 2014b; Herath et al. 2012). The inhibition of the degrading enzymes by specific and non-specific inhibitors is effective in producing analgesia.

Table 12.1 Opioid peptides and their precursor proteins and receptor selectivity (Luca et al. 2007; Coward et al. 1998)

Endogenous peptide	Amino acid sequence	Receptor affinity	Precursor
β-Endorphin	YGGFMTSEKSQTPLVTLFKNAIIKNAYKKGE	$\delta = \mu$	Proopiomelanocortin
[Met]-enkephalin [Leu]-enkephalin Metorphinamide	YGGFM (YGGFMRF, YGGFMRGL) YGGFL YGGFMRRV-NH_2	$\delta >> \mu$	Proenkephalin
Dynorphin A Dynorphin A(1-8) Dynorphin B α-neoendorphin β-neoendorphin	YGGFLRRIRPKLKWDNQ YGGFLRRI YGGFLRRQFKVVT YGGFLRKYPK YGGFLRKYP	$\kappa >> \delta = \mu$	Prodynorphin
Nociceptin	FGGFTGARKSARKLANQ	*ORL	Pronociceptin
Endomorphin-1 Endomorphin-2	YPWF-NH_2 YPFF-NH_2	μ	Unknown

*ORL means orphan opioid-receptor-like

12.2.2 Physiological Role of Opioid Peptides

12.2.2.1 Sites of Action of Opioid Peptides

Opioids act at two sites on the neurons, *viz.* the presynaptic nerve terminal and the postsynaptic neuron. The postsynaptic actions of opioids are usually inhibitory, while presynaptically they inhibit the release of neurotransmitters. Thus, the central nervous system (CNS) effect of the opioids is an overall result of their actions at the multiple presynaptic sites of both inhibitory and excitatory neurons and also includes their actions at postsynaptic sites. As a consequence, the overall effect of opioids depends upon the location and density of opioid receptors on the neurons. For instance, presynaptic inhibition of neurotransmitter release leads to the excitatory effects in target neuron if the neurotransmitter produces an inhibitory effect. On the other hand, if the opioid also has a postsynaptic inhibitory effect on the target neuron, then excitatory effects may not occur (Winters et al. 2017; Fields and Margolis 2015). Morphine acts on μ-receptors and inhibits the discharge of numerous diverse neurotransmitters including acetylcholine, noradrenaline and the neuropeptide substance P (Kerage et al. 2019; Commons 2010; Dickenson 1994).

12.2.2.2 Opioid Peptides and Their Effect on Analgesic/Pain Pathways

Pain is usually considered to be associated with augmented activity in primary sensory neurons provoked either by strong thermal or mechanical stimuli or by the chemicals released by inflammation or tissue damage (Yam et al. 2018). The primary sensory neurons implicated in the pain sensation releases glutamate and substance P in the dorsal horn of the spinal cord principally (Zieglgänsberger 2018; Lembeck 2008). The nociceptive signal is then communicated *via* the spinothalamic tracts to the brain, thereby activating the descending pathways from the periaqueductal gray area in the midbrain which then exerts inhibitory control over the dorsal horn (Venkatraman et al. 2017; Mendell 2011).

Opioid receptors are located at various regions of the nervous system that are involved in pain transmission and control, including primary afferent neurons, spinal cord, midbrain, and thalamus. Though the physiological function of endogenous opioids toward the pain transmission is still unclear, under pathological conditions, the endogenous opioid system gets activated. The opioid drugs, however, produce analgesia by inhibition of neurotransmitter release from the primary afferent terminals in spinal cord and activation of descending inhibitory controls in the midbrain (Allouche et al. 2014; McDonald and Lambert 2005; Waldhoer et al. 2004).

Any modulation in the nociceptive pathways may result in profound alterations in levels of neurotransmitters in primary afferent neurons, thereby causing changes in sensitivity to opioid analgesia (Yam et al. 2018). Accordingly, neuropathic pain is related to reduced sensitivity to opioids; on the contrary, inflammatory pain is linked with increased opioid sensitivity. Moreover, the alterations that occur in pain sensitivity during the states of chronic pain have been ascribed to the activation of glutamate NMDA (*N*-Methyl-D-aspartate) receptor (Greenwald and Shafritz 2018; Latremoliere and Woolf 2009; Petrenko et al. 2003; Bennett 2000).

12.2.2.3 Activity of Opioid Peptides

Opioid peptides are produced by different tissues, including mainly pituitary and adrenal glands (Przewlocki 2013). Enkephalins, for instance, are also released by heart, skeletal muscle, kidney, and intestinal cells, thus playing an important role in behavior, pain, cardiac function, cellular growth, immunity, and ischemic tolerance (Denning et al. 2008). The expression of prodynorphin is mainly observed in the cerebral cortex and basal ganglia in addition to reproductive tissues of testis, uterus, and ovary (Collard et al. 1990; Douglass et al. 1987).

Opioid peptides are neuromodulators rather than neurotransmitters—that is, OPs can alter the release and neuronal response to neurotransmitter by changing the hyperpolarizing neuronal cell membrane (North and Williams 1983; Loose et al. 1990; Wu et al. 2007). The activation of ORs by endogenous peptides transmits or modulates signal transmission for other neurotransmitters. Therefore, the peptides have a wide range of activities that include central and peripheral antinociception, endocrine, immune, motor activity, feeding, sexual behavior, regulation of body temperature, respiration and cardiovascular and gastrointestinal functions (Przewlocki 2013).

The β-endorphin is regarded as the most important opioid peptide. The peptide is involved in interneuron communication through synapses as well as extracellular spaces and cerebrospinal fluid (volume transmission, VT) (Veening and Barendregt 2015). The peptide showed central and peripheral analgesic activities (Sprouse-Blum et al. 2010). Both ACTH and β-endorphin are stress hormones released during painful stimulations. The release of ACTH and β-endorphin from a culture of pituitary tumor cells can be evoked by epinephrine (Mains and Eipper 1981). Therefore β-endorphin is responsible for stress-induced analgesia (Rubinstein et al. 1996). The *N*-terminal amino acids of β-endorphin are likely accountable for analgesic activity, while the C-terminal amino acids are more related to potency. The removal of eight amino acids from *N*-terminal abolishes the analgesic activity of β-endorphin (Deakin et al. 1980). Although the analgesic potency of β-endorphin is higher than [Met]-enkephalin, the potency is reduced by *N*-α-acetylation (Deakin et al. 1980).

Enkephalins are another group of opioid peptides that include mainly [Met]-enkephalin and [Leu]-enkephalin. The affinity of enkephalins for MOR is similar to that of morphine and is ten times lower then affinity for DOR. Besides analgesia, enkephalins may be involved in emotional and motivational behavior (Nieto et al. 2005) and sexual activities for male (Rodríguez-Manzo et al. 2002) and controls gastrointestinal motility and secretions (Mitznegg et al. 1977; Holzer 2009).

Dynorphin is an opioid peptide which is released at the level of the spinal cord and augments the afferent pain signal (Podvin et al. 2016), thus provoking allodynia, pain sensation for usually non-painful stimuli. Therefore, dynorphin has a significant role in the mediation of chronic pain (Podvin et al. 2016) as well as tolerance to antinociceptive opioids (Vanderah et al. 2001). Moreover, dynorphin promotes anxiety, stress, and dysphoria-driven cravings for another dose in addiction through its action on KORs (Chavkin and Koob 2016; Knoll and Carlezon 2010).

Endomorphins (1 and 2) are reported to be the only discovered opioid peptide that are selective for MOR (Zadina et al. 1997). The two peptides differ from other opioid peptides in having NH_2-Tyr-Pro-Trp(Phe)-Phe-CO-NH_2, that is, having carboxamide (aminocarbonyl) terminal. Some studies showed that endomorphin-1 produces higher analgesia and without reward effect compared to morphine (Wilson et al. 2000). Although the reward effect is known to be mediated by binding MOR, it is limited to a specific conformational set of receptors; thus it is ligand-dependent property. However, the potential of respiratory depression, urinary retention, tolerance, addiction and cardiac side effects and the low intrinsic bioavailability restrict the clinical use of endomorphins (Gu et al. 2017). Glycosylation through succinamic acid linker at the *N*-terminal enhances metabolic stability and membrane permeability of endomorphins while maintaining potency and efficacy (Varamini et al. 2012).

Nociceptin is structurally related to dynorphin; however, it binds to opioid receptor-like (ORL) while having no affinity for other ORs. Similar to dynorphin, nociceptin antagonizes the analgesic effect of other opioid ligands (Mika et al. 2011). Activation of ORL inhibits adenylyl cyclase and Ca^{+2} channels while activating K^+ channels like opioid receptors (Calo et al. 2000). However, the pharmacological behavior observed upon activation by nociceptin produces potent anti-analgesic action supra-spinally and analgesic action spinally (Mogil and Pasternak 2001).

12.3 Receptor (Types, Subtypes, Localization, Down Signaling Pathways, Functions, Agonist, Antagonist)

Endogenous opioids produce effects on the neurons by binding to three types of receptors located on neuronal cell membranes: namely, mu (μ), delta (δ) and kappa (κ) receptors. Other receptors such as opioid receptor-like-1 (ORL1) have sequence similarity to ORs, however not classified as ORs since being unresponsive to classical opioids ligands (Snyder 2004). Naturally occurring opioids, β-endorphins interact preferentially with μ-receptors, while the enkephalins and dynorphin interact respectively with δ-receptors and κ-receptors (Ghelardini et al. 2015; Al-Hasani and Bruchas 2011; Dhawan et al. 1996). The relative selectivity for endogenous and exogenous ORs ligands are listed in Table 12.2. The crystal structures for opioid receptors are available for mu, delta, and recently kappa receptors. The structures are for inactive conformations that are stabilized by bound antagonists (Manglik et al. 2012; Wu et al. 2012; Granier et al. 2012) and for active conformations that are stabilized by either bound agonist or conformation-specific nanobody or G-protein (Huang et al. 2015; Koehl et al. 2018; Che et al. 2018). The further sorting of main OR classes into subtypes is only approved from the anesthetic perspective in a way that μ1 is responsible for analgesia and dependence, μ2 is for euphoria, dependence and respiratory depression, and μ3 is for vasodilation. However, currently, there is no clear evidence for the existence of subtypes for these receptors (Dietis et al. 2011).

Table 12.2 Selectivity of naturally occurring endogenous opioids and opiates for opioid receptors

	μ-receptor	δ-receptor	κ-receptor
Opioid peptides (endogenous opioids)			
β-endorphin	+++	+++	+++
[Leu]-enkephalin	+	+++	–
[Met]-enkephalin	++	+++	–
Dynorphin	++	+	+++
Opioid drugs (agonists)			
Morphine	+++	+	++
Codeine	+	+	+
Pethidine	++	+	+
Fentanyl	+++	+	–
Opioid drugs (partial/mixed agonists)			
Pentazocine	+	+	± ±
Buprenorphine	± ± ±	–	–
Opioid drugs (antagonists)			
Naloxone	+++	++	++
Naltrexone	+++	++	++

+ indicates agonist; ± indicates partial agonist; number of + or ± indicates potency

Opioid receptors are classical G protein-coupled receptors (Fig. 12.2), thus interacting with intracellular G-protein that has GTPase activity to mediate intracellular signaling. The G-protein is a heterotrimeric protein composed of Gα, Gβ, and Gγ. The activation of ORs leads to the dissociation of G-protein. The Gβγ subunits may interact directly with the membrane ion channels, thereby causing inhibition of voltage-gated calcium channels (VGCCs) or rectifying potassium channels (Kir). The Gβγ subunits also contribute to the activation of inositol triphosphate kinase (IP3K), phospholipase C—phosphatidyl inositol triphosphate (PLC-IP3), or mitogen-activated protein kinase (MAPK) (Khan et al. 2013; Smrcka 2008). With respect to Gα subunit, it is classified into four families: $G\alpha_s$ (activates adenylyl cyclase), $G\alpha_{i/o}$ (inhibits adenylyl cyclase), $G\alpha_{q/11}$ (activation of phospholipase Cβ) and $G\alpha_{12/13}$ (activation of GTPase activating protein for Ras)(Ras, a small GTP-binding protein named after Rat sarcoma) (Neves et al. 2002). The inhibition of adenylyl cyclase by $G\alpha_{i/o}$ subunit leads to reduced production of cyclic adenosine monophosphate (cAMP). Decreased concentration of cAMP further modulates membrane sodium or calcium channels (Law 2011; Catterall 2011; Scheuer 2011). Additionally, cAMP may also interact with and modulate the inward rectifying potassium channels (Li et al. 2013; Butt and Kalsi 2006; North 1993). Chronic consumption of opiates inhibits the production of cAMP; however, this inhibition is offset in the long run by other cAMP production mechanisms (Ramaswamy and Langford 2017; Al-Hasani and Bruchas 2011; Kosten and George 2002). When no opiates are available, this increased cAMP production capacity comes to the force and results in neural hyperactivity, thus causing a sensation of craving the drug (Al-Hasani and Bruchas 2011; Bie 2005; Kosten and George 2002).

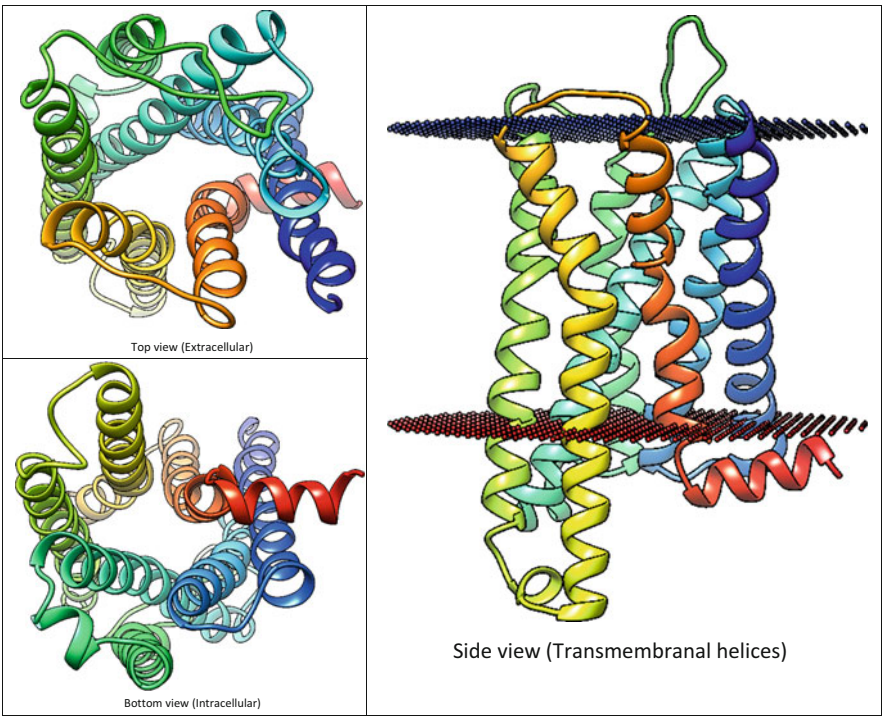


Fig. 12.2 Crystal structure of active conformation of Mu opioid receptors (PDB ID: 6DDE) with missing H8 being added from PDB 5C1M and the position within cell membrane is optimized using OPM database

Besides interaction with G-protein, ORs interact with other regulatory proteins and may end up with its sequestration or desensitization. Specific phosphorylation of ORs by GPCR kinases leads to interaction with β-arrestin and subsequent internalization of the receptor. Probably, $G_{i/o}$ mediates the antinociceptive effect produced by activation of MOR (Lamberts et al. 2011; Connor and Christie 1999), while respiratory depression and tolerance is mediated by activation of β-arrestin. Specific phosphorylation of ORs by GPCR kinases leads to interaction with β-arrestin and subsequent internalization of the receptor further causes tolerance to morphine analgesia (Bohn et al. 1999). Avoiding activation of β-arrestin-2 pathway reduces internalization of MOR and consequently tolerance; however, it has little effect on the up-regulation of adenylyl cyclase activity that is correlated to physical dependence (Bohn et al. 2000).

Opioid receptors may exist as homo- or heteromers to provide a cellular specific response for the same ligand. The ORL-1 or nociceptin/orphanin FQ (N/OFQ) receptor undergoes heteromerization to form heteromers with μ-opioid receptor (MOR), δ-opioid receptor (DOR), and κ-opioid receptor (KOR) (Evans et al. 2010). Additionally, MOR may also form heteromers with DOR in small dorsal

root ganglion neurons (DRGn) and in homologous expression systems (Gendron et al. 2015).

All three ORs produce analgesia when opioid binds to them; however, it is higher for μ then κ and δ subtypes and is associated with euphoria, dysphoria, and anxiolytic effects, respectively (Lutz and Kieffer 2013). Activation of κ-receptors produces comparatively less physical dependence as activation of μ-receptors (Machelska and Celik 2018; Feng et al. 2012). Each of these receptors is coupled to intracellular mechanisms *via* G-protein and mediates its effect through the second messengers, thereby influencing the probability of opening of the ion channels, which in some instances may reduce the excitability of the neurons (Machelska and Celik 2018, Feng et al. 2012). This reduced excitability contributes as a potential source of the euphoric effect of opiates and probably appears to be mediated by μ- and δ-receptors (Machelska and Celik 2018; Reisine and Bell 1993).

Like many GPCRs, ORs have binding sites for orthosteric and allosteric ligands. While orthosteric ligand can modulate receptor conformation independently, allosteric ligand depends on orthosteric ligand for modulating receptor conformation. The orthosteric active opioid peptides (Livingston and Traynor 2018) share a common *N*-terminal sequence of Tyr-Gly-Gly-Phe that starts with an ionized amine. The peptides are proposed to have two pharmacophores named as message and address pharmacophores. In other words, the address leads the peptide for opioid receptor subtype, while the message stabilizes particular receptor conformations to mediate specific set of intracellular signals. This proposal has been used to design highly potent and selective non-peptide DOR antagonists (Portoghese et al. 1988). The extracellular loops of opioid receptors have long been thought to involve in controlling ligand selectivity to receptor subtypes by interaction with the address part of the ligand (Metzger and Ferguson 1995). On the other hand, allosteric ligands (or modulators) are classified as positive (PAM), negative (NAM) and neutral/silent allosteric (SAM) modulators, which increases, decreases, and has no effect on orthosteric ligand activity, respectively (Mahmod Al-Qattan and Mordi 2019). Most of the allosteric ligands require bound orthosteric ligand in order to exert activity.

The conformational ensembles stabilized by orthosteric ± allosteric ligand(s) modulate the downstream signaling of the receptor (i.e. interaction with intracellular second messengers). Therefore, any ligand binding to ORs has a 2D fingerprint of intracellular signaling paradigm as affinities versus efficacies. Accordingly, some ligands are biased toward activating a particular group of second messengers over others, which is referred to as biased agonism. Biased signaling (also known as differential efficacy or functional selectivity) is the way of separating wanted pharmacological effect from other unwanted effects mediated by the same receptor (Bologna et al. 2017). The biased activation of ORs was observed among endogenous as well as exogenous ligands. Unlike other endogenous OPs, α-neoendorphin, [Met]-enkephalin, [Met]-enkephalin-RF, endomorphin-1 and endomorphin-2- showed biased agonistic activities compared to reference DAMGO (a synthetic peptide with high μ-opioid receptor specificity and its structure is H-Tyr-D-Ala-Gly-N-MePhe-Gly-ol) across multiple signaling pathways as shown in Fig. 12.3 (Thompson et al. 2015).

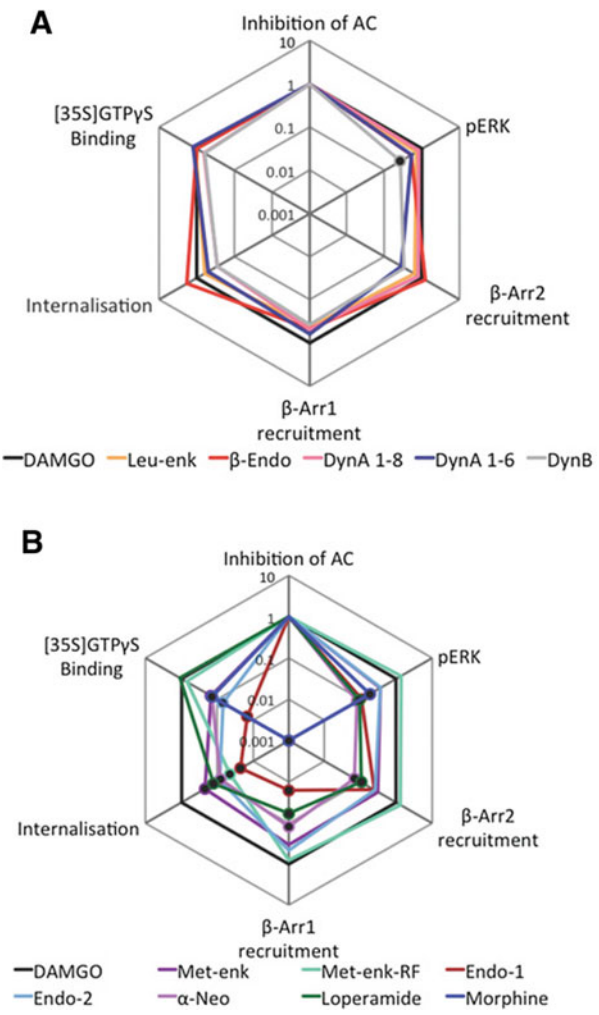


Fig. 12.3 Webs of bias of endogenous opioid peptides and reference ligands at the MOP. (**a**) Ligands with profiles similar to DAMGO. (**b**) Ligands with profiles that differ from that of DAMGO. The score of τ/K_A (or efficacy/affinity) values was normalized to the reference ligand DAMGO and to the cAMP assay. Statistically significant differences ($P \leq 0.05$) are denoted by black circles as determined by two-tailed t test. For the purposes of visualization only, a τ/K_A for Met-enk-RF in the internalization assay was estimated using the incomplete concentration response curve for plasma membrane marker (Thompson et al. 2015)

With respect to exogenous ligands, biased activation of MOR toward G-protein was observed for a fungal peptide, which is opposite to the biased activity produced by endogenous endomorphin-2 that is toward β-arrestin-2 (Dekan et al. 2019). Biased agonist at MOR opens a new opportunity of agonists with lower side effects (Madariaga-MazÓN et al. 2017). Oliceridine is a biased agonist at MOR, which provides lower β-arrestin binding leading to lower receptor internalization and thus lower respiratory depression and tolerance compared to unbiased morphine agonist (Dewire et al. 2013). Similar natural products such as mitragynine and 7-hydroxymitragynine (Kruegel et al. 2016) and salvinorin-analogues (Groer et al. 2007) also showed a biased effect with lower β-arrestin binding. The PZM21, a molecule obtained by structure-based screening and design, showed selective MOR agonistic effect with minimal β-arrestin activation (Manglik et al. 2016). Biased agonist at KOR was developed to produce antinociception and anti-itching activities

without the concomitant dysphoria and sedation usually associated with this receptor (Brust et al. 2016). Several biased agonists at KOR were successfully developed, and some are being used clinically (Mores et al. 2019; Brust et al. 2016). Despite the previous successes, there are unresolved experimental limitations of measuring differences in biased factor among various ligands in addition to the implications of cellular environments (Mores et al. 2019; Ho et al. 2018). Although beneficial results observed by using biased agonists to stabilize receptor conformational ensembles that said to activate intracellular Gαi over β-arrestin-2 recruitment (Ranjan et al. 2017), such simplification of the story might not be precise (Conibear and Kelly 2019; Bermudez et al. 2019).

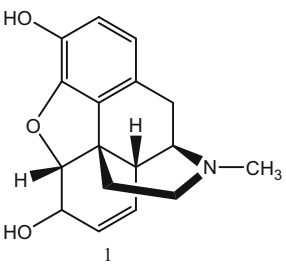
Like other GPCRs, the allosteric sites of ORs are therapeutically promising to get biased signaling (Livingston and Traynor 2018; Livingston et al. 2018). Moreover, allosteric ligands can enhance efficacy/affinity for endogenous opioid peptides as well as provide selectivity toward particular OR type, which usually is difficult to achieve using orthosteric ligands (Livingston et al. 2018). Interestingly, some endogenous compounds act as PAM for ORs, for example, oxytocin which is a peptide hormone principally involved in labor and lactation act as PAM for orthosteric endomorphin-1, β-endorphin, and morphine by enhancing efficacy and not affinity toward MOR (Meguro et al. 2018). The tuber of the species Aconitum is traditionally used in Japan to relieve pain, currently shown to have ignavine, that has selectivity for MOR over KOR and acts as PAM for endomorphin-1 and morphine (Ohbuchi et al. 2016). Biased signaling can also be produced using allosteric modulators. The BMS-986187, a synthetic compound discovered by high-throughput screening (HTS), can function as PAM selectively for DOR (Burford et al. 2015) and produce biased signaling toward G-protein over β-arrestin-2, which is elicited by lower receptor internalization (Stanczyk et al. 2019).

12.4 Opioid System as Potential Target for Neurological Disorders

The ongoing developments, including a plethora of available drugs and various promising investigational molecules, suggest the potentiality of opioid receptors as viable drug targets for therapeutic interventions of numerous disorders. Targeting and modulating these molecular switches mediates the alleviation of Alzheimer's disease (AD) (Torres-Berrio and Nava-Mesa 2019), mood disorder (Lutz and Kieffer 2013; Lalanne et al. 2014; Ehrich et al. 2014; Browne and Lucki 2019) and psychiatric disorders (Tejeda et al. 2011; Carlezon and Krystal 2016; Guerrero et al. 2019) and treatment of alcohol and opioid use disorder (AUD and OUD) (Niciu and Arias 2013; Schuckit 2016). Besides, the target-specific treatment intervention related to bowel disorders (Lacy et al. 2016; Corsetti et al. 2019), chronic pain (Günther et al. 2017; Markman et al. 2019), ischemic stroke (Wang and Subedi 2020) and respiratory disorders (Zebraski et al. 2000) have also been demonstrated amongst others. This section focuses on the current updates on targeting the opioidergic system in an effort to treat neurological disorders.

12.4.1 Alzheimer's Disease (AD)

Numerous evidential researches suggest the possible association of the opioidergic system and pathogenesis of AD. The opioid receptors are innervated extensively in the specific region of the central nervous system that is vital for cognition and memory and includes hippocampus and cortex. Any abnormality within the opioid system homeostasis may result in the hyperphosphorylation of *tau* proteins and subsequent generation of amyloid-beta (Aβ) proteins. This event cause neuroinflammation followed by cholinergic neurons' deterioration and impairment of cognitive functions (Mathieu-Kia et al. 2001). Alterations in the level of G-protein coupled opioid receptors (μ, δ, and κ) indicate a significant association of the endogenous opioid in AD pathology (Nandhu et al. 2010). The ORs can modulate the imbalance of various neurotransmitters of cholinergic, adrenergic, GABAergic, serotonergic, and glutaminergic systems that are involved with the progression of AD (Cai and Ratka 2012). Studies on morphine (**1**) and endogenous opioids such as endomorphin-1 (**2**) and endomorphin-2 (**3**) showed substantial protection against intracellular Aβ toxicity in both human and rat brains *in vivo*. Morphine mediated its activity by stimulating estradiol release in the hippocampus and increases the activity of P450 cytochrome aromatase. This chain of events stimulates the heat shock protein 70 (Hsp70) and confers protection against neurodegeneration (Cui et al. 2011). Furthermore, morphine attenuates the Aβ induced neurotoxicity by activation of MOR and upregulates the mammalian target of rapamycin (mTOR) signaling as evaluated by cell viability and neurite outgrowth assay (Wang et al. 2014).



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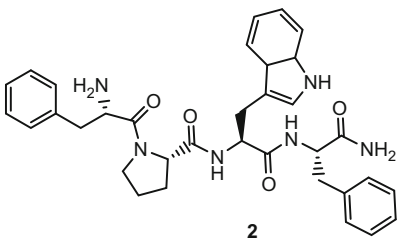

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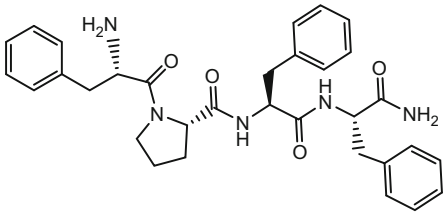

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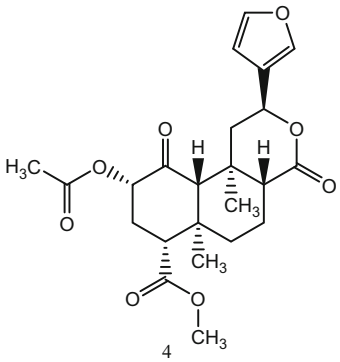

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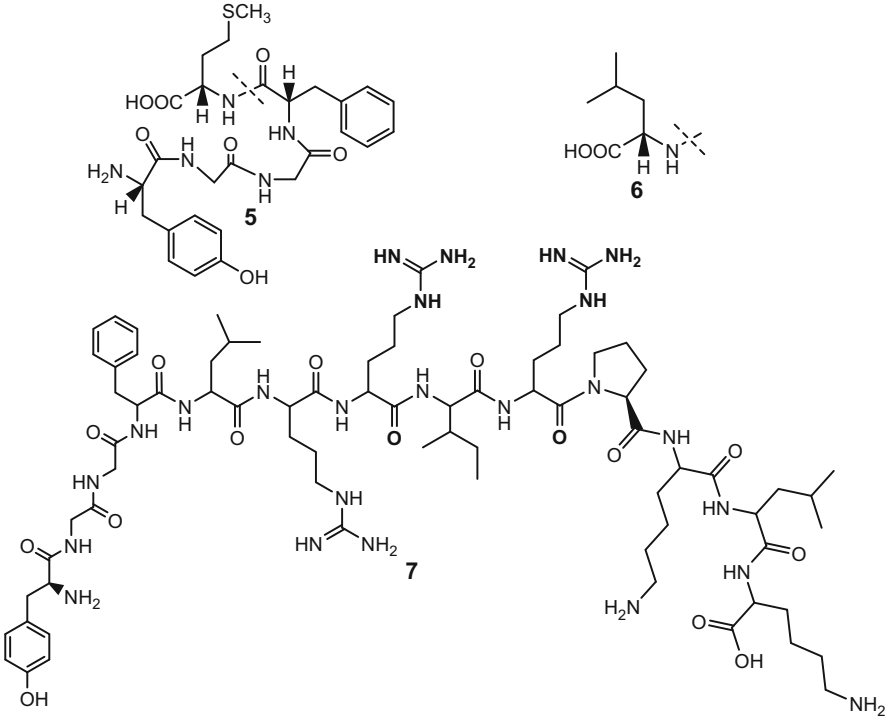
Unlike morphine, salvinorin A (**4**), a novel non-nitrogenous hallucinogenic neoclerodane diterpene isolated from *Salvia divinorum* (Roth et al. 2002), exhibited potent hallucinogenic property by selective agonism at κ-opioid receptor (KOR). This activity opens up new avenues and insights for the development of selective KOR antagonists. Targeting and blocking KOR will reverse the hallucinosis and altered perception as observed in AD and Pick's and Huntington's diseases (Cunningham et al. 2011; Sheffler and Roth 2003). Besides, salvinorin A also displayed modulatory activity on cholinergic systems and may signify a valuable molecular entity for alleviating the progression of AD (Motel et al. 2013). Research on an elevated level of dynorphin A (**5**) (Yakovleva et al. 2007) and enkephalins, namely [Met]-enkephalin (**6**) and [Leu]-enkephalin (**7**), observed that they induce neurodegeneration in transgenic mouse models and AD patients (Meilandt et al. 2008).



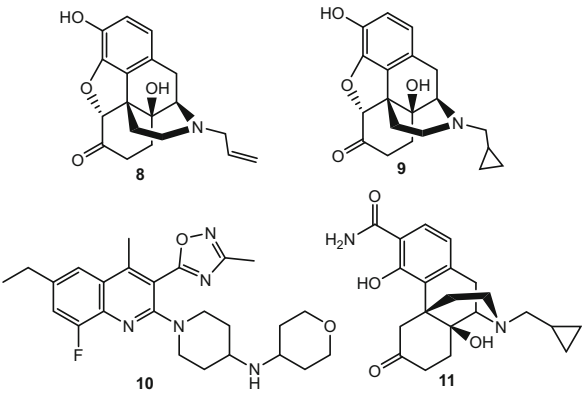
The dynorphins were profoundly expressed during the aging process and AD and have been reported to stimulate the KOR and thought to induce stress-related memory impairments. Additionally, they also affect glutamate neurotransmission and perturb their function of synaptic plasticity essential for memory (Ménard et al. 2013).

The rate-determining step in the production of Aβ is the proteolytic breakdown of Amyloid Precursor Protein (APP) by β-secretase (BACE1). The activation of DOR

by an agonist has been shown to increase the secretase activity probably through the post-translational mechanism and causing an increased formation of Aβ (Sarajarvi et al. 2015). The cascade of reaction makes DOR and BACE1 as prospective targets in the design of DOR antagonist (Zhao et al. 2015) and BACE1 inhibitors (Coimbra et al. 2018) for ameliorating the neurodegeneration underlying AD. The hypermethylation of opioid receptor δ 1 (OPRD1) promoter was also linked with the risk of AD (Ji et al. 2017). Subsequent studies suggest that in addition to OPRD1, elevated methylation of opioid receptor κ1 and opioid receptor μ1 genes are also involved in the progression of AD. Therefore, the genes of the opioid receptors could serve as potential biomarkers for AD diagnosis (Xu et al. 2018).

12.4.2 Schizophrenia

Schizophrenia is a multifaceted, diverse mental disorder that affects the behavior and cognitive function and has genetic or environmental predisposition, or both. Antipsychotics, along with psychological therapies, are the primary line of management available to alleviate the disorder. In recent years, much research to gain insight into the pathophysiology of schizophrenia has been undertaken (Owen et al. 2016; Patel and Shulman 2015) and identification of novel targets is under investigation (Gill et al. 2018; Yang and Tsai 2017). The cardinal features of schizophrenia are negative symptoms (reduced enthusiasm and withdrawal from society), cognitive symptoms (disruption of attentiveness and dementia), and positive symptoms (hallucinations accompanied by delusions). KOR agonists could elicit these specific symptoms and drugs blocking this receptor might result in a fruitful therapeutic outcome. Antipsychotics are capable of effectively combating the positive symptoms; however, presently, efficient therapeutic managements for controlling the symptoms (negative or cognitive) of schizophrenia are not available. The potentiality of a pan-opioid antagonist either naloxone (**8**) or naltrexone (**9**) could make KOR a promising target option for overall treatment benefits in schizophrenia (Clark and Abi-Dargham 2019; Shekhar 2019). Alongside, contemporary research on the discovery of novel KOR antagonist by Guerrero et al. has shown encouraging results. The promising drug candidate BTRX-335140/CYM-53093 (**10**) exhibited potent (IC_{50}-0.8 nM) and selective KOR antagonistic activity with a favorable pharmacokinetic profile. Currently, the compound is undergoing phase I clinical trials for the possible treatment of various psychiatric disorders (Guerrero et al. 2019). A recent double-blind phase II study found that a combination of olanzapine and fixed dose of MOR antagonist samidorphan (**11**) demonstrated clinically and statistically significant reduction of weight gain and adverse metabolic effect of olanzapine without compromising the antipsychotic efficacy of olanzapine. The combination was considerably tolerated and comparable to that of olanzapine-placebo in terms of safety (Chaudhary et al. 2019; Martin et al. 2019).

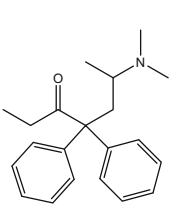


12.4.3 Post-traumatic Stress Disorder (PTSD), Opioid Use Disorder (OUD), and Alcohol Use Disorder (AUD)

Post-traumatic stress disorder (PTSD) is a psychiatric disorder that may arise after a single encounter or exposures to life-threatening chronic events. PTSD deteriorates physical health and is mostly accompanied by cardiorespiratory, musculoskeletal, gastrointestinal, immunological, endocrine, and metabolic problems. It is also associated with psychiatric comorbidity and an increased suicidal tendency (Bisson et al. 2015; Yehuda et al. 2015). Existing approaches in the treatment of PTSD involve cognitive behavioral therapy and the use of anxiolytic/antidepressant agents to ameliorate the symptoms (Shalev et al. 2017). Interestingly, studies suggest that endogenous opioid peptides exhibited a placebo effect in PTSD, and the mood-enhancing effects of the peptides may be initiated by exercise and light therapy to relieve the stress. It is suggested that the interaction between dopaminergic pathways and the endogenous opioids may be responsible for the placebo effect (Sher 2004), although it would not be a stand-alone option and may be concomitantly utilized along with standard drugs. As discussed earlier, the dynorphin mediates its action *via* KOR and the dynorphin/KOR interrelationship is associated in several brain disorders (De Lanerolle et al. 1997; Mathieu-Kia et al. 2001; Mello and Negus 2006). Various works of the literature suggest that in PTSD, there is a substantial expression of the KOR and mediate the symptoms of anxiety. Therefore, in line of the evidence, targeting the KOR might be a viable option in the management of PTSD (Bailey et al. 2013). The opioid analgesics prescribed in PTSD often result in comorbidity between PTSD and OUD, and they are frequently considered as two sides of the same coin (Elman and Borsook 2019; Hassan et al. 2017).

Centrally acting competitive MOR antagonist opioid receptor antagonists such as naloxone is the ideal choice in emergencies related to opioid overdose. On the contrary, naltrexone, which mediates its action *via* KOR antagonism, is employed mainly in OUD and AUD for maintaining abstinence by decreasing the cravings

(Theriot et al. 2019). However, both opioid agonist and antagonist are utilized in substance abuse therapies to combat the withdrawal syndromes and for the inhibition of return usage. Agonists such as morphine and methadone (**12**), partial agonist buprenorphine (**13**) and opioid antagonist such as extended-release injectable naltrexone are recommended for overall treatment and tackling the relapsing of OUD. The mechanism by which opioid antagonist maintain abstinence in OUD and AUD is by reducing the mesolimbic dopaminergic neurotransmission (McCarty et al. 2018; Williams et al. 2008).



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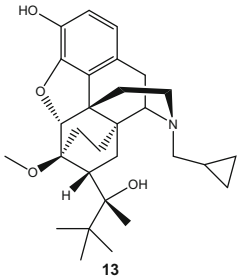

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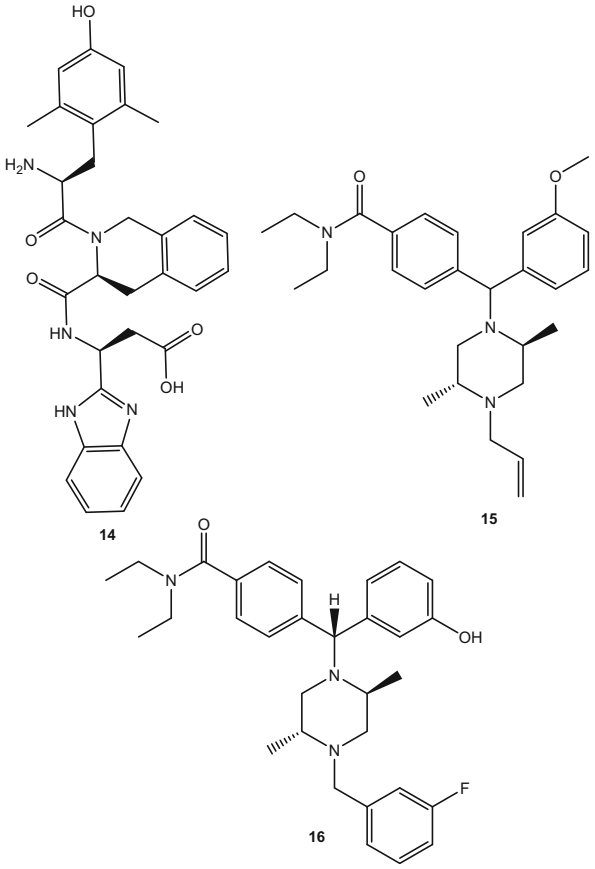
12.4.4 Parkinson's Disease (PD)

It is a chronic neurodegenerative disease having marked motor (rigidity, tremor, bradykinesia, firmness, and defective gait) and nonmotor features (hyposmia, sleep disorders, depression, etc.) (Schapira et al. 2017; Xia and Mao 2012). The disease is related to formation of Lewy bodies and dopaminergic neuronal damage in the substantia nigra. The main challenge in the treatment of Parkinson's disease (PD) is the failure to make a conclusive diagnosis at the earliest stages and difficulties in late-stage management of symptoms. Presently, there are no effective treatments for slowing down the neurodegenerative process and involve significal physical and mental co-morbidity (Demaagd and Philip 2015; Kalia and Lang 2015). The currently available pharmacotherapeutic options in PD are dopamine precursor (first line), dopamine agonist and monoamine oxidase B inhibitors (second line), the antiviral drug amantadine (third line) and a newer United States Food and Drug Administration (USFDA)-approved second-generation antipsychotic pimavanserin (Young and Mendoza 2018). The increasing perspective of the opioid receptors as a promising target in numerous brain disorders is well established. The treatment with levodopa is almost always associated with dyskinesia. Recent data suggest that selective opioid antagonists (MOR and DOR) can efficiently improve the dyskinetic side effect in animal models (Pan and Cai 2017; Sgroi and Tonini 2018).

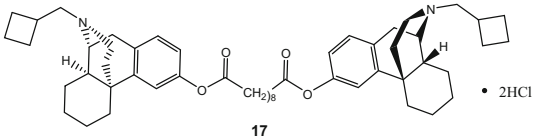
Nevertheless, a study on DOR agonist UFP-512 to mitigate motor insufficiencies in hemilesioned rodents resulted in a mixed response. At a low dose involving rotarod test, it largely improved the gait, whereas at high dose UFP-512 (**14**) was found inefficacious or to exacerbate the symptoms of Parkinsonism. Further, when

locally microinjected in the globus pallidus (GP), increased akinesia was observed, and vice versa, when injected in the substantia nigra pars reticulate. Adverse effects such as convulsions may restrict the use of DOR agonist in Parkinsonism. The convulsion was avoided by a synergistic combination of a DOR agonist, SNC-80, (**15**) and J-113397, an N/OFQ antagonist (Mabrouk et al. 2009; Mabrouk et al. 2014). Conversely, UF-512 showed encouraging activities as anxiolytic/antidepressant and treatment against chronic and neuropathic pain (Polo et al. 2019; Vergura et al. 2008). Recently, a new mixed DOR agonist/MOR antagonist, DPI-289 (**16**), in combination with levodopa elicited improved activity without increasing dyskinesia, and it was superior when compared to high dose levodopa (Johnston et al. 2018).



12.4.5 Mood Disorder

Mental illness such as bipolar disorder (BD) and major depressive disorder (MDD) are recalcitrant to treat due to the chronic nature and due to inter-individual variation (Jeon et al. 2016). Studies found that opioid analgesic demonstrated potent mood-elevating effect on patients with bipolar disorder and involve positive interaction between the opioid and the dopaminergic systems (Schaffer et al. 2007). Preclinical evaluations revealed that the dynorphin system is related to mood, motor, cognitive, and endocrine functionality and subjects with MDD and BD showed a decreased level of prodynorphin mRNA expression (Hurd 2002). Besides, a novel KOR antagonist MCL-144B (**17**) displayed antidepressant activity in the forced swim test (Reindl et al. 2008). Berrocoso et al. reported that a combination of a selective serotonin reuptake inhibitor (SSRI) with a weak MOR agonist, (+)-tramadol (**18**), produced better antidepressant activity than SSRI alone (Berrocoso and Mico 2009). As previously discussed, UF-512, a DOR agonist, showed anxiolytic/antidepressant properties (Polo et al. 2019). However, a combination of opioid-based samidorphan (MOR antagonist) and buprenorphine (ALKS 5461) in phase III trials was recently rejected by USFDA because of inadequate data to prove its effectiveness.



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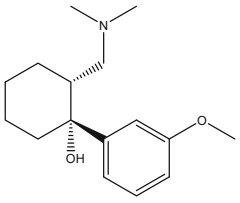

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12.5 Effect of Opioids on Various Ion Channels

The body of human beings normally generates substances similar to opiates and utilizes them as neuromodulators. These opiate-like substances comprise of β-endorphins, dynorphins, and enkephalins, and are frequently conjointly called as opioid peptides or endogenous opioids (Corder et al. 2018; Li et al. 2012; Pathan and Williams 2012). Opioid peptides are implicated in the regulation and/or control of various body functions such as tolerance and drug dependency, stress and pain,

cognition, immunological, muscle-related, cardiovascular (CVS) and endocrine. Not only these, endogenous opioids also play a vital role in monitoring various sensory functions (Bodnar 2018). Opioids are enormously expressed in several parts of the brain including non-neuronal tissues as well, namely central nervous system (CNS) and peripheral nervous system (PNS). Within the CNS, opioids exert their actions in spinal cord; while in PNS, they have been found to act not only in myenteric but also in submucous plexus situated within the stomach wall and are accountable for producing vigorous constipation. Besides, opioids have been entailed in reduction of pain stimuli and inflammation in several peripheral tissues like joints (Iwaszkiewicz et al. 2013).

12.5.1 Calcium Channels

The entry of Ca^{2+} ions through the voltage-gated Ca^{2+} channels (VGCCs) results in depolarization of nerve terminals that further causes the discharge of neurotransmitters from the nerve cells. Three types of voltage-gated Ca^{2+} channels are reported, namely, T-type channels which are capable of showing small conductance, N-type channels which are inept to demonstrate intermediate conductance, and L-type channels which illustrate large conductance. Opioids act through the inhibition of N-type voltage-gated Ca^{2+} channels and reduce the passage of Ca^{2+} ions inside the cell, thereby inhibiting the release of neurotransmitters (Zamponi et al. 2015; Seseña et al. 2014; Catterall et al. 2013; Zamponi and Currie 2013). However, this action of opioids exclusively is not accountable for the total cumulative effect of opioids on neurotransmitter release (Chieng and Bekkers 2001).

12.5.2 GABA Channels

The euphoric effect of opioids may be due to another mechanism in which the GABA inhibitory interneurons of the ventral tegmental area (VTA) are involved (Listos et al. 2019; Creed et al. 2014; Xi 2002). By attaching to the μ-receptors, the exogenous opioids like morphine and heroine decrease the amount of GABA (a neurotransmitter) released. More often than not, GABA reduces the amount of dopamine released in the nucleus accumbens (NAcc). Hence, by inhibiting GABA, the opiates eventually increase the concentration of dopamine produced and consequently the amount of pleasure felt (Dubhashi 2018; Nuechterlein 2016; Shirayama and Chaki 2006). Opiates also have dopamine-independent effects within the NAcc, which play an important role in opiate reward (Ting-A-Kee and Van Der Kooy 2012; Tomkins and Sellers 2001; O'malley et al. 1992; Shippenberg and Elmer 1998; Koob and Bloom 1988).

Besides this, the periaqueductal gray (PAG) in the midbrain region being rich in endogenous opioids and opioid receptors is a major target of analgesic action in CNS (Tsagareli et al. 2012; Pathan and Williams 2012; Mansour et al. 1995). The analgesic action of opioids on PAG is exerted by the suppression of inhibitory

influence of neurotransmitter GABA on neurons that form part of a descending antinociceptive pathway (Tsagareli et al. 2012; Basbaum and Fields 1984). Opioids inhibit GABA-mediated (GABAergic) synaptic transmission in the PAG and other brain regions by reducing the probability of presynaptic neurotransmitter release (Wilson-Poe et al. 2017; Vaughan and Christie 1997), but the mechanisms involved remain uncertain. Literatures have reported that opioid inhibition of GABAergic synaptic currents in the PAG is controlled by a presynaptic voltage-dependent potassium conductance. Opioid receptors of μ-type in GABAergic presynaptic terminals are specifically coupled to this potassium conductance by a pathway involving phospholipase A_2, arachidonic acid and 12-lipoxygenase. Additionally, opioid inhibition of GABAergic synaptic transmission is also found to be potentiated by inhibitors of the enzymes cyclooxygenase and 5-lipoxygenase, presumably because more arachidonic acid is available for conversion to 12-lipoxygenase products (Zhang and Pan 2011; Heinke et al. 2011; Finnegan et al. 2006; Ingram et al. 1998). These mechanisms account for the analgesic action of cyclooxygenase inhibitors in the PAG and their synergism with opioids (Leith et al. 2007; Vaughan 1998; Vaughan et al. 1997; Tortorici and Vanegas 1995; Maves et al. 1994).

12.5.3 Sodium Channels

Voltage-gated sodium channel plays a critical role in nociception by interacting with the δ-opioid receptor. The dorsal root ganglia (DRG) neurons being rich in voltage-gated sodium channels (Wang et al. 2011; Rush et al. 2007; Wang and Wessendorf 2001; Zhang et al. 1997) can be correlated with the emergence of pain-related behavior characteristic of painful diabetic neuropathy (PDN). Activation of presynaptic δ-opioid receptor by enkephalin prevents the increase in neuronal Na^+ in DRG through inhibition of protein kinase C (PKC) and p38 mitogen-activated protein kinase. This can be implicating presynaptic receptors of primary sensory afferents in modulating the amount of voltage-gated sodium channels and can be a useful therapy for PDN (Chattopadhyay et al. 2008).

12.6 Conclusion

Opioid peptides are endogenous ligands for opioid receptors. Proteolytic processing of larger precursor proteins generates the peptides. The peptides are stored in dense vesicles within neurons and released upon activation. The release is not restricted to synaptic space; thus peptides may signal other neurons by volume transmission. Opioid peptides inhibit the release of neurotransmitters by the affected neurons, thus modulating their signal propagations. The action of opioid peptides is mediated by binding GPCR group of receptors (opioid receptors) by binding to the orthosteric binding site. However, the affinity/efficacy of orthosteric ligand is affected by cooperation with ligand at allosteric site. Interestingly, many orthosteric and allosteric ligands show biased activation of intracellular second messengers, which

provide an opportunity of separating the desired pharmacological properties from a myriad of unwanted effects. Due to the interaction between opioid receptors and other types of receptors and ion channels, opioid peptides find a wide range of applications in managing several types of neurological disorders besides their primary use as analgesics.

12.7 Remark

The authors declare that theory of biological evolution and its related terms mentioned in this chapter and in references are not considered per se by them.

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Pharmacology of Endocannabinoids and Their Receptors

13

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Abstract

The identification of cannabinoid (CB) receptors has contributed to the state-of-the-art on the endocannabinoid system and its elements. Endocannabinoids (eCBs) are the endogenous agonists, derived from the conjugation of arachidonic acid with either ethanolamine (i.e. anandamide) or glycerol (i.e. 2-arachidonoylglycerol) acting as a lipid signaling mediator via two types of cannabinoid receptors (i.e. CB_1 and CB_2). Introduction of selective CB antagonists, inhibitors of eCB transport and metabolism, cannabinoid receptor-deficient mice and highlights on amidohydrolase have greatly facilitated the subsequent investigation of the eCB system. Moreover, modulation of the eCB system holds a promising therapeutic potential in the management of a myriad of pathophysiological conditions such as anxiety or mood disorders, neuropathic pain, multiple sclerosis, neurodegenerative diseases, osteoporosis, obesity and cancer

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among others. This chapter is comprehensively focused on the signal transduction and metabolic pathways, physiological roles, pharmacology and therapeutic potential of the endocannabinoids, with particular emphasis on cannabinoid addiction.

Keywords

Cannabinoids · Cannabinoid receptors · Biosynthesis of endocannabinoids · Pharmacology of endocannabinoids · Phytocannabinoids · Therapeutic potentials of cannabinoid receptors · Addiction of cannabinoids

Abbreviations

Abh4	α/β-Hydrolase 4
2-AG	2-Arachidonoylglycerol
CB	Cannabinoid
CB1R	Cannabinoid 1 receptor
CB2R	Cannabinoid 2 receptor
CBD	Cannabidiol
CBN	Cannabinol
DAG	Diacylglycerol
eCBs	Endocannabinoids
FAAH	Fatty acid amide hydrolase
GPCRs	G protein-coupled receptors
MAGL	Monoacylglycerol lipase
MAPK	Mitogen-activated protein kinase
NAPE	N-arachidonoyl phosphatidylethanolamine
PA2	Phospholipase A2
pCBs	Phytocannabinoids
PLA1	PI-explicit phospholipase A1
PLC	Phospholipase C
THC	Δ^9-Tetrahydrocannabinol

13.1 Introduction

The medicinal use of *Cannabis sativa* or marijuana has a long and rich history dating back to the sixth century B.C. and it was introduced into western medicine during the nineteenth century (Gaoni and Mechoulam 1964). Being the oldest source of textile fibers, cultivation of cannabis originated in Western Asia and Egypt, and later expanded to Europe and America. Under federal laws in the United States, the cultivation, use and possession of cannabis is illegal (1982). The legalization of cannabis for medical and recreational use is supported by many countries, and it has been legalized in some states of America.

Cannabis sativa is a dioecious species, that is, there is a distinct male and female flower on separate plants, and it belongs to the *Cannabinaceae* family (Small et al. 2002). It is generally reported that the psychomimetic property of cannabis is

Fig. 13.1 Cannabis sativa plant

associated with the female plant and the sticky resins are secreted from the glandular hair located on the female flowers and their adjacent leaves (Fig. 13.1). A number of constituents are found in the plant possessing their crucial role in the treatment of various diseases, among which Δ^{13}-tetrahydrocannabinol (THC) is the most active constituent (Baron 2018). *Cannabis sativa* also consists of a hempseed fixed oil which is found in the seed part of the plant.

This plant is therapeutically indicated in the management of nausea, pain, glaucoma, neuralgia, cardiovascular disorder, epilepsy, inflammation, cancer, neuropsychological disorder, neurodegenerative diseases, addiction, arthritis, depression and headache (Adler and Deleo 2019; Akram et al. 2019; Alipour et al. 2019). Recent data also suggests the use of phytocannabinoids in the management of multiple sclerosis and HIV/AIDS symptoms.

The compounds identified or isolated from the plant have been continuously increasing over time. Approximately 565 compounds have been identified from *C. sativa*, among which approximately 120 are called cannabinoids. Cannabinoids (CBs) are the compounds possessing the typical C_{21} terpenophenolic ring or its derivatives/transformation products. CBs can be classified into two broad categories on the basis of the location where they are found (Baron 2018). The CBs found in the plant are known as phytocannabinoids (pCBs), whereas the CBs obtained from animals are known as endocannabinoids (eCBs). Phytocannabinoids are either found in the cannabis plant or in agricultural hemp (Gertsch et al. 2010). CBs are lipophilic in nature due to which previously it was speculated that the drug directly disrupts the cellular membrane without any specified pathway. But after the discovery of some phytocannabinoids, the presence of certain receptors was observed showing an affinity toward the CBs. CBs show their pharmacological effect by binding with a specific cannabinoid receptor (CBR) found in the animal body, which can be classified into two types: cannabinoid 1 receptor (CB_1R) and cannabinoid 2 receptor (CB_2R). These CBRs are G protein-coupled receptors (GPCRs). Endocannabinoids are lipid-based endogenous cannabimimetic neurotransmitters which bind to the CBR and CBR-proteins found in both the central nervous system (CNS) and the peripheral nervous system (PNS) (Andrade et al. 2019; Borsoi et al. 2019; Breit et al. 2019). The eCBs form the endocannabinoid system, which

is involved in the maintenance of the homeostasis of the human body (Battista et al. 2012). The development of the endocannabinoid system depends upon the intake of nutritional and dietary co-factors. The formation of eCBs occurs according to the necessity and requirement of the body, and can bind with the receptors after being produced. The endocannabinoids found in the human body are N-arachidonoyl-ethanolamine (AEA; anandamide) and 2-arachidonoylglycerol (2-AG). They serve as the endogenous agonists of CBRs and regulate the CNS as well as the PNS, thus controlling various physiological functions of the body including the immune system. Therefore, any changes in the endocannabinoid system will result in various disorders, from neurodegenerative disorder to arthritis. Homeostasis is maintained due to the constant enzymatic degradation of eCBs. Anandamide shows higher affinity but less efficacy toward the CB_1R, whereas 2-AG exhibits less affinity but high efficacy for both the receptors (Carr et al. 2019; Chye et al. 2019; Cohen et al. 2019). The eCBs are hydrophobic in nature and exhibit slower diffusion. The degradation of eCBs inside the cell occurs either by oxidation or by hydrolysis. Anandamide is hydrolyzed by fatty acid amide hydrolase into free arachidonic acid and ethanolamine, whereas 2-AG is hydrolysed into arachidonic acid and glycerol in the presence of monoacylglycerol lipase (MAGL). Cyclooxygenase-2 and several lipoxygenases are responsible for hydrolysing the eCBs by the process of oxidation. Apart from maintaining homeostasis, the eCBs are also responsible for recovery and repair of cells. This may confer various properties including anti-oxidant, anti-inflammatory, anti-anxiety, anti-psychotic, anti-epilepsy, anti-cancer, anti-nausea, anti-bacterial, anti-diabetic, anti-arthritis, pain relief, bone stimulant, immune modulator, neuroprotective and cardio protective activities. Some endogenous fatty acid derivatives are also found in the body, which are known as eCB-like compounds. These substances are known to promote the activity of classic eCBs by the entourage effect (Dale et al. 2019; Diao and Huestis 2019; Dinis-Oliveira 2019). Apart from the above two eCBs, other reported eCBs are noladin ether (2-arachidonylglyceryl ether), arachidonoyl dopamine and virodhamine. The pharmacological profile and biochemical properties of these eCBs are not known. This chapter discusses the signal transduction and metabolic pathways, physiological roles, pharmacology and therapeutic potential of the endocannabinoids.

13.2 Cannabinoid Receptors: Signaling Pathway and Distribution

Cannabinoid receptors are a class of cell membrane receptors that belong to the family of rhodopsin-like G protein-coupled receptors (GPCR). There are thus two cannabinoid receptor classes, CB_1 and CB_2 receptors, which are both coupled to G_i or G_o protein, via negative coupling to adenylyl cyclase (AC) and positive coupling to the mitogen-activated protein (MAP) kinase family. CB_1 receptors are also coupled to ion channels through the $G_{i/o}$ proteins. There is precisely positive coupling to the A-type inward rectifier potassium channels with negative coupling to N-type, P/Q-type voltage-gated calcium channels and to D-type potassium channels (Pertwee et al. 2010). Moreover, it is presumed that CB_1 also activates adenylate cyclase types II, IV and VIII via Gsα (Rhee et al. 1998). Both receptors are

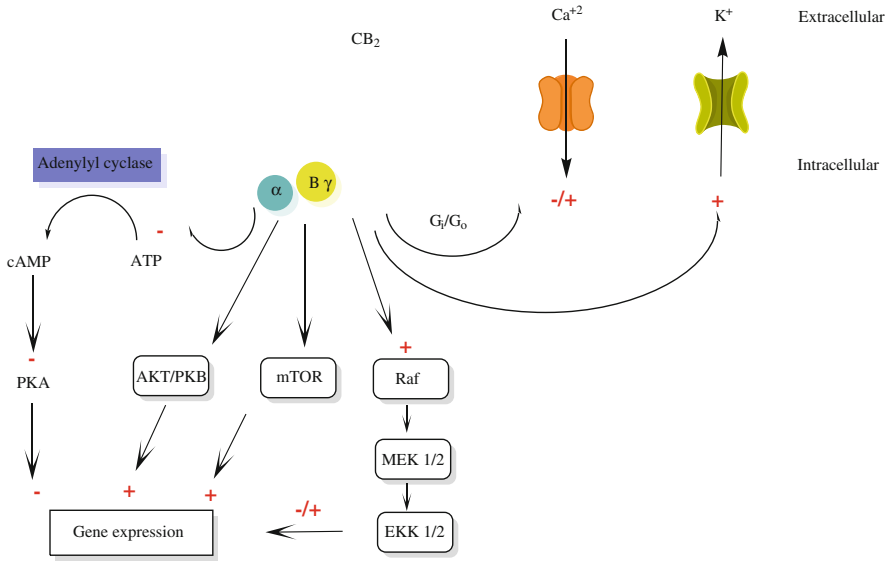


Fig. 13.2 Cannabinoid receptor signaling. *CB* Cannabinoid receptor, *mTOR* Mechanistic target of rapamycin, *Akt* Protein kinase B, *PI3K* Phosphatidylinositol-3-kinase, *PKA* Protein kinase A, *ERK* Extracellular signal-regulated kinase

located pre-synaptically and modulate neurotransmitter release. Different impacts ascribed to the CB receptor seem to include actuation of the PI3K/AKT pathway. Activation of the anandamide/CB/PI3K pathway was known to be associated with protection of rodent brain from cocaine-initiated neurotoxicity (Fig. 13.2) (Crowley et al. 2018; Curran et al. 2019; Cyr et al. 2018; Da Silva et al. 2018; Daris et al. 2019).

However, one of the essential differences between the receptor subtypes is their distribution, whereby the CB_1 is expressed on the presynaptic peripheral and central nerve terminals as well as in the peripheral organs. It is presumed to be distributed throughout the brain, with the highest concentration of these receptors located in the cerebellum (motor control and cognitive function), cerebral cortex (sensation, information processing and cognitive function), hippocampus (short- and long-term memory), hypothalamus (hormone release, metabolic process and sexual orientation), basal ganglia (voluntary movements control, learning and emotion), amygdala (memory and emotions), nucleus accumbens (motor and reward system), spinal cord (transmission of neural signals) and to a lesser extent the cardiopulmonary center of the brainstem, which seemingly justifies its lack of respiratory depression as opposed to the opioids (Pertwee 1997). Several pronounced effects of (-)Δ^9-THC can be accounted for by the distribution pattern of CB_1 receptors within the CNS. Some of these effects include the ability of (−)Δ^9-THC to decrease motor activity, as manifested in rodents by catalepsy and hypokinesia, to stimulate food intake, and

to produce analgesia (Pertwee et al. 2010; Matsuda et al. 1990; Herkenham et al. 1991).

CB_2 receptors are predominantly associated with expression in the peripheral cells derived from the immune system. Expression of the CB_2 receptor gene transcripts was detected in the thymus, mast cells, spleen, tonsils and blood cells (Galiègue et al. 1995; Munro et al. 1993), where they tend to modulate inflammatory and immunosuppressive activity and control cytokines release.

In contrast, the CB_1 was also detected in several peripheral tissues including the reproductive system, cardiovascular system and gastrointestinal tract. Recently, the CB_2 was reported to be located in the CNS, for example, in the microglial cells (Svíženská et al. 2008). It has been demonstrated that CB_2Rs expression level in the cerebrum is much lower than CB_1Rs in healthy subjects (Onaivi et al. 2008, 2006; Nunez et al. 2004). In the brain, CB_2A is the significant transcript isoform, while both CB_2A and CB_2B transcripts are available in larger amounts in the peripheral tissue such as spleen, skeletal, cardiovascular, thymus and renal systems (Jordan and Xi 2019; Rossi et al. 2018) and in immune cells, especially cells of macrophage, lineage thymus, tonsils, T-lymphocytes, monocytes, natural killer cells, and polymorphonuclear cells and B-lymphocytes (Howlett et al. 2002; Schatz et al. 1997; Galiegue et al. 1995). This prevalent distribution of cannabinoid receptors explains their wide therapeutic applications in almost every system in the human body.

It has been reported that the CB_1 receptor conserves its identity across different species such as mammals, amphibians and fish (Yamaguchi et al. 1996; Soderstrom et al. 2000). In contrast, cannabinoid CB_2 receptors are more varied. Mukherjee and co-workers (2004) and Bingham et al. (2007) reported that rat CB_2, mouse CB_2 and human CB_2 receptors show different pharmacological profiles, although all of them are considered CB_2 receptors (Mukherjee et al. 2004; Bingham et al. 2007). There is an 81% amino acid sequence in rodent and human CB_2 receptors, contrasted with 93% amino acid identity among rodent and mouse CB_2 receptors (Griffin et al. 2000).

Notwithstanding CB_1 and CB_2 receptors, pharmacological studies recommend the presence of non-CB_1, non-CB_2 receptors interceding the impacts of CB. Although a few proteins have been examined as contenders for a potential "CB_3" receptor, the reality is questionable and not yet established (Chen 2016).

13.3 Cannabinoid Receptor Agonists and Antagonists

13.3.1 Endocannabinoids

The extensively studied endocannbainoids (eCB) are the arachidonic acid derivatives anandamide and 2-AG. The first putative endocannabinoid to be identified is known as Anandamide, where 'Ananda' is the Sanskrit word for 'bliss' and 'amide' describes the 'amide linkage' in its chemical structure, which was detected in porcine brain (Devane et al. 1992).

This ligand behaves as a CB_1 and CB_2 receptor partial agonist and demonstrates higher intrinsic activity toward CB_1 than CB_2 (Mackie et al. 1993). Gonsiorek and co-workers reported that anandamide and not its metabolite arachidonic acid antagonizes hCB_2 activation by 2-AG in CHO-hCB_2 and anandamide can function as an endogenous antagonist at the peripheral cannabinoid receptor. This strengthens the hypothesis that the *in vivo* immunosuppressive effects of 2-AG or other cannabinoids are dependent on the local concentration of anandamide and 2-AG (Gonsiorek et al. 2000).

2-AG was the second endogenous cannabinoid receptor ligand to be discovered following anandamide (Mechoulam et al. 1995) and better illustrates selective binding toward CB_1 than CB_2 and functions as a full agonist. Anandamide and 2-AG show similar affinity toward hCB_2. Some other substances have been found to function as endocannabinoids. These substances comprise *N*-dihomo-γ-linolenoylethanolamine, *N*-docosatetraenoylethanolamine, arachidonoylethanolamine (virodhamine), oleamide, *N*-arachidonoyl dopamine (NADA), Arachidonoyl-serine (ARA-S) and Noladin etherand *N*-oleoyl dopamine (for a review, see Pertwee et al. 2010). Table 9.1 represents various eCBs and their type of interaction with CB_1R and CB_2R, respectively.

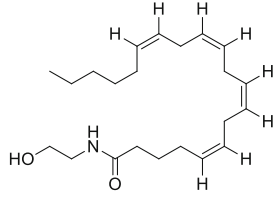
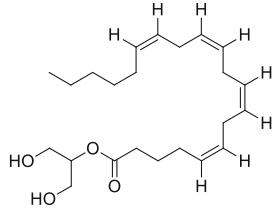
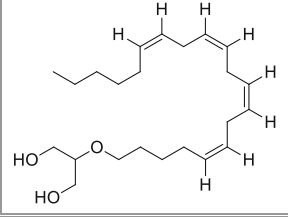
13.3.1.1 Biosynthesis of Endocannabinoids

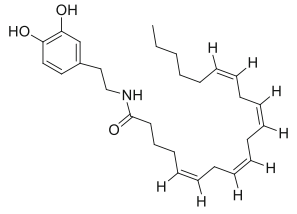
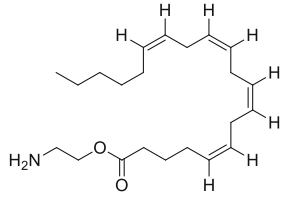
Two classes of eCBs have been recognized and completely contemplated. Anandamide and 2-AG are bioactive lipids, having a place with the subdivisions called monoacylglycerols and N-acylethanolamines, respectively.

As opposed to the majority of the intercellular signaling systems where neurotransmitters are synthesized in advance and stored in vesicles for future use, endocannabinoids are synthesized and used immediately. The endocannabinoids are synthesized on demand in a stimulus-dependent pathway, activating the cannabinoid receptor where it is required. The action of the cannabinoids is terminated once they enter the cells, where they are metabolised by enzymatic hydrolysis (Varma et al. 2001).

Several mechanisms have been postulated for the synthesis of AEA from its corresponding *N*-acyl phosphatidyl ethanolamine (NAPE) precursor. The most widely studied pathway is based on NAPE-phospholipase D (PLD) interaction (Okamoto et al. 2004; Schmid et al. 1983). However, two additional parallel pathways have recently been proposed. One pathway involves deacylation of NAPE by α,β-hydrolase 4 (ABHD4) followed by the cleavage of glycerolphosphate producing anandamide (Liu et al. 2008). The other pathway involves phospholipase C-mediated hydrolysis of NAPE-producing phosphoanandamide. The latter is then dephosphorylated by phosphatases such as tyrosine phosphates PTPN22 and the inositol 5` phosphatase SHIP1. The functional relevance of these different pathways has not yet been confirmed; however, it is quite certain that synthesis of AEA depends on the tissues in which it is synthesized (Liu et al. 2008). Biosynthesis of 2-AG involves a two-step mechanism whereby the first step involves the generation of 1-acyl-2-arachidonoylglycerol (diacylglycerol, DAG) from phosphatidylinositol

Table 13.1 Nature of endocannabinoids with receptor

S. No	Name of eCB	Chemical Name	Chemical Structure	CB1R	CB2R
1.	Anandamide	*N*-arachidonoyl-ethanolamine		Partial or full agonist	Low efficiency as an agonist and may act as an antagonist
2.	2-Arachidonoylglycerol	2-arachidonoylglycerol		Agonist	Agonist
3.	Noladin ether	2-arachidonylglyceryl ether		More affinity	Less affinity than CB1R

4.	Arachidonoyl dopamine	*N*-arachidonoyl dopamine		Antagonist	Partial Agonist
5.	Virodhamine	*O*-arachidonoyl ethanolamine		Antagonist	Agonist

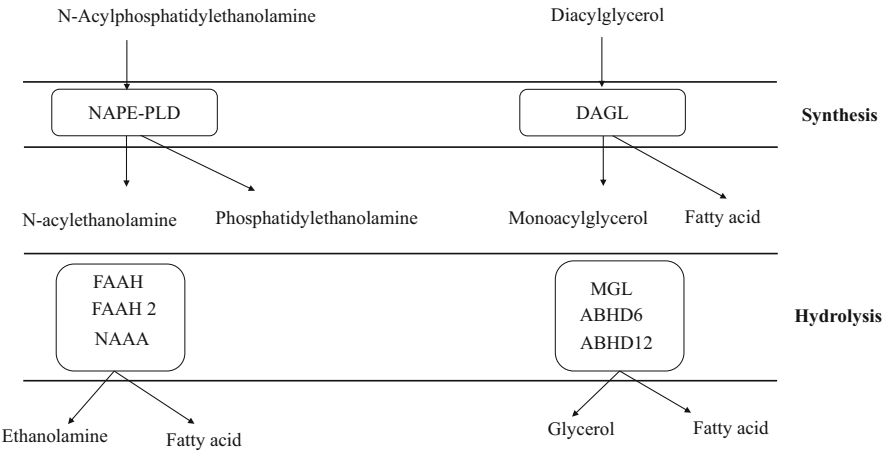


Fig. 13.3 Schematic representation of the endocannabinoid synthesis and hydrolysis. *DAGL* Diacylglycerol lipase, *NAPE-PLD* N-acylphosphatidylethanolamine phospholipase D, *FAAH* fatty acid amide hydrolase, *NAAA* N-acylethanolamine-hydrolyzing acid amidase, *MGL* Monoacylglycerol lipase, *ABHD6* α,β-hydrolase 6, *ABHD12* α,β-hydrolase 12

by PLC activity and the second step comprises the hydrolysis of DAG by diacylglycerol (Stella et al. 1997).

13.3.1.2 Hydrolysis of Endocannabinoids

The chief pathways involving the metabolism of endocannabinoids specifically, AEA and 2-AG, are the hydrolase and oxygenase pathways (Alexander 2016). AEA hydrolysis is mainly mediated by fatty acid amide hydrolase (FAAH) through the hydrolytic cleavage of the amide linkage to form arachidonic acid and ethanolamine (Giang and Cravatt 1997). Two other enzymes have also been identified, namely FAAH2 and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA). The hydrolysis of 2-AG is mainly directed by monoacylglycerol lipase (MGL). Other 2-AG hydrolases such as ABHD12 and ABHD6 have been identified (Blankman et al. 2007). AEA is also converted by cyclooxygenase type-2 (COX-2) to prostamides (prostaglandin-ethanolamides) while 2-AG is converted by COX-2 to prostaglandin glycerol esters (Fowler 2007). Biosynthesis and the hydrolysis mechanism of eCBs are presented in Fig. 9.3.

13.3.1.3 Pharmacodynamics of Endocannabinoids

Anandamide and 2-AG are understood as two noteworthy endogenous agonists of CBRs. 2-AG alone is a full agonist for CB_1 and CB_2 and intercedes retrograde signs at the neural connection, strongly suggesting that 2-AG is physiologically more significant than anandamide, which is essentially a partial agonist for the CB_1 receptor and CB_2 receptors, but has higher affinity for the CB_1R (Howlett et al. 2002). Both eCBs produce biological activity through initiation of Gi/o G proteins, coupled to calcium channel (N-and P/Q-type) hindrance and potassium channels

opening in the cell membrane, which are associated with the regulation of synapse discharge (either restraint of glutamate or GABA). Through hindrance of AC and a decrease of cAMP, or through MAP kinase pathways, eCB could likewise prolong cell action (Miller and Devi 2011; Tsuboi et al. 2018).

13.3.1.4 Physiological Role of Endocannabinoids

The eCB opened up new methodologies in the treatment of pain, obesity and neurological diseases including multiple sclerosis and other mental disorders such as drug addiction (Bilbao et al. 2004). Motor work, control of tremor and spasticity, psychological capacity (e.g. learning and memory), thermogenesis, regulation of sleep/wake cycle, adult neurogenesis, stress reaction by regulating the hypothalamic-pituitary-adrenal axis (HPA), conceptive capacity through hypothalamic-pituitary-gonadal pivot (HPG) and sex conduct, retinal neurotransmission from the retina to the essential visual cortex (Chianese and Meccariello 2016) may improve by endocannabinoids. The well-recorded inhibitory impacts of the CB_1 receptor agonists on the arrival of GABA, glutamate, acetylcholine and noradrenaline were investigated in different preclinical settings solely as clinical examinations (Schlicker and Kathmann 2001; Piomelli 2003).

13.3.1.5 Pharmacology of Endocannabinoids

More than 4000 years ago, the eCB system was first reported in India, as well as the remedial and psychotropic activity of the plant *Cannabis sativa*. The eCB system involves the CB receptors and endogenous lipid ligands. Restorative uses of antagonists of CB receptor for obesity treatment as well as control of eating conduct, intake of food and vitality metabolism have been examined in several studies. The excessive medicinal, religious and recreational utilization of marijuana in all ages was clearly not adequate to start cautious and broad research up to a few decades of 20th era on CB. The endocannabinoid system is associated with a wide range of physiological activities, a considerable number of which are identified with stress recuperation systems and the support of homeostatic equalization. Among other capacities, the endocannabinoid system is associated with neuroprotection, adjustment of nociception, regulating motor-related activities and the control of specific points of the memory process. Likewise, the endocannabinoid system is associated with balancing the resistant and provocative reactions. It additionally impacts the cardiovascular and respiratory system by controlling pulse, pressure and bronchial capacities. Endocannabinoids are known to exhibit significant antiproliferative activities in tumor cells (Elliott et al. 2019; Friedman et al. 2019; Goncalves et al. 2019; He et al. 2019; Henschke 2019).

In 1992, the first endogenous CB, AEA, additionally known as anandamide, was distinguished. Later, a second endocannabinoid, 2-arachidonoyl glycerol (2-AG), was found (Devane et al. 1992). Both these compounds are arachidonic acid derivatives and bind to CB_1 and CB_2 receptors with contrast in affinities and initiation efficacies. Neuropsychopharmacology has portrayed the eCB system activity alongside the distinguishing proof of CB_1R and CB_2R and their endogenous ligands. Nerve center, amygdaloid complex, hippocampus, mesencephalic

structures, substantia nigra, periaqueductal gray, superior colliculus and inferior colliculus comprise a complex neural circuit that handles the dread and resistance responses. For the control of anxiety and delirium states, substantia nigra pars reticulata act as a key structure in the modulator circuit. Recently, eCB system in the brain has developed as a significant subject of experiments focusing on stress-related reactions. Endocannabinoids have a wide scope of neuronal reactions such as perception, suffering, nervousness and anxiety-related symptoms. *N*-arachidonoylethanolamide and 2-arachidonyl glycerol are the two most examined eCBs that have been found to bind with CBRs. Both CB_1R and CB_2R, being the GPCR, share similar signaling nature (Pertwee 1997). CB_2R manages the constrained restriction in the brain and furthermore controls the processes that are not quite the same as CB_1R. CB_1R is associated with both glutamatergic excitatory pathways and GABAergic inhibitory neurons. CB_1R is otherwise called 'cerebrum type' CBR as it is primarily located in the cerebrum, whereas CB_2R is widely distributed in immune and blood cells. Several studies conducted on striatonigral neurons and the nigrostriatal pathway have demonstrated the presence of CB receptors in the presynaptic terminals of striatonigral neurons (Ho et al. 2019; Huestis et al. 2019; Khuja et al. 2019; Krebs et al. 2019).

In highly evolved creatures, the capacity of the ECS is to control a wide assortment of physiological procedures at both central and peripheral level. However, the potential job of the endocannabinoid system in skeletal muscle tissue remains obscure. Recently, it has been demonstrated that the muscle dimensions of 2-AG are diminished amid both myotube arrangement *in vitro* from C2C12 myoblasts and mouse muscle advancement *in vivo*. It has been additionally revealed that in primary human myoblasts the activation of CB_1 by endogenous 2-AG or synthetic agonists, for example, arachidonoyl-2-chloroethylamide (ACEA), invigorates myoblast expansion while checking myoblast separation. Inverse impacts were seen with rimonabant (SR141716) or AM251, two CB_1 antagonists/reverse agonists. Several experiments showed that the activation of CB_1 actuates a commonplace GPCR-intervened signaling component, that is, the hydrolysis of 4,5-bisphosphate, along these lines causing the hindrance of myogenesis-advancing voltage-gated potassium Kv7.4 channels (Kumar et al. 2019; Lattanzi et al. 2019). The development of diacylglycerols and inositol trisphosphate is suggested as occurring as an outcome of protein Gq-interceded PIP_2 hydrolysis. CB ligands likewise bind to GPR55, a vagrant GPCR, suggesting that this receptor may show a novel focus of CB activity. CB_1R is the standout among the most plentiful GPCR in the human brain. ECBs are lipophilic and therefore cannot be stored in vesicles such as different neurotransmitters (Lossignol 2019; Lowin et al. 2019).

Thus, the endocannabinoid signaling regulation is firmly constrained by its synthesis, release, uptake and degradation. A few other stimuli, including layer depolarization and expanded intracellular Ca^{2+} and receptor activation, can initiate complex enzymatic machineries, which lead to the cleavage of membrane phospholipids and finally to eCB synthesis. Significantly, various proteins are associated with the synthesis of particular eCB, showing an autonomous inclusion of eCB in various conditions. After synthesis, eCB can activate CB receptors, either

after the past discharge into the extracellular space or straightforwardly moving inside the cell membrane. Endocannabinoid flagging is restricted by extremely proficient degradation processes, including encouraging the uptake from the extracellular space into the cell and enzymatic catabolism interceded by explicit intracellular compounds. The molecular nature of the transporter protein(s) associated with endocannabinoid uptake has not yet been illustrated. Nonetheless, the compounds' capacity to degrade eCB is quite well characterized (Bara et al. 2018; Baron 2018; Bonini et al. 2018). They are FAAH for anandamide and related eCBs, and monoglycerollipase for 2-AG, albeit different chemicals may be in part engaged with the degradation of this last compound. An intriguing part of endocannabinoid function is the quick induction of their synthesis, receptor activation and degradation. The endocannabinoid framework has in this manner been proposed to act on interest, with a firmly controlled spatial and temporal selectivity. The framework applies its modulatory activities only when and where it is required. It represents a significant refinement among physiological elements of the endocannabinoid framework and the biological activities of exogenous CB receptor agonists, which need such selectivity. With regard to regulation of endocrine, it is intriguing to note here that hormonal incitement with glucocorticoids can prompt eCB synthesis in the nerve center by quick nongenomic systems (Bonn-Miller et al. 2018; Bramness et al. 2018; Cardenia et al. 2018). It was likewise demonstrated later that phospholipase C communicates to an intracellular event finder of membrane depolarization and receptor incitement, prompting the combination and, potentially, the arrival of eCB in the hippocampus. This information uncovers a novel component for activation of the endocannabinoid framework, which could be engaged with the regulation of endocrine frameworks. This also concerns eCB degradation, which communicates to a significant regulatory part of the movement of the endocannabinoid framework (Citti et al. 2018; Colizzi et al. 2018).

13.3.2 Phytocannabinoids

More than 500 compounds have been identified from *Cannabis sativa*, which are phytocannabinoids in nature as well as non-phytocannabinoids. A total of 104 phytocannabinoids have been isolated to date, classified into 11 chemical classes as follows: (–)-delta-9-trans-tetrahydrocannabinol (Δ^9-THC), (–)-delta-8-trans-tetrahydrocannabinol (Δ^8-THC), cannabidiol (CBD), cannabinodiol (CBND), cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL), cannabielsoin (CBE), cannabitriol (CBT) and miscellaneous-type cannabinoids (Bilbao et al. 2004). Miscellaneous cannabinoids represent compounds that are composed of different chemical structures and these compounds tend to differ in their psychoactive properties. In particular, the psychoactive properties of cannabis have been attributed to the most abundant constituent, Δ^9-THC, which was initially discovered in 1964 by Ganoi and Mechoulam (Gaoni and Mechoulam 1964), while CBD is the major non-psychotropic cannabinoid found in cannabis. The other noncannabinoid constituents that have been isolated from cannabis since 2005 belong to 8 major chemical classes, steroids, flavonoids, fatty acids,

phenanthrenes, spiroindans, xanthones, biphenyls and nitrogenous compounds (Baron 2018).

13.3.3 Examples of Cannabinoids

13.3.3.1 Δ^9-Tetrahydrocannabinol (THC)

Cannabis sativa and its subsidiary, that is, marijuana, are among the best-known substances utilized by humans for centuries and are still among the most mishandled substances around the world. Δ^9-THC is a partial agonist at both the CB_1 and CB_2 receptors and the major psychoactive component of cannabis. Δ^9-THC was isolated and the structure was characterized by Gaoni and Mechoulam in 1964. Δ^9-THC induces reactions such as excess craving, decrease in the severity of sickness, reduction of intraocular stress, euphoria and dysphoria (Volkow et al. 2014; Wang et al. 2008; Moreira and Crippa 2009). Increasingly serious antagonistic impacts include respiratory problems, hypertension, tachycardia, chest pain, muscle jerks, intense renal disappointment, nervousness, disturbance, psychosis, self-destructive ideation and intellectual impedance (Cohen and Weinstein 2018). A purified active compound of Δ^9-THC is (-)trans-Δ^9-tetrahydrocannabinol (dronabinol), as of late affirmed for the treatment of chronic pain and chemotherapy-induced nausea and vomiting (Kowal et al. 2016; May and Glode 2016). Nabilone, a more established commercial Δ^9-THC, is used in the management of chemotherapy-induced nausea and vomiting (Ware et al. 2008).

13.3.3.2 Cannabidiol (CBD)

Recent studies suggest that cannabidiol (CBD) might be helpful for the treatment of various neuropsychiatric disorders. Cannabidiol does not show tranquilizing abuse potential in experiments on mice. The researchers demonstrated that CBD, the second most significant component of cannabis, has reduced misuse potential (Viudez-Martinez et al. 2019).

13.3.3.3 GW405833

CB receptor, especially cerebrum CB_2R, expression, shows dynamic and inducible profiles under different neurotic conditions. GW405833 sub-atomic compound is under preclinical investigation showing a particular CB_2R agonistic activity. A recent report revealed that CB_2R agonist GW405833 secures liver cells and shows therapeutic impact by lessening serum aminotransferase levels, and diminishes hepatocyte apoptosis against intense concanavalin A-initiated poisonous quality through the CB_2 receptors communicated in liver resistant cells (Huang et al. 2019).

13.3.3.4 URB597 ([3-(3-carbamoylphenyl) phenyl]-*N*-cyclohexylcarbamate)

The compound URB597 is an FAAH inhibitor which prompts delay in the degradation of anandamide, leading to increased centralization of anandamide accessible for its natural movement by means of CB_1 receptor. In addition, URB597 has been

exhibited to be dynamic in discouragement (Gobbi et al. 2005), inflammation (Holt et al. 2005), neuropathy (Russo et al. 2007), and acute pain and anxiety (Kathuria et al. 2002).

13.3.4 CB Receptor Blockers

Medications that modify the endogenous eCB levels by blocking CB receptors intervene in downregulatory signaling and may give another medication focus to treating different neuropsychiatric and obesity issues. Receptor blockers that have been broadly explored include Rimonabant, Taranabent, AM4113SR141716A and SR144528.

13.3.4.1 Rimonabant and Taranabent

Rimonabant (Sanofi-Aventis) and Taranabant (Merk pharmaceutical) can block agonist-induced activation of cannabinoid CB_1R2 stuck an aggressive way and imbroglio with fundamentally more noteworthy partiality to cannabinoid CB_1 than cannabinoid CB_2 receptors (Pertwee et al. 2010).

In spite of the fact that these mixes are lacking in their capacity to activate CB_1 receptors when regulated alone, there is proof that in some CB_1 receptor-containing tissues, they can initiate inverse reactions from those evoked by a CB_1 receptor agonistic; they are CB_1 receptor inverse agonists (Pertwee 2005; Fong et al. 2007). They are suggested for the treatment of obesity by creating activity on CB_1 receptors in CNS to control hunger. Other proposed components are activities on receptors in the GIT that may tweak satiety as a peripheral means of controlling nourishment intake or on those communicated in the fat tissue that may improve metabolic confusions regularly found in those who are overweight thus diminishing insulin opposition, coronary artery disease and dyslipidemia (Kaur et al. 2013). A recent investigation revealed knowledge on the anti-tumor adequacy of Rimonabant, firmly recommending that it could be a novel lead compound for colorectal cancer treatment (Fiore et al. 2018). Alongside the clinical preliminaries in obesity that produced the information submitted to regulatory authorities, Rimonabant was additionally examined in clinical preliminaries as a potential treatment for different conditions, including diabetes, atherosclerosis and smoking cessation (Hollander et al. 2010; Dol-Gleizes et al. 2009; Steinberg and Foulds 2007). Psychiatric adverse events such as depression and nervousness were observed to be increasingly regular with Rimonabant (20 mg/day), which was tested by the US Food and Drug Administration through distributed and non-distributed preliminaries (Dol-Gleizes et al. 2009). In addition, two deaths from suicide were accounted for in patients who consumed Rimonabant. Moreover, 10% of individuals experienced sickness and upper respiratory tract infections and around 1–10% of individuals suffered from unfavorable impacts such as gastroenteritis, tension, sleeping disorder, hot flashes, loose bowels, heavy, dry or irritated skin, tendonitis, muscle aches, fatigue and flu-like symptoms.

Rimonabant was never endorsed in the United States for the treatment of obesity. The marketing endorsement for the drug was canceled by the European Regulatory Authorities in 2009 (Sam et al. 2011; Moreira and Crippa 2009).

13.3.5 Therapeutic Potential of Targeting Cannabinoid Receptors

The undesired psychotropic effects of cannabinoid agonists represent a drawback in the therapeutic development of compounds for direct activation of CB_1 receptors. On the other hand, activation of CB_2 receptors has no psychotropic side effects such as hypolocomotion or catalepsy (Volkow et al. 2014). Currently, there is an approach to developing medications that activate CB_2 receptors at doses that have little or no CB_1 receptor activation. This approach appears tempting because there is much evidence that the undesired effects induced by CB_1/CB_2 receptor agonists are attributed to CB_1 rather than CB_2 receptor activation. This indicates that there are important potential therapeutic applications for CB_2 selective agonists. For instance, therapies based on agonists targeting CB_2 receptors have been proposed for the treatment of a wide range of conditions. Table 13.2 represents therapeutic potentials of cannabinoids or their derivatives.

13.3.5.1 Pain

Therapies based on agonists targeting CB_2 receptors have been proposed for the management of an array of painful conditions. Such conditions include acute pain, nociceptive, neuropathic pain, and chronic inflammatory pain (Whiteside et al. 2007). CB_2 agonists exerted analgesic effects in animal models of neuropathic pain such as partial sciatic nerve ligation model, spinal nerve ligation model and chemotherapy-induced neuropathy. In addition, cannabinoids also showed analgesic effects in diverse persistent inflammatory pain models such as carrageenan, capsaicin, complete Freund's adjuvant, formalin and arachidonic acid (Guindon and Hohmann 2008). The mechanisms through which cannabinoids alleviate pain involve decreasing the sensitivity of transient receptor potential channel vanilloid 1 (TRPV1) to noxious stimuli (Jeske et al. 2006), inhibiting NF-κB activity and microglial production of IL-1β, IL-6 and TNFα (Klegeris et al. 2003).

In clinical trials, there were controversial findings regarding the effect of cannabinoids in pain management, since some qualitative systematic reviews reported that cannabinoids are no more effective than codeine in pain management and the risks associated with their use outweigh their benefit because of their depressant effects (Campbell et al., 2001).

13.3.5.2 Metabolic Disorders

A hypothesis proposed that when mice are exposed to cannabinoids, CB_1 receptors inhibit hypothalamic pro-opiomelanocortin (POMC) neurons which participate in feelings of satiety resulting in the release of beta-endorphins (Koch et al. 2015). The first human study assessing the effect of cannabinoids on appetite was reported in

Table 13.2 Summary of certain diseases that could be targeted by cannabinoids or their derivatives

Biological Disease	Targets	Therapeutic Potentials
Pain	• Decrease sensitivity of TRPV1 to noxious stimuli • Inhibit NF-κB activity and microglial production of IL-1β, IL-6 and TNFα	Acute pain, nociceptive, neuropathic and chronic inflammatory pain
Metabolic disorders	• CB_2R blockade	Insulin resistance associated with obesity and obesity-associated fatty liver
Asthma	• Inhibitory effects on mast cells and eosinophils in lung tissue • Reduce levels of cytokines involved in the immune response to an allergen	Immunosuppressive, anti-inflammatory and bronchodilator effects
Glaucoma	• Neuroprotective and vasorelaxant properties via CB_2R activation, increased aqueous humor outflow via enhancing the p42/44 MAP kinase	Decrease intraocular pressure, and hence decrease optic nerve damage due to inadequate blood supply
Autoimmune diseases	• Enhance levels of anti-inflammatory mediators while decreasing the levels of pro-inflammatory cytokines	• Maintain normoglycemia • Decrease inflammation associated with rheumatoid arthritis • Decrease neurodegeneration in multiple sclerosis • Improve the symptoms of Crohn's, multiple sclerosis
Bone diseases	• Stimulation of the CB_2R	• Prevent osteoclast formation • Decrease arthritis progression • Enhance fracture healing
Cardiovascular disorders	• CB_2R activation	• Diminish infract size
Gastrointestinal disorders	• Targeting the CB_1 and CB_2 receptors	• GastricB58 ulcers • Gastroesophageal reflux • Irritable bowel syndrome, • Secretory diarrhea, • Crohn's disease, • Paralytic ileus • Hepatitis C
Mood and anxiety disorders	• CB_2R stimulation	• Bipolar disorders • Drug abuse • Post-traumatic stress disorder
Neurodegenerative diseases	• Selective targeting of the CB_2R	• Multiple sclerosis • Amyotrophic lateral sclerosis • Parkinson's disease • Huntington's disease

(continued)

Table 13.2 (continued)

Biological Disease	Targets	Therapeutic Potentials
Cancer	• Down-regulated Id-1 gene expression and increase in the generation of reactive oxygen species leading to induction of apoptosis and autophagy • Induce apoptosis via pro-caspase-3 cleavage to caspase-3 • Inhibition of angiogenesis by the reduction of pro-angiogenic factors VEGF, inhibiting forskolin-induced cAMP formation and activation of RAF1 translocation and MAPK activity	• Breast and prostate cancer • Glioblastoma, glioma • Pancreatic and oral cancer • Liver and colorectal cancer • Thyroid, ovarian and cervical cancer • Skin and gastric cancer

1971 in which there was a reported increase in food intake following use of *Cannabis* (Hollister 1971). Another study also presented that oral Δ^9-THC doses of up to 15 mg/day stimulated appetite and produced significant weight gain in advanced cancer patients (Regelson et al. 1976). Later on, a more comprehensive study demonstrated clearly that smoking *Cannabis* leads to a substantial increase in food intake (Foltin et al. 1986). The expression of CB_1 receptor, MAGL and FAAH in the human pancreas was reported (Kim et al. 2011) and it was recognized that CB_1 suppresses β-cell proliferation by hindering insulin secretion. As a result, CB_1 receptor blockade leads to elevated β-cell mass in diabetic mice and enhanced insulin sensitivity. Additionally, the contribution of cannabinoids to the pathogenesis of diabetic neuropathy, retinopathy and nephropathy has been explored (Horváth et al. 2012).

CB_2 receptor stimulation enhanced insulin resistance associated with obesity and obesity-associated fatty liver and was improved in CB_2 knock-out mice, suggesting that CB_2 blockade might be beneficial for the treatment of insulin resistance and fatty liver (Deveaux et al. 2009).

13.3.5.3 Asthma

Several studies postulated that targeting cannabinoid receptors might be promising for treating patients with asthma. Cannabinoids demonstrated immunosuppressive, anti-inflammatory and bronchodilatory effects. *In vivo* models showed that cannabinoids exerted inhibitory effects on mast cells and eosinophils in lung tissue (Giannini et al. 2008) and reduced the levels of cytokines involved in the immune response to an allergen (Vuolo et al. 2015). Moreover, cannabinoids demonstrated antibacterial effects against *Staphylococci* and *Streptococci* in broth (Van Klingeren and Ten Ham 1976). CB2 receptors also regulate the function of natural killer cells by inhibiting cytokine production in a murine model of asthma (Ferrini et al. 2017).

In human studies, early studies found that *Cannabis* smoke, unlike cigarette smoke, caused bronchodilatation rather than bronchoconstriction and, unlike

opiates, did not cause central respiratory depression (Vachon et al. 1973). Another study also demonstrated that doses of THC provided by aerosol caused bronchodilatation as measured by the enhancement of lung function (Hartley et al. 1978).

13.3.5.4 Glaucoma

Recent studies have demonstrated that the neuroprotective and vasorelaxant properties of cannabinoids might be effective in reducing intraocular pressure (Tomida et al. 2004). CB_2 receptor activation increased aqueous humor outflow by enhancing the p42/44 MAP kinase activity in cultured porcine trabecular meshwork cells (Zhong et al. 2005). According to the American Glaucoma Society, cannabinoids can decrease intraocular pressure briefly, and reduce blood pressure and hence can decrease optic nerve damage due to inadequate blood supply (Jampel 2009).

13.3.5.5 Autoimmune Diseases

CB_2 is primarily expressed in peripheral tissues of the immune system (leukocytes, spleen, tonsils, thymus, bone marrow), and it has been established that cannabinoids display immunosuppressant effects and hence can be beneficial in treating autoimmune diseases. They can amend immune balance by enhancing levels of anti-inflammatory mediators while decreasing the levels of pro-inflammatory cytokines (Nagarkatti et al. 2009). Recently, the immunomodulatory effect of THC has been linked to its ability to affect epigenetic regulation by modifying histones (Yang et al. 2014).

In animal studies, cannabinoids produced a decreased risk of hypoglycemia along with a significant reduction in beta-cell damage in autoimmune diabetes (Li et al. 2001). This, in turn, helps in maintaining normoglycemia (Weiss et al. 2008). Moreover, cannabinoids were found to contribute to decreasing inflammation associated with rheumatoid arthritis (Costa et al. 2004), as well as arresting the neurodegeneration in multiple sclerosis (Pryce et al. 2003). In clinical studies, there has been evidence that cannabinoids can improve the symptoms of Crohn's disease (Naftali et al. 2014), fibromyalgia (Schley et al. 2006) and multiple sclerosis (Collin et al. 2007). A correlation has been established between the negative regulation of the cAMP signaling pathway, leading to less cAMP response element-binding protein affecting gene expression and immunomodulatory effects mediated by cannabinoids (Kaminski 1998), suggesting that selective targeting of CB_2 receptors can cause immunosuppressive effects without eliciting psychotropic effects (Turcotte et al. 2016).

13.3.5.6 Bone Diseases

Multiple studies have recognized the involvement of cannabinoids in osteoporosis as CB receptors are substantially expressed in osteoblasts, osteoclasts and osteocytes. A correlation between CB_2 receptor expression and bone density, as well as polymorphism of the gene responsible for coding CB_2 receptor and post-menopausal osteoporosis in humans, has been reported, and thus it has been found that stimulation of

CB_2 prevents osteoclast formation (Ofek et al. 2006; Karsak et al. 2005). Other studies involving *in vivo* models demonstrated that cannabinoids might improve fracture healing (Kogan et al. 2015) and prevent the progression of arthritis (Malfait et al. 2000).

13.3.5.7 Cardiovascular Diseases

In animal and human studies, cannabidiol has been found to exert anti-arrhythmic, vasodilator, antioxidant and anti-inflammatory effects (Stanley et al. 2013), while acute administration of low doses of THC before ischemia also produced cardioprotective effects by diminishing myocardial damage (Waldman et al. 2013).

CB_2 receptor stimulation has been demonstrated to diminish the infarct size of myocardium in ischemia/reperfusion (I/R) injury by inhibiting apoptosis and enhancing Akt phosphorylation (Li et al. 2013).

13.3.5.8 Gastrointestinal Disorders

The most commonly used indication of cannabinoids is nausea and vomiting since former studies have revealed that THC and its analogues were effective in managing chemotherapy-induced nausea and vomiting (Shiling et al. 1981). Paradoxically, cannabis can be problematic if used chronically and has been reported in very few cases to lead to cannabinoid hyperemesis syndrome (Lu and Agito 2015). Stimulation of CB_1 receptors has been shown to suppress gastrointestinal motility, gastric acid secretion and intestinal secretion.

CB_2 receptors are also involved in modulating the inflammatory response in the gastrointestinal system by reducing the secretion of proinflammatory cytokines (Wright et al. 2008). Thus, targeting cannabinoid receptors can be effective in multiple conditions including, gastric ulcers, gastroesophageal reflux disease, irritable bowel syndrome, secretory diarrhea, Crohn's disease, paralytic ileus and hepatitis C (Izzo and Camilleri 2008).

13.3.5.9 Mood and Anxiety Disorders

Numerous investigators suggested that CB_2 agonists can be beneficial in the management of bipolar disorders, personality disorders and drug abuse disorders (Navarrete et al. 2012). Moreover, recent evidence demonstrated that cannabinoids can alleviate the symptoms of post-traumatic stress disorder (Korem and Akirav 2014). CB_2 receptors have also been reported to modulate the midbrain, ventral tegmental area, and dopamine activity responsible for reward and addiction (Zhang et al. 2014).

13.3.5.10 Neurodegenerative Diseases

The undesired psychotropic side effects of CB_1 receptor agonists represent the downside in drug discovery. Activation of CB_2 receptors, however, has no psychotropic side effects such as hypolocomotion and catalepsy (Volkow et al. 2014). Moreover, CB_2 receptors are present in limited amounts in the brain, and this indicates that selective targeting of CB_2 receptors could be useful in treating diseases with neuroinflammatory or neurodegenerative components. Such diseases include multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease, Huntington's

disease and inflammatory peripheral disorders (Pertwee 2010). Recent evidence acknowledged the involvement of the endocannabinoid system in neuroprotection induced by minocycline against brain edema, microglial stimulation and neurological damage in traumatic brain injury in mice (Lopez-Rodriguez et al. 2013).

The down-regulation of CB_2 receptors has been described in patients with Parkinson's disease. Hence, the activation of CB_2 receptors has been postulated to delay the progression of the disease (Javed et al. 2016).

13.3.5.11 Attention-deficit Hyperactivity Disorder (ADHD)

THC has been reported to reduce hyper impulsivity associated with ADHD by binding to CB_1 receptors and enhancing retrograde signaling inhibition in the neuronal synapses, thus decreasing hyperactivity (Adriani et al. 2003). A clinical case reported in Germany showed that THC improved cognitive performance in individuals suffering from ADHD similar to stimulant medications and with limited side effects when combined with CBD (Strohbeck-Kuehner et al. 2008). Overactivation of mTOR signaling has been implicated in autism spectrum disorders by inhibiting autophagy that results in autism-like behaviors (Tang et al. 2014), suggesting that targeting mTOR signaling might provide a new therapeutics for autism spectrum disorders.

13.3.5.12 Cancers

In addition to palliative effects, preclinical studies demonstrated that cannabinoids exert antitumor, antiproliferative, antiangiogenic or proapoptotic effects both *in vitro* and *in vivo* against several types of cancer. Such cancers include glioblastoma (Guzman et al. 2006), glioma (Massi et al. 2004), pancreatic cancer (Carracedo et al. 2006), oral cancer (Whyte et al. 2010), breast cancer (Caffarel et al. 2010), prostate cancer (De Petrocellis et al. 2013), lung cancer (Preet et al. 2011), blood cancer (Gustafsson et al. 2006), liver cancer (Knowles et al. 1980), colorectal cancer (Kogan et al. 2007), thyroid cancer (Portella et al. 2003), ovarian cancer (Afaq et al. 2006), cervical cancer (Lukhele and Motadi 2016), gastric cancer (Park et al. 2011) and skin cancer (Blázquez et al. 2006).

In breast cancer cells, cannabinoids have shown the ability to down-regulate Id-1 gene expression and increase the generation of reactive oxygen species leading to induction of apoptosis and autophagy (Śledziński et al. 2018). In addition, the antitumor effects of cannabinoids, phytocannabinoids, and synthetic and endogenous ligands have been reported in various types of breast cancer including hormone-dependent and hormone-independent breast cancer cell lines. These antitumor effects include induction of apoptosis via pro-caspase-3 cleavage to caspase-3 and cell cycle arrest, inhibition of angiogenesis by the reduction of pro-angiogenic factors VEGF, inhibiting forskolin-induced cAMP formation, and activation of RAF1 translocation and MAPK activity (Kisková et al. 2019).

One of the primary advantages of the potential use of cannabinoids in cancer management is selectivity and lack of cytotoxicity to normal cells (Guzman 2003). These effects were observed in cultured cells originating from human, mouse and

rodent tumor models. Clinical trials are needed to demonstrate the effectiveness and safety of cannabinoids in cancer patients.

13.3.5.13 Cannabinoids and their Potential for Addiction

According to the National Institute on Drug Abuse, marijuana (cannabis) is the most commonly used illicit substance. One of the key issues associated with the use of cannabis is the manifestation of psychological effects. Former clinical studies investigating the effects of marijuana were conducted by Moreau and it was concluded that consuming large doses causes personality changes and hallucinations (Wilkinson et al. 2014).

It has been found that the subjective effects of marijuana consumption are partially mediated by CB_1R. The effects of cannabis differ broadly depending on many factors including the product, its route of administration, dosage form, duration of use, drug–cannabis interaction, pharmacokinetics and pharmacogenetics properties. The acute effects of cannabis tend to be transient and dose dependent and can be both physiological and psychological. At low doses, cannabis use can cause difficulties in concentration and thinking, lack of coordination, temporal distortion, restlessness, memory impairment, tachycardia and increase in blood pressure. On the other hand, higher doses can lead to anxiety, visual hallucinations, panic attacks and sensory distortion (Chopra and Smith 1974). These effects are mainly attributed to the psychoactive component of cannabis, THC. CBD, on the other hand, the nonpsychoactive constituent of cannabis, produces the opposite effects, such as anti-anxiety, anti-psychotic, neuroprotective and bradycardia.

Due to the highly lipophilic and protein-bound nature of cannabis, it has a prolonged half-life and the retention of THC can persist from several hours to days depending on the amount consumed and its route of administration. Therefore, abrupt cessation of cannabis is generally tolerated. Marijuana Abstinence Syndrome usually commences within 48 h of cessation and can last up to 2 weeks and manifests in the form of craving, anger or aggression, fatigue, anxiety, shakiness, sweating, insomnia, vivid dreams, decreased appetite, irritability and depression. Substantial clinical data is still required to confirm the safety and efficacy of cannabis consumption (Abood and Martin 1992).

13.3.6 Conclusion

The underlying disclosure and ensuing thorough research of the endocannabinoid system over the last three decades have uncovered presumably the most notable retrograde neurotransmission system. As the fundamental mediator of the psychoactive impact of THC, CB_1R has gained remarkable attention over these years. Its widespread expression and versatile functions not only increase its promising potential as a medication focus for different illnesses, but additionally make the undesired reactions almost inescapable. This hindrance drives specialists to give more consideration to the science that quite a while ago disregarded CB_2R and other endo-/phyto-cannabinoids. In addition, as a neuromodulator, the crosstalk among the

endocannabinoid and other synapse systems, by means of nearby neural circuits, or receptor heteromerization, or downstream pathway, has been accentuated. Productive investigations have been carried out, unraveling the intricacy of the entire endocannabinoid system. It is essential to remember that the investigation of the endocannabinoid system should be region- and condition-explicit, alongside the consideration of other neurotransmission systems.

Even though cannabis has existed for more than 5000 years, there is anecdotal evidence that cannabis is therapeutically beneficial for a variety of human diseases. Nevertheless, we still have insufficient data to accept or reject the use of cannabinoids in many clinical conditions. Few randomized controlled trials exist and the majority of the data is based on case reports and small retrospective reviews. The availability of a varied range of cannabinoids products, doses and routes of administration renders it difficult to compare studies and derive appropriate conclusions.

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Hormones and Steroids as Neurotransmitters

14

Sarapynbiang Marwein, Satyajeet Biswal, and Pratap Chandra Acharya

Abstract

Neurotransmitters are chemical messengers synthesized by neurons, which enable interconnection of nerve fibers within their vicinity. Neurotransmitters traditionally consist of amino acids and their derivatives, chains of amino acids, peptides or proteins. However, several studies report that steroids and hormones also exert an acute effect on the physiology of neuronal activity and the expression of behavior that can happen within minutes. Those steroids that can bind to the neurotransmitter receptors and modulate the neurotransmission signal are included together within the term neurosteroids or neuroactive steroids. The examples of neuroactive steroids include progesterone, estradiol, testosterone, DHEA, glucocorticoid, allotetrahydrodeoxycorticosterone (THDOC), androstanediol (AD), ganaloxone, androsterone, pregnenolone, allopregnanolone and their sulfate esters. Additionally, several synthetic steroids such as alphaxalone and 3α-hydroxy-5β-pregnan-20-one hemisuccinate possess similar characteristics of modulating neuronal activities to the endogenous steroids. These hormonal steroids exert their neuronal excitability functions through various receptors and ion channels such as the estrogen receptor, progesterone receptor, androgen receptor, $GABA_A$, AMPA and NMDA receptors. These neuroactive steroids are also involved in the pathology and physiology of various neurological disorders such as epilepsy, schizophrenia and traumatic brain injury. Additionally, these neuroactive steroids have agonistic or antagonistic effects toward the neurotransmission action of various other neurotransmitters some of which have undergone clinical trials for the treatment of various neurological disorders. Thus, these steroids and hormones can act as neurotransmitters, exert either agonistic or antagonistic effects on receptors and have potential benefits in the treatment of neurological disorders.

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Keywords

Neurotransmitter · Neuroactive steroids · DHEA · Estradiol · Ganaloxone · Neurological disorders

Abbreviations

AD	Androstanediol
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANT	Adenine nucleotide transporter protein
ARH	Arcuate nucleus of the hypothalamus
BLSA	Baltimore Longitudinal Study of Aging
CB1	Type-1 cannabinoid
CNS	Central nervous system
CREB	cAMP response element binding protein
D1	Dopamine 1
D2	Dopamine 2
DHEA	Dehydroepiandrosterone
DHP	Dihydroprogesterone
ERα	Estrogen receptor α
ERs	Estrogen receptors
ERβ	Estrogen receptor β
ET	Essential tremor
GABA	Gamma-aminobutyric acid
GAMSA	$GABA_A$ receptor modulating steroid antagonists
GPER	G protein-coupled estrogen receptor
GPER1	G protein-coupled estrogen receptor 1
GSK3b	Glycogen synthase kinase 3b
HPA	Hypothalamic-pituitary-adrenal
IP3	Inositol 1,4,5-trisphoshate
LXRs	Liver X receptors
MAPK	Mitogen-activated protein kinase
MDD	Major depressive disorder
mERα	Membrane-associated estrogen receptor α
mERs	Membrane-associated estrogen receptors
mERβ	Membrane-associated estrogen receptor β
mGluR	Metabotropic glutamate receptor
MPN	Medial preoptic nucleus
mPRs	Membrane-bound progesterone receptors
MPTP	1-Methyl 4-phenyl-1,2,3,6 tetrahydropyridine
NADPH	Nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartate
$P450_{7\alpha}$	Cytochrome P450 7α-hydroxylase

$P450_{C11\beta}$	Cytochrome P45011β-hydroxylase
$P450_{C17}$	Cytochrome P450 17α-hydroxylase
$P450_{C21}$	Cytochrome P450 21-hydroxylase
PGRMC1	Progesterone receptor membrane component 1
PI3K	Phosphoinositide-3 kinase
PNS	Peripheral nervous system
PPD	Postpartum depression
PPT	Propyl-pyrazoletriol
PREs	Progesterone response elements
PTZ	Pentylenetetrazol
PXR	Pregnane X receptor
SERM	Selective estrogen receptor modulator
Src	Src kinase
SRSE	Super refractory status epilepticus
StAR	Steroidogenic acute regulatory protein
THDOC	Tetrahydrodeoxycorticosterone
THP	Tetrahydroprogesterone
TSPO	Translocator protein of 18kDa
VDAC	Voltage-dependent anion channel protein
17PA	(3α5α)-17-Phenylandrost-16-en-3-ol
17β-HSD	17β-Hydroxysteroid dehydrogenase
3α-HSD	3α-Hydroxysteroid dehydrogenase
3α-diol	5α-Androstane-3α,17β-diol
3α-HSOR	3α-Hydroxysteroid oxidoreductase
3β-diol	5α-Androstane-3β,17β-diol
3β-HSD	3β-Hydroxysteroid dehydrogenase
3β-HSOR	3β-Hydroxysteroid oxidoreductase
6-OHDA	6-Hydroxydopamine

14.1 Introduction

Neurons in the brain signal to their neighbors *via* the aid of endogenous chemicals called neurotransmitters. Neurotransmitters are chemical messengers synthesized by neurons, which enable interconnection of nerve fibers within their vicinity. In a broader sense, neurotransmitters are chemical messengers which communicate signals between chemical synapses including neuromuscular junctions, from one neuron to another "target" neuron, muscle cells or gland cells (Badgaiyan 2011). Till the very recent past, neurotransmitters belonging to the categories of amino acid and their derivatives, chains of amino acids, peptides or proteins was a very stated fact (Rudolph et al. 2016). Molecules such as steroids, cholesterol derivatives and hormones were never believed to be involved in the neurotransmission system since they easily pass through cell membranes and distribute themselves in tissue. It was found that steroids exert an acute effect on physiology (Szego and Davis 1967), neuron activity (Kelly et al. 1976) and the expression of behavior (Hayden-

Hixson and Ferris 1991) that can happen within minutes. Their ability to modulate the neuronal function within minutes and to rapidly stimulate cognitive functions and behaviors suggest their neurotransmitter-like action. The understanding of the role of neuroactive steroids was conceived from the study by Etienne-Emile Baulieu on the levels of dehydroepiandrosterone (DHEA) sulfate in the brain of adult male rats (Corpechot et al. 1981; Baulieu 1998). In another study, Corpéchot and his group attained a significant finding which describes the endogenous production of DHEA sulfate within the brain, without considering the steroids secreted from the adrenals and the gonads (Corpechot et al. 1981). The steroids produced within the nervous system were referred to as neurosteroids (Corpechot et al. 1981). The documentation of other classes of steroid receptor such as the pregnane X receptor (PXR) that can be stimulated by steroids like pregnenolone and progesterone (Kliewer et al. 1998; Moore et al. 2000) additionally augments to the element of brain action modulating activity of steroids. From the 1970s onward, the potential effects of steroids such as estradiol, a cholesterol derivative, in the nervous system were appreciated owing to their ability to cause rapid alteration of the electrical impulse fired by specific neurons within the brain. This rapid change and the response time are not achievable by the binding of estrogen to the nuclear steroid receptor within the neurons, which is a slow process that takes hours to be initiated (Majewska et al. 1986; Paul and Purdy 1992). This was the initial evidence of the possible mechanism that certain steroids and hormones can modulate excitability of neurons by interacting with specific cell surface neurotransmitter receptors (Lambert et al. 1995; Rupprecht 1997; Rupprecht and Holsboer 1999). The modulatory effects of steroids in between the neurons occurs rapidly, ranging from milliseconds to seconds, which is distinguishable from the steroid's action at the genome, since it involves an event ranging from minutes to hours depending on the proportion of protein biosynthesis (McEwen 1991). Therefore, the activity of steroids within the brain that can be either their genomic or non-genomic effects establishes the understanding at the molecular level of a comprehensive effect of steroids on neuronal excitability and plasticity. Several studies demonstrate that steroids can and do function in ways that are "neurotransmitter-like," as they are produced locally in a specific region within neural circuits whereby they can exert their modulating activity locally within minutes to stimulate neuronal functions such as cognition and behavior-related activity (Balthazart et al. 2006; Dewing et al. 2007; Saldanha et al. 2011; Remage-Healey 2014).

Thus, steroids which have the capability of binding to the neurotransmitter receptors and modulate the neurotransmission signal are included together within the term neurosteroids or neuroactive steroids. However, neurosteroids are actually metabolic products of cholesterol which are produced continuously inside the brain from the readily available metabolic precursors and enzymes and they initiate instant effects on neuronal excitability (Carver and Reddy 2013). Neuroactive steroids are steroids synthesized in the endocrine gland that circulates through the bloodstream into the brain to exert their effects on brain function. Therefore, neuroactive steroids include neurosteroids and other steroids that are produced within the adrenal, testis

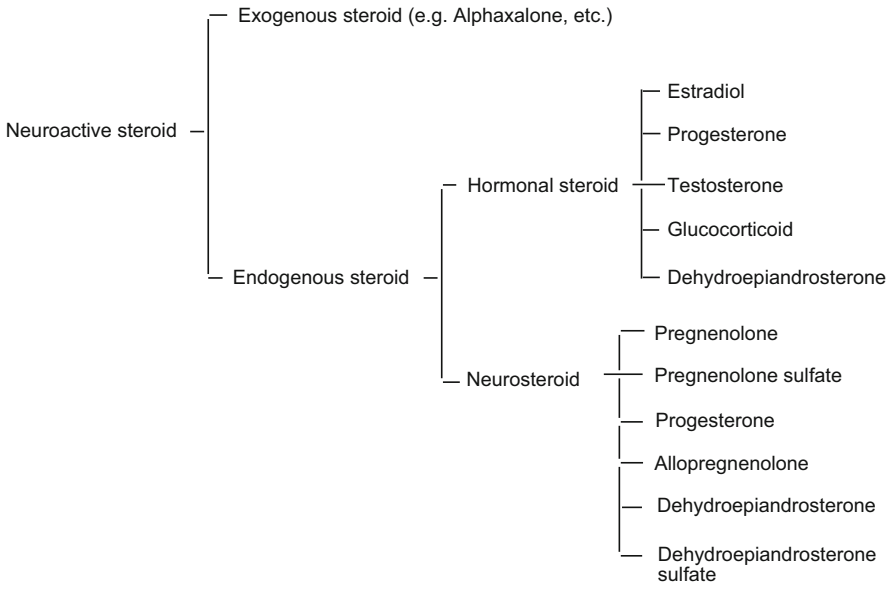

Fig. 14.1 Broad classification of neuroactive steroids

and ovary, and are transported into the brain through the blood-brain barrier to stimulate brain action in a similar way to the neurosteroids (Girdler et al. 2012).

Neuroactive steroids have the ability to regulate neuronal excitability function and are of different categories depending on their source and site of production (Zheng 2009). Neuroactive steroids are divided into two categories on the basis of the source and production site, that is, the exogenous steroids (these include the synthetic steroids) and endogenous steroids (Fig. 14.1). Further, based on the site of production, endogenous steroids are subdivided into hormonal steroid (steroids synthesized within the endocrine glands) and neurosteroids (steroids synthesized within the brain) (Melcangi et al. 2008). The examples of hormonal steroids mostly consist of steroids synthesized in the ovary such as progesterone and estradiol, in the testis such as testosterone, and in the adrenal gland including DHEA and glucocorticoid (Rhodes et al. 2004; Scharfman and MacLusky 2006), whereas the neurosteroids include steroids produced by the neuronal and glial cells, with DHEA, pregnenolone, allopregnanolone, progesterone, and their sulfate esters as the well-known examples (Baulieu 1998). Additionally, several synthetic steroids such as alphaxalone and steroid-3α-hydroxy-5β-pregnan-20-one hemisuccinate possess similar characteristics of modulating neuronal activities to the endogenous steroids (Melcangi et al. 2008). Neuroactive steroids also include allotetrahydrodeoxycorticosterone (THDOC), and androstanediol (AD), ganaloxone, androsterone, etiocholanolone (Akk et al. 2005, 2007), and 17-α and 17-β estradiol (Nguyen et al. 2017).

14.2 Synthesis, Storage, Release of Hormonal and Steroidal Neurotransmitters

The synthesis of neuroactive steroids occurs in the brain, the gonads, the adrenal glands and even in the fetoplacental unit within the body (Midzak et al. 2011). In peripheral tissues, the gonads and adrenal gland are the main site of neuroactive steroid production. Further, the metabolization process into active steroid metabolites occurs in tissues including the endocrine tissues and liver (Akk et al. 2009). The synthesis of neuroactive steroids within the body is catalyzed with the aid of various enzymes. For instance, the steroid 5α-reductase, an NADPH-dependent enzyme, catalyzes the reduction of testosterone to the neuroactive steroid, dihydrotestosterone (Reddy and Rogawski 2010). These neuroactive steroids are then circulated into the bloodstream, crossing through the blood-brain barrier into the brain (Haage and Johansson 1999; Baulieu et al. 2001). 5α-Reductase enzyme also catalyzes the reduction of steroids such as deoxycorticosterone, progesterone and various 3-keto-pregnane steroids and is available in tissues such as the brain, liver, prostate, genitalia and testis. The enzyme 5α-reductase exerts its activity in brain regions such as the neurons, glial cells, neocortex, subcortical white matter and the hippocampus (Appelgren 1967). Other enzymes necessary for the biosynthesis of neuroactive steroids include aromatase, sulfotransferase sulfatase, cytochrome 7α-hydroxylase ($P450_{7\alpha}$), 11β-hydroxylase ($P450_{C11\beta}$), cytochrome P450 17α-hydroxylase ($P450_{C17}$), cytochrome P450 21-hydroxylase ($P450_{C21}$), 3α-hydroxysteroid dehydrogenase (3α-HSD), 3β-hydroxysteroid dehydrogenase (3β-HSD), 5α-reductase and 17β-hydroxysteroid dehydrogenase (17β-HSD) (Do Rego et al. 2009).

Within the nervous system neuroactive steroids are produced locally, mainly from cholesterol (Ellsworth et al. 1998), in specific brain tissues such as the pineal gland which is the main neurosteroidogenic organ, glutamatergic neurons, cortex and the hippocampal region (Appelgren 1967). These sites are steroidogenic sites and produce steroidogenic enzymes such as 5α-reductase, aromatase, sulfotransferase sulfatase, cytochrome 7α-hydroxylase, 11β-hydroxylase and many other enzymes necessary for steroid production. Neuroactive steroids are synthesized directly from cholesterol via a progressive A-ring reduction process (Melcangi et al. 2008). The primary stage in steroid synthesis is the metabolization of cholesterol into pregnenolone. This step involves the transportation of cholesterol from the outer to the inner mitochondrial membrane with the aid of molecular complex comprising proteins such as the translocator protein of 18 kDa (TSPO), the adenine nucleotide transporter protein (ANT), the steroidogenic acute regulatory protein (StAR), and the voltage-dependent anion channel protein (VDAC) (Morohaku et al. 2014; Papadopoulos et al. 2018; Selvaraj and Stocco 2015; Selvaraj et al. 2015). It is located inside the inner mitochondrial membrane, where cholesterol is metabolized into pregnenolone in the presence of the steroidogenic enzyme P450scc (Midzak et al. 2011). Pregnenolone then undergoes transformation to other steroids via sequent stages inside the endoplasmic reticulum. For instance, pregnenolone when catalyzed by 3β-HSD enzyme or cytochrome $P450_{C17}$ is

changed into progesterone or dehydroepiandrosterone respectively. Consequently, DHEA acts as a precursor for the synthesis of other neuroactive steroids like testosterone. DHEA and progesterone can be converted further into other neuroactive steroids with the help of the enzyme 5α-reductase. Specifically, testosterone is metabolized into dihydrotestosterone and progesterone into dihydroprogesterone (DHP), which can be further metabolized into other neuroactive metabolites in the presence of 3α-HSOR or 3β-HSOR enzymes. For example, DHP is converted into tetrahydroprogesterone (THP) or isopregnanolone, while testosterone is converted into 5α-androstane-3α,17β-diol (3α-diol) or into 5α-androstane-3β,17β-diol (3β-diol). Testosterone can also be converted in to 17β-estradiol with the help of aromatase enzyme (Giatti et al. 2019). The reduction of the A-ring in steroid hormones such as progesterone, testosterone and deoxycorticosterone results in the production of other neuroactive steroids (Reddy and Rogawski 2010). The synthesis of sulfate-conjugated steroids such as DHEA sulfate and pregnenolone sulfate from their respective neuroactive steroids is catalyzed by two enzymes, that is, sulfotransferase and sulfatase (Do Rego et al. 2009). Moreover, the steroidogenic enzymes such as 3α-HSOR and 5α-reductase can convert the steroid precursors present in peripheral tissues such as liver to produce androstanediol, allopregnanolone and tetrahydrodeoxycorticosteone (THDOC), which can be circulated systematically into the brain as they are lipophilic in nature and have an effect on neuronal brain function (Do Rego et al. 2009; Kushida and Tamura 2009; Yagishita et al. 2012). Figures 14.2 and 14.3 depict the biosynthetic pathway of different neuroactive steroids.

14.3 Receptors and Channels Involved in Neurotransmitter Action of Hormones and Steroids

Primarily, steroids and hormones were conceived to act solely through the traditional genomic pathway by binding to the known steroid receptors (Evans 1988; Paul and Purdy 1992). However, the documentation of new binding sites for neuroactive steroids such as progesterone, testosterone (Ramirez and Zheng 1996), glucocorticoids (Orchinik et al. 1991), estradiol (Pappas et al. 1995) or aldosterone (Wehling 1997) in the membrane of cells and tissues with different signal transduction pathways that differ from the conventional transduction pathway involved in steroid action (Wehling 1997) has shed new light on the primarily known concept.

The neuroactive steroids exert several physiological activities within the brain facilitated by their interaction with the nuclear/membrane steroid receptors (estrogen and progesterone receptors), androgen receptors and glucocorticoid receptors and through their modulating activity toward the ion channels, which include the $GABA_A$ (gamma-aminobutyric acid), NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptors (Ogden and Traynelis 2011). The neuroactive steroids also exert their neuronal modulation function through various voltage-dependent ion channels which include the T-type Ca^{2+} channel, Na^+ channel, Ca^{2+} activated K^+ channel and anion

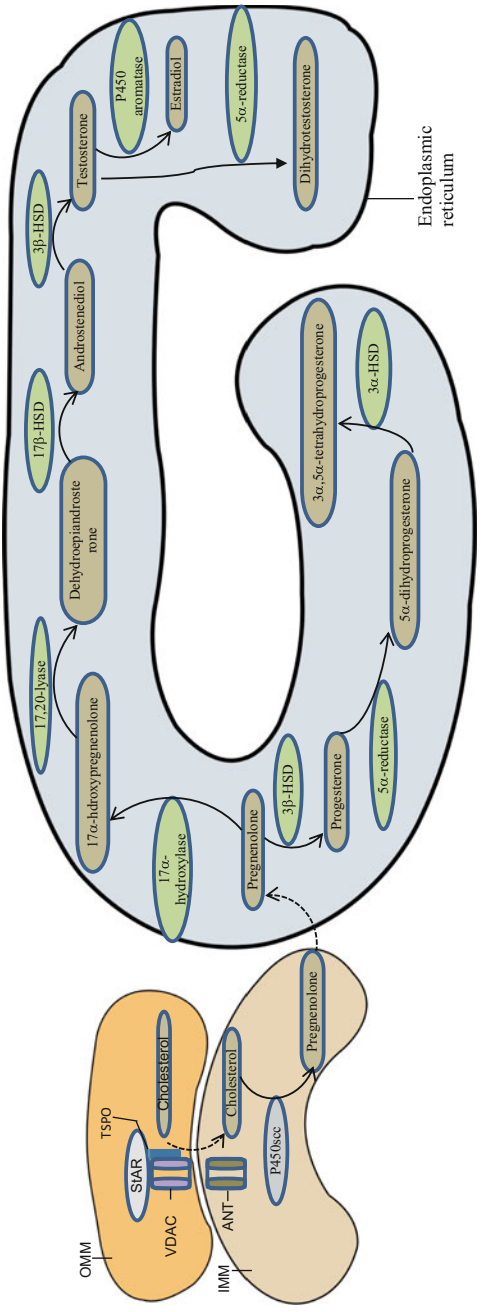

Fig. 14.2 Synthesis of neuroactive steroids within the brain

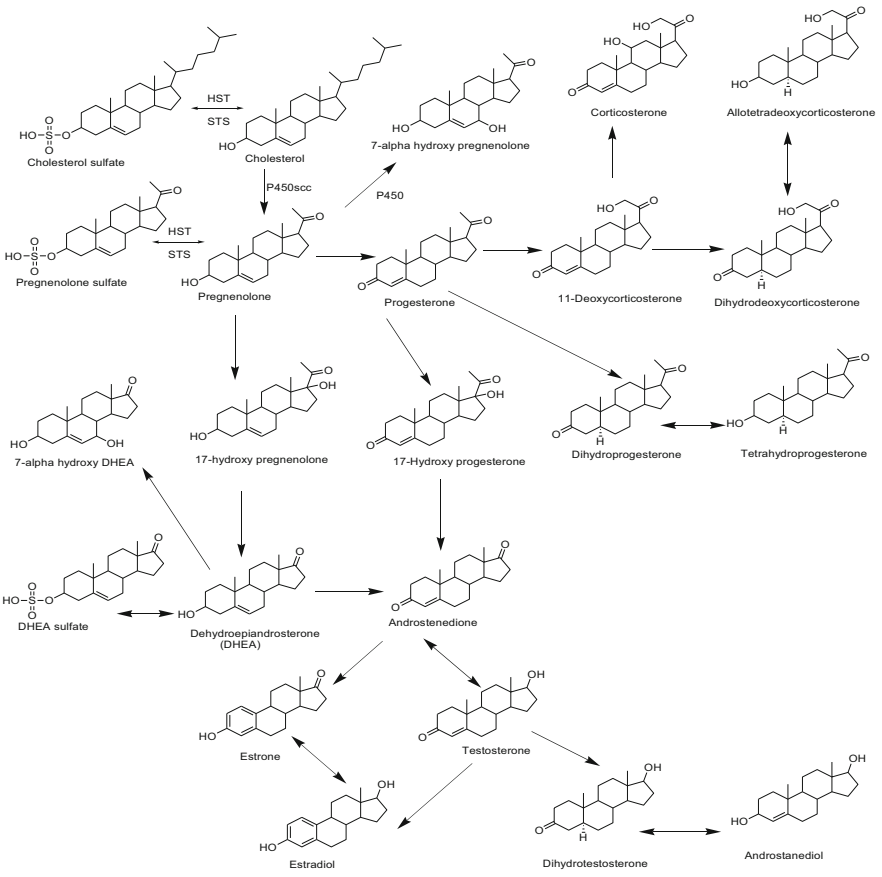

Fig. 14.3 Biochemical pathways for the synthesis of neuroactive steroids in the brain. AROM, aromatase; HST, sulfotransferase; STS, sulfatase; P450scc, cytochrome P450 side-chain cleavage; $P450_{7\alpha}$, cytochrome 7α-hydrolase; $P450c_{11\beta}$, 11β-hydrolase; $P450_{C17}$, cytochrome P450 17α--hydrolase/C17,20-lyase; $P450_{C21}$, 21-hydrolase; 3α-HSD, 3α-hydroxysteroid dehydrogenase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 5α-R, 5α-reductase; 17β-HSD, 17β-hydroxysteroid dehydrogenase

channels, and the high-voltage activated Ca^{2+} channel (Carrer et al. 2003; Todorovic et al. 2004; Pathirathna et al. 2005; Joksovic et al. 2007; Cheng et al. 2008). It is understood that the physiological effects of neuroactive steroids result from their interaction with definite receptors. Therefore, it is necessary to study the mechanism by which these steroids activate these receptors and affect brain physiology and the impact of the neurotransmitter receptors on neuroactive steroid actions (Rossetti et al. 2016).

14.3.1 Estrogen Receptors

The discovery of estradiol binding sites in different rat tissues by Elwood V. Jensen in the early 1950s led to the proposition of the occurrence of an explicit receptor for estradiol (Jensen 1962). Thereafter, Toft and Gorski (1966) isolated an estrogen receptor from rat uterus, which was later identified as estrogen receptor α (ERα) shortly after the discovery of the additional estrogen receptor known as estrogen receptor β (ERβ). These two functionally similar estrogen receptors (ERs) originated from different genes (Kuiper et al. 1996). Both the ERα and ERβ receptors are also expressed on the cell membrane (Coleman and Smith 2001; Pedram et al. 2007; Boonyaratanakornkit 2011; Meitzen et al. 2013), but there is still a lack of understanding of their cell membrane association. However, it is suggested to be involved in post-translational lipid modification and communication with membrane/cytoplasmic scaffolding proteins (Boonyaratanakornkit 2011; Meitzen et al. 2013; Pedram et al. 2007). In addition to the membrane-associated mERα and mERβ, one more membrane-associated estrogen receptor is also known to be involved in the neurotransmission function of the neuroactive steroids, G protein-coupled estrogen receptor 1 (GPER1) (Carmeci et al. 1997). All these estrogen receptors were found to be commonly expressed in the neuronal and glial cells within the central nervous system (Cardona-Gomez et al. 2000; Chaban et al. 2004; Quesada et al. 2007). Brain regions such as astrocytes were also found to express ERα, ERβ, GPER and Gq-mER (Kuo et al. 2010; Almey et al. 2012), and all these receptors are activated by neuroactive steroids such as estradiol and contribute to the regulation of the astrocytic functions (Pawlak et al. 2005; Kuo et al. 2010; Karki et al. 2014). For instance, triggering membrane-associated estrogen receptor by neuroactive steroid can alter membrane permeability (Fu and Simoncini 2008), initiate second messenger cascades (Coleman and Smith 2001) and hyperpolarize neurons in the preoptic area (Bologa et al. 2006; Prossnitz et al. 2008). Additionally, cumulative data from different studies indicate that the dihydrotestosterone metabolite, 3β-diol, can interact with and trigger the ERβ, thereby exerting its oestrogenic effects on various pathophysiological brain functions, including anxiety, depression, affection disorders and cognition (Fugger et al. 2000; Krężel et al. 2001; Österlund et al. 2005; Kudwa et al. 2006; Handa et al. 2008).

14.3.2 Progesterone Receptors

The progesterone receptors are important for the neuronal function of steroids such as progesterone, metabolites of progesterone and sulfated progesterone. Comparable to the estrogen receptors, progesterone receptors are of two isoforms, that is PR-A and PR-B, and unlike ERα and ERβ originated from an identical gene (Pratt and Toft 2003). Bound progesterone receptors dissociate from the chaperone proteins, and undertake conformational alteration leading to their dimerization, thus resulting in the direct interaction with progesterone response elements (PREs) in promoter regions of targeted genes through their binding with the steroid receptor coactivators

(Leonhardt et al. 2003). Furthermore, several membrane-bound progesterone receptors (mPRs) have also been identified (Lösel et al. 2005; Tang et al. 2005; Thomas et al. 2007). Additionally, progesterone also binds to membrane progesterone receptors and to the membrane-associated protein PGRMC1 (Progesterone receptor membrane component 1), termed 25-Dx (Schumacher et al. 2007; Brinton et al. 2008; Guennoun et al. 2008). Progesterone receptor PR-B has been reported to have rapid neurotransmitter-like actions by inducing sexual receptivity when progesterone binds to this receptor (Mani et al. 1994; Serey et al. 2014). Rapid actions of PR-B signaling are involved in a subcircuit regulating lordosis, which originates in the arcuate nucleus of the hypothalamus (ARH) and projects to the medial preoptic nucleus (MPN) (Sinchak and Wagner 2012; Sinchak et al. 2015). This phenomenon was verified by a study which described that instillation of progesterone into the ARH of estradiol-primed ovariectomized rats enables receptivity within 30 min by inhibiting ARH β-endorphin neurons that project to the MPN, which induces sexual receptivity (Huss et al. 2011). Although most evidence of neurotransmitter-like actions of steroids has focused on estrogens, progesterone and reproduction, other steroids have also been shown to act via non-classical mechanisms.

14.3.3 Glucocorticoid Receptors

Neuroactive glucocorticoids such as corticosterone and cortisol are also known for their stimulating activity in various brain-related functions and exhibit their effect mainly through the glucocorticoid receptors. Glucocorticoid receptors are nuclear receptors located in the cytoplasm that can be included in the group of receptors by which the neuroactive steroids (glucocorticoids) facilitate their neuronal signaling activity (Aranda and Pascual 2001). Classical glucocorticoid receptor signaling requires ligand binding to initiate glucocorticoid receptor dimerization and translocation to the nucleus (Oakley and Cidlowski 2013) where they bind directly with DNA and other transcription factors to regulate gene transcription. Glucocorticoids can activate these receptors at the membrane level to alter the physiology functioning more like neurotransmitters. The enzymes necessary for corticosterone production are widely distributed throughout the brain (MacKenzie et al. 2000), and *de novo* corticosterone synthesis from pregnenolone has been observed in the hippocampus of adrenalectomized rats (Higo et al. 2011). This suggests the synthesis of glucocorticoids, the expression of their receptors within the nervous system and their responsibility in modulating brain function.

14.3.4 Other Neurotransmitter Receptors

The neuroactive steroids usually bind with the steroid receptors to exert their physiological actions. However, they are also known to modulate various other ion channels including the voltage-dependent ion channels, especially $GABA_A$, NMDA and AMPA receptors. There is ample evidence indicating how neuroactive

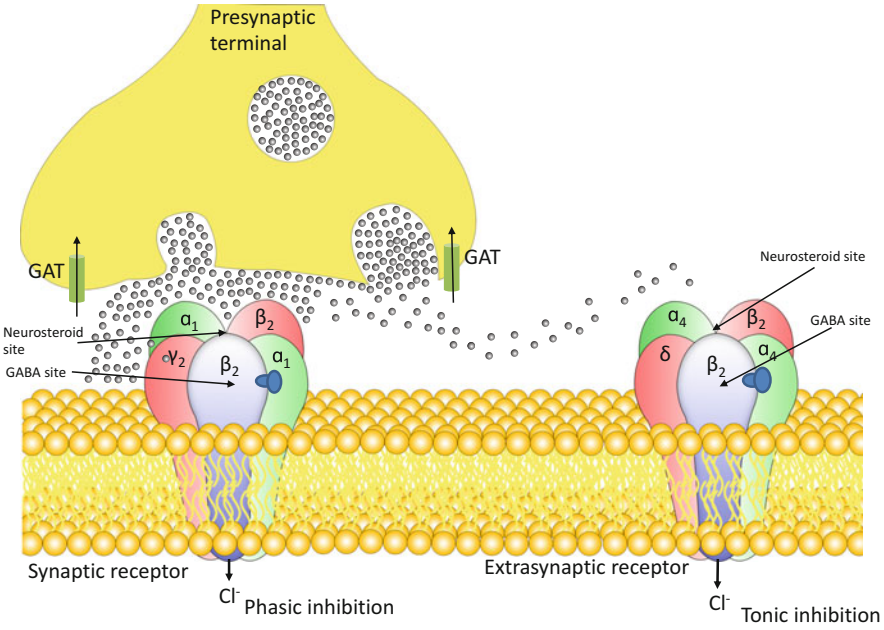

Fig. 14.4 Schematic illustration of neurosteroid-sensitive synaptic and extrasynaptic $GABA_A$ receptors in the brain

steroids modulate neuronal excitability by their direct interaction with $GABA_A$ receptors. Due to their high affinity and inherent modulating activity toward the $GABA_A$ receptor, numerous steroids are often mentioned as endogenous modulators of $GABA_A$ receptors (Majewska et al. 1986; Reddy 2003). These receptors also act as the primary neurotransmitter receptor in the brain for several other neuroactive steroids (Mellon and Griffin 2002). $GABA_A$ receptors are ligand-gated chloride channels which when activated hyperpolarize the neurons through the influx of chloride ions. $GABA_A$ receptors can be grouped into two classes: the synaptic and extrasynaptic $GABA_A$ receptors (Fig. 14.4). However, the synaptic and extrasynaptic $GABA_A$ receptors possess different degrees of affinity and sensitivity toward neuroactive steroid neurotransmitters (Brown et al. 2002; Bianchi and Macdonald 2002; Mortensen et al. 2012), desensitization rates and agonist efficacy (Bianchi and Macdonald 2002, 2003). For instance, the measure by which various progesterone derivatives modulate the $GABA_A$ receptors depicts the importance of these receptors for the neuroactive steroids to mediate their neuronal excitability actions. Studies show that fluctuations in the level of neuroactive steroids within the brain and fluctuations in GABAergic signaling correlate with the manifestation of pathophysiological brain conditions, such as depression, anxiety, seizure, stress and epilepsy (Charalampopoulos et al. 2008; MacKenzie and Maguire 2013; Murphy et al. 1998; Pluchino et al. 2015), suggesting that $GABA_A$ receptors play a

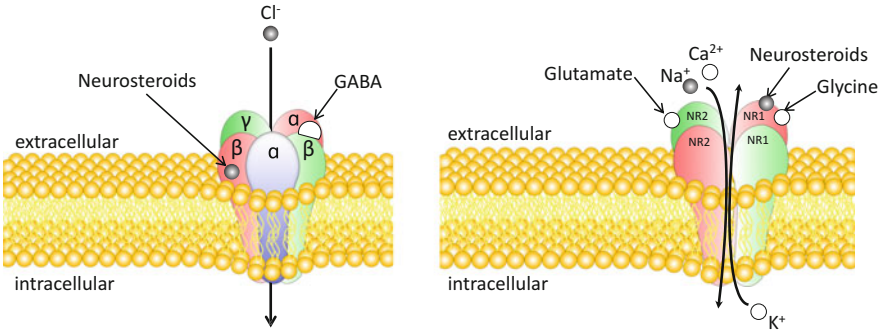

Fig. 14.5 Schematic illustration of neuroactive steroid actions mediated by $GABA_A$ receptor and NMDA receptor

significant role in mediating the neuronal excitability function of these neuroactive compounds.

The neuroactive steroids also have an affinity with the NMDA receptors in addition to the $GABA_A$ receptors (Fig. 14.5). These receptors are tetrameric ion channels consisting of two GluN1 and GluN2 subunits (formerly designated as NR1 and NR2). The GluN2 subunits of the NMDA receptors are the main contributor to the multiple functionality of the NMDA receptors and they are specifically expressed only during development in the brain regions (Ogden and Traynelis 2011). Some neuroactive steroids such as estradiol act as negative modulators, whereas DHEA, pregnenolone and their sulfate esters act as positive allosteric NMDA receptor modulators (Charalampopoulos et al. 2008; Mellon and Griffin 2002). Although $GABA_A$ and NMDA receptors seem to be the main ion channel receptors that the neuroactive steroids interact with and bind to, other kinds of receptors such as AMPA receptors have also been documented (Mellon and Griffin 2002; Niitsu et al. 2012). Neuroactive steroids such as pregnenolone sulfate, DHEA sulfate and progesterone have been known to interact allosterically with the AMPA, and their involvement in the cental nervous system functions and pathologies has been established.

In addition to the $GABA_A$, NMDA and AMPA receptors, studies also demonstrate that neuroactive steroids have an affinity with the kainate, serotonin and X-receptors of the liver, sigma-1, nicotinic receptors (Rupprecht et al. 2001; Dubrovsky 2006; Strous et al. 2006) and various voltage-dependent ion channels such as T-type Ca^{2+} channel, high-voltage activated Ca^{2+} channel, Na^+ channel and anion channels (Kelly et al. 2002; Carrer et al. 2003; Darbandi-Tonkabon et al. 2004; Todorovic et al. 2004; Pathirathna et al. 2005; Joksovic et al. 2007; Cheng et al. 2008). Neuroactive steroid metabolites can also trigger non-classical steroid receptors such as the dopamine 1 (D1) receptor, the α_1 adrenergic receptor and L-type Ca^{2+} channels (Giatti et al. 2015; Vega-Vela et al. 2017). Studies have demonstrated the neuroactive steroids' ability to modulate the neuronal excitability

which results in brain function stimulating activity such as regulation of mood and learning via their indirect involvement with the T-type voltage-gated Ca^{2+} channels (Smith and Woolley 2004; Benarroch 2007; Grassi et al. 2007; Fatehi and Fatehi-Hassanabad 2008). They have also been demonstrated to modulate mitochondrial function and synaptic plasticity and to play a vital role in neuroprotective activity due to their interaction with the voltage-dependent anion channel receptors (Tuem & Atey 2017). Furthermore, neuroactive steroids are described to have modulating effects on every synaptic transmission, namely GABAergic, glutamatergic, dopaminergic, cholinergic, serotonergic and noradrenergic synaptic transmission, through their ability to alter the receptivity of postsynaptic receptors or to alter presynaptic neurotransmitter release (Belelli et al. 2006; Gibbs et al. 2006; Mitsushima et al. 2007; Pérez-Neri et al. 2008; Laconi et al. 2007). Though with less efficacy, the neuroactive steroids tetrahydroprogesterone and 3α-diol (Carver and Reddy 2013) indeed stimulate neurotransmission via the $GABA_A$ receptor, thereby mediating their effects on affection and behavior (Belelli and Lambert 2005). Similarly, sulfate-conjugated neuroactive steroids such as pregnenolone and DHEA also exert a weak antagonistic effect on the NMDA receptor at the micromolar level, thereby producing a stimulating neuronal excitatory action (Rupprecht 2003). Neuroactive steroids including progesterone can also bind with the membrane receptors such as the membrane-associated protein PGRMC1, also known as 25-Dx, and the membrane progesterone receptors (mPRs) (Singh et al. 2013; Giatti et al. 2016). The recently identified nuclear pregnane-X receptor (PXR) presents an additional site for neuroactive steroids to bind and exert their actions (Frye 2011).

It is clear that neuroactive steroids exert various types of physiological activity in the brain (Lapchak and Araujo 2001; Belelli et al. 2006; Strous et al. 2006) either through interaction with nuclear steroid receptors such as the estrogen receptors, androgen receptors and progesterone receptors or through interaction with membrane receptors such as membrane progesterone receptors (Fig. 14.6). In particular, neuroactive steroids interact with various ion channels (Fig. 14.6) and act as allosteric modulators of the $GABA_A$ receptors (Covey et al. 2001; Belelli and Lambert 2005), NMDA receptors (Mameli et al. 2005; Monnet and Maurice 2006), kainate receptors (Costa et al. 2000), AMPA receptors (Dubrovsky 2005), sigma receptors (Maurice et al. 2006), serotonin receptors (Shannon et al. 2005a, b), nicotinic receptors (Paradiso et al. 2000) and muscarinic receptors (Horishita et al. 2005). Additionally, it has been reported that neuroactive steroids may directly trigger G protein-coupled membrane receptors (Meyer et al. 2002; Schiess and Partridge 2005) or indirectly control the binding of neuropeptides to their receptors (Torres and Ortega 2003).

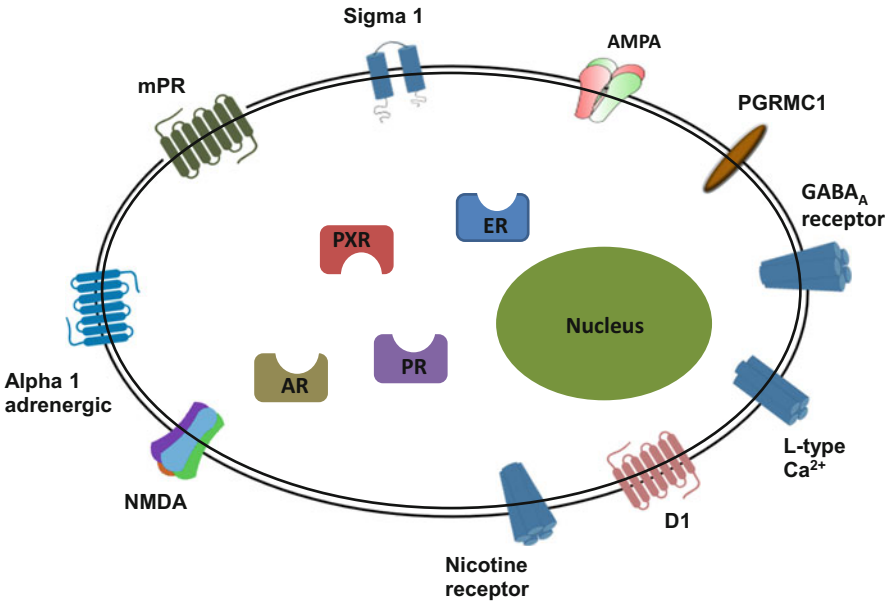

Fig. 14.6 Schematic illustration of different receptors involved in the neuronal function of the neuroactive steroids

14.4 Mechanism of Action and Signaling Pathways for Neuroactive Steroids

Neuroactive steroids usually exhibit their mechanism of action through:

1. Classical intracellular binding to steroid receptors that facilitates interaction with specific nucleotide sequences stimulating gene expression (Cato et al. 2002).
2. Effect on membrane receptors and ion channels (i.e., $GABA_A$, NMDA, AMPA, sigma 1, kainate receptors) described as a rapid non-genomic effect of neuroactive steroids (Rupprecht et al. 1993; Le Mellédo and Baker 2002) or
3. Their metabolic interconversion to other neuroactive steroids in the brain or through their interaction with certain neurotransmitter receptors (Rupprecht et al. 1993; Le Mellédo and Baker 2002).

The general down signaling pathway for most of the neuroactive steroids occurs through a series of processes as depicted in Fig. 14.7. First, neuroactive steroids might bind to ion channels (i.e., $GABA_A$, NMDA, AMPA, sigma 1, kainite receptors) associated with neurotransmitter receptors (1). Second, they can bind and activate the known steroid receptors widely distributed in the plasma membrane (2), or they may bind to the nuclear plasma membrane-associated steroid receptors (3) or bind with the traditional cytoplasmic-associated nuclear steroid receptors (4).

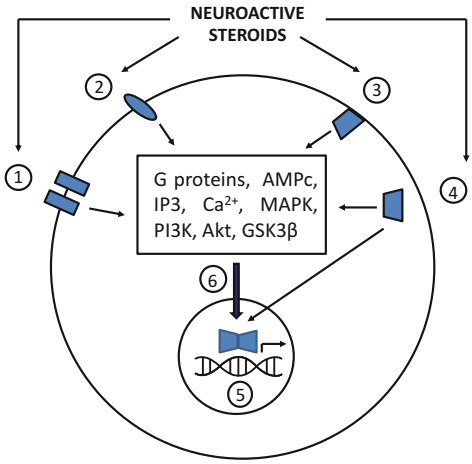

Fig. 14.7 Different potential mechanisms of action of neuroactive steroids, including hormonal steroids in the nervous system

The binding of neuroactive steroids to the membrane and cytoplasmic-associated receptors regulates intracellular signaling by a series of steps which include G protein activation, alteration of intracellular levels of cAMP, inositol 1,4,5-trisphoshate (IP3) and Ca^{2+} and modulation of kinases activity such as mitogen-activated protein kinase (MAPK), phosphoinositide-3 kinase (PI3K), and Akt or glycogen synthase kinase 3b (GSK3b). However, the binding and triggering of cytoplasmic nuclear receptors by neuroactive steroids (4) result in their dimerization followed by binding to steroid-responsive elements in the promoter region of discrete genes and subsequent transcriptional activity (5). Moreover, neuroactive steroids, modulative ability regarding both the membrane and cytoplasmic transduction signaling pathway exerts an effect on transcriptional activity (6) (Melcangi and Mensah-Nyagan 2008).

The rapid neurotransmitter-like effects of steroids such as progesterone and estrogen have been described to change the activity of neuronal systems through several types of receptors (Micevych and Mermelstein 2008; Mani and Oyola 2012; Micevych et al. 2011; Valadez-Cosmes et al. 2016). Signaling pathway stimulation is usually initiated via the extranuclear or membrane steroid receptors mediated through the G proteins or other secondary messengers (Boulware et al. 2005; Zuloaga et al. 2012; Valadez-Cosmes et al. 2016). Nevertheless, classical nuclear steroid receptors containing the palmitoylation sequences can be translocated to the plasma membrane to rapidly change cellular action (Mani and Oyola 2012; Meitzen et al. 2013; Schwartz et al. 2016). These nuclear transcription factors could initiate their signaling at the plasma membrane level only by direct interactions with other proteins (Boonyaratanakornkit et al. 2007; Micevych and Mermelstein 2008; Boulware and Mermelstein 2009). Subsequently, intracellular signaling pathways which involve effectors (e.g., MAPK, PI3K, Akt) are initiated via the transactivation of receptors bound to the cell surface (Micevych and Mermelstein 2008; Boulware and Mermelstein 2009).

Neuroactive steroid estrogens trigger faster signaling mechanisms by interacting with the membrane-bound estrogen receptors, ERα and ERβ (Milner et al. 2001; Razandi et al. 2004), and on steroid binding, these receptors simultaneously also trigger intracellular signaling cascades (Chaban et al. 2004; Cheskis 2004; Song et al. 2005). The estrogen membrane-initiated signaling causes subsequent activation of cAMP response element binding protein (CREB) to affect transcriptional activity. For example, estradiol is capable of evoking immediate effects by inducing protein kinase A activity (Gu et al. 1999) and MAPK pathway in neurons, or protein kinase A and MAPK/Src pathway in astrocytes (Pawlak et al. 2005; Chen et al. 2014). Remarkably, the membrane-initiated signaling activity of estradiol can also be stimulated by the binding of estradiol with the metabotropic glutamate receptors (Boulware 2005; Boulware et al. 2013; Kuo et al. 2010; Seredynski et al. 2015). In addition, estrogen-stimulated signaling may also be activated through the membrane-associated estrogen receptors (mERs) counting the G protein-coupled estrogen receptor (GPER or GPR30) situated in intracellular membranes. These receptors also mediate the quick initiation of various signaling pathways by estradiol, which include the MAPK pathway (Filardo et al. 2000) and the Akt/PI3K pathway (Ruiz-Palmero et al. 2013).

Similarly, progesterone can initiate rapid responses in the CNS by triggering various membrane-bound receptors. These membrane receptors mediate the intracellular signaling pathways via transactivation of Src kinase (Src) (Micevych and Mermelstein 2008; Boulware and Mermelstein 2009). These receptors are widely expressed in the neurons and glial cells and they include the progesterone receptor membrane component-1 (Pgrmc1) and the membrane progesterone receptors, that is, mPRα, mPRβ, mPRγ, mPRδ and mPRε (Guennoun et al. 2008, 2015; Guerra-Araiza et al. 2003; Labombarda et al. 2010; Meffre et al. 2013). The cytoplasmic-associated progesterone receptors (Waters et al. 2008) and the membrane-bound progesterone receptors (mPRα and Pgrmc) (Labombarda et al. 2003, 2010) widely expressed in astrocytes also initiate rapid responses in the CNS and mediate the induction of intracellular signaling pathways via transactivation of Src kinase.

In the case of androgens, the signaling pathway is mediated by initially binding to the androgen receptors, followed by their interaction with the membrane and cytoplasmic signaling proteins (Foradori et al. 2008; Michels and Hoppe 2008), which eventually leads to an increase in intracellular Ca^{2+} levels (Gorczynska and Handelsman 1995) or regulation of MAPK (Cheng et al. 2007; Fix et al. 2004). In the case of glucocorticoids, studies find that they possess membrane as well as intracellular actions through the glucocorticoid receptors. This was evident in primary hippocampal neurons and in hypothalamic slices when rapid activation of kinase pathways was initiated subsequent to BSA-conjugated glucocorticoids infusion (Qi et al. 2005; Malcher-Lopes et al. 2006; Yang et al. 2013). Similar kinase pathway activation occurring in intracellular glucocorticoid receptors is not present, suggesting the regulation of the pathway via other receptors including the membrane-associated glucocorticoid receptors that also signal via G protein and protein kinase pathways (Xiao et al. 2005). The intracellular glucocorticoid receptors

can also be trafficked, thereby stimulating rapid membrane-initiated glucocorticoid signaling (Di et al. 2016).

Furthermore, neuroactive steroids also modulate the ligand-gated ion channels or G protein-coupled receptors, leading to alteration of the intracellular kinases activity, which subsequently changes the transcription outlines of the downstream genes, for example via the cyclic AMP-protein kinase A-cAMP responsive element binding protein pathway (Wehling 1997; Zakon 1998).

14.5 Physiological Role of Hormones and Steroids in Brain Function as Neurotransmitters

Neuroactive steroids encompass both endogenous and exogenous steroids that control brain function by interacting with receptors including the steroid receptors, ion channels, ligand-gated ion channels and their respective membrane receptors (Cai et al. 2018). Being neuroactive in nature, these steroids naturally regulate various physiological functions in the human body (Simerly 2005), which is not limited to sexual functions, reproductive biology and the development of accessory reproductive organs, but also affect brain physiology and pathophysiological functions (Frick et al. 2015; Maeng and Milad 2015; Diamanti-Kandarakis et al. 2017). Under physiological conditions, neuroactive steroids can illustrate significant modulatory effects on brain-behavioral functions such as behavioral affection, stress, memory, cognition and emotion (Vallée et al. 1997; Serra et al. 2000; Frye 2001; Darnaudery et al. 2002; Johansson et al. 2002; Melcangi and Mensah-Nyagan 2008). They also demonstrate significant roles in the etiology and management of neurological disorders such as epilepsy, schizophrenia, anxiety, depression, multiple sclerosis, premenstrual syndrome and other brain function-related disorders (Bäckström et al. 1983; Landgren et al. 1987; Pisu and Serra 2004; Marx et al. 2006; MacKenzie et al. 2007; Morrow 2007).

14.5.1 Physiological Role of Estrogens as Neurotransmitters

The neuronal activity of estrogens can stimulate various brain-related activities and behavior. Abundant evidence describing the relationship of estrogens with brain disorders has been attained through different investigations in female animals or women. These studies have demonstrated the role of estrogens in brain-related disorders (Walf and Frye 2006). Women are observed to be susceptible to depression when there are fluctuations in the concentration of sex hormones, especially estrogen, which is believed to be responsible for disorders including post-partum depression, premenstrual dysphoric disorder, and perimenopausal or postmenopausal depression (Thorpe et al. 2001). On the other hand, estrogens can display neuroprotective effects when abundant amounts of endogenous estrogens are produced within the brain (Carswell et al. 2000; McCullough et al. 2003). Estrogens are reported to display a vital role in processes such as neuronal plasticity and spine

synapse formation (Herrick et al. 2006; McEwen et al. 2012). Additionally, numerous studies have revealed the significant effects of estrogens on cognition (Fortress and Frick 2014; Vahaba and Remage-Healey 2015; Sheppard et al. 2018). It has also been found that estrogen shows protective effects on neurons to counteract the toxicity of amyloid plaques in Alzheimer's disease patients (Thomas et al. 1999). However, this finding is contradicted by the results from the Women's Health Initiative Memory Study, which reported that combination therapy of estrogen with progestin increased the possiblity of acquiring dementia in postmenopausal women (Shumaker et al. 2003; Webber et al. 2005).

Estrogens also exhibit modulation action on dopaminergic signaling via their D1 receptors potentiation activity and D2 (Dopamine 2) receptor antagonizing activity (Soares et al. 2003; Hedges et al. 2010). Through their antagonistic effects on D2 receptors, estrogens can lessen the severe symptoms of psychotic disorders. On the other hand, they may intensify addictive behaviors through their agonistic actions on D1 receptors (Hedges et al. 2010). Estrogens have modulating activity on other signaling systems, which includes their stimulating effect on glutamatergic activity (Cyr et al. 2001), and their negative effect on GABAergic activity (Wójtowicz et al. 2008; Wójtowicz and Mozrzymas 2010). These modulating effects of estrogens on D1 and D2 receptors are believed to trigger their positive stimulation in mood management, cognition improvement (Amin et al. 2005) and attention (Soares et al. 2003; Soares and Frey 2010). Other neuroactive steroids belonging to this class such as estradiol also display their enhancing effects on the development and care of the central nervous system (McEwen and Alves 1999). This is clearly evident in the presence of estrogen receptors in the wide array of brain structures (McEwen and Alves 1999). Studies have shown that estrogen receptors expressed on GABAergic neurons may play a key role by which estradiol exerts an excitatory effect on the nervous system (Schultz et al. 2009). Additionally, it is demonstrated that positive modulation of ERα on GABAergic neurons decreases the release of GABA, the inhibitory neurotransmitter (Xiao et al. 2003).

14.5.2 Physiological Role of Progestogens as Neurotransmitters

Progestogens such as progesterone exhibit neuronal excitability function directly by acting at their own receptors or when they are converted into other neuroactive steroids. Allopregnanolone and pregnenolone are two metabolites of progesterone and effective allosteric modulators of the $GABA_A$ receptor (Djebaili et al. 2005; Gibson et al. 2007). The neuroactive steroid progesterone and the neuroactive metabolites with sex steroids including estrogens and testosterone have been linked to neuronal disorders such as schizophrenia (Koenig et al. 2002; Corcoran et al. 2003; Marx et al. 2011; Melcangi et al. 2011). There are additional studies which highlight the role of progesterone as a main player in schizophrenia through its direct interaction with it own receptors and also through an indirect pathway by first being converted to glucocorticoids or allopregnanolone followed by subsequent binding of

these neuroactive metabolites with specific receptors to elicit their modulating activity (Marx et al. 2011).

The physiological role of progesterone in brain function and diseases has been clearly demonstrated by various studies in animal models. For instance, infusion of exogenous progesterone to traumatic-induced brain injury and ischemia-induced animal models lessens the lesion volume in the brain and stimulates improvement in cognition function of those animal models (Djebaili et al. 2005; Gibson et al. 2007). Furthermore, the effect of progesterone has been broadly evaluated by Frye and colleagues for improvement of depression, anxiety and cognition in animal models (Frye and Lacey 2000; Frye and Sora 2010; Frye and Walf 2010; Frye 2011). For instance, ovariectomized rats when treated with a high level of progesterone show enhanced brain function performance such as improvement in spatial memory, working memory and avoidance memory (Frye and Lacey 2000). Similarly, progesterone administration shows better performance in cognitive tasks in intact aged mice as assessed through object recognition, T-maze, water maze, inhibitory avoidance and contextual fear experimental conditions (Frye and Walf 2008). Even in progesterone receptor knock-out mice, progesterone was still able to stimulate enhanced performance in various learning and memory tasks, signifying the possibility of progesterone's metabolites being responsible for the memory enhancement effect (Frye and Walf 2010).

Other neuroactive steroids and allopregnanolone (progesterone metabolites) have a significant influence on brain physiology and its system. Allopregnanolone has been found to exhibit an inhibitory effect on serotonergic neurons of the raphe nucleus (Genazzani et al. 2000; Birzniece et al. 2006). Progesterone has also been described to antagonize sigma-1 receptor activation, thereby inhibiting the release of norepinephrine (Genazzani et al. 2000; Pluchino et al. 2006). Furthermore, progestogens act as strong agonists of the GABA-receptor, particularly allopregnanolone (Bäckström et al. 2011), and are implicated in the decline of dopaminergic tone in the nucleus accumbens (Quinones-Jenab and Jenab 2010)

14.5.3 Physiological Role of Androgens as Neurotransmitters

Neuroactive steroids including testosterone and DHEA have a great modulating impact on brain functional activity and brain-related behavior (Carré et al. 2011). These androgenic neuroactive steroids are found to play an influential role in the management of disorders such as anxiety and depression, particularly for menopausal women and men suffering from hypogonadism (Seidman 2006). Fluctuations of testosterone levels have accompanied pathophysiological conditions such as mood disorders, depression and psychosis (Talih et al. 2007; van Wingen et al. 2011; McHenry et al. 2014). Then again, these neuroactive steroids do exhibit a neuroprotective role as evident in an investigation which demonstrates that continuing exposure to these steroids stimulates neurogenesis in the hippocampus in addition to increased survival of new neurons (Galea et al. 2013). They are also

known to play a vital role in the process of synapse formation in the spine (Leranth et al. 2004; Romeo et al. 2005).

Several studies have demonstrated testosterone's ability to induce positive modulatory effects on serotonergic signaling indirectly when it is metabolized to estradiol, thereby activating the estrogen receptors (Ebinger et al. 2009). Testosterone also synergistically enhances the effect of noradrenergic antidepressant agents through its androgen receptor activating activity (Martínez-Mota and Fernández-Guasti 2004; Ebinger et al. 2009). Similarly, DHEA and its sulfated derivative have an affinity with a variety of receptors including $GABA_A$, sigma-1 and metabotropic glutamate receptor (mGluR) (Maninger et al. 2009). Owing to its versatility of action toward various receptors, DHEA and its sulfated derivative can induce various synaptic transmissions including GABAergic, dopaminergic, cholinergic and glutamatergic synaptic transmission (Yoon et al. 2010; Xu et al. 2012). In the nervous tissues, DHEA is believed to affect neuronal excitability via its modulating effect on the NMDA receptor (Baulieu 1997) and its positive allosteric modulating effect on the $GABA_A$ receptor (Marx et al. 2006). This is confirmed through an animal model study which demonstrated that DHEA and its sulfated derivative can bring about excitation of neuronal function in the nervous system of rodents mainly by their ability to activate the glutamate and GABA-releasing neurons (Wolf and Kirschbaum 1999; Meyer et al. 2002; Dong et al. 2007). DHEA also displays neuroprotective and anti-glucocorticoid effects, thereby aiding in the improvement of depression, anxiousness, psychotic symptoms and cognitive deficits (Maninger et al. 2009). DHEA and its sulfated derivative have a protective effect against NMDA-induced neurotoxicity. The pathophysiological activity of DHEA and its sulfated derivative also include neuroprotection, enhancing neuronal survival, neurogenesis and neurite growth (Pluchino et al. 2015). Despite its modulating effect on various synaptic transmissions and their neurotransmitter production, DHEA sulfate can also activate the postsynaptic receptors, thereby, encouraging various significant brain activities, including antidepressant and anxiolytic effects and enhancement of memory, especially in patients suffering from brain function disorders such as Alzheimer's disease and addiction (Kaminska et al. 2000; Balashov 2010; Dong and Zheng 2012).

14.5.4 Physiological Role of Glucocorticoids as Neurotransmitters

Glucocorticoids such as corticosterone and cortisol are also involved in modulation of brain activities and various synaptic neurotransmissions. Additionally, their concentration level has been associated with various pathophysiological conditions of the brain functions such as stress, cognition, memory and psychotic behavior (Whitaker et al. 2016; Jentsch et al. 2019; Kinlein et al. 2019). An organism's capacity to cope with stressful experiences is dependent on its ability to appropriately engage central and peripheral systems, such as the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a primary neuroendocrine mediator of neural and behavioral responses to stress, and dysfunction of this system is linked to

increased risk for developing mental health disorders including anxiety, post-traumatic stress disorder and depression. Numerous studies has revealed that corticosterone pretreatment before stress, as observed in rodent models, helps in proper functioning of the HPA axis, which would reduce stress and stimulate improved emotional stability (Whitaker et al. 2016). Glucocorticoids such as cortisol have been demonstrated to enhance regulatory activity in the ventro-lateral prefrontal cortex. Research also suggests that delayed cortisol release in response to a stressor facilitates cognitive emotion regulation processes that might be beneficial for restoring emotional stability in the aftermath of stressful events (Jentsch et al. 2019). In recent studies it has been found that cortisol concentration level is high in patients suffering from psychotic disorders including schizophrenia. Cortisol concentration level has also been found to be closely linked to disorders such as cognitive dysfunction, mild cognitive impairment and Alzheimer's disease (Mondelli et al. 2015; Pietrzak et al. 2017; Hubbard and Miller 2019). The positive results of various investigations advocate the possibility of therapies that target depressing cortisol and Aβ levels, which can be employed in extenuating cognitive impairment in Alzheimer's disease. Additionally, in the case of the commencement of psychosis, cortisol can act as a predictor of treatment response or can be considered as a target for the design of new therapeutic agents (Mondelli et al. 2015; Hubbard and Miller 2019).

The physiological role of other neuroactive steroids, for example pregnenolone and pregnenolone sulfate, also has implications for their role in neuronal excitability and plasticity. Pregnenolone, the precursor to neuroactive steroid progesterone, possesses the ability to boost the neurotransmitter activity of GABA and to directly activate the $GABA_A$ receptor, as observed in studies conducted using bovine preparations (Callachan et al. 1987). Pregnenolone action on GABA receptor can either bring about prolonged temporal influx of chloride ions or can directly open the ion channel when there is elevated plasma concentration of pregnenolone. On the other hand, pregnenolone sulfate has an inhibitory action toward the GABA receptor and acts as an antagonist, as evidenced in an investigation utilising rodent cell cultures (Mienville and Vicini 1989). The mechanism of pregnenolone-sulfate antagonistic activity is due to its ability to cause a decline in the frequency of the chloride channels opening on the GABA receptor, thereby reducing inhibition of neuron discharge, ultimately producing an excitatory effect on the nervous system (Mienville and Vicini 1989).

The neuroactive steroid pregnenolone has also been described to modulate molecular targets such as sigma1 receptors and the type-1 cannabinoid (CB1) receptor (Vallée 2016). It has a positive modulating effect on the sigma1 receptors but exhibits a positive modulative effect on type-1 cannabinoid receptor. Pregnenolone's positive modulating activity toward brain activity advocates the possibility of pregnenolone involvement in processes that are activated through the CB1 receptor, including anxiety, memory, cognition and reward-related behavior (Fujiwara and Egashira 2004; Gardner 2005). Some of the neuroactive steroids which have been marketed or are under investigation for their action against various neuronal disorders are listed in Table 14.1.

Table 14.1 List of marketed and investigational neuroactive steroid drugs for the treatment of neurological disorders

Sl. no	Drug	Indication	Brand name
1	Pregnenolone	Used for the treatment of Alzheimer's disease, depression, seizures	Pregnenolone, Life-Flo Pregnenolone, etc.
2	Allopregnenolone	Used for the treatment of postpartum depression	Zulresso
3	DHEA	Used for the treatment of Alzheimer's disease, schizophrenia	Intrarosa
4	Ganaloxone (Investigational drug)	Under development for the treatment of postpartum depression, uncontrolled seizures in female children and other rare genetic epilepsies	NCT02358538, NCT03865732
5	Zuranolone (SAGE-217) (Investigational drug)	Under development for the treatment of major depressive disorder, postpartum depression, essential tremor, Parkinson's disease, insomnia and seizures	NCT04007367, *NCT02978781*, *NCT03000569*
6	Mifepristone (Investigational drug)	Under development for the treatment of psychotic major depression and other depressive disorders	NCT00867360, NCT00637494, NCT00146523

14.6 Receptors as Potential Target for Neurological Disorders

14.6.1 Agonists to the Neuroactive Hormone and Steroid Receptors and Their Biological Activities

Receptors involved in the neuronal signaling of various neuroactive steroids include the steroid receptors and many other receptors by which the conventional neurotransmitters exert their function. Thus, agonists to these receptors encompass molecules that activate any of these receptors, and these agonists have been employed in the management of various clinical neurological disorders such as behavioural and biochemical alterations in Parkinson's disease (Baraka et al. 2011), negative symptoms and cognition suffered by schizophrenic patients (Usall et al. 2011), post-partum depression (Smith et al. 2007), Dravet syndrome (Hawkins et al. 2017), essential tremor (ET), major depressive disorder (MDD) (Martinez Botella et al. 2017), epilepsy (Reddy and Rogawski 2010) and anxiety disorders (McKernan et al. 2000). Examples of agonists to the neuroactive hormone and steroid receptors are shown in Fig. 14.8.

Several investigations have reported the neuroprotective effects of estrogenic compound 17β-estradiol (**1**). One of the studies was performed on mice administered with MPTP (1-methyl 4-phenyl-1,2,3,6 tetrahydropyridine) to induce striatal dopamine depletion. The investigated mice were given the estrogenic compounds continuously for a period of 5 days before MPTP treatment and this was continued until the

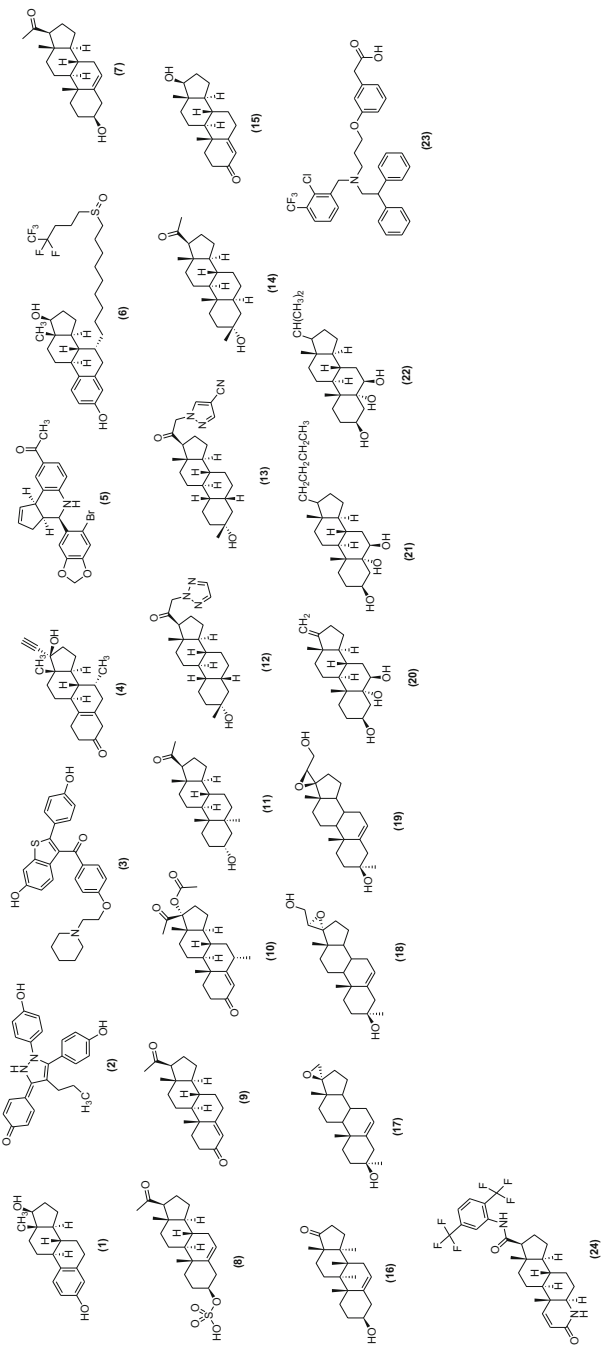

Fig. 14.8 Examples of agonists to the neuroactive hormone and steroid receptors and their biological activities

14th day. When dopamine markers in the mice were assessed the results showed that 17β-estradiol inclusion in the therapy prevents the depletion of striatal dopamine and that MTTP could not cause reduction in the dopamine level in mice (Callier et al. 2001; Grandbois et al. 2000). Likewise, studies have reported that unilateral administration of 6-hydroxydopamine (6-OHDA) in an ovariectomized female rat model with Parkinson's disease can induce similar biochemical alteration and abnormal behavioural patterns to that seen in human Parkinson's disease (Baraka et al. 2011). The administration of 17β-estradiol has been reported to prevent these biochemical and behavioral alterations induced by 6-OHDA in ovariectomized rats, as observed in an MPTP-induced mouse model (Quesada et al. 2008; Baraka et al. 2011). Estradiol when given as an adjuvant has been reported to alleviate psychotic-like behavioural disorders via its neuroprotective ability (Akhondzadeh et al. 2003; Begemann et al. 2012; Kulkarni et al. 2008a, b, 2011, 2015).

Likewise, the neuroprotective effects of other estrogen receptor modulators such as the ERα agonist, propyl-pyrazoletriol (PPT) (**2**) and raloxifene (**3**), which acts as a selective estrogen receptor modulator (SERM) on MPTP-treated mice, are also described (Grandbois et al. 2000; Callier et al. 2001; Bourque et al. 2019). These two compounds prevented MPTP-induced striatal dopamine depletion in mice when the drug treatment was continued for 5–14 days. Similarly, a study on a 6-OHDA lesion-induced rat model of Parkinson's disease has shown that raloxifene and compound **2** prevented the induction of biochemical alterations and altered behavioural function by 6-OHDA (Baraka et al. 2011).

The examination of raloxifene (**3**) as a selective modulator of the estrogen receptor and its ability to prevent the estrogenic stimulation of the gonadal tissue caused by the long-term use of estradiol has been reported (Chlebowski et al. 2009). Raloxifene's ability to selectively modulate the estrogen receptors has also been reported to enhance memory (Yaffe et al. 2005). In a clinical trial using raloxifene as an adjuvant therapy for schizophrenia (Kulkarni et al. 2010) it was described that raloxifene stimulates enhancement of the positive symptoms and improvement of the negative symptoms in postmenopausal women within a dose of 120 mg/day. Consequent investigations with a dose of 60 mg/day of raloxifene as an adjunct displayed significant enhancement of cognitive ability and improvement in negative symptoms (Usall et al. 2011).

The synthetic steroid tibolone (**4**) is considered as a selective tissue estrogenic activity regulator due to its ability to convert into neuroactive metabolites that could bind directly to steroid receptors and regulate enzymes needed for its metabolization into other neuroactive metabolites depending on the tissue involved (Kloosterboer 2004; Reed and Kloosterboer 2004; Cummings et al. 2008). There are reports which describe the neuroprotective activity of tibolone in neurons (Belenichev et al. 2012; Pinto-Almazán et al. 2012; Farfán-García et al. 2014). Tibolone has also been reported to display protective activity in a human astrocyte cell model whereby it is first metabolized into 3α-hydroxy tibolone and 3β-hydroxy tibolone followed by the binding of these metabolites to the ERα and ERβ receptors in a human astrocytes. It thus results in the activation of these receptors to further bring about the desired neuronal protective activity (Guzman et al. 2007; Avila Rodriguez et al. 2014).

The G protein-coupled estrogen receptor 1 (GPER1) agonist G1 (**5**) is another example with neuroprotective properties and has been reported for its efficacy in improving brain injury in several experimental models. It has been demonstrated that G1 enhances the level of protein expression of GPER1, which strengthens the neuroprotection ability of estrogen in the case of spinal cord injury. There are also reports that describe the ability of G1 to prevent neurons from NMDA and glutamate toxicities (Lebesgue et al. 2010; Hu et al. 2012; Liu et al. 2012; Tang et al. 2014; Chen et al. 2015).

The neuroprotective profile of the estrogen agonist ICI182,780 (Fulvestrant) (**6**) in rat primary hippocampal neurons, verifying its neuroprotective efficacy against neurodegenerative symptoms linked with Alzheimer's disease and other similar disorders, has been reported. Compound **6** greatly enhances neuron survival in a concentration-dependent manner when the rat hippocampal neurons are exposed to excitotoxic glutamate. The neuron survival is evidenced even when exposed to neurodegenerative-induced amyloid$_{1\text{-}42}$. It has been shown that compound **6** directly interacts with ion channels and brings about rapid intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) oscillations in a similar manner to that of neurons treated with 17-β-estradiol. Furthermore, compound **6** also stimulates better activation of signal-regulated kinase 1/2 and Akt (protein kinase B) and greatly augments the production of spinophilin and Bcl-2. Altogether, these findings deliver a thorough understanding of the agonistic ability of compound **6** toward estrogen receptors in rat hippocampal neurons (Zhao et al. 2006).

The role of the neuroactive steroid pregnenolone (**7**) in the regulation and improvement of anxiety and depression has been evidenced in several preclinical trial studies. Pregnenolone could greatly diminish the sedative side effects of diazepam when given together. This significant reduction of sedation suggests a possible therapeutic advantage of pregnenolone for the management of psychiatric conditions that could be useful in reversing the unwanted sedative-hypnotic actions of benzodiazepines (Meieran et al. 2004). Further, data from preclinical studies and human trial studies propose the beneficial effect of pregnenolone for the management of bipolar depression (Marx et al. 2009; Ritsner et al. 2010). Consequently, a recent clinical study reports the positive result of pregnenolone in alleviation of depressive symptoms in bipolar disorder patients when exposed to the pregnenolone therapy for a period of 12 weeks (Brown et al. 2014). Furthermore, pregnenolone is also known for its ability to increase the synapse strength that ultimately increases learning and enhances memory retention. Its positive modulating effect on brain cholinergic synaptic neurotransmission also boosts the learning process and memory enhancement in aged rats (Bu and Zu 2014).

Pregnenolone sulfate **(8)** is another excitatory neuroactive steroid that acts as an NMDA receptor agonist and plays a role in memory-enhancing effects. Pregnenolone sulfate has been shown to restore memory impairment in cirrhotic rats as compared to rats treated with AP5, a competitive antagonist of NMDA receptor, which shows no significant effect on memory performance. This shows that acute pregnenolone sulfate therapy could enhance memory in cirrhosis memory deficit through its NMDA receptor activating effect (Dastgheib et al. 2015).

The neuroactive steroid progesterone (**9**) has also been reported for its neuroprotective effects and its ability to enhance neuronal survival in males and females (Wei and Xiao 2013). This activity of progesterone was reported by Schumacher et al. (2004) whereby they described the neuroprotection action of progesterone for the dopaminergic neurons in rats. In this study progesterone displayed protectective activity for the dopaminergic neurons against MPTP-induced degeneration, an outcome which is closely related to hormonal-induced weakening of dopaminergic signaling caused by age and the onset of Parkinson's disease (Schumacher et al. 2004). Additionally, the beneficial effects of progesterone for the treatment of multiple sclerosis is evidenced in the experimental autoimmune encephalomyelitis model. Progesterone exerts its action through its involvement in repairing of the myelin sheath and by increasing the concentration of endogenous oligodendrocyte precursor cells (Zawadzka and Franklin 2007).

Progestin (**10**), a synthetic form of progesterone, is also known for its association with physiological processes including preservation of myelin sheath integrity, formation of synapses and neuronal survival (McEwen and Woolley 1994; Mellon 2007; Zhang et al. 2010; Schumacher et al. 2012). Progestin has been reported to display neuroprotection action in the case of spinal cord and peripheral nerve injury (Labombarda and Garcia-Ovejero 2014). There are also data which support the neuroprotective effect of progestin in brain disorders that include demyelinating disease, motor neuron diseases, epilepsy and depression (Frye and Lacey 2000; Walf et al., 2006; Deutsch et al. 2013; Li et al. 2013).

Allopregnanolone (**11**), a metabolic product of progesterone, is a well-known endogenous neurosteroid with inhibitory action. This neuroactive steroid acts as an allosteric modulator toward the $GABA_A$ receptor and exerts its modulative properties at the synaptic and extrasynaptic $GABA_A$ receptors. Allopregnanolone is well known for its anticonvulsion property, and numerous experimental animal models of epilepsy such as the pentylenetetrazol (PTZ)-induced seizure model and picrotoxin-induced and bicuculline-induced seizure models have helped to establish this fact (Kaminski et al. 2004; Reddy and Rogawski 2012; Rogawski et al. 2013). The anticonvulsant activity of allopregnanolone is observed to fully suppress limbic seizures and status epilepticus in animal models induced by pilocarpine and kainic acid (Reddy 2010, 2011; Reddy and Rogawski 2012). A phase III clinical trial on the anticonvulsant activity of allopregnanolone revealed that continuous parenteral infusion of allopregnanolone formulation represents a better therapy regime for the management of persistent seizure, a deadly brain condition known as super refractory status epilepticus (SRSE), that does not respond to usual treatments (Vaitkevicius et al. 2013; Bialer et al. 2015). Allopregnanolone has also been reported to have better effects in the management of postpartum depression (PPD) and essential tremor (ET) in a phase II clinical trial and exploratory study, respectively (Ellenbogen et al. 2016; Kanes et al. 2017a). In the case of postpartum depression it is observed that with the delivery of the placenta, allopregnanolone levels decrease rapidly, and this is believed to cause the onset of postpartum depression in susceptible women (Smith et al. 2007). This is not the case in a healthy women, as the rapid decline in allopregnanolone levels activates GABA receptor

expression (MacKenzie and Maguire 2013). Studies on the efficacy of brexanolone, a synthetic form of allopregnanolone, in the management of postpartum depression present hopeful outcomes. It acts as a positive allosteric modulator of $GABA_A$ receptors. This result can be further testified through a phase II randomized placebo-controlled trial where brexanolone treatment showed 70% efficacy in decreasing the severe state of postpartum depression (Kanes et al. 2017b).

The compound SGE-516 (**12**) is a 1,2,5-triazole analog of allopregnanolone which can be administered orally while still retaining similar modulative activity toward the $GABA_A$ receptors (Martinez Botella et al. 2015). The 1,2,5-triazole analog was reported to display comparable pharmacological properties to allopregnanolone with better aqueous solubility and enhanced pharmacokinetic profile (Hammond et al. 2017). This potent allosteric modulator of both synaptic and extrasynaptic $GABA_A$ receptors has been reported to exhibit anticonvulsant activity as demonstrated in a seizure rodent model (Hammond et al. 2017). Furthermore, the anticonvulsant activity of SGE-516 on an experimental epilepsy animal model employing the Scn1a+/− mouse with symptoms similar to those of Dravet syndrome has been evaluated for efficacy on hyperthermia-induced seizure. The results revealed that SGE-516 prevents seizures induced by hyperthermia, reduces the seizure frequency and increased the survival rate of the Scn1a+/− mice (Hawkins et al. 2017).

Certain classes of neuroactive steroids such as 5β-nor-19-pregnan-20-one analogs conjugated with substituted pyrazoles and triazoles at C-21 exhibit strong allosteric modulation activity for the synaptic and extrasynaptic $GABA_A$ receptors. The pyrazole substituted derivative, 3α-hydroxy-3β-methyl-21-(4-cyano-1H-pyrazol-1′-yl)-19-nor-5β-pregnan-20-one (SAGE-217, **13**), is one such example of an allosteric modulator of $GABA_A$ receptor with brilliant oral pharmacodynamic and pharmacokinetic properties. SAGE-217, also known as Zuranolone, is an investigational medication and has undergone a successful phase I clinical study and is presently animated for a parallel phase II clinical trial to investigate its effectiveness for the management of essential tremor, postpartum depression and major depressive disorder (MDD) (Martinez Botella et al. 2017). A phase II clinical trial on SAGE-217 for its implication toward Parkinson's disease is also currently ongoing (NCT02978781 n.d., B245).

Ganaxolone (**14**) is another example of an allosteric modulator of the $GABA_A$ receptors with comparable properties to those of allopregnanolone. This neuroactive steroid has been verified for its anticonvulsant potential from ample of supporting data attained from numerous preclinical studies on epileptic animals such as the *pentylenetetrazole*-induced seizure model and the amygdala kindling model (Gasior et al. 2000; Reddy and Rogawski 2010). The synthetic neuroactive steroid, ganaxolone, induces its inhibitory action by activating both the synaptic and extrasynaptic $GABA_A$ receptors (Reddy and Rogawski 2010, 2012). Reportedly, a large number of clinical studies have been conducted to verify the efficacy and safety of ganaxolone meant for the management of epilepsy. One such study includes the phase III multicenter, randomized, double-blind, placebo-controlled study of ganaxolone as an adjunctivant for the treatment of uncontrolled partial-onset

seizures in adult patients (NCT01963208 n.d.). This study established safety and tolerability issues with the revelation of some adverse events such as drowsiness and fatigue. Another ongoing clinical study on ganaxolone is the phase II multicenter, open-label, proof-of-concept study for the management of epilepsy in children (NCT02358538 n.d.).

The neuroactive steroid testosterone (**15**) acting as an agonist to the androgen receptors was also reported to have a neuroprotective effect (Moffat et al. 2002). The Baltimore Longitudinal Study of Aging (BLSA) reported significant results regarding the neuroprotection action of testosterone in the case of deterioration of cognitive ability in Alzheimer's disease due to aging. The study reports that elevated endogenous testosterone concentration can stimulate verbal memory enhancement in young patients which is otherwise absent in elders and in men with testosterone deficiency (Moffat et al. 2002). Studies also report that low testosterone production in the case of older men increases the risk for the onset of Parkinson-like disorders (Okun et al. 2006). Accordingly, many investigations have been conducted which have described the benefit of including testosterone in therapy meant to correct Parkinson disease-like symptoms due to its ability to directly effect and improve motor symptoms (Okun et al. 2002). Mitchell and group noted the substantial decrease of resting tremor and improved motor symptoms in a Parkinson's patient with testosterone deficiency only after the administration of testosterone (Mitchell et al. 2006).

The neuroactive steroid DHEA (**16**) and its role in alleviating depressive symptoms have been studied widely. Recent studies have revealed that higher DHEA and DHEA sulfate levels prevent the commencement of depressive symptoms in both males and females (Veronese et al. 2015). However, low-plasma level DHEA sulfate was described to alleviate severe depression in men but not in women (Veronese et al. 2015). Additionally, a longitudinal cohort study revealed that low levels of DHEA sulfate improve depressive symptoms irrespective of gender and age (Souza-Teodoro et al. 2016).

DHEA analogs conjugated with spiro-epoxy functional group at C-17 were found to induce substantial protective activity on neurons. Compounds under this class act as good positive modulators of ion channel receptors such as the NMDA and $GABA_A$ receptors. The spiro-epoxy steroidal derivatives **17–19** reported by Calogeropoulou et al. (2009) were found to be the most potent neuroprotective agents against induced apoptosis when tested in neural PC12 cells. In addition, treatment of PC12 cells with neuroactive steroid derivatives **17–19** also up-regulates the production of dopamine from the dopaminergic neurons. A further structure activity relationship study showed that introduction of an epoxide moiety at C-17, as in the case of the DHEA analogs **17–19**, facilitates their easy transportation across the cell membrane, whereby they can bind directly to estrogen receptors ERα and ERβ. The structure activity relationship study also revealed that the presence of the hydroxy group at C-21 in **18** and **19** encourages better hydrogen bond formation and better interaction with the NMDA or the $GABA_A$ receptors that can be credited with enhancing neuroprotective activity of these spiro-epoxy conjugated steroids (Calogeropoulou et al. 2009).

Alternative hydroxy derivatives of DHEA (**20–22**) that are known to bind to the membrane receptors, NMDA and $GABA_A$ receptors with high affinity, were studied for their neuroprotective effects against hypoxia-induced neurodegeneration in the cortical neurons. The triol derivatives of DHEA could easily cross any physiological membrane, then directly bind to the membrane receptors and display significant neuroprotection of the neurons. Supplementary structure activity relationship studies revealed that addition of a methylene functional group at C-17 as in the derivative **20** produces less neuroprotective effect, while incorporation of the alkyl moiety at C-17 just as in the derivatives **21** and **22** presented improved dimensional flexibility and enhanced interaction with NMDA and $GABA_A$ receptors, leading to better neuroprotective effects (Long et al. 2016).

The compound 1OP-2198 or XEN 1101 (undisclosed structure) represents a new class of neuronal allosteric modulator of the Kv7 (KCNQ) potassium channel. The Kv7 neuronal channels have been described for their function of slowly activating and deactivating voltage-gated M-current. Studies validate the role these channels play in the management of hyperexcitability disorders such as epilepsy (Large et al. 2012). 1OP-2198 has been described to exhibit anticonvulsant effect in the maximal electroshock seizure, in pentylenetetrazol-induced, picrotoxin-induced, bicuculline-induced, and 6 Hz seizure animal models (Hoffmann et al. 2017). Studies reported that the 1OP-2198 mechanism of action is comparable to that of other Kv7 channel modulators such as retigabine; however, it is more potent than the other modulators (Roeloffs et al. 2008). 1OP-2198 lessens the state of convulsion, thereby exerting antiepileptic action through a mechanism which involves opening of the neuronal Kv7 channels through their direct interaction with the ion channel (Roeloffs et al. 2008).

Neuroactive steroids also exert a protective activity on the diabetic-induced degeneration of neurons. But these steroidal compounds can trigger endocrine side effects when administered into the physiological system (Moran et al. 2013; Mehlig et al. 2014). However, other physiological pathways could be targeted so as to induce the neuroactive steroid production directly in the nervous tissues specifically; for example, the liver X receptors (LXRs) could be targeted instead of the conventional steroid receptors. In this regard, the LXR agonist GW3965 (**23**) was evaluated and reported to increase the neuroactive steroid level specifically in nervous tissues such as the cerebral cortex, cerebellum and spinal cord of the diabetic-induced rat model (Mitro et al. 2012). Studies also demonstrated that the LXR agonist GW3965 stimulates an upsurge production of progesterone metabolites that include dihydroprogesterone, tetrahydroprogesterone and isopregnanolone, and these metabolites themselves are potent $GABA_A$ receptor modulators. In particular, isopregnanolone does not interact or modulate the $GABA_A$ receptor activity, but it exhibits an antagonistic effect against the tetrahydroprogesterone-activation of the $GABA_A$ receptor (Bäckström et al. 2005). These findings suggest that LXR agonist GW3965 can improve neuronal synaptic activity in diabetic patients through its indirect activation of $GABA_A$ receptors (Mitro et al. 2012).

Some of the neuroactive compounds exert their neuronal activity not by their interaction with any receptors but by targeting the enzymes that are necessary for the

biosynthesis of steroids rather than acting directly on the receptors involved in the neurotransmission functions of the neurotransmitters. For instance, the 5α-reductase inhibitor dutasteride (**24**) has been described for its ability to activate dopaminergic signaling and as a drug beneficial in the management of motor neuron disorders and other neurodegenerative disorders including Parkinson's disease (Paba et al. 2011). Current research shows that dutasteride prevented the MPTP-induced damage of several dopaminergic markers in male mice (Litim et al. 2015). The study was conducted on mice which were previously exposed to MPTP so as to reduce the striatal dopamine in the mice brain up to 50%. However, the MPTP-exposed mice that received dutasteride showed a significant increase in striatal dopamine and its metabolite concentrations. Dutasteride prevented MPTP toxicity on dopamine metabolism in MPTP-treated mice (Litim et al. 2015). The neuroprotective activity of dutasteride could be facilitated by its 5α-reductase inhibitory action, which in turn stops the conversion of these steroid precursors into their metabolites, leading to buildup of the precursors such as progesterone, estrogens and DHEA which are known to possess a neuroprotective effect against MPTP-induce neurodegeneration (Bourque et al. 2009; Bourque et al. 2019).

14.6.2 Antagonists to the Neuroactive Hormone and Steroid Receptors and Their Biological Activities

The antagonists to the hormone and steroid receptors include drugs and derivatives of various structural functionality, acting on the different receptors, namely, $GABA_A$ receptors (Johansson et al. 2015), NMDA receptors (Hu et al. 2014), progestin receptors (Sun et al. 2018), AMPA glutamate receptors (French et al. 2012) and various ion channels including the voltage-gated sodium channels (Bialer et al. 2013), T-type calcium channels (Tringham et al. 2012) and Na(+)-K(+)-2Cl(−) cotransporter (Löscher, Puskarjov & Kaila 2013) that are involved in the neurotransmitter signaling of the hormone and steroidal neurotransmitter. Through their antagonistic effects, they exert various therapeutic effects on various neurological disorders. Examples of antagonists to the neuroactive hormone and steroid receptors are shown in Fig. 14.9.

There are a group of neuroactive steroid compounds that exhibit an antagonistic effect toward the $GABA_A$ receptor and these are categorized as the $GABA_A$ receptor modulating steroid antagonists (GAMSA). They selectively inhibit neuroactive steroid-mediated enhancement of GABA-evoked currents at the $GABA_A$ receptor. These compounds exert an antagonistic action to the activation effect of the $GABA_A$ receptor by the neurosteroid but do not hamper the neurotransmitter GABA to induce chloride flux via the receptor (Johansson et al. 2016). The most common example of GAMSA is the neuroactive steroid isoallopregnanolone (**25**), similar to allopregnanolone, whereby the difference is in the hydroxyl group orientation at C-3 in the A-ring of the steroid. Its antagonistic action toward the $GABA_A$ receptors has been extensively explored through various experimental models. For instance, the voltage-clamp model using the recombinant $GABA_A$ receptors containing rat

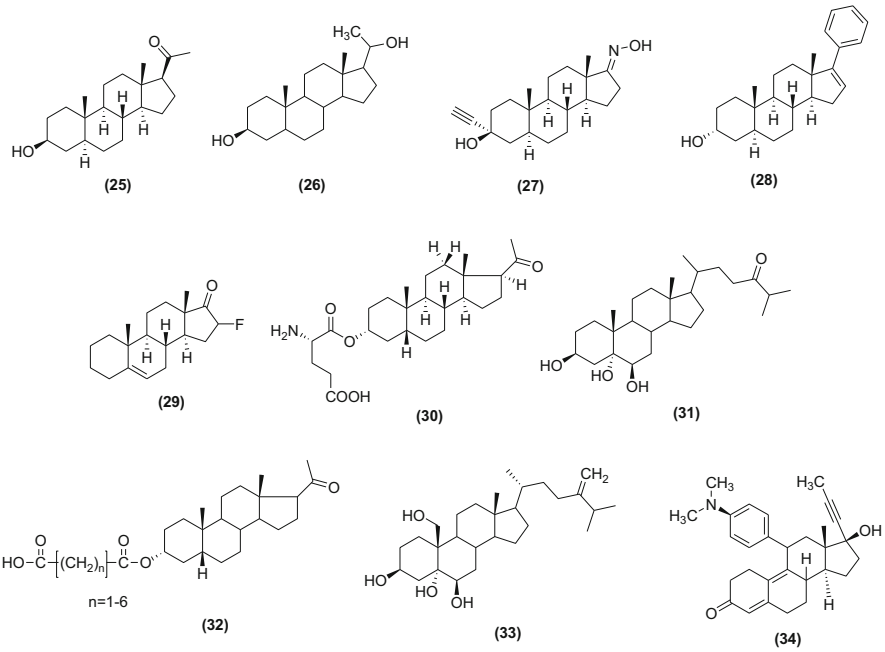

Fig. 14.9 Examples of antagonists to the neuroactive hormone and steroid receptors and their biological activities

revealed the inhibitory action of isoallopregnanolone toward the $GABA_A$ receptor activation by allopregnanolone (Wang et al. 2002). In the same way, isoallopregnanolone shows an antagonistic effect toward allopregnanolone-induced neuronal inhibition of the CA1 pyramidal neurons when it is applied directly to the pyramidal cell obtained from the hippocampal slices of rats (Wang et al. 2000, 2018). The specificity of the antagonistic effect of isoallopregnanolone was established through a demonstration whereby it antagonizes allopregnanolone-induced chloride ion flux even in the presence of GABA, without affecting the chloride ion uptake induced by GABA alone (Lundgren et al. 2003). Additionally, isoallopregnanolone treatment was not accompanied by any adverse effects as reported by the findings of the phase I/II clinical trial on isoallopregnanolone for the treatment of premenstrual dysphoric disorder (Bixo 2014).

The antagonists of the $GABA_A$ receptor modulator include numerous other 3β-hydroxy-steroids (Strömberg et al. 2006; Wang et al. 2002). UC1011 (3β-20-β-dihydroxy-5α-pregnane) (**26**) is another $GABA_A$ receptor modulating steroid antagonist which has been reported to antagonize the allopregnanolone activation of GABA-induced chloride ion uptake as observed in isolated hippocampal and cortical microsac models (Turkmen et al. 2004). The compound GR3027 (**27**) also comes under the category of $GABA_A$ receptor modulating steroid antagonists through its antagonizing effect on tetrahydrodeoxycorticosterone activation of the

$GABA_A$ receptors as seen in HEK-293 cells (Johansson et al. 2015). Another example includes 17PA ((3α5α)-17-phenylandrost-16-en-3-ol) (**28**), which exerts its effect by selectively antagonizing the effects of allopregnanolone. The compound17PA was also described to antagonize $GABA_A$ receptor activation by 5α-reduced steroids as demonstrated in isolated rat hippocampal neurons and in homogenized fractions of mouse brain cortex (Mennerick et al. 2004; Kelley et al. 2007).

Fluasterone (DHEF) (**29**) is yet another compound with possible NMDA receptor inhibition activity that is testified to be beneficial in the treatment of traumatic brain injury and in the management of various behavioral symptoms including changes in affection, memory, coordination and loss of motor function suffered by many traumatic brain injury patients. In research carried out by Mallik et al. in an animal model, DHEF significantly improved behavioral recovery after 3 days when given prior to 12 h post injury (Malik et al. 2003).

The synthetic neuroactive steroid derivative 20-oxo-5β-pregnan-3α-yl-L-glutamyl-1-ester **(30)** has been described to possess a strong antagonist effect toward the NMDA receptor and was reported to be a neuroprotective agent by Kapras and his co-workers. This derivative by-passes the fast metabolic process catalyzed by sulfatase enzyme and possesses a improved bioavailability profile in comparison to the sulfated derivative of pregnanolone. Furthermore, this derivative exerts its inhibitory effects on the NMDA receptor in a dose-dependent manner (Kapras et al. 2012).

The metabolite of cholesterol, cholestane-3,5,6-triol (Triol) **(31)**, was also noted as a major endogenous neuroprotectant by Hu and his colleagues (Hu et al. 2014). Derivative **31** was found to exhibit a protective effect on the neurons from injury as observed in *in vitro* models. In an *in vivo* animal study, the triol derivative **31** prevents neuronal injury as observed in ischemic-induced rabbits and rats. Different studies notified that triol treatment greatly decreases cellular calcium ions concentration and directly antagonizes the NMDA receptors, thereby inducing its neuroprotective effect (Hu et al. 2014).

Most of the therapeutically active compounds of NMDA receptor antagonists exert activity through their competence in selectively blocking the NMDA receptors. But it is also known that overexpression of NMDA receptors causes excitotoxicity, leading to neurological disorders (Zhou and Sheng 2013). Some neuroactive steroids including pregnanolone sulfate can selectively inhibit the tonically activated NMDA receptors. With this concept in mind, the development of pregnanolone derivatives **32** (n = 1–6) was achieved by incorporating carboxylic acid functionalities at the terminal of the alkyl chain with varying carbon number substituted at C-3 of the parent steroid. These derivatives were evaluated *in vivo* for their potential to inhibit the NMDA receptors and the results revealed that the derivative with the longest chain, pregnanolone hemipimelate (n=6), exhibits the maximum inhibitory effect against the tonically activated NMDA receptors with no psychotogenic side effects. This result offers a better understanding of the action of synthetic neurosteroids on neuronal function that can be employed in the design of novel neurosteroid-based ligands (Jiang et al. 2012).

The neuroactive steroid of marine origin c 24-methylenecholestane-3β,5α,6β,19-tetrol (**33**) has been explored for its neuroprotective profile on neurons induced with toxic concentration of glutamate in an animal model. The outcome of the study represents enhanced neuron survival of cerebellar granule neurons when the neuronal cells are incubated with tetrol. This marine steroid derivative can also attenuate NMDA-induced intracellular calcium [Ca^{2+}] and inhibit the NMDA currents in cortical neurons, thus demonstrating the neuroprotective effects (Yan et al. 2015).

RU486 (Mifepristone) (**34**), an efficient antagonist of progestin receptor (Sun et al. 2018), has shown therapeutic activity on major psychotic depressions and meningiomas. It also exhibits protective effects against traumatic neuronal alterations such as protection of cerebellar Purkinje cells. This agent, which is also an antiglucocorticosteroid, can be orally administered and has demonstrated anti-neurosteroidal effects on inappropriate neuroprogesterone and neuroglucocorticosteroid pathologies (De Nicola et al. 2006; Rakotomamonjy et al. 2011).

14.7 Future Perspectives

The nervous system can be described as a site of neuroactive steroid production whereby these steroids target the neurons and glial cells. These hormonal steroids that are synthesized locally in the neuronal or glial cells, as well as the synthetic steroids, have been found to exhibit neuronal activity and are involved in various brain functions through their interaction with different receptors. Their ability to alter neuronal excitability has been investigated in different clinical trials and some of these steroids have been used in clinical practice (Giatti et al. 2019). For instance, estradiol was employed in clinical trials for the treatment of schizophrenia. One of the clinical trials has reported a dose-finding pilot study on estradiol and its ability to alleviate schizophrenic symptoms by employing a transdermal implant of estradiol (Kulkarni et al. 2001), while the other clinical trial has described the activity of transdermal estradiol at a dose of 100 mcg (Kulkarni et al. 2008a, b). In both studies, estradiol displayed excellent effectiveness, thus suggesting its usefulness for the management of psychotic sypmtoms in women suffering from schizophrenia. Another clinical trial as reported by Kulkarni and his group also represents results that support the efficacy of adjunctive transdermal estradiol for the treatment of schizophrenia (Akhondzadeh et al. 2003; Kulkarni et al. 2015). The estradiol treatment was described to have a positive effect on both male and female patients. This was presented through a 14-day trial in men suffering from schizophrenia, whereby patients with abundant estradiol concentration showed rapid recovery from psychotic symptoms (Kulkarni et al. 2011).

The modulative effect of neuroactive steroids on neuronal function has the drawn attention of many researchers to study further their effects as well as the effects of their analogs. Recently, Hogenkamp patented his method that describes the development of the novel 17β-heteroaryl-substituted steroid compounds **35–37**. According to his findings, these compounds act as $GABA_A$ receptor modulators

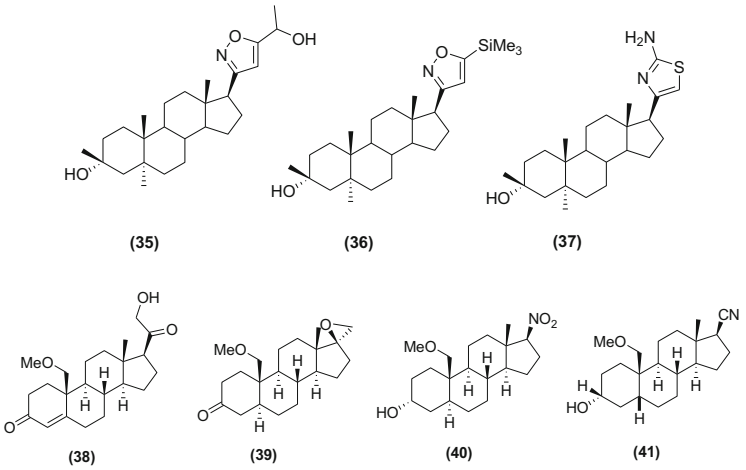

Fig. 14.10 Examples of patented neuroactive steroids and their biological activities

and are considered valuable in the treatment and/or prevention of various CNS disorders including epilepsy, cognitive dysfunction, depressive or bipolar disorders, stroke and multiple sclerosis (Hogenkamp 2014). Similarly, Covey and Robichaud patented their invention of the neuroactive 19-alkoxy-17-substituted steroids **38–41** for their effectiveness against CNS disorders through their action on the GABA receptors regulating the excitation of neuron, mood disorder and decreasing stress level (Covey and Robichaud 2014). Examples of patented neuroactive steroids are shown in Fig. 14.10.

Neuroactive steroids embodied various protective therapeutic agents against CNS (central nervous system) and PNS (peripheral nervous system) disorders and diseases. Nevertheless, attention must be given to other aspects that are associated with the application of neuroactive steroids. It is important to point out that systematic administration of neuroactive steroids can bring about endocrine side effects. Undeniably, the receptors for neuronal excitability, including the steroid receptors themselves, are extensively distributed in several nervous tissues. The study describing the SERMs provides an example of alternative approaches to selectively activate only those receptors expressed in the nervous tissues. SERMs are molecules that exert regulation of estrogen receptor and transcriptional activity in specific tissues, especially in nerve cells, and may not exert any effects or even have an antagonistic effects toward the estrogen receptors expressed in other cell types (Arévalo et al. 2015). However, the use of synthetic steroids may not fully evoke the desired effects as the actions of neuroactive steroids depend on their conversion into active metabolites. Most of the synthetic steroidal receptor ligands cannot be metabolized in other active metabolites and are ineffective or induce partial effects. This occurs with medroxyprogesterone acetate whereby it is not converted to the progesterone metabolites (i.e., dihydroprogesterone and tetrahydroprogesterone) in the CNS as is natural progesterone (Ciriza et al. 2006).

14.8 Conclusions

The understanding about the physiological activity of neuroactive steroids, the complex process involved in their metabolism, the complex mechanisms of action and their multiple roles within the nervous system is still emerging. Thus, further studies could be an interesting endeavour. It can be noted that neuroactive steroids are mainly produced within the neuronal tissues and these steroids do exert effects other than the conventional well-known genomic effects. These steroids, especially those produced within the brain, also exerts neuronal excitability action, myelin sheath protection and many other activities related to brain function. They bind to the neurotransmitter receptors, for example $GABA_A$ receptors, the NMDA glutamate receptors, the sigma 1 receptors and the steroid receptors and produce physiological effects within the nervous system. It is also well known that their levels in the nervous system are affected by pathophysiological conditions, indicating neuroactive steroids as a potential therapeutic intervention. However, fluctuations of steroid concentration within the brain do not correspond to the peripheral steroid levels. Therefore, employing neuroactive steroids in the treatment of neuronal disorders might bring about unanticipated effects on brain steroid levels. Thus, they may cause more negative effects than positive. Lastly, novel steroidal derivatives that are found to have neuronal excitability functions may be used to modulate brain steroidogenesis and could be a therapeutic possibility that ought to be assessed further to establish them as future drug candidates.

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Pharmacology of Neuropeptides: Substance P, Vasoactive Intestinal Peptides, Neuropeptide Y, Calcitonin Peptides and Their Receptors

15

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Abstract

Neuropeptides are responsible for the regulation of various biological activities and mediate various regulatory mechanisms associated with every organ system. They control and regulate communication between endocrine and nervous systems and are also involved in signaling between cells found in the central and peripheral nervous system. Certainly, they can modulate immunomodulation, neuroprotection, and physiological homeostasis (e.g., feeding behavior, balance of water, blood pressure, breakdown of glucose, cognition, stress response, and pain) by working as peptide hormones.

Keywords

Calcitonin peptide · Neuropeptides · Neuropeptide tyrosine · Neuropeptide Y · Substance P · Tachykinins · Vasoactive intestinal polypeptides

Abbreviations

CD	Circular dichroism
CNS	Central nervous system
DAG	Diacylglycerol
GDP	Guanosine diphosphate
GLP	Glucagon-like peptide
GPCRs	G protein-coupled receptors
GTP	Guanosine triphosphate
IL	Interleukin

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IP3	Inositol triphosphate
NF-κB	Nuclear factor kappa B
NK_1R	Neurokinin one receptor
NK_2R	Neurokinin two receptor
NK_3R	Neurokinin three receptor
NKA	Neurokinin A
NKB	Neurokinin B
NMR	Nuclear magnetic resonance
PACAP	Pituitary adenylate cyclase activity peptide
PHI	Peptide histidine isoleucine
PRL	Pituitary prolactin
SP	Substance P
TAC	Tachykinin
TNF	Tumor necrosis factor
VIP	Vasoactive intestinal polypeptide

15.1 Introduction

Neuropeptides are molecules similar to a protein, which are created and discharged by neurons. They regulate the secretory tract and act on neural substrates (Russo 2017). Holmgren and Jensen defined neuropeptide as a peptide discharged by neuron as a signaling molecule. This signaling molecule will impact other excitable cells as a modulator or a transmitter (and actually affecting its own cells sometimes) (Belzung et al. 2006). A considerable point is that neuropeptide is not only just a peptide existing in neurons but also acts as a medium of communication within the cells, which means it stimulates other cells through high-affinity binding toward particular targets (receptors). These molecules are articulated in peripheral nerves like sensory and motor nerves and also in the central nervous system (CNS).

15.2 Definition of the Neuropeptides

In terms of pleiotropic potential, the concept was best demonstrated by Candace Pert, who stated: "Similar to the change in the feelings, the peptides mixture are regulated around the body as well as the brain. This leads them to alter the chemistry throughout the body." In the general sense, neuropeptides are basically transmitters, which are made up of amino acids, where a peptide bond combines them. In terms of size, they are quite large and hold amino acids in the range of 3–36. These are released into the synaptic cleft together with other neurotransmitters, extracted from the large 90 amino acids, i.e., inactive precursors. Bioactive peptide is produced when the eradication of an individual sequence occurs through neuropeptide antecedent. The same bioactive neuropeptide is also produced in few neuropeptide precursor peptides, in various forms. These are synthesized into the cell part of the neuron, following its lumen sequestration as well as transition to the axon, during the

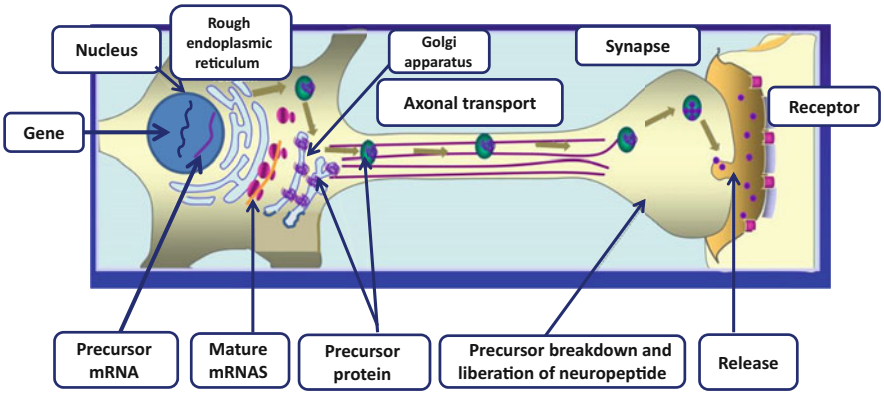

Fig. 15.1 Neuropeptide synthesis

Table 15.1 Origin and examples of neuropeptides

Origin of neuropeptides	Examples
Pituitary peptides	PRL, vasopressin, GH, TSH, ACTH, α-MSH, β-endorphin, and oxytocin peptides creating an impact on the brain and gut
Hypothalamic releasing hormones	GIHH (somatostatin pituitary peptides, RH and LRH)
Impact of peptides on the gut and brain	Insulin, nerve GF, CCK, subs P, leucine enkephalin, brain-related neurotrophic factors, methionine enkephalin, neurotensin, glucagon from other tissues
From other tissues	Bradykinin, sleep peptides, ag-II, carnosine, calcitonin

processing events such as signal peptide cleavage. These are placed in a large dense-core vesicles (LDCVs). Subsequent to the LDCV exocytosis, the re-internalization of the LDCV components occurs in the component part of the membrane. Thus, no neuropeptide is re-used in the synapse. Generally, neuropeptides are released at low cytosolic Ca concentrations, though LDCV exocytosis is usually stimulated by Ca ions, whereas the same ion may be utilized for exocytosis when derived through other important sources such as transmembrane internal stores (Fig. 15.1; Table 15.1).

Along with it, neuropeptide also imposes certain alteration on behavior. The alteration in the peptide effect behavior is demonstrated by the gene manipulation experiments either by transgene mutations or viral gene transfer, exhibiting changes in the behavioral traits which are part of the expression of peptide receptor (Ludwig 2011). This shows that the peptidergic neurons in the brain do not determine the behavior but the peptide distribution. The human neurological disorder, such as depression, autism spectrum disorder, schizophrenia, and social anxiety, has been associated with vasopressin and oxytocin. In addition, there are neuropeptides involved in appetite regulation, mood disorder, and libido disorder, which are included in the peptide-based therapies.

15.3 Research Focusing on Neuropeptides and Opportunities for Drug Discovery

In the last 40 years, neuropeptides and their receptors with similarity have been steadily increasing. At first, the isolation of peptides from the brain and guts can be observed (e.g., substance P, somatostatin). This can be done by targeted mining in a particular region that includes orexin in the brain, cortistatin, etc., as well as G protein-coupled receptors that are deorphanized (GPCRs including ghrelin receptors and orexin). Moreover, the accomplishment of the Human Genome Project can also be seen (Schalla and Stengel 2018). The distribution of neuropeptides is being regionally restricted in the central and peripheral nervous system. The signaling of neuropeptides and their receptors is more distinct in terms of spatial than signaling with the classical as well as low-molecular-weight neurotransmitters. These neurotransmitters seemed to be widely expressed due to the assumption that drugs that act on neuropeptide receptors possess more selective actions pharmacologically with a few side effects. However, drugs reacting on glutamatergic, cholinergic, monoaminergic, and GABAergic systems are less selective pharmacologically. The functions of neuropeptides include neurotransmitting and acting as growth factors. They are observed in the hormones found in the endocrine system such as glial cells; they also act as the messengers in the immune system. When the nervous system faces any complications (e.g., by stress, injury, or drug abuse), these neuropeptides seemed to be important. This leads to a massive number of opportunities to produce new drugs to treat disorders belonging to the nervous system. As a result of this, subtypes of receptor agonists and certain antagonists as well as the substance P receptor (neurokinin-1) were discovered. This has proved its clinical efficiency while treating depression and in chemotherapy induction. Additionally, several other neuropeptides are under clinical trials for different indications (Hoyer and Bartfai 2012).

These receptors are assumed to contribute to the discovery of potential drugs linked to various neurological disorders. According to the researchers, there is a dire need for antiepileptic drugs (AEDs) which act in several ways than the drugs already present in the market. A large number of AEDs block the sodium channels to act and also increase the GABAergic transmission. The therapeutic options are also expanded by a completely new generation of AEDs; however, these are not as effective as the older drugs (Hanaya and Arita 2016). To these medications, patients having mesial temporal epilepsy (mTLE) seemed to be the most pharmacoresistant (Pati and Alexopoulos 2010). With the aim of rectifying the dilemma, finding the new mechanism of AEDs is not the only need of neuropharmacologists but to also focus on the information shared on the pathophysiology of epilepsy. Moreover, several mechanisms can be observed during the complex procedure of epileptogenesis; therefore, new compounds should be identified ideally that can target the other pathways simultaneously. While studying the pathogenesis of epilepsy, a number of neuropeptides including somatostatin, galanin, and neuropeptide Y were briefly studied. However, these neuropeptides are poor and are seen as the main hurdle for developing the brain drug due to their poor ability to penetrate

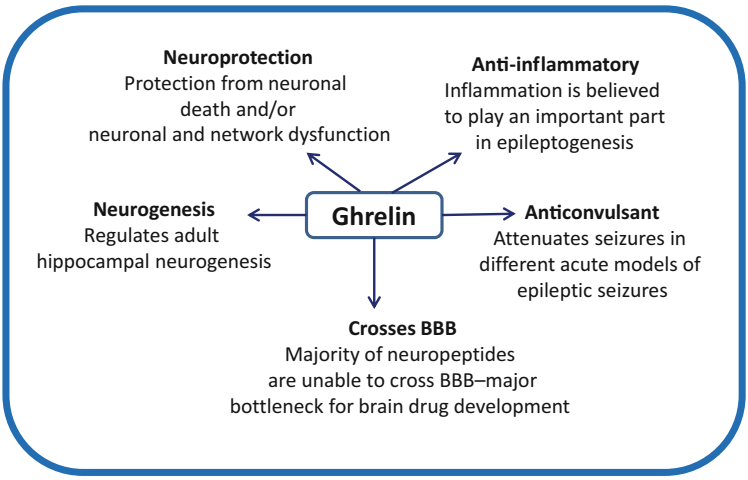

Fig. 15.2 Benefits of ghrelin

the blood-brain barrier (BBB) (Robertson et al. 2011). The discovery of ghrelin in 1999 brought great excitement to all scientists (Chen et al. 2017). It has been produced both in peripheral and central terms in the field of epilepsy (Fig. 15.2).

While comparing the well-established anticonvulsant neuropeptides, ghrelin is recognized to possess a large number of advantages. This neuropeptide has the ability to cross the BBB while also possessing the characteristic to affect the physiological processes. The physiological procedures range from the neuroprotective properties and potent anti-inflammatory along with BBB protection and integrated hippocampal neurogenesis (Portelli et al. 2012). Besides, various research studies indicated that physiological procedures are affected negatively during the process of epileptogenesis (Coremans et al. 2010; Vezzani et al. 2011; Marchi et al. 2012; Parent and Kron 2012; Vezzani et al. 2013). The recent phase III clinical trial of ghrelin receptor agonist JMV1843, which highlighted the lack of hormone among humans, seems to be completed, and the drugs were observed to be well effective. A company Aeterna Zentaris is successfully developing this drug now. Moreover, through clinical trials, orexin receptor seemed to be developed and progressed to treat sleeping disorders. Almorexant completed the phase III clinical trials (Hoever et al. 2012) and serves as unrecognized orexin receptor antagonist (Owen et al. 2009). The production of this drug has stopped to a greater extent, as provided through a brief review of data that was achieved through clinical studies. This further served as an avenue to demonstrate the advantages of almorexant (Actelion 2011). The next drug includes suvorexant (MK-4305) which has also accomplished the phase III clinical trials to identify its effects on different sleeping patterns (Cox et al. 2010). Based on the classical system of neurotransmitter, majority of the drugs were approved for treating obesity, while the potential ability of the system which provides important side effects was ignored. Lorcaserin (Belviq), a 5-HT2C receptor, seems to receive FDA approval recently for treating

obesity in selected obese patients. Weight loss can be seen to be greater than 5% by placebo-subtraction which was achieved by 27.2%. Maintenance of weight loss by following the same treatment is observed in 68% of patients (Fidler et al. 2011). However, the impact of lorcaserin cannot be understood completely specifically for body weight. However, by mediating it with hypothalamic POMC, activation can be accomplished (Halford et al. 2007). Mild side effects are revealed in the clinical trials of lorcaserin while treating for a headache (Smith et al. 2010).

Neuropeptides are also recognized as the molecules that are significant in determining neurological disorders such as epilepsy and depression. The integration of the peptides as pharmacological components serves as a great proposition given its decreased level of toxicity related to the functioning of the metabolism, which also escalates its potency. Reflecting on an in-depth understanding of the neurological disorder, it has been found that their use in the therapeutic methods has remained limited particularly for the treatment of clinical problems. The main contributing factor for their deficiency of delivering adequate results in the clinical treatment is their intact peptide delivery to particular regions of the brain, which is essential for treating the neurological disorder. Generally, the delivery of the drug, which is derived from the peptides, is confined to two factors: the first includes the issues concerning the general bioavailability, while the second deals with providing information regarding blood-brain barriers. Several factors have been recognized in creating an impact over pharmaceutical bio-presence of the brain. The factors comprise the distribution, which takes place in the cardiovascular space, its total volume, the disappearance of the half-life, as well as the drug capacity for causing the biological effect by reaching the target. All these factors contribute differently to each of the drugs, and in terms of the neuropharmaceutical peptide, this serves as a likely problem, which can be associated with the delivery of the drug.

Depending on the distribution of the cardiovascular system, the component can be divided into three fractions, namely, protein, blood cells, and protein free fraction (PFF). In the latter category, the existence of peptide drugs is usually achieved when it is being transported to the brain. The dynamic equilibrium is maintained by these three components for their peptide distribution. Generally, the core objective of this equilibrium is to evolve peptide in PFF, as it is in continuous depletion due to the action of the metabolic enzymes, its excretion as well as its uptake. Moreover, the cardiovascular compartment is of great importance as numerous neuropharmaceutical peptide areas are available in the analogous form of the endogenous peptides along with its capacity to interact with two other carriers such as proteins and peptidases. Moreover, it must be emphasized that the level, which these comprise, can be regulated by enzymes, as well as proteins as the change in the disease takes place along with the variation in species. Insulin-integrate growth factor (IGF)-1 that is usually comprised of approximately 70 amino acids is further assimilated into the binding locations found within the plasma; each location however has its own different constant for binding. One of the IGF-binding proteins has demonstrated changes due to its IGF level regulation as well as changing disease state. The efficiency of the peptide is affected by various endogenous peptides, which are present in the serum as well as in the blood vessels.

Example includes the peptide of amino peptidase A, which exists in the serum, to beat the high level in pregnant women than non-pregnant women. The presence of BBB also sets parameters for the peptide delivery to the brain. It is because it exists at the brain micro-vascular capillaries at the endothelial cell level. The resistance exists because of its integration of the increased level of resistance from the transendothelial electrical resistance, i.e., 2000 cm in comparison with the peripheral vessels where it is 3–30 cm. Moreover, the number of endothelial cells in BBB is low, which reduces the vesicular transportation. BBB also causes neuropeptide degradation due to its inclusion of the proteolysis enzymes encompassing amino peptidase A, amino peptidase M, and angiotensin-converting enzyme. In addition, alkaline phosphatase, glutamyl, transpeptidase, and monoamine oxidase enzymes are also present in it, which increase the micro-vessel level in the brain and may or may not be expressed in the peripheral vessels at low levels. It also contributes to the significant role in the brain homeostasis as well as in several transport systems allowing the entrance of the substance toward the brain, where large substrates are present in each of these systems inclusive of several peptides, which can be transported actively.

Peptide holds relatively strong potential for therapeutic regulation for prevention against gene and treatment, though it remains limited as a drug due to various obstacles. In addition, the drug formation of peptide specifically developed for the disorders related to the central nervous system has remained limited, as a result of BBB. The peptide drug development and its delivery are being enhanced, which improves the delivery of the drugs. Previously it was not possible, such as the concept of the particularly directed antibody vectors as well as glycosylation as its effect is not only confined to the enhancement of the pharmacological profile of the drugs but also increases the transportation of the BBB to a particular region in the brain. The drug delivery of the neuro-peptide can be demonstrated by the utilization of the vector-mediated delivery, which is a promising area for improving the peptide targeting, along with gene, where the possibility of success remains high. The application of these methods is of great assistance in chemotherapeutic treatment of disease related to the chronic CNS such as Parkinson disease, along with the effective delivery of neuroprotective agents in the case of acute disorders such as strokes. To enhance the bio-distribution of the brain, glycosylation is found to be an effective approach, which improves the stability and mitigated clearance as well as enhance the transport of the BBB. The enhancement of the opioid peptide related to the analgesia serves as a great area for recognizing the potential engraved in the glycosylation strategy. The strategy is also reflected as a source, which can improve the drug administration for a long period of time, for treating depression or patients suffering from chronic pain. It is projected that peptide-based CNS drug may prove to be a composite of more similar methods, of which its discovery through more research and development in this area continues.

15.4 Classification of Neuropeptides

It is often tricky to classify these types of peptides with the discovery of endocrine peptides and neuropeptides, as well as their area of production and their target. This gives rise to various approaches to the classification of neuropeptide; some classification of neuropeptide are illustrated below (Table 15.2).

15.4.1 Hypothalamic Hormones

The hypothalamus is a part of the brain containing various neurons responsible for the release of numerous hormones. It is located just above the brainstem, below the thalamus. The hypothalamus is present in all vertebrates and in humans, and its size almost the same as that of an almond. The hypothalamus is involved in various regulatory mechanisms and other related functions of the autonomic nervous system; it produces and releases neuropeptides and neurohormones. Due to the presence of receptors, there is an increased interest in extra-pituitary actions of these neurohormones in several non-pituitary tissues. Furthermore, the presence of

Table 15.2 Classification and examples of neuropeptides

Classification of neuropeptides	
Pituitary hormones	• LH: luteinizing hormone • β-Endorphin • PRL: prolactin • GH: growth hormone • FSH: follicle-stimulating hormone • TSH: thyrotropin [thyroid-stimulating hormone] • αMSH: α-melanocyte-stimulating hormone • ACTH: adrenocorticotropic hormone
Neurohypophyseal peptides	• Oxytocin • Vasopressin
Circulating peptides	• Angiotensin • Bradykinin
Hypothalamic releasing factors	• GHRH: growth hormone that is used to release another hormone • Somatostatin • CRH: corticotropin-discharging hormones • TRH: thyrotropin-releasing hormone • GnRH: gonadotropin-discharging hormone
Neuronal and endocrine peptides	• Vasopressin • Oxytocin
Opiate peptides	• Dynorphin • Leu-enkephalin • Met-enkephalin • β-Endorphin
GI and pancreas peptides	• Glucagon • PP: pancreatic polypeptide

receptors in immune cells suggests a paracrine or autocrine role throughout the immune system (Quintanar and Guzman-Soto 2013). These kinds of peptides are usually called hypothalamic or releasing hormones, which accelerate or restrain the release of pituitary hormones. The function of the hypothalamus includes regulating the temperature of the body, parenting and attachment behaviors, hunger, thirst, sleep, tiredness, and the circadian clock. The released peptides are discharged into the blood through the network of capillaries, which route quickly to portal veins present in the second capillary bed located in the pituitary gland anterior lobe, where it exerts its effects. The neuropeptides are secreted in periodic spurts, and that is the reason why replacement hormone therapy with the help of these hormones fails because they do not work unless the replacements are done with spurts as well. It is important for notifying the number of identified neuropeptides, which far exceeds the number of classical neurotransmitters (Siegel 1999).

15.4.2 The Pituitary Gland and the Related Hormones

The pituitary gland is placed beneath the brain, in the midline pocket of fossa, which is a small space or cavity in the sphenoid bone. This cavity is also called the "sella turcica" or the "Turkish Chair"; it is a saddle-figured cavity found in the sphenoid bone of the human skull and also in other Hominidae skulls, which are the great family of primitive apes, chimpanzees, gorillas, and also orangutans. The pituitary gland in humans is made up of two lobes, namely, posterior and anterior lobes. However, the posterior lobe includes one third of the given area, while the anterior lobe is based on two thirds of the area. The plain radiography has been replaced with the availability of modern radiological techniques of the sella turcica to examine hypothalamo-pituitary abnormalities (Tekiner et al. 2015).

The posterior lobe of the pituitary gland is a lump located underneath the hypothalamus on the bottom of the brain. The neurons present in the hypothalamus gland are launched directly to the posterior lobe which are associated with the pituitary gland. Approximately 100,000 axons are projected from the hypophyseal nerve tract. The nerve terminals and axons from hypothalamic neurons constitute the posterior lobe of pituitary gland. The hormones stored in the terminals are secreted by electrical excitation. Moreover, the altered astrocytes which are also called pituicytes surround the nerve terminals. The modified astrocytes, known as pituicytes, surround the nerve terminals, which assume to have a significant role in the local control of hormone release (Nussey and Whitehead 2013). Various hormones are produced by pituitary cells and secreted into the bloodstream affecting other organs of the body. It is also referred to as "the master gland" because it releases various kinds of peptide hormones which regulate the functions of many other mechanisms.

The pituitary gland is responsible for sensing the needs of the body, where it sends signals to different organs and glands throughout the body to regulate their function and maintain an appropriate environment after sensing. The hormones secreted by pituitary gland act as messengers after being released in the bloodstream.

These messengers are responsible for transmitting information from the pituitary gland to distant cells, regulating their activity. Spontaneous electrical activity is enough for driving the intracellular calcium concentration exceeding the threshold for stimulus transcription and stimulus secretion coupling in some cells. The function of these action potentials is to retain the cells with cytosolic calcium in a response state near the threshold phase (Stojilkovic et al. 2010). Some of the pituitary gland hormones have been indicated below with their functions:

- Prolactin—acts on the breasts and stimulates the mammary glands to produce milk.
- Other hormones are secreted to stimulate the adrenal gland, thyroid gland, testes, and ovaries that in turn stimulate other hormones.
- The hormones released by the pituitary gland are responsible for controlling metabolism, sexual maturation, blood pressure, reproduction, growth, and other vital physical processes and functions.

15.4.3 Tachykinins

The tachykinin peptides are related to an extended family of neuropeptide, which is found in a large number of species in both mammals and amphibians. The name tachykinins is derived from their ability to timely stimulate the contraction of the gut tissues. Its family can be distinguished by C-terminal alignment of "Phe-X-Gly-Leu-Met-NH_2." Here, X represents the aromatic amino acid. For instance, neurokinin A (NKA), neurokinin B (NKB), first neuropeptide discovered in mammals, and substance P (SP) are included in these neurotransmitters (Majkowska-Pilip et al. 2019). The contraction of the bladder muscle and gut, secretion of saliva in mammals, and hypotension are caused by tachykinin peptides. The preprotachykinin genes that are involved in encoding the precursor proteins are intertwined randomly to create diverse set of peptides. The processing of the precursor protein is done with the protease assistance to create smaller peptide units. The neurokinins are also included in the family of tachykinin including pysalaemin, neurokinin B, substance P, and eledoisin. Initially, the porcine spinal cord is the source that was used to derive neurokinin A and B. The neurokinin receptors that include NK1 and NK3 and neurokinins (neurokinin A, neurokinin B, and substance P) are articulated extensively in the nucleus of the solitary tract (NST), where they are a part of the central regulation of visceral mechanism. Hematopoietic regulation is the function of neurokinin A, while neurokinin B is involved in mediating the pain of transmission. Moreover, the structure of neurokinin A is very identical to the structure of substance P, and it also has nearly similar biological activities like substance P. However, neurokinin A is a potent bronchoconstrictor, and it is created by a complicated nervous system that is found in the gut (Majkowska-Pilip et al. 2019).

15.4.4 "Novel" Neuropeptides

The latest advancement in neuropeptides technology is used in proteomics and genomics, which has led to the discovery of neuropeptides and also newly found functional peptides which are present in the neuron systems of various species as well as humans. The exploitation of nanoscale chromatography merged with mass spectrometry has even enabled the structural identification of neuropeptides in a variety of lower-level species, which was not previously possible. A number of bioactive peptides comprise a proline residue in the second or third position from the N-terminus. This aspect also supports the peptide at the N-terminus from peptidase activity. Across the animal kingdom, all these structural attributes can be widely reported in neuropeptide sequences (Hayakawa et al. 2019). As peptides produced in the nervous system function as both communicators and regulators of various biological processes, they are essential in identifying how these peptides function and how they are created. It is also important to identify the structure of naturally produced neuropeptides to interpret the ways in which neuropeptide precursors emerge and how they operate, as faulty production of neuropeptides or inaccurate cell signaling can cause organism dysfunctionality and even death.

15.4.5 Putative Neuropeptides

Putative neuropeptides refer to the neuropeptide precursors and encoding genes that are attained through a biologically active process. Not all members of the cerebellin family meet the definition of a neuropeptide; in particular, the regulated discharge has not been recognized for each member of this family. The names of the genes are thus abbreviated using the standard nomenclature for genes and their given localization in the human genome. Neuropeptides and their receptors are concerned in behaviors, specifically in mammals, which include sleep and feeding. Neuropeptide functions are yet not understood, despite their clear roles in synaptic behavior and signaling (Nathoo et al. 2001).

15.5 Tachykinin-Related Peptides: Substance P

The tachykinin family is made up of approximately 12 amino acid peptides characterized by a carboxyl terminus, represented as Phe–X–Gly–Leu–Met–NH_2. Here X can be referred to as branched aliphatic particle (Schöppe et al. 2019; Pennefather et al. 2004). Tachykinin further involves different peptides like neurokinin B (NKB), substance P (SP), neurokinin B (NKB), and endokinins that are structurally related to peptides. The peptides are found in abundance and are expressed in the cell system in the body (Lorente et al. 2018) and thus help in regulating multiple cell activities including activation of postsynaptic receptor and neurotransmitter release (Chi et al. 2018). In particular, they serve as agonists with different binding forces for three different receptors of neurokinin (NK_1R, NK_2R,

and NK_3R) that belong to the grand categories of G protein-coupled receptors (GPCRs) (Yin et al. 2018). Therefore, NK_1R represents a complicated structure of neuropeptide (Steinhoff et al. 2014; Schöppe et al. 2019). Lorente et al. (2017) indicated that the peptides belonging to the category of tachykinin play a fundamental role in various psychological processes of different mechanisms including gastrointestinal, respiratory, immune and nervous system and dermal and urogenital system. This further creates a significant impact on nociception pain, muscle contractility, gastrointestinal proliferations, and epithelial secretions. These peptides are further involved in generating various health-related problems including chronic pains and inflammations, functional disparities of the urinary bladder and intestine, cancer injuries, bronchial asthma, etc.

In mammals, substance P was identified as the prime member of the tachykinin family. The given peptide was first discovered in 1931 from the intestine and brain of a horse and thus was provided in a study that discusses the impact of intestinal contractility (Chi et al. 2018). Such pieces of evidence are significant in identifying the role and significance of NK_1R and SP in the process of generating pain and neurogenic inflammation in the intestinal muscles (Lorente et al. 2018). The study further went on to provide the major cause of peripheral vasodilation.

15.5.1 Synthesis and Degradation of Substance P

In various cases, tachykinins are extracted in the form of an enzyme from the existing proteins. This helps in the formation of products that are biologically active and functional. The neuropeptides can be achieved through the alternate processing of three significant *TAC* genes. The genes are further encoded by preprotachykinin genes, where the preprotachykinin A (*TAC1*) gene helps in encoding the sequences of neurokinin A (NKA) and SP. In contrast to the two, the third category (*TAC3*) helps in the uniformity of neurokinin B (Kalina et al. 2018). Besides, α-amidation is generated through the bio-activation of SP that is integrated through α-amidating monooxygenase and peptidylglycine. The α-amidation is functionally limited to provide α-amidated peptides. Other enzymes such as the angiotensin and endopeptidase help in altering or minimizing the impact of α-amidation (Scholzen and Luger 2004; Mendlewicz et al. 2005).

The degradation and synthesis of substance P is shown in Fig. 15.3. Plasma levels of SP in humans ranges between 30 pg/ml and 500 pg/ml (Bondy et al. 2003; Lee et al. 1997; Reynolds et al. 1988).

15.5.2 Structure of Substance P

Schank and Heilig (2017) illustrated substance P as an 11 amino acid undecapeptide. However, the sequence of the given amino acid is provided as H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (Chang et al. 1971). SP shares a carboxyl terminal with other tachykinins in the same family; however, it differs from those

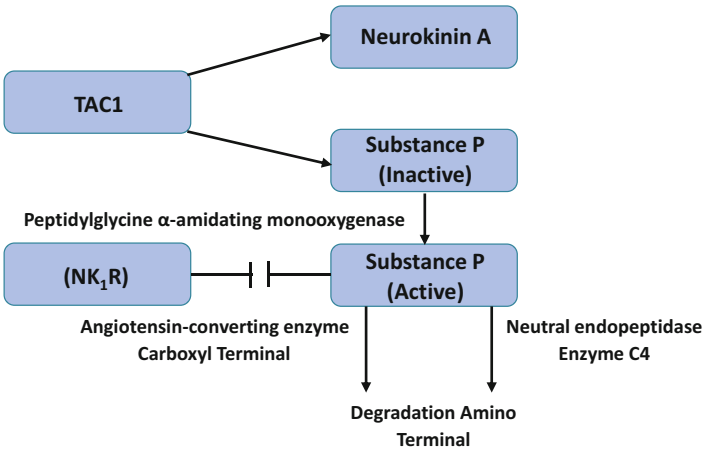

Fig. 15.3 Synthesis and degradation of substance P

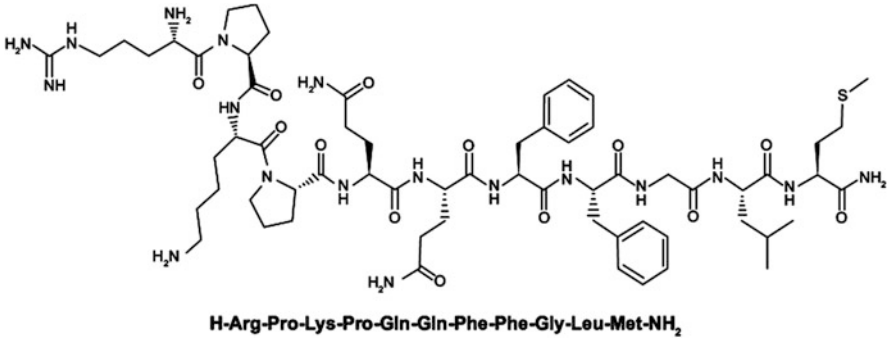

Fig. 15.4 Molecular structure of substance P (Lu et al. 2015)

tachykinins in terms of its pathophysiologic activity and receptor subtype affinities (Lu et al. 2015; Chi et al. 2018). The molecular formation of substance P is illustrated in Fig. 15.4.

15.5.3 Substance P Receptors

Substance P binds to NK_1R, NK_2R, and NK_3R receptors in target tissues (Yin et al. 2018), having the highest affinity to NK_1R; the biological effects of SP are thus mainly mediated through this receptor (Chi et al. 2018).

There are two isoforms of NK_1R, which are derived from differences in the tachykinin receptor gene and are different depending on the cytoplasmic tails of the C-terminal. The number of amino acids is also different, as one of the categories consists of 407 amino acids. The second, i.e., the truncated receptor, is based on

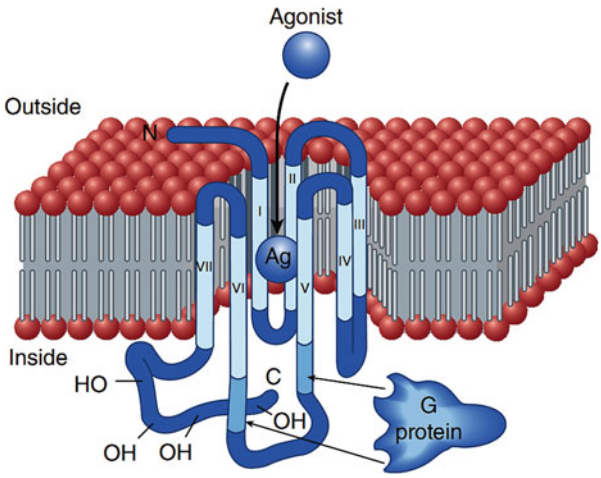

Fig. 15.5 Full-length isoform NK_1R

311 receptors. The bonding power of SP is ten times higher for the receptor available in full length in comparison to the truncated receptor (Chi et al. 2018). The isoforms are different depending on their impact over the metabolic functions of cell and thus result in separate pathways (Steinhoff et al. 2014; Lai et al. 2008). The long isoform takes just 1–2 min to phosphorylate extracellular-regulated protein kinases, while this cellular response is not observed until considerable time, at least 20 min, has elapsed after exposure to SP in cells expressing truncated NK_1R (Chi et al. 2018).

The mediation of complete NK_1R helps in cyclic adenosine monophosphate (cAMP), while Ca^{2+} helps in the formation of binding protein. It further helps in increasing the mechanism of gene transmission. The independent mechanism of Ca^{2+} helps in signaling the truncated NK_1R of the C-terminal (Lai et al. 2008).

The formation of the transmembrane domains is initiated through a NK_1R polypeptide chain that is functional by seven times providing seven different transmembrane domains. It is further connected to extracellular and intracellular loops (i.e., EL1, EL2, and EL2 and C1, C2, C3). However, the extracellular terminal is found in amino (N) terminal of the receptor, while intracellular terminal is located at the carboxyl (C) terminal of the receptor. G protein interacts with cytoplasmic regions of the receptor, especially around loop C3, connecting the fifth and sixth transmembrane domains. The binding sites of antagonists and agonist are found in the second and third transmembrane domains (Chi et al. 2018). Figure 15.5 illustrates the layout of full-length NK_1R.

15.5.4 Signaling Pathways Integrated Through Tachykinins and NK1R

Different types of G proteins ($G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha 12/13}$, $G_{\alpha 0}$, $G_{\alpha q}$) that are attached to the receptor are functional in demonstrating the intracellular pathways through

substance P (Garcia-Recio and Gascon 2015). Besides, the replacement of guanosine diphosphate (GDP) on α-subunit takes place through the activation of G protein. The process however is integrated through the allocation of SP at extracellular surface. This helps in releasing the Gα and Gβγ units through the separation of G protein. The process further modulates different enzymes to liberate the secondary source of messengers such as DAG and IP3. These secondary messengers are responsible for further metabolic activities in the cell. The terminal tail of the cytoplasmic receptors comprises various threonine residues. However, the hydroxyl (-OH) groups of the residues can be phosphorylated and are connected to the diminished receptor G protein to promote receptor endocytosis (von Zastrow 2018).

15.5.5 Physiological and Pathological Role of Substance P

Tachykinin-related peptides such as SP participate in multiple important physiological processes, and its major purpose is to regulate different functions related to gastrointestinal, urogenital, immune, and nervous system. It further includes the mechanism of epithelial secretion, inflammations, and contractility of muscles along with proliferation (Lorente et al. 2018). The molecular bases of different processes that are related to the human pathologies include the mechanism of SP-NK_1R that helps in maximizing the amount of serum. Moreover, a significant contribution in various pathological activities including chronic or acute inflammation such as functional disparities of urinary bladder and intestine can be provided through receptors that are found in different cells (Lorente et al. 2018). Other pathological activities include infectious diseases (respiratory syncytial receptors and acquired immune deficiency syndrome), migraine, cancer, anxiety, depression, etc. (Campbell et al. 2006).

15.5.5.1 Central Nervous System

The central and peripheral systems are rich with the distribution of receptor and SP (Satake and Kawada 2006). SP is however located at unmyelinated primary sensory neurons of the nervous system. It is further released through the brain stem and the spinal cord; this helps in developing the slow excitatory potential in the target neurons. The sensory fibers further help in the transmission of nociception and noxious stimuli while releasing different enzymes and peptides such as glutamate and substance P. Therefore, SP serves as a fundamental functional position in terms of binding NK_1R and functions as the transmitter of neuronal sensors that helps in providing different responses to stress-related problems. It is further important in performing other functions, as it is located at the central nervous system that is not associated with pain pathways. In addition, SP/NK_1R system plays a role in the pathophysiology of depression and anxiety disorders, epilepsy, and migraine (Yin et al. 2018; Wang et al. 2017; Chi et al. 2018; Gray 2018).

In various cases, SP serves as a neuromodulator that is functional in the transmission of sensory neurons associated with anxiety, stress, emesis, and depression. Other important regions of the brain (area postrema and the nucleus tractus

solitarius) help in providing spontaneous responses related to vomiting (Garcia-Recio and Gascon 2015).

15.5.5.2 Cardiovascular System

The cardiovascular cell system consists of substance P that helps in creating a significant impact over neurophysiological activities that include systematic regulation of heart rate. The information however is provided through different activities, such as blood pressure monitoring, stimulation of cholinergic neurons, and elasticity of vessels. It further plays a crucial role in atrial myocardial electrophysiology, and intact SP levels protect against the development of atrial fibrillation. Besides, the frequent occurrence of atrial fibrillation helps in reducing the SP levels. It also helps patients that have undergone coronary bypass surgery in generating the atrial fibrillation that is also initiated through the low levels of SP serum (Veldkamp et al. 2018a). Other pieces of evidence provide the implication of SP in different stress responses integrated through reperfusion, ischemia, and cardiovascular disorders. In the past, SP has been implicated in inflammation, angiogenesis, and the transmission of pain (Eliska et al. 2016).

In various studies of rabbits, potassium currents that are mediated through the activation of NK_3R by SP help in increasing the duration of potential functions. In contrast, ventricular action potential duration was unaffected by NK_3R activation. The stimulation of NK_3R helps in extending the overall duration of atrial repolarization of the rabbit's heart, and as a result, the duration of arrhythmia is decreased. A similar case was observed when the functions are implied in the human system. This shows that the stimulation of NK_3R helps in the representation of anti-arrhythmic concepts including the non-functionality of atrial fibrillation (Veldkamp et al. 2018a; b).

SP played a beneficial role in the improvement of left ventricular ejection fraction recovery after myocardial infarction and led to a reduction of infarct size, reducing inflammation and ischemia-reperfusion injury after acute myocardial infarction (Sim et al. 2018). The findings are associated with the porcine model of myocardial infarction.

15.5.5.3 Gastrointestinal Tract

The enteric nervous system serves as the fundamental source of SP, which is also present in the immune and enterochromaffin cells of gastrointestinal tract mucosa (Holzer 2013). It is mainly functional in the gastrointestinal tract; therefore, it can be perceived that changes occurring in SP might create a significant impact in different gastrointestinal functions. In cases where SP is incorporated through different functions including inflammation and other pathological processes, the resulting impact over gastrointestinal functions is usually high (Vilisaar and Arsenescu 2016; Zalecki 2019). The open distribution of NK_2R suggests that the substance is majorly involved in ensuring smooth functioning of human colonic activities (Jaafari et al. 2008).

In addition, SP and its receptors are highly functional in managing smooth regulation of the stomach muscles through the enteric nerve. Other major responses

include acute antral ulcerations of the stomach tissue. The increasing amount of SP-immunoreactive Auerbach's response in the porcine stomach helps in proving the significant value of the given neurons for neuroplasticity, since the inflammatory problems are highly responsive to ulcer diseases. This, however, promoted the indirect functioning of SP in the gastric nerve pathways and submucosal neurons to provide a massive supply of pyloric sphincters in various animals with antral ulcerations. This indicates that majority of the issues in the given regions are often connected to inefficient functioning of SP providing no significant contribution in regulating the myenteric neurons of the stomach (Zalecki 2019).

15.5.5.4 Musculoskeletal System

The skeletal growth period also includes various functions of SP, provided in the form of homeostasis of cartilage, and autocrine functions along with the regulation of the physiological mechanism of chondrocytes (Grässel and Muschter 2017). Various subclasses of SP including the receptors and neuropeptides are further represented by chondrocytes and other bone cells. SP further helps in increasing the proliferation of chondrocytes, indicating anabolic effects (Opolka et al. 2012; Grässel and Muschter 2017). Besides, the formation rate of bones is further affected through the reduction or loss of SP. The information thus shows that SP further helps in regulating the osteoclast differentiation that significantly affects the remodeling of bone (Wang et al. 2009). The function is common in the different cell types including the mesenchymal stem cells of bone marrow, osteoblasts, B or T lymphocytes, and synovial fibroblastic cells (Nilsson et al. 1985; Liu et al. 2007). In murine studies, costal chondrocytes express SP and NK_1Rs, and SP stimulation dose-dependently potentiates the chondrocyte proliferation rate and induces the formation of focal adhesion contacts (Niedermair et al. 2014).

Neuropeptides are also significant in the pathogenesis of osteoarthritis and osteoporosis. The pathogens are important in creating a negative influence over the microstructure of bone and may cause severe pain (Xiao et al. 2016).

Additional mechanical load increases the expression of NK_1R and endogenous production of SP, promoting cartilage degradation (Pirosa et al. 2018). The stimulation of synovial cells and SP helps in releasing the immune-regulatory cytokines and inflammatory mediators (Campbell et al. 2006). This suggests that SP serves as an important mediator of inflammation acting by stimulating the secretion of prostaglandin E2, reactive oxygen species, IL-1β, TNF-α, and SP. Thus, it further leads to cartilage degradation, when it is incorporated in chondrocytes. The functionality of SP along with the associated receptors was significantly high in chondrocytes and extracellular matrix. The following functions were integrated through low exercise, which helps in providing significance to its role that helps in providing fundamental response of chondrocytes to the mechanical functionality. Blockades of SP signaling using NK_1R antagonists were similarly found to prevent chondrocyte responses to mechanical stimulation (Grässel and Muschter 2017).

15.5.5.5 Respiratory System

Respiratory diseases such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease are stimulated through the involvement of substance P (Atanasova and Reznikov 2018). In asthmatic airways, mucus content was found to be positively related to SP expression, with NK_1Rs also elevated based on extensive expression detected on goblet cells (Chu et al. 2000). Patients suffering from chronic obstructive pulmonary disease were provided with the maximum concentration of SP (Tian et al. 2000).

Restoring SP-mediated signaling by means of tachykinin agonists has shown to be an ineffective strategy in cystic fibrosis. As per the pieces of evidence, it is suggested that the deformity of cystic fibrosis transmembrane serves as the major cause of the undue functionality of the glandular secretion that is mediated through SP. The cystic fibrosis transmembrane plays a crucial role in the absorption and secretion of transepithelial salt, which is integrated through different epithelial tissues (Ianowski et al. 2008).

15.5.5.6 Immune System

SP and other neuropeptides, when released through sensory nerves and other inflammatory cells, usually result in mast cells (Black 2002; Rosa and Fantozzi 2013) and further modulate the interactions between the immune and nervous systems and trigger neurogenic inflammation (Manak et al. 2010).

In most of the immune disorders, substance P plays a fundamental role in stimulating the mast cells that are often functional in pro-inflammatory activities and thus plays various roles to regulate the immune system (Amin 2012). This produces allergic, immune, and inflammatory responses. Mast cell stimulation occurs either through high-affinity NK_1R or low-affinity Mas-related G protein-coupled receptors (Zikou et al. 2018). The stimulated mast cells play an active role in allergic and non-allergic disorders based on the secretion of histamine and tryptase alongside cytokines and chemokines (Petra et al. 2018a; b). Interleukin (IL)-33 belongs to the IL-1 family of cytokines, which helps in increasing the functionality of mast cells by involving SP. This however results in the secretion of tumor necrosis factor (TNF) (Zikou et al. 2018). Besides, cytokine interleukin (IL)-31 also serves as an important factor for various inflammatory diseases that are related to pruritus, including the mastocytosis and atomic dermatitis. Besides, various mast cells that are regulated through both allergic and non-allergic triggers help in the secretion of tryptase, histamine, chemokines, and cytokines (Petra et al. 2018a, b). Besides, SP is functional in improving the production of inflammatory cytokine (TNF-α, IL-1, and IL-6). The process is integrated through immune cells including macrophages that are activated due to the nuclear characteristic of kappaB (NF-κB) (Manak et al. 2010).

SP and its receptor (NK-1R) also participate in HIV infection of monocyte-derived macrophages. HIV infections are found in human immune cells, and SP shows a significant association, since the HIV replicated through the isolated mononuclear phagocytes is often augmented through SP, whereas the HIV infection found in the cell is regulated through the nonpeptide SP antagonist (CP-96,345) (Lai et al. 2001).

The SP/NK_1R system potentiates numerous pathways involved in the proliferation and formation of cancerous colonic epithelium cells from normal cells (Koon et al. 2004), and truncated -NK_1R contributes to colorectal adenoma growth, with possible participation in adenoma-carcinoma growth (Gao and Wang 2017).

15.5.6 NK_1R Antagonist Pharmacology and Therapeutics

The crystal structure of human NK_1R bound to a high-affinity antagonist reveals the molecular basis of antagonist interactions and offers information toward understanding ligand binding selectivity for this pharmacologically therapeutically important family of GPCRs (Yin et al. 2018). Recently, SP and NK_1R have gained attention based on the development of complex NK_1R antagonists that have many promising therapeutic indications for issues such as depression, pain, and emesis (Campbell et al. 2006). It has been shown that NK_1R is significant in creating the impact on different activities of the brain including emesis centers. Aprepitant which is an oral antagonist is proved to be an impactful agent in preventing acute and delayed chemotherapy-induced nausea and vomiting (Manak et al. 2010; Muñoz and Coveñas 2013; Schank and Heilig 2017; Yin et al. 2018). Maximum exposure of aprepitant is important in creating a significant impact over the physiological activities that include the unbalance metabolism of cholesterol and cell trafficking, inflammation, and apoptosis. Plasma levels of SP decrease when treated with aprepitant due to a significant impact of over 176 plasma proteins and other pathways that are important for metabolisms including lipid metabolism and inflammation. The agent is further related to a significant increase in HDL cholesterol. Also significant impact is provided to different metabolic and hematologic markers, which are further helpful in restoring the baseline levels after 30 days of aprepitant treatment (Spitsin et al. 2017).

The ability of aprepitant treatment is significant in coping with the blood-brain complexities; its approval as drug to reduce chemotherapy complexities provided by FDA helps in giving it a significant status in treating infections related to human immunodeficiency virus (Manak et al. 2010). Cases that include the NK_1R as an antagonist help in suppressing cholesterol that in return provides significant macrophages. This helps in protecting the metabolism from atherosclerosis (Spitsin et al. 2017).

Other oral NK_1R antagonists, including netupitant and rolapitant, cross the blood-brain barrier and bind brain NK_1R. They are highly selective and have no affinity for serotonin, dopamine, or corticosteroid receptors. However, netupitant (300 mg) is available only in a combination formulation with palonosetron (0.5 mg). Fosaprepitant is an intravenous pro-drug of aprepitant that is converted within 30 min after infusion (McQuaid 2018). These NK_1R antagonists are safe and well tolerated, and most adverse events are minor and well tolerated, such as headaches (Muñoz and Coveñas 2013), fatigue, and dizziness (McQuaid 2018).

An increasing number of studies on NK_1R antagonist have been conducted which focuses on psychiatric processes and disorders like depression, stress, and anxiety.

The clinical developments, however, indicated the failure of the given treatment. Several studies in the given framework have indicated the importance of taking NK_1R drug as it has impact on the treatment of stress-related problems. Despite greater efforts, studies remain unsuccessful in increasing the usage of drugs among the given populations. The study further demonstrated that the inefficiency of NK_1R antagonists in treating alcohol addictions is due to the insufficient presence of receptors. As a result of this, several developments are being made on the functions of NK_1R to complete the target of providing new pharmacotherapeutics in treating addiction problems (Schank and Heilig 2017).

With regard to pain transition, SP receptor antagonists do not block responses to certain types of pain, though they can modify some responses where glutamate, which is often extracted through SP, provides a greater significance in eradicating pain (Gray 2018). As a therapeutic agent for treating epilepsy, NK_1R antagonists reduce kainic acid-induced seizure activity, though their therapeutic potential for treating epilepsy has not yet been fully exploited (Wang et al. 2017; Chi et al. 2018). Additionally, human NK_1R modulators have shown promise in clinical trials for migraine and depression (Yin et al. 2018). In particular, there is a greater similarity found between SP receptor antagonist MK-86 and paroxetine due to their anxiolytic and anti-depressant effect that is usually attained in treating patients with unipolar disorder (Kramer et al. 1998). The models related to the chronic obstructive pulmonary disease have significantly provided decrease in inflammation in the respiratory tract when treated through tachykinin receptors (De Swert et al. 2009). The insufficient evidence provided in this regard demonstrates that the antagonists are important to minimize the airway responses while improving the function among patients with asthma (Ramalho et al. 2011).

In terms of addressing tumor genesis, there is also evidence to indicate that NK_1R antagonists may have value in treating patients with colorectal adenomas (Gao and Wang 2017).

15.6 VIP-Glucagon Family: Vasoactive Intestinal Polypeptide (VIP)

Vasoactive intestinal polypeptide (VIP) belongs to superfamily of glucagon that are similar to neuro and endocrine peptides. VIP further consists of 28 amino acids. Furthermore, the given peptides are present in the human body and are expressed through their functionality. Also, they are important in performing significant biological functions in the form of muscle relaxation, secretion of electrolytes and water in intestine, and cerebral and coronary artery vasodilation along with increased myocardial contractile performance (Dickson and Finlayson 2009; Henning 2013).

At an initial level, VIP was characterized as the intestinal hormone, which represents its isolation from digestive tract, while acting to be fully functional in the intestinal tract for the secretion of electrolytes. Later, it was shown that VIP is extensively distributed in multiple types of tissue as a neurotransmitter. Besides, the structural formation of VIP occurs when released from different sources including

the endocrine cells, the digestive tract, and the central nervous system. VIP is further important as it regulates secretion and blood flow in both the intestine and pancreas. This helps in increasing the gastrointestinal motility due to the display of similar effects in urological, respiratory, and cardiovascular system. Reports provided for this neuropeptide have recently identified a wider range of activities, highlighting its neuroprotective and immunological effects (Dickson and Finlayson 2009; Hisato Igarashi et al. 2011). The ligands along with the Class II G protein-coupled receptors have a great therapeutic potential, as suggested by clinical data along with similar investigations (Chapter et al. 2010). Still, it is important to analyze the developments of applications in various ongoing disease and pathological disorders, along with the structural and functional value of neuropeptides and its receptors (Hisato Igarashi et al. 2011). The existing information related to VIP and its receptors involves several pharmacological and clinical findings that are relevant to the existing developments in the given problem. Therefore, the following sections provide a detailed description about the given idea.

15.6.1 Structure of Vasoactive Intestinal Polypeptide

As mentioned earlier, VIP acts as a linear peptide that is composed of 28 different amino acids. The molecular weight of VIP is 3326 which is provided by neural tissues; however, the sequence of amino acid is illustrated in Fig. 15.6. VIP is based on the structural family that includes various neuropeptides and hormones such as glucagon, growth hormones, secretin, helodermin, glucagon peptide 1 and 2, etc. However, the most homologous peptide to VIP is pituitary adenylate cyclase-activating peptide (PACAP), based on 70% identity at the amino acid level (Vaudry

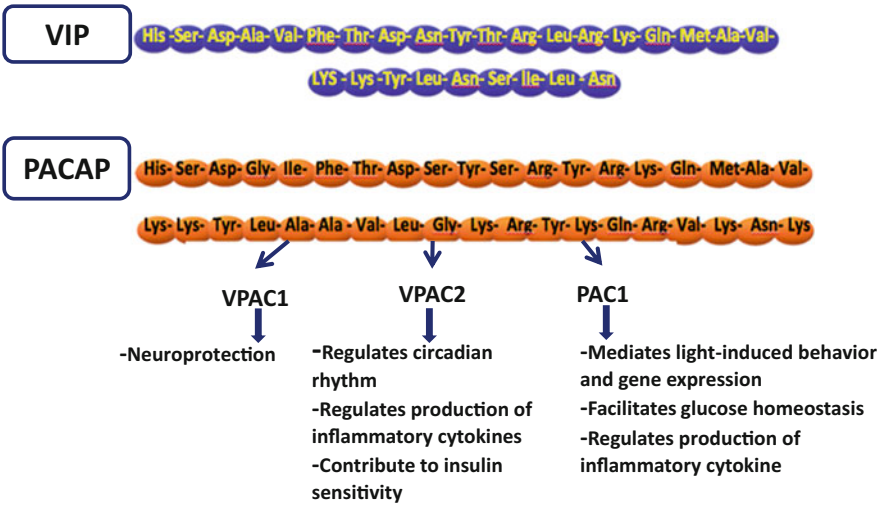

Fig. 15.6 Overview of VIP and PACAP and their receptors

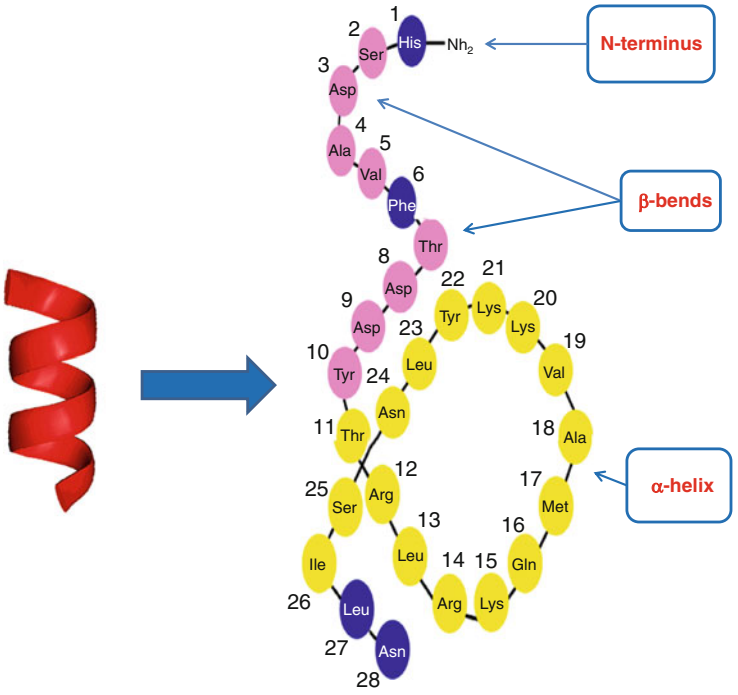

Fig. 15.7 Helical conformation of the secondary structure of VIP

et al. 2009; White et al. 2010). Figure 15.6 demonstrates the high degree of the amino acid sequences homology shared by both VIP and PACAP peptides.

Analyses of VIP using nuclear magnetic resonance spectroscopy (NMR) provided a helical conformation with α-helix and two β-bends provided at the given residues of 11–26 and 2–5 and 1–10, respectively. The helical conformation is located at the N-terminus as indicated in Fig. 15.7. The two domains including the C-terminal and N-terminal are important, as they play a significant role in managing bio-activity and receptor recognition (Igarashi et al. 2011).

In 1995, cloning and mapping of a VIP gene was identified in humans and is placed at chromosome 6q25. The structural formation of a human VIP gene is based on 7 exons that are incorporated with 6 introns along with the spans of 9 kb. The gene is further significant in providing the two peptides that are biologically active and highly functional. The formation occurred through the incorporation of 170 amino acid preproteins tailored in a proteolytical manner. The two resulting peptides are known as peptide histidine methionine (PHM) and VIP (Dickson and Finlayson 2009; Dorsam et al. 2011).

15.6.2 Vasoactive Intestinal Polypeptide Receptors

VIP is based on two definite receptors including $VPAC_1$ and $VPAC_2$ that are mutually connected through PACAP and VIP to a certain extent. The given receptors are important and thus belong to a class B group of the G protein that are further coupled through seven different transmembrane receptors. It further consists of approximately 437–459 residues of amino acids followed by a long chain of N-terminal (Laburthe et al. 2007; Vaudry et al. 2009). The availability of N-terminal with a structural representation of N-cap indicates that it is highly involved in the stimulation of receptors and could be implicated in providing important developments in terms of treatment design that targets different membranes of B GPCRs and VPAC receptors (Neumann et al. 2008). However, the α-helical conformation is usually indicated through the binding of peptides along with certain specific receptors (Laburthe et al. 2007; Vaudry et al. 2009).

The examination regarding the distribution patterns of $VPAC_1$ and $VPAC_2$ is provided in abundance. The allocation of $VPAC_1$ specific to certain regions of the central nervous system includes the piriform cortex, putamen, choroid plexus, lateral amygdaloid nucleus, etc. Besides, the functionality of $VPAC_1$ is provided in kidneys, small intestine, liver, mucosa of the stomach, prostate gland, spleen, lymphocytes, mammary glands, and many other tissues. Contrary to this, $VPAC_2$ is extensively found in smooth muscles of various organs and vascular walls that specifically include the large intestinal mucosa, heart, retina, pancreas, adrenal medulla, alveolar epithelium, testis, and adipose tissue. $VPAC_2$ when functioning in the central nervous system is usually found in the cerebral cortex, amygdala, hippocampus, olfactory brain, paraventricular cortex, and thalamus (Hisato Igarashi et al. 2011).

VIP and PACAP receptors are stimulated by several endogenous peptides that vary in their affinities such as VIP, PACAP-38, PACAP-27, peptide histidine methionine amide (PHM), peptide histidine isoleucine amide (PHI), and peptide histidine valine (PHV). Similar binding strengths have been identified in VIP and PACAP by both receptors. However, PACAP27 and PACAP38 with 1000-fold reflected maximum affinity in comparison to VIP that serves as the antagonist of isoforms found in the PAC1 receptor (Henning 2013). VIP and PACAP are important in maintaining the physiological mechanism of several systems that contribute to important pharmacological effects in the human body as described in the sections below.

15.6.3 Mechanisms of Physiological Action of Vasoactive Intestinal Polypeptide

The peripheral and central nervous systems and gastrointestinal tract are comprised of VIP neuropeptides that are found in abundance. The given neuropeptide is crucial in functioning as a neurotransmitter and neuromodulator for different physiological functions including ion transportation, neuronal functionality, and mucosal secretions. It further helps in vasodilation in endocrine, cardiovascular, pancreatic,

Table 15.3 Some actions of VIP in different biological systems

Biological system	Effect
Cardiovascular system	It helps in coronary and peripheral vasodilation and thus creates a positive impact in terms of chronotropic and inotropic effects
Respiratory system	Homeostasis of the respiratory system and exerts a potent vasodilatory and bronchodilatory effect
Endocrine system	It helps in the regulation of pituitary functions such as releasing of growth hormones, FSH, T3 and T4 thyroid, PRL, ACTH, adrenal and testosterone, TSH, vasopressin, and PRL
Pancreas	It releases hormone from the pancreas and further serves as a stimulator to insulin
Immune system	It controls the homeostasis of the immune system. Furthermore, it is indicated as a potent anti-inflammatory factor

respiratory, and urological systems. VIP neuropeptide is highly important as it regulates the mucosal inflammatory response, promotes gastric acid secretion, moderates hemodynamic regulations, etc. (Vu et al. 2015; Takei et al. 2016). Some of these physiological activities are summarized in Table 15.3.

VIP basically mediates three proven major physiological effects including (Abba Kastin 2013):

- Relaxation of blood vessels and smooth muscles by inducing the release of nitric oxide (NO)
- Enhancement of electrolytes and diaresis by increasing urine volume and enhancing fractional excretion of sodium, chloride, and potassium
- Regulating the neuroendocrine and endocrine functions

The vasodilatory impact generated by VIP in vascular muscles is based on the ability of VIP to provide increased concentration of vascular cyclic AMP. This helps in triggering the process of protein kinase A and thus promotes the sequestration of Ca^{2+} through sarcoplasmic reticulum. Besides, it is important to maximize the extrusion of Ca^{2+} into the extracellular space. According to previous studies, vasorelaxant impact of VIP is based on endothelium that is found in the aorta, uterine artery, and intrapulmonary artery. It is further mediated through the functioning of lipoxygenase found in the aorta of rat. Other important components include nitric oxide that helps in the activation of guanylyl cyclase that is usually found in the uterine artery of humans (Henning and Sawmiller 2001). Several pieces of evidence indicated that neuropeptide VIP is significant in regulating the cardiac rhythm of the heart. The abnormality of the cardiac function results in cardiac pathology. Therefore, VIP serves as the major tool in regulating the physiological process of the heart that includes the suprachiasmatic nucleus that helps in regulating the cardiovascular function (Schroeder et al. 2010).

Immunocytochemical evidence suggests that VIP functions as a vagal neurotransmitter in pancreatic nerve fibers and plays a potential role in triggering the release of

hormones from the pancreas. It is further important in increasing the flow of blood through vasodilation (Dickson and Behrman 2013; Chandra and Liddle 2015). VIP and its close relative PACAP also promote insulin and glucagon release through several ways, such as the secretion of insulin through cAMP coupling metabolism, extracellular signal-regulated kinase (ERK) pathway along with signaling of P13K, β-adrenoceptors. Others include the mobilization of Ca^{2+} that is integrated through the intracellular stores of the gastrin-releasing peptide (Röder et al. 2016).

Studies further indicated that the anterior pituitary and hypothalamus are used to secrete and synthesize VIP. Also, it helps in making accurate regulations of the pituitary functions including FSH, LH, TSH, and prolactin, T3 and T4 thyroid, and gonadal and adrenal hormones, in response to acute inflammation provided through LPS (Bik et al. 2004). Immunoreactive VIP that is found in the hypothalamus and pituitary usually increases due to the treatment provided through estrogen and adrenalectomy. Moreover, the immunoreactive VIP decreases followed by the hyperprolactinemic states. During the duration of sexual maturation and lactation, the levels of VIP mRNA in the hypothalamus are significantly high. Results however provided the physiological role of pituitary and hypothalamic VIP gene expression in relation to its fundamental function as neuroendocrine hormone. Moreover, VIP stimulates the release of luteotropic hormone called pituitary prolactin (PRL) from the pituitary at different levels that can easily be obtained from the hypophyseal-portal blood, while including other pituitary functions, such as release of growth hormone, ACTH, and vasopressin, and stimulates the release of catecholamine from the adrenal medulla.

The control of the PRL secretion is integrated through the endogenous VIP found at multiple locations. This further helps in maintaining firm interaction with other moderators of the PRL secretion that include prostaglandins, cholecystokinin, serotonin, and oxytocin (Chaiseha et al. 2010; Blanco et al. 2013).

VIP serves as the fundamental regulator of neuropeptide that is extensively distributed in both peripheral and central nervous system. The representation of the significant type of endogenous sponsors to the formation and regulation of the immune variances in various CNS immune organ, further helps in controlling acute inflammation in various peripheral immune organs (Ganea et al. 2014). Provides an important recognition to VIP in terms of serving as a valuable anti-inflammatory factor in adaptive and innate immunity. The production of pro-inflammatory cytokines and chemokines from dendritic cells, microglia, and macrophages is integrated through VIP. The process is highly common in innate immunity. Also, VIP diminishes the expression of costimulatory molecules on the antigen-presenting cells and consequently decreases the activation of T cells that are highly specific to antigens. In the case of adaptive immunity, VIP promotes the responses provided by Th2 and helps in reducing the Th1 type of pro-inflammatory responses (González-Rey et al. 2004).

VIP is significantly functional in the homeostasis of the respiratory system and employs a potent vasodilatory and bronchodilatory consequences in the system. It further encourages the secretion of mucous that is headed through the tracheobronchial submucosal glands. Through several clinical trials and research studies, it can

be concluded that VIP-inhaled agonists are significantly functional in respiratory therapy (Mathioudakis et al. 2013).

15.6.4 Role of Vasoactive Intestinal Polypeptide in Pathogenesis of Disease

Vasoactive intestinal polypeptide has a significant biological role in health and disease and may serve as an important determinant in the pathogenesis of neurodevelopmental, allergic, gastroesophageal, and osteoarthritis diseases. Several studies indicated that up-regulation of VIP can counteract the effect of pro-inflammatory stimuli and is valuable in minimizing the pain in osteoarthritis (OA) which is a chronic and degenerative bone disease that is commonly represented through disabilities in aged population. The important knowledge regarding VIP is that it is effective in preventing the damage of chronic cartilages along with remodeling activities of the joints. Through various pieces of evidence, it is indicated that VIP is regulated at minimal levels in the synovial fluids of OA. This helps in increasing the level of production related to pro-inflammatory cytokines that are significant in contributing toward OA pathogenesis (Jiang et al. 2016).

Past studies have shown that VIP is important in the pathogenesis of bronchial obstruction throughout the period of exacerbation of the disease (increase in the severity of a disease or its signs and symptoms). Elevated levels of VIP and other neuropeptides were found in the serum of asthmatic patients during exacerbation period of the disease, indicating its important clinical significance in asthmatic patients (Semernik and Lebedenko 2015). Recent review provides an updated understanding on the functions and the value of VIP in treating various allergic diseases including asthma, allergic rhinitis, and dermatitis. The immunomodulatory impact of VIP is reflected in the mononuclear leukocytes of the peripheral blood. Moreover, various subjects suffering from diseases such as asthma and allergic rhinitis were found to have various neuropeptides such as morphine, SP, VIP, and ACTH. The symptoms were only prominent in the form of VIP in both allergic and normal subjects. Other than this, the current evidence is significant in providing practical information of the integration of VIP in various skin inflammatory disorders reflecting the pathophysiological background of such diseases. The participation was evaluated through improvised proliferation and keratinocyte production of cytokine. The pathogenesis of inflammatory dermatosis indicates the significance of cytokine structures around keratinocytes (Verma et al. 2017).

The most recurrent gastrointestinal disorder, i.e., the gastroesophageal reflux disease (GERD), is developed through acid reflux and heartburn. GERD pathogenesis is also developed through the increasing levels of VIP serum. Relaxants that are facilitated through VIP often have a significant impact on lower esophageal sphincter (LEP), which interacts with the formation of harmful acids. These acids in return increase the level of nitric oxide (NO) that is responsible for providing low pressures for LEP (Kassim et al. 2002).

Recently, in vivo test was used to test the interneurons that function through VIP and are mostly found in post-natal development of the cortical circuits. The removal of the early postnatal ErbB4 helped in dysregulation of the VIP interneurons. In some cases, it appears during the growth period which is visible in creating digressions in their functioning. It further leads to high dysregulations in the relationship between cortical, temporal, and state dependence. The data however provides valuable insight about the function of VIP interneurons in the formation of cortical circuit and is also important in creating a significant impact over the pathophysiology of neurodevelopmental disorders (Batista-Brito et al. 2017).

15.6.5 Pharmacology of Vasoactive Intestinal Polypeptide

Vasoactive intestinal peptide belongs to the superfamily of various peptides that are structurally related and thus includes growth hormone-releasing hormones, secretin, gastric inhibitory peptide, glucagon, and glucagon-related peptides.

The peptide is important as it exerts its action through $VPAC_1$ and $VPAC_2$ secretin receptor-like. Progress in characterizing the functions of these receptors has been delayed due to the insufficient availability of drugs. Literatures identify several antagonists and agonists related to the VIP receptors. [$Ala^{11,22,28}$] VIP and [Lys^{15}, Arg^{16}, Leu^{27}] VIP (1–7)/GRF (8–27)-NH_2 are certain agonists of the $VPAC_1$ receptor and PG 97–269 is a selective antagonist. The most selective identified $VPAC_2$ agonist is Ro 25–1392; but on the other hand, myristoylation provides a -K-K-G-G-T sequence of the amino-terminus for VIP (1–26) [K (12)]. It further provides the extended carboxyl-terminal of Ro 25–1553 that is a specific antagonist of $VPAC_2$ receptor antagonist. The molecule provides a $VPAC_2$ receptor antagonist that is found in humans and is widely present (Moreno et al. 2000; Dickson et al. 2006; Harmar et al. 2012).

Cardiopulmonary system highly reflects the activities of VIP through various pharmacological functioning that includes; anti-inflammatory actions of, potent improved blood circulation within the region of lungs and heart, potent airway and dilatory actions, etc. (Wu et al. 2011). Since VIP functions as a neurotransmitter, a significant impact is created in the form of vasodilatory and potent bronchodilatory functioning. Also, it helps in the secretion of mucus through submucosal glands. VIP functions as immunomodulator, for instance, in suppressing the humoral immune response, inhibition of bronchial and vascular inhibition, and diminishing the extracts of cigarette smoke resulting in the loss of L2 alveolar cells. Recent investigation indicates a potential therapeutic role of a novel stabilized inhaled VIP agonist with minimized side effects dealing with a variety of lung diseases such as pulmonary hypertension, cystic fibrosis, asthma, chronic obstructive pulmonary diseases, etc. (Mathioudakis et al. 2013).

VIP plays a fundamental role followed by various functions such as the regulation and control of timing of circadian rhythms, memory, and learning along with various stress responses. The present study relates $VPAC_2$ receptor in providing vulnerability to schizophrenia. VIP, in various peripheral regions, plays a significant role in

dealing with inflammation and immunity controls and the emancipation of catecholamines from the adrenal medulla and functioning as a co-transmitter in both sensory and autonomic neurons (Harmar et al. 2012).

The endogenous VIP is further important in controlling the composition of body mass and secretion of important hormones that regulate the overall metabolism. VIP plays a significant role in maintaining body weight and reducing fat mass. This indicates that VIP may play a crucial role in treating issues related to obesity by moderating the appetite and body mass (Vu John et al. 2013). Important roles are played by VIP and PACAP in controlling inflammation and immunity, the release of catecholamines, and the control of pancreatic insulin secretion and as co-transmitters in sensory and autonomic neurons (Harmar et al. 2012).

15.6.6 Vasoactive Intestinal Polypeptide: Therapeutics

By providing a firm consideration toward pharmacotherapeutics and drug formation, the present study focused on the function of Class II G protein-coupled receptors (GPCRs) to provide a significant knowledge regarding the development of new therapeutic drugs. The development of information in the study involved recent clinical findings. Ligands including VIP help in stimulating the given receptors and are characterized as effective therapeutic targets to develop treatment methods regarding neurological disorders such as autism spectrum disorder and Alzheimer's and Parkinson's disease (White et al. 2010). The development of the drug may provide important treatments for stroke, sleep disorder, age-related loss of memory, and neurodegenerative disorders. Other than this, VIP is classified as an effective source of treatment for various cardiopulmonary disorders like PAH, asthma, and COPD. Clinical application of VIP has been limited in the past for a number of reasons, including its short plasma half-life and difficulty in routes of administration. The development of long-acting VIP analogs, in combination with appropriate drug delivery systems, may provide clinically useful agents for the treatment of PAH, asthma, and COPD (Wu et al. 2011). Despite all the advantages of VIP as a promising candidate in pharmacotherapeutic targeting, VIP is still regarded as highly reactive to peptidases (DPP-4) that is found in most tissues. Thus, the therapeutic impact of VIP can be identified through high-dose injections, when provided multiple times (Pezzilli 2006).

VIP is highly functional in exhibiting the neuroprotective response against various cell deaths and serves as the inflammatory modulator for the inflammatory immune responses. It further offers promise as a therapeutic approach for the treatment of Alzheimer's disease and has been regarded as an efficient treatment for neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Here, the first is a growing disease that has affected more than 35 million people worldwide and is diagnosed at the aging phases of life. The disease consists of fibrillar β-amyloid (Aβ) in the brain senile plaques, which is later activated and thus becomes functional through the release of pro-inflammatory chemokines and cytokines from microglia and astrocytes. This initiates the inflammatory progression, resulting in

neurodegeneration over time. In a recent study, VIP was shown to effectively limit injurious stimulation of Aβ-concentrated microglia and helps in discharging the neurotoxins including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and nitric oxide (NO). Thus, VIP is crucial in preventing the neuronal cell death leading toward AD pathology in the brain (Delgado et al. 2008; Querfurth and LaFerla 2010).

Publications have resulted in providing significant relationship between variations of gene encoding including schizophrenia susceptibility and VPAC2 receptor. These results have generated some enthusiasm in the field of new antipsychotic drug developments (Levinson et al. 2011).

The suppression of Th1 immune response along with the activation of T cells is integrated through VIP, where it serves as the anti-inflammatory mediator to provide immune balance. In the present era, VIP acts as a therapeutic agent for various autoimmune diseases such as ulcerative colitis, multiple sclerosis, and rheumatoid arthritis (Pezzilli 2006).

Current literature reviews show that vasoactive intestinal peptide receptors (VIPRs) are over functional in some malignant tumors. Also, they provided information related to the progression of several malignancies. The analogs of the agents are labeled through radionuclide and are used for imaging of tumor receptor. The images are important in visualizing the VIPR surface of protein functioning. The images were visualized via in vivo test that provides information regarding the properties of molecular drugs when used for treating tumor. Besides, the relationship between VIPRs and angiogenesis and malignant transformation is important in the treatment of cancer (Bo Tang et al. 2014).

The function of VIP specifically in the gastrointestinal tract indicates that neurotransmitters and its receptors help in dealing with several disorders related to the secretion of gastric acid along with their impact on gastrointestinal motility including functional bowel syndrome (Mawe and Hoffman 2013).

15.7 Neuropeptide Y and Related Peptides: Neuropeptide Tyrosine (NPY)

Deficiency of nutrients (or caloric restriction) can accelerate the orexigenic peptide neuropeptide Y (NPY) and autophagy in cortical and hypothalamic neurons (Catalani et al. 2017). The NPY plays a very essential role in various physiological functions like energy homeostasis, cognition, and neuroprotection and neurogenesis, intake of food, stress response, and circadian rhythm; therefore, it serves as an important neuropeptide that is widely present in the brain (Diaz-delCastillo et al. 2018).

A distinguished hypothalamic neural cell in rat and hypothalamic neuronal cell line in mouse enhance the flux of neuronal autophagy, which is exhibited by the study of LC3-II turnover, the rise in the number of autolysosomes and autophagosomes, and decline in the amount of p62. This impact is exercised by the triggering of Y1 and Y5 receptors. The protein kinase stimulation such as PKA,

PI3K, and ERK1/2-MAPK by NPY results in the signaling of track linked with the induction of autophagy. By in vivo over manifestation of NPY in the arcuate nucleus, the flux of NPY-induced stimulation was verified in the hypothalamus of mice. Furthermore, in cortical neurons of rat, the autophagy is accelerated by NPY (i.e., the decline of p62 expression and growth of LC3-II) possibly by the prevention of mTOR activity. In mice, which are nourished with a diet containing a high level of fats, the removal of AMPK activity within the arcuate nucleus of hypothalamus reduced NPY expression and autophagy, hence decreasing body weight and intake of food as a result. Therefore, in the hypothalamic cell lines, increased levels of NPY, the stimulation of AMP-activated protein kinase, and regulating of mTOR signaling result in the induction of autophagy. In both, the NPY levels and autophagy keep declining with age; research is being carried out to enhance the level of NPY and increase autophagy including caloric restriction, as a result of which it was recommended to develop defending effects that postpone deficiencies related to prolonged existence. By regulating hypothalamic autophagy, one might also be able to eliminate the prospects of obesity and other metabolic disorders associated with aging.

Lastly, the NPY exercises a neuroprotective effect on the cerebellum and striatum of spinocerebellar ataxia type 3 of two mouse models, which is a disorder symbolized by autophagy defects. Therefore, it was recommended that this action might be associated with the stimulation of the clearance process of protein like autophagy; however, more data is required to support this hypothesis or assumption. Generally, the NPY ability to prolong neuro-degeneration by activation of autophagy as an approach to a free abnormal and misfolded protein that results in neurodegenerative disease is necessary to be studied in detail.

15.7.1 Composition of Neuropeptide Tyrosine Receptors

Neuropeptide Y (NPY) was first discovered in 1982 and was found in the porcine brain. The information suggested that formation of neuropeptide Y is possible through the amalgamation of 36 amino peptides and thus is generally functional in the peripheral nervous system (PNS) and central nervous system (CNS) of mammals. This neuropeptide comprises pancreatic polypeptide (PP) and peptide YY (PYY) as it shares its amino acid sequence homology in 70% and 50%, respectively. The similarity of the sequence in terms of their molecules represents them in a U-shaped molecule, which is formed in a structure similar to the hairpin, along with a tertiary structure comprising of an amphiphilic α-helix, which are joined by the β-turn with a type II helix and similar to polypro line. This structure of the neuropeptide represents the tridimensional structure, which constitutes peptide affinity and efficacy connecting them to their several receptors. The NPY biosynthesis translates the molecule of the pre-pro-NPY in which translocation occurs directly with the endoplasmic reticulum, where the mitigation of the peptide takes place. Through the prohormone convertases, cleaving of the pro-NPY precursor takes place in the NPY along with its C-terminal NPY peptide (CPN), where the NPY peptide

goes through two truncations, i.e., through a peptidyl glycine alpha-amidating mono oxygenase and a carboxypeptidase for the NPY maturation as well as its activation. The amidation of the C-terminal serves as the final characterization of the molecule, which impedes the degradation and takes place through the action of the carboxypeptidases. With NPY immense consistent distribution of the CNS and PNS, there are several biological impacts as well as brain disorder, which encompass disorder like obesity, anorexia, depression, drug addiction, itching, pain, and energy homeostasis (Loh et al. 2015; Gonçalves et al. 2015; Farzi et al. 2015; Bourane et al. 2015; Arcourt et al. 2017).

15.7.2 Mechanisms of the Physiological Action of Neuropeptide Tyrosine

The physiological action pertaining to the neuropeptide tyrosine is more prominent in the up-regulation of the peripheral injury of the nerve that provides cell bodies in various diameters. These cell bodies are associated with the neurons of the primary sensors, which innervate with the spinal cord dorsal horn. Its two receptors, i.e., Y1R and Y2R, are located in DRG in the form of CGRP—positive neurons which indicate the mediating role of NPY in nociception. This is due to NPY supplies in a physical circuit, which permits the effect of autocrine or paracrine NPY on the modulation of the pain. The Y1R receptor is found in neurons, which are small and medium, whereas the location of the Y2R is generally found in large and medium neuronal somas. The use of the optogenetic method for Y2R neuron targeting shows that the Y2R is mostly expressed as a peptidergic A-fiber subset in the activation of the nociceptors, which is done through the mechanical stimulus belonging to the noxious range (Arcourt et al. 2017). In addition, 40% of the positive neurons of the NPY is located in the DRG, followed by the mechanical injury of the nerve, which also indicates Y1R as well as Y2R. The exertion of NPY actions has remained controversial, but some expressed it as pro-nociceptive, while some categorized it as anti-nociceptive in the functional form. Studies have suggested that the differences in action are based on the deliverance path. Moreover, the peripheral administration of NPY is pronociceptive. The subcutaneous injection of the peptide or Y2R agonist and subsequent sciatic nerve injury intensifies the hyperalgesia both mechanically and thermally. However, divergent results are obtained when the Y1R agonist is used for administration in a similar manner, which leads to the improvement of mechanical hyperalgesia, whereas it is low for thermal hyperalgesia. The NPY receptor has been observed to have a mediating effect on the pronociceptive, which is part of the sympathetic terminals of the nerve, following the increase of the affinity or intercellular effect on the injury of the nerve, and its expression. In addition, the expression of the de novo NPY synthesis is located at the dorsal root ganglia along with the terminal regions of the spinal cord, where the neurons inhibit nociceptive pathway where it serves as the compensatory mechanism for adaptation in response to increase in the excessive excitatory signaling. The two NPY receptors such as Y1R and Y2R are viewed to form a mediating effect, which is primarily

related to the spinal cord in the dorsal horn and encompasses the likely targets for the drug in the treatment of chronic pain.

15.7.3 Functional Value of Neuropeptide Tyrosine in the Pathogenesis of Disease

Concerning the pathogenesis of a disease, the characterization of NPY of the cardiovascular system is primary. In line with norepinephrine, the involvement of NPY is extensive in the regulation of cardiovascular sympathetic responses. The presence of NPY is significant in the heart and is also recognized as a cardiac peptide at the time of its isolation and sequencing. The plasma levels of NPY are in correlation with the activity of the sympathetic nervous system, which is observed in various pathophysiological situations, i.e., during exercise, whether mild or heavy; the activation of the sympathetic nervous system is related to the reduced sympathetic nervous system responses, as well as in heart-related disease or pheochromocytoma patients. At the moment, the NPY also shares a link with coronary artery disease (CAD) such as the single nucleotide polymorphisms of the NPY genome which is related to the human CAD and more significantly on the first-onset patients. Moreover, the role of NPY is also related to that of the gastrointestinal (GI) tract involving adaptation to diet, electrolyte balance, intestinal growth, water uptake, and gastric emptying.

15.7.4 Pharmacology of Neuropeptide Tyrosine

In recent times, the association of neuropeptide tyrosine (NPY) has been found to be in neurological responses related to ethanol and drug abuse, which is highlighted from genetic, pharmacological, and molecular indication (Ciafrè et al. 2016). The pharmacology of neuropeptide tyrosine has initially been examined from the peripheral sympathetic control of the neurons related to the blood vessels, spleen, heart, as well as vas deferens. The release of the NPY largely takes place when the stimulation constitutes of high frequency or activation of the strong sympathetic reflex. NPY has a particular receptor mechanism, which is present at the pre-junction level as well as post-junction levels, where the existence of an enormous amidated C-terminal portion of NPY is required to bind the receptor and results in vasoconstrictor effects as well as the inhibition of cyclic AMP formation. Moreover, the release of the NPY is also impacted by various pharmacological agents encompassing clonidine, guanethidine, yohimbine, nicotine, angiotensin, angiotensin II, and desipramine. Among the NPY receptors, the Y_5 receptor constitutes the pharmacological profile particularly for the peripheral tissues, though its mRNA expression is greatly confined to the CNS (Dan Larhammar et al. 2015).

15.7.5 Neuropeptide Tyrosine Receptors

The fragments of the NPY C-terminal serve as the source, which characterizes the NPY receptors. It is due to its NPY and PYY fragment imitation of the responses which are given by the NPY such as in the twitch responses of the pre-junctional inhibition as it occurred in rat vas deferens; however, they are not observed in vasoconstriction of guinea pig iliac vein. In the context of the NPY receptor, it has been suggested that Y_1 are the receptors that are activated by holopeptides, whereas Y_2 are the receptors that are activated by the fragment of the C-terminal and holopeptides. Although the synthesis of the various C-terminal fragments occurs, the NPY and PYY of the 3–36, 13–36, and 18–36 fragments are often utilized irrespective of their obvious benefit between these fragments. The discrimination of the receptors such as Y_1 and Y_2 is done using the NPY C-terminal fragment, though these are not only confined to the Y_2 selection as Y_5 receptors can also be activated using the same concentration. It should be noted that the selection of the receptors is based on the difference between the availability of tools and the substantial difference concerning the affinity of the receptor in relation to their cognate ligands which prevails between the species. The recognition of the receptor is based on the various combination of the complementary agents which are used. The application of this condition is more momentous for in vivo, whereas the less characterization of the compounds occurs in contrast to in vitro.

15.7.6 Neuropeptide Tyrosine: Therapeutics

The neuropeptide tyrosine is significantly used for the therapeutic of various diseases. One of such disease is a gastrointestinal disease where NPY receptor performs in the capacity of the modulators in cases of constipation, diarrheas, as well as IBD. The use of the NPY agonist in the position of a non-specific activating agent is said to assist in IBD treatment. This idea gains further momentum to the fact that the application of the therapeutic agent does not require integration in the blood stream. For instance, the earlier study by Litvak et al. (1999) demonstrated that in the case of diarrhea, the presence of two stable PYY peptide analogs is found (BIM-43073D and BIM-43004C), which resulted in increase in the absorption of water when dogs were treated with these two analogs. This further indicates that the health of the gut is promoted by the assimilation of a selective Y1R agonist. On the contrary, there is another fact that the use of Y1 receptor knockout mice which comprises lower IBD susceptibility also highlights the fact that the use of the antagonist is more effectual in constipation and IBD treatment. This elucidates the use of Y2R and Y4R receptors in overcoming the discrepancy and finding more treatment.

In addition, the use of NPY is also linked to the administration of chronic pain. This is due to the NPY receptors' heterogeneity, their application site, as well as the various chronic pain models for the chronic pain, which allow the NPY to exert various effects. The use of central NPY contributes to the production of analgesia

which causes mediating influence on the spinal as well as supraspinal level (Taylor et al. 2014). In the chronic pain modulation, the role of NPY along with its Y1R and Y2R receptors is found to be crucial, where the NPY receptors can also be regarded as a target for a drug where they are used for devising new drug design. However, various problems exist, which are difficult to overcome with the use of this targeted treatment. The route of administration of these receptors, such as the NPY agonists which are peripherally applied, results in the behavior that emerges due to a halt, and it appears that the analgesic effect occurs only when the peptide or drug formulation takes place following the blood-brain barrier (BBB). However, the lumbar intrathecal drug delivery is often found to be linked with some patients' sort of discomfort and inconvenience, which can be mitigated through increasing the treatment efficiency as well as persistence of its effects, which are still lacking. Another difficulty associated with the determination of the severe pain in NPY agonist when found in clinical practices is linked with the deficiency of the non-peptidic small molecule of the NPY receptor agonists (Mittapalli and Roberts 2014). To treat these cases, different strategies are adopted such as targeted agonist delivery by using the therapy related to the nanoparticles or gene (Chandrasekaran 2013).

15.8 Calcitonin Peptides and Others

Huang et al. (2006) initially discovered calcitonin (CT) which is a kind of polypeptide hormone (Masi and Brandi 2007). This identification occurred when the regulatory hormone for calcium level was being discovered. This peptide is able to reduce the blood calcium level through the direct inhibition of the mediation of the bone resorption, which also improves the kidney exertion of calcium. There are 32 amino acid residues present in the single chain of human CT peptide, which has its molecular mass as 3418 Da. The cysteines are linked through the disulfide bridge, which connects them from positions 1 to 7 to form a ring structure that includes seven amino acids located at the amino terminus. Moreover, the CT precursor, pre-procalcitonin (PreProCT), withholds 141 amino acids (Fig. 15.8).

The stem cells from neural crest as well as the rostral are transported into the ultimo-branchial glands of lower vertebrate animals and into the para-follicular cells in humans. The C cells found in the ultimo-branchial glands of lower vertebrates and in the thyroid gland of mammals produce calcitonin (CT) and release them into circulation, which works in a similar manner to other hormones in the body. Through this advance movement, it is assumed that concentration of C cells is required in ultimo-branchial body and thyroid gland (Johansson et al. 2015). However, it is often noticed that some C cells might not be able to make their way forward toward the thyroid and end up in the extra-thyroidal tissues during their forward movement (Giovanella et al. 2013). Calcitonin is considered as endogenous controller of calcium homeostasis and protects the skeleton especially in "calcium stresses" by acting primarily on the bone. Calcitonin also acts directly on gastrointestinal and kidney secretion activity by creating an indirect impact on the central nervous system (CNS) to lessen the pain. Studies have found that the role of calcitonin is not limited

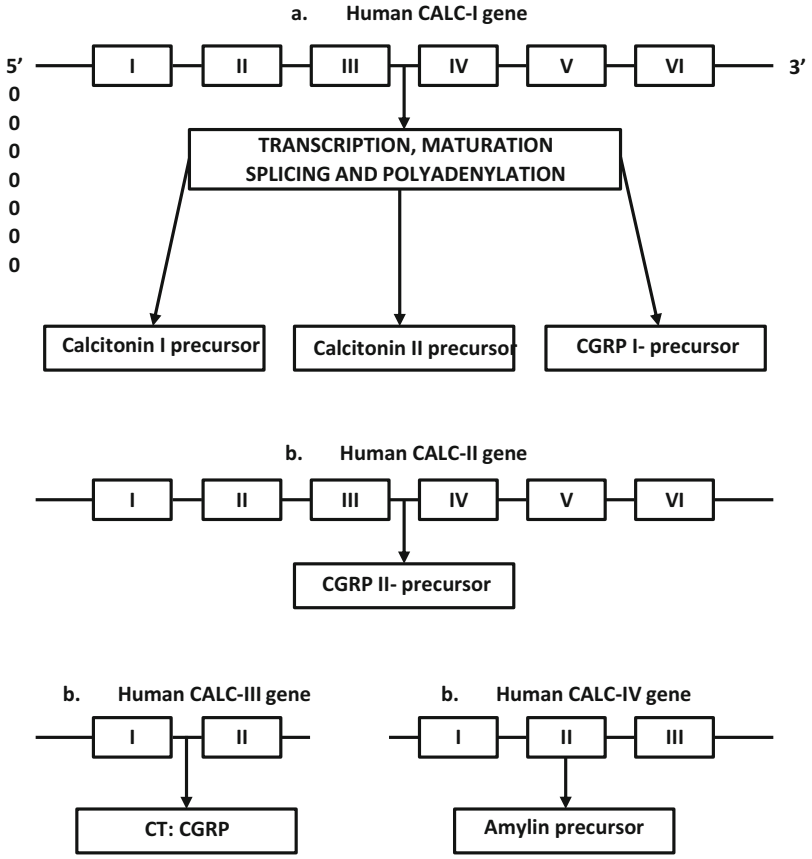

Fig. 15.8 Human calcitonin gene family (Source: Wimalawansa 2010)

to intrinsic analgesic effects on CNS and thus creates a significant impact over various neuronal activities, like moderating them directly on their existing location or indirectly moderating them by a process which is yet to be explained on other locations. Presently, the main indications for using calcitonin in therapies are disorders, which include high-bone turnover osteoporosis, acute pancreatitis, osteoporosis linked to pain or metastases of bone, hypercalcemia, Sudeck's atrophy, and Paget's disease (osteitis deformans). Many kinds of calcitonin are commonly consumed such as synthetic human calcitonin (SCT), analog (Elcatonin), synthetic salmon calcitonin (SCT) also known as salcatonin, natural porcine calcitonin (PCT), and synthetic eel calcitonin (ECT). One of the problems with its treatment is that the only imaginable way for its administration is through an injection, although other ways for its dosage are being explored.

15.8.1 Structure of Calcitonin

During the evolution, calcitonin has been well preserved. Calcitonin from 9 distinctive species has been recognized with the identification of 12 different sequences. The remains of six unchangeable amino acids are found at the amino terminal, while two others are obtained through the end of the carboxyl terminal of the peptide molecule.

Moreover, at C-terminal, all the calcitonins have 1–7 disulfide bridge and proline amide. For the osteoclast inhibitory functions and for hypocalcemic bioactivity, all the 32 amino acids are needed. The substitution of certain amino acids during the synchronization of synthetic calcitonin (like synthetic eel-CT) helps in increasing their lifetime to a certain extent by struggling against degradation, hence improving their circulatory half-life and its biological function. The method involves certain risks as it may result in allergenicity. Calcitonin is prepared as a precursor molecule. Before the release of the fully developed form of biologically active calcitonin, various C-terminal amidation and post-translational changes, such as cleavage, take place. Glucagons, theophylline, cholecystokinin, dibutyryl cyclic AMP, gastrin, and cations like Mg^{2+} and Ca^{2+} accelerate the secretion of calcitonin from C cells. The impact of calcitonin over osteoclasts can be represented through dibutyryl cyclic AMP. However, the dose dependency to eliminate the cyclic AMP is integrated through calcitonin.

The quantification of plasma i-CT levels (i.e., for hypertension) is being utilized for diagnostic and inspection tests for C cell hyperplasia for the diagnosis of pre-malignant and malignant disorders that are related to C cell (i.e., patients' families suffering through medullary thyroid carcinoma (MCT)) (Wimalawansa 2010). Currently, there is the availability of various diagnostic-stimulation tests for the diagnosis of MTC like an infusion of pentagastrin and injecting calcium (Fig. 15.9).

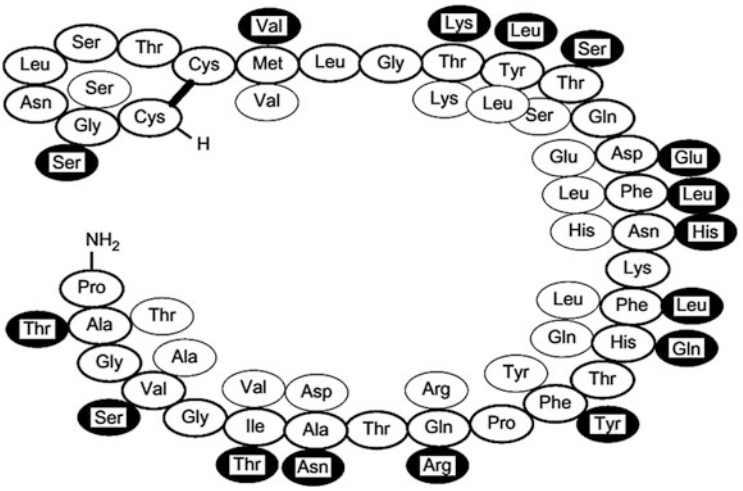

Fig. 15.9 Human calcitonin amino acid sequence (Source: Wimalawansa 2010)

15.8.2 Mechanisms of the Physiological Action of Calcitonin

The calcitonin physiological concentration is expected to have an energizer effect to limit the resorption of osteoclastic bone. Physiologically, the function of calcitonin is to sustain skeletal form throughout the time of calcium stresses like pregnancy, lactation, and growth (i.e., when the skeleton is required to be sustained). During lactation and pregnancy, the calcium is released into the milk, and the retention of calcium by the fetus happens at the cost of prolactin and the skeleton of the mother (i.e., activation through parathyroid hormone-related protein (PTHrP)). Therefore, in these conditions, it is mandatory to provide solutions that may act against the regulatory impact to secure the mineral contents of maternal skeletal. The release of calcitonin is expected to have an impact by regulating unnecessary resorption of bone. Estrogen and testosterone, which are sex steroid hormones, activate calcitonin production by C cells.

Though the calcitonin's primary function is to regulate calcium homeostasis, which acts mainly on bones, it also has a direct effect on the gastrointestinal tract and kidneys. The receptors of calcitonin are abundantly present in osteoclasts. Other than this, the brain, kidney, and hypothalamus are other sources of these receptors. Calcitonin is further evident in creating both direct and indirect impact on CNS and is involved in regulating the neuromodulatory functions and pain. However, the osteoclast serves as the basic cell that is targeted for calcitonin with more than a million calcitonin receptors. The osteoclast withdraws its pseudopodia under just a few minutes of the organization of calcitonin, and as a result, it diminishes the resorption function of bone, cell size, and motility. The cells become steady for hours and stop bone resorption. Throughout bone excavation, the osteoclasts release acids (HCl) and enzymes (like acid phosphatases and metalloproteinases), which later leads to several pits in the bone matrix through hydrolysis. The information thus revealed that physiological integration of plasma calcitonin is significant in performing functions related to osteoclast (Fig. 15.10).

15.8.3 Role of Calcitonin in the Pathogenesis of Disease

Calcitonin has been observed to play a significant role in the treatment of pathogenic diseases. The effectiveness of calcitonin is well-established for providing treatment to patients who are suffering from Paget disease. The raised Pagetic bone turnover indices are decreased when the calcitonin is administered along with the subcutaneous injection of about 50% (Pondel 2000).

The symptoms related to the Paget disease are also reduced with the use of CT which encompass disease comprising pain in the bones and complications related to the neurology comprising nerve root compression, headache, and spinal stenosis. Along with it, osteoporosis is characterized as the systemic skeletal disease which comprises low bone mass as well as bone tissue deterioration which result in fragility in the bone along with fracture susceptibility. The decrease in the levels of estrogen which takes place at menopause results in increase resorption of the osteoclastic

	Man	Rat	S-1	S-2	S-3	Eel	*Chiek	Bov	Pore	Ovi	*Man[2]
1	Cys	-	-	-	-	-	-	-	-	-	Tyr
2	Gly	-	Ser	Ser	Ser	Ser	Ala	Ser	Ser	Ser	Ser
3	Asn	-	-	-	-	-	Ser	-	-	-	-
4	Leu	-	-	-	-	-	-	-	-	-	-
5	Ser	-	-	-	-	-	-	-	-	-	-
6	Thr	-	-	-	-	-	-	-	-	-	-
7	Cys	-	-	-	-	-	-	-	-	-	-
8	Met	-	Val	-	Val	Val	Val	Val	Val	Val	Leu
9	Leu	-	-	-	-	-	-	-	-	-	Gln
10	Gly	-	-	-	-	-	-	Ser	Ser	Ser	-
11	Thr	-	Lys	Lys	Lys	Lys	Lys	Ala	Ala	Ala	-
12	Tyr	-	Leu	Leu	Leu	Leu	Leu	-	-	-	-
13	Thr	-	Ser	Ser	Ser	Ser	Ser	Trp	Trp	Trp	Leu
14	Gln	-	-	-	-	-	-	Lys	Arg	Lys	-
15	Asp	-	Glu	-	-	Glu	Glu	-	Asn	-	Tyr
16	Phe	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu	Leu
17	Asn	-	His	His	His	His	His	-	-	-	Lys
18	Lys	-	-	-	-	-	-	Asn	Asn	Asn	Asn
19	Phe	-	Leu	Leu	Leu	Leu	Leu	Tyr	-	Tyr	-
20	His	-	Gln	Gln	Gln	Gln	Gln	-	-	-	-
21	Thr	-	-	-	-	-	-	Arg	Arg	Arg	Met
22	Phe	-	Tyr	-	-	Tyr	Tyr	-	-	Tyr	-
23	Pro	-	-	-	-	-	-	Ser	Ser	Ser	-
24	Gln	-	Arg	Arg	Arg	Arg	Arg	Gly	Gly	Gly	Gly
25	Thr	-	-	-	-	-	-	Met	Met	Met	Ile
26	Ala	Ser	Asn	Asn	Asn	Asp	Asp	Gly	Gly	Gly	Asn
27	Ile	-	Thr	Thr	Thr	Val	Val	Phe	Phe	Phe	Phe
28	Gly	-	-	-	-	-	-	-	-	-	-
29	Val	-	Ser	Ala	Ala	Ala	Ala	Pro	Pro	Pro	Pro
30	Gly	-	-	-	-	-	Glu	Glu	Glu	Glu	Gln
31	Ala	-	Thr	Val	Val	Thr	Thr	Thr	Thr	Thr	Ile
32	Pro	-	-	-	-	-	-	-	-	-	-

Fig. 15.10 Predicted calcitonin sequences of amino acid

bone, up surging the overall loss of the skeletal bone mass. In addition, CT is also recognized as the steady inhibitor related to the osteoclast-mediated bone resorption which makes it effective to be used in the therapeutic treatment related to

osteoporosis. The osteoclastic bone resorption which is inhibited by the calcitonin reduces serum calcium (antagonized parathyroid hormone).

15.8.4 Pharmacology of Calcitonin

The inhibition of bone resorption which emerges as a result of calcitonin causes a direct inhibitory influence on the osteoclast. The use of calcitonin results in the mitigation of the osteoclast number, when it is provided for some months. Though the mitigation of the osteoclast number is not apparent, it occurs as a result of the influence of calcitonin on osteoclasts and has an independent influence on associated precursor cells. The consequence of this effect is observed to be crucial for treating various diseases related to bone metabolism. The impact of calcitonin regarding the lowering of calcium is not observed in the average individual due to its overall slow bone turnover rate. Thus, calcitonin administration causes either minor or no influence on the plasma calcium present in an average individual.

Though when bone turnover is high such as in children, or in various disease conditions, i.e., hypercalcemic statuses, Paget's disease, and more, following calcitonin regulation, decrease in calcium level in the circulatory system can occur. Moreover, the pharmacological activities of calcitonin are also reported for the gastrointestinal (GI) system, where it leads to increase in intestinal secretion of sodium, water, and chloride as well as inhibition of acid secretion and gastric emptying. Furthermore, it is also linked with the secretion of various gastrointestinal regulatory peptides, encompassing insulin, motilin, pancreatic glucagons, pancreatic polypeptide, gastrin, and possibly gastric inhibitory peptide. Moreover, the secretion of pituitary hormones is also inhibited by calcitonin, which constitutes growth hormones, thyroid-stimulating hormone, as well as luteinizing hormone.

15.8.5 Calcitonin Receptors

The CT activities are regulated with the use of calcitonin receptors which possess high affinity. The calcitonin receptors are a member of G protein peptides of seven transmembranes (Masi and Brandi 2007). All the members of the family have identical structure along with seven other transmembranes spanning domain G protein-coupled receptors. The cloning of porcine CTR (pCTR) cDNA was initially done in the year 1991 by Lin et al. (1991). The characterization of the receptor is based on its constituent of the extracellular long NH_2-terminal domain. It shares similarity with the parathyroid hormone which is linked with peptide receptor as well as secretin receptor. Later, pCTR gene cloning showed that its length is about 70 kb which also contains a minimum of 14 axons, in which 12 axons were inclusive of protein. The cloning of the human CTR was done from ovarian cancer cell line, i.e., BIN-67. The different isoforms of CTR are the outcome of the gene splicing change which has been indicated in various species of animals comprising divergent transcripts of tissue expression as well as divergent signaling properties. Concerning

the large tumor cell of the bone, two kinds of isoforms have been explained, where the first comprises similar design like GC-10, which is divergent from the earlier explained human ovarian cancer CTR gene in the 5′ region as it is devoid of the 71-bp segment while being almost similar to the 3′ region. However, the second is the CTR cDNA variant of the large human tumor cell, which is highlighted as GC-2, devoid of the 71-bp 5′ insert, though it comprises 48 encoded nucleotides part in the first intercellular domain. The difference in the expression of CTR isoforms may be the result of the biological regulatory mechanism which provides responses to the calcitonin. The significant shift in the CTR isoforms can be explained as the responsiveness of the variable related to calcitonin of the patients who have increased turnover of the metabolic bone ailment. Moreover, the outlying of the CTR gene is based on the chromosome 7q21.3.

15.8.6 Calcitonin: Therapeutics

With the several bone disorders, patients are being treated with calcitonin for the last four decades, characterized on the basis of people that have high bone resorption issues. The therapeutic function of calcitonin can be indicated through the patients with disorders such as hypercalcemic states, i.e., bony metastases, Paget's disease, pain in osteoporotic fractures, toxicity of vitamin D, Sudeck's atrophy, and osteoporosis with high bone turnover. Until a few years ago, injection was the only possible means of management. On the contrary, dosage forms are being produced, and a calcitonin nasal spray is now commercially available using other routes (Wimalawansa 2010). It seems difficult to administer calcitonin orally as it is a peptide. However, in line with research studies into several delivery systems as well as the increased improvisation of calcitonin molecule, it is highly probable to administer the analogs of calcitonin orally. The process of oral administration can be integrated through the buccal mucosal route. Bone pain that includes vertebral fractures/osteolysis because of neoplasms is observed to be an indication for calcitonin therapy. Calcitonin seems to be successful for patients with Sudeck's atrophy and algoneurodystrophy. These are the syndromes caused by several factors such as reflex dystrophy, post-traumatic osteoporosis, and iatrogenic neuropathy. Moreover, apart from the developed uses of calcitonin, the advantageous outcomes of using calcitonin can also be observed, especially in the prevention of osteoporosis. In case of osteoporosis that is associated with pregnancy, immobilization, and increased bone catabolism, osteogenesis imperfecta, controlling loss of bone while administering prednisone and heparin and severe renal insufficiency and excessive osteoclastic activity is associated with these mentioned disorders. Calcitonin is observed to be an additive therapy, although the growth of bone mass should be increased when it comes to the ideal treatment for osteoporosis. Calcitonin and bisphosphonates are important in controlling bone loss. In various osteoporosis patients, albeit, that type of calcitonin serves as a less functional agent that is used to provide stability to bone mass. In different types of osteoporosis, it seems to have the greatest advantages when enhanced resorption is considered to be a feature. It has

also been employed for post-menopausal osteoporosis. For effective prevention of bone loss, it has been observed that calcitonin dose of 50 IU twice a week is really effective. Moreover, the hormone replacement therapy (HRT) along with calcitonin seems to be effective. Calcitonin is highly efficient in preventing bone loss. However, its effectiveness in reducing osteoporosis seems to be less than bisphosphonate.

Calcitonin has been used in the treatment of complex issues related to Paget's disease of the bone over the last 40 years. The osteoclastic activities are important to provide the moderate effects of calcitonin, whereas the long-term response can be seen with the decrease of osteoclasts. A symptomatic response can be observed in patients having Paget's disease under 1–2 weeks' therapy, and the response could be increased after 12 weeks. Until maximum symptomatic relief, the therapy seems to be continued till at least the next 6 months. Calcitonin can be useful (e.g., a dose of 100 IU that is provided through injections) for patients with radiological evidence of an osteolytic lesion. It is only when osteolytic lesion can be seen in the bone that it becomes a burden due to extensive body weight which may result in fracture in the future. Calcitonin along with intravenously administered bisphosphonate will accelerate the process of recovery. Patients having skeletal metastatic disease can also be treated with calcitonin that is secondary to malignancy. s-CT works as an analgesic when calcitonin is injected in the subarachnoid space. It seems the main location for the action is within the CNS, whereas the mechanism is still not understood when it is analgesic. By using a number of mechanisms, calcitonin and its analgesic effects have been suggested. A direct, as well as indirect, action can be observed by the interference with the neurotransmitters that include prostaglandin, serotonin, and peripheral action. All these can be mediated through hindrance of inflammatory cytokines as well as chemical factors. There is an association between bone pain and hormone-sensitive tumors that reveals the great response along with some lung tumors that secrete ectopic hormones. These sensitive tumors include breast, thyroid, and prostate. Moreover, a possibility can be seen for the effects of calcitonin on the release of β-endorphins. Calcitonin can also be combined with calcitonin gene-related peptide receptors (CGRP) found in the hypothalamus of CNS. Suggestions are given when CGRP along with its receptors are distributed in the dorsal view of the spinal cord and brain. The suggestions include the involvement of processes of pain sensation. In several studies, calcitonin has been shown to interact with CGRP and the associated sites in the kidney and nervous system that involved the hypothalamus. Therefore, in cases where the calcitonin doses are required, CGRP neuromodulatory receptors serve as highly functional in CNS. This happens specifically in the hypothalamic region that alters the sensory neurotransmission.

15.9 Conclusion

Neuropeptide proteolytic processing contributes significantly in terms of its regulatory function along with the resulting fragment peptide which emerges due to the degradation of the enzyme exerting vital physiological roles. The generation of proteolytic processing is not limited to the biologically inactive fragments but also

expands to those fragments which modulate and at times counteract the parent peptide response. Generally, the integration of the peptide fragments is frequent for those receptors which are unidentified by the parent peptides. This chapter explains the concepts related to the tachykinins, VIP-glucagon family, neuropeptide Y, and calcitonin peptides which are present with the bioactive degradation processes. The chapter demonstrates their related mechanisms of physiological action, pathogenesis of the disease, pharmacology, receptors, and therapeutics. The chapter provides insight that these neuropeptides can further be explored for their utilization in drugs to improve the efficiency of the drugs as well as drug-like development of the substance, which can serve as a rich source of development of new pharmaceuticals.

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Neuropeptides and Neurotransmission

16

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Abstract

Over the past four decades, many neuropeptides, that is, 3–100 amino-acid-long polypeptides, have been identified in the central nervous system and the peripheral nervous system which can act on either neural substrates such as neurons and glial cells or other target cells. Neuropeptides mediate neuronal communication by acting on neuropeptide receptors. Neuropeptide receptors include over 44 receptor families, of which most are G protein-coupled receptors. Neuropeptides and their cognate receptors are involved in various physiological and pathophysiological functions, such as pain regulation, blood pressure, body

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temperature, feeding behavior, reproduction, sleep, learning and memory. Therefore, neuropeptide transmission is an attractive area for drug discovery in several therapeutic areas, including inflammatory conditions, epilepsy and psychiatric diseases targeting neuropeptide receptors. This chapter elaborates and expounds on the role of various small neuropeptides such as substance P (SP), vasoactive intestinal peptides (VIP), neuropeptide Y (NPY) and endogenous opioid neuropeptides, along with their physiology, pathophysiology and pharmacology. This chapter also describes the various neuropeptide receptor agonists and antagonists and their possible roles in the treatment of various disorders.

Keywords

Neuropeptide · VIP · Substance P · NPY · Endorphin · Enkephalin · Dynorphin

Abbreviations

5-HT	Serotonin
ACE	Angiotensin-converting enzyme
Ach	Acetylcholine
ACTH	Adrenocorticotrophic hormone
a-MSH	Melanocyte-stimulating hormone
CAMP	Cyclic Adenosine Monophosphat
CGRPs	Calcitonin gene-related peptides
CINV	Chemotherapy induced nausea and vomiting
CNS	Central Nervous System
DAG	Diacylglycerol
DOR	δ opioid receptor
EPI	Epinephrine
GABA	Gamma amino butyric acid
GIP	GPCR-interacting proteins
GLP-1	Glucagon-like peptide-1
GPCR	G protein-coupled receptors
HA	Histamine
IBD	Inflammatory bowel disease
IP_3	Inositol triphosphate
KOR	κ opioid receptor
MAPK	Mitogen-activated protein kinase
MEC	Moderately emetogenic chemotherapy
MOR	μ-opioid receptors
NE	Noradrenaline
NK	Neurokinin
NKA	Neurokinin A

NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
PACAP	Pituitary adenylate cyclase-activating polypeptide
PC2	Pro-protein convertase-2
PENK-A	Proenkephalin-A
PHM	Peptide histidine methionine
POMC	Precursor proopiomelanocortin
PP	Pancreatic polypeptide
PPT-A	PreprotachykininA
PYY	Peptide YY
RAMPs	Receptor activity modifying proteins
SP	Substance P
VIP	Vasoactive intestinal peptides

16.1 Introduction

Research on the underlying cellular and molecular mechanisms involved in various activities of the brain composes a leading area in neuroscience that started long before (Fan and Markram 2019). Neuropeptides are small protein-like molecules that act as neuronal signaling molecules (Burbach 2011). These help the neurons to communicate with one another and regulate various types of biological actions and mediate many regulatory functions involving all organ systems, particularly brain function such as food intake, social behavior, memory and learning, reproduction and analgesia (Russo 2017). They are responsible for cellular signaling in the CNS, PNS and the endocrine system (Catalani et al. 2017). Interestingly, humans have a broad collection of neuropeptides that can influence a multitude of activities. To date, there are over 100 neuropeptides that have been identified and it is predicted that many more are present that are yet to be identified from the over 1000 predicted peptides encoded by the genome (Russo 2017). The neurotransmitters are involved in the transmission of electrical stimuli between nerve cells and mediate neuron-to-neuron communication transmission. Neuropeptides and neurotransmitters are quite different when monitoring their mode of communication in the nervous system. Neurotransmitters are generally smaller than neuropeptides. Interestingly, neurotransmitters may be a single amino acid, for example L-Glu, NMDA and gamma aminobutyric acid (GABA), or any other molecules with small size such as noradrenaline (NE), epinephrine (EPI), serotonin (5-HT), acetylcholine (ACh) or histamine (HA). A signaling molecule is called a neuropeptide when it is compiled with more than three amino acids (Purves et al. 2001). Thus the present chapter describes the general overview of various small neuropeptides such as substance P (SP), vasoactive intestinal peptides (VIP), neuropeptide Y (NPY) and other neuropeptides and their related physiology, pathophysiology and pharmacology (Fieber 2017).

16.2 Neuropeptides and Neurotransmission

Neurotransmitters are endogenous substances that are released from the neuron. These molecules are responsible for the transmission of information on chemical synapses. Neurotransmitters are released from the presynaptic axonal membrane to the synaptic cleft and bind to the receptors present in the postsynaptic membrane of other neurons and conduct signal transfer across the synapse. A molecule can be considered a neurotransmitter if it fulfills the following criteria. It must present in the vesicles along with the suitable enzymes that help in their synthesis and breakdown. The substance should be released from the nerve ending in a chemically or pharmacologically identifiable form as an effect of presynaptic nerve stimulation. They must have action in the synapse and its actions must be prevented by receptor blockers. Thus the neurotransmitters are chemicals that are released from the neuron on nerve stimulation by generation of action potential and communicate with the muscle, other organs and other neurons (Purves et al. 2001; González-Espinosa and Guzmán-Mejía 2014; Fieber 2017).

The process of neurotransmission takes place in the synaptic cleft of the nervous system composed of the basic cells called neurons. Neurons, in spite of having unimaginable variation in their structure and functions, possess many common features among them, and they do the work of cellular communication (Patri 2019; Lodish et al. 2000). The communication between two neurons starts when electrical impulse or action potential is carried in only one direction by the long slender projection called the axon (Hormuzdi et al. 2004). The action potential reaches the axon terminal but cannot cross the synaptic space and thus triggers the neurotransmitter release from their storage vesicles present in synaptic terminals (presynaptic membrane) into a space known as the synapse. The neurotransmitters then bind with special proteins called the receptors present in the postsynaptic membrane of another neuron, which triggers the action potential to move toward the cell body to the axon of the postsynaptic neuron (Lodish et al. 2000). The neurotransmitters then release a related message from the receptor into the synaptic space. Some of the neurotransmitters degraded by the local enzymes are taken back by the transporter proteins present in the presynaptic membrane (Lodish et al. 2000; Forehand 2009). The neurotransmitters that are taken back are repackaged into a vesicle which is released again when an action potential reaches the axon terminal. The entire process is repeated when an action potential reaches the axon terminal (Forehand 2009).

16.3 Substance P

Substance P (SP), alongside Neurokinin A (NKA) and Neurokinin B (NKB) and others, is a member of the tachykinin family, which is known to be one of the largest neuropeptide families. It has marked biological activity in different systems across species. It was originally identified in the early years of the twentieth century in the course of investigating the tissue distribution of acetylcholine by researchers experimenting on a rabbit's isolated intestine, though the substance was initially

extracted from equine horse brain and gut tissues (V Euler and Gaddum 1931). This substance was later named Substance P, P as in the powder obtained during the procedure. Substance P is an undecapeptide, composed of a chain of 11 amino acid residues (Arg-Pro-Lys-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2). It shares the same carboxyl terminal sequence, Phe-(Phe or Val)-Gly-Leu-Met-NH_2, with the rest of the tachykinin family members; however, it has a net positive charge. The N-terminus holds the positively charged residues, while the C-terminus is confined to hydrophobic residues, rendering it an amphiphilic peptide. The biological actions are mediated by neurokinin (NK) G protein-coupled receptors acting on smooth muscle contraction, vasodilation, nociception and modulation of inflammatory responses (Mashaghi et al. 2016a, b).

16.3.1 Physiology

16.3.1.1 Biosynthesis and Metabolism

Substance P is encoded by the TAC1 gene, which is found in the central nervous system, peripheral afferent sensory neurons, cardiovascular system and other body parts. The gene expresses four mRNA isoforms (α, β, γ and δ), α being more abundant in the brain and β and γ in peripheral tissues, all generating substance P (Dehlin and Levick 2014). The peptide precursor was designated as preprotachykinin A (PPT-A), which in turn is cleavaged by the actions of proteases. The C-terminus undergoes amidation executed by the enzyme peptidyl-Gly-α-amidatingmonoxygenase, using glycine as an amide donor. Produced peptide fragments are then bound by a pair of basic amino acids (Lys-Arg) on both sides. The synthesis occurs in ribosomes and is then packed into large dense-core storage vesicles and transported to nerve endings for enzymatic processing and converted into the active form and stored in vesicles ready for release (Nakanishi 1987; Steinhoff et al. 2014). A number of enzymes are responsible for the metabolism of substance P, including substance-P-degrading enzyme, angiotensin-converting enzyme (ACE) and endopeptidases (Matsas et al. 1984), among many others.

16.3.1.2 Action on Target Tissues

Neurokinin (NK) receptors belong to the class I rhodopsin-like G protein-coupled receptors family, consisting of seven hydrophobic transmembrane domains, connected by extracellular and intracellular loops and coupled to G proteins. Substance P exerts its effects as a neurotransmitter and neuromodulator principally by subsequent activation of a second messenger system. It binds majorly to the NK1 receptor, which is most abundant in body tissues. However, it also activates the NK2 and NK3 receptors in certain conditions, that is, high peptide concentration and receptor availability. The peptide has two distinguishable binding sites: one part is inserted in to the hydrophobic ligand binding site, while the remainder interacts with amino acid residues on the extracellular sites of the receptor. C-terminal of the receptor holds threonine and serine amino acids, which are sites of phosphorylation

of the GPCR. This results in the activation of phospholipase C which catalyzes the hydrolysis of phosphatidylinositol and the production of inositol triphosphate (IP_3) and diacylglycerol (DAG), elevating cytosolic calcium concentration. Then phospholipase A induces an increase in arachidonic acid movement while adenylyl cyclase invokes cAMP accumulation. The rise in the intracellular calcium concentration then activates a calcium-dependent chloride channel that leads to cell response. It has been proposed that SP can employ negative feedback via activation of autoreceptors. This finding is particularly substantial concerning inflammation and nociception, and the possibility of designing a drug affecting its functions (Takeda et al. 1992; Malcangio and Bowery 1999; Yin et al. 2018).

16.3.2 Pathophysiology

Due to its wide distribution in the body, substance P has numerous physiological implications in the CNS, cardiovascular, respiratory, gastrointestinal systems and modulation of the immune response. Additionally, it plays a critical role in the regulation of the excitability of dorsal horn nociceptive neurons (Christofi 2018). It is associated with the regulation of mood disorders, anxiety, stress, neurotoxicity and pain. In the gastrointestinal tract, SP and other tachykinins act as neurotransmitters that regulate motor activity, secretion of ions and vascular functions. Therefore, the elevation of the neuropeptide or its receptor has been linked to several chronic pathological conditions such as inflammatory disorders (e.g. irritable bowel syndrome) (Sohn et al. 2013), Alzheimer's disease (Severini et al. 2016), Parkinson's disease (Thornton and Vink 2015), and mood and mental disorders. Nerve fibers producing substance P were detected both in atrial and ventricular myocardia, where efferent and afferent sensory functions of the peripheral neurons innervating the heart and coronary arteries are mediated by tachkykinins and calcitonin gene-related peptides (CGRPs). It has been pointed out that substance P possesses binding sites surrounding coronary arteries and cardiac fibers and on mitral and tricuspid valves. It has negative inotropic and chronotropic effects due to an increase in the activity of cholinergic neurons, leading to a weaker heart rate and a change in the force of contractions (Mistrova et al. 2016). SP and its receptors' distribution in the brain was first described in the 1970s. It has been demonstrated that high levels of SP immunoreactivity have been spotted in areas associated with regulation of stress and anxiety reactions, mainly in the hippocampus, amygdala and some areas of the hypothalamus. In these aforementioned regions, the peptide coexists in the same neuron terminal with various neurotransmitters and neuropeptides such as glutamate, GABA, histamine, acetylcholine, dopamine and serotonin (Ebner and Singewald 2006).

Substance P initiates the expression of various known immunological chemical messengers. Primary lymphoid organs such as the bone marrow and thymus and secondary ones such as the spleen and lymph nodes have been observed to be innervated by neurons containing SP. This suggests that substance P may act as a

mediator crosslinking the nervous and immune systems. Activated T lymphocytes are shown to express PPT-A, thus producing the neuropeptide, which may act in an autocrine fashion to regulate T cell proliferation (Mashaghi et al. 2016a, b). It has been demonstrated that substance P contributes to pain transmission and neurogenic inflammation, both mediated through the NK1 receptor. Pain-transmitting receptors are present in the dorsal horn of the spinal cord. SP is released from the peripheral terminals of the primary afferent nerves after peripheral nerve injury, contributing to the induction of neuropathic pain.

16.3.3 Pharmacology of Substance P

Different organs and multiple neurotransmitters are involved in the response to emetic triggers. Afferent nerve impulses transmitting from the cerebral cortex, chemoreceptor trigger zone and vagal afferent fibers of the gastrointestinal (GI) tract travel to the vomiting center, which is located in the medulla oblongata of the nervous system. Efferent impulses then travel from the vomiting center to the abdominal muscles, salivation center and respiratory center, causing vomiting. Other areas of the CNS are also involved, such as the limbic and vestibular systems due to the states of vertigo, motion sickness and pain. Predominant neurotransmitter and neuropeptide receptors involved in this kind of signaling include serotonin (5-HT3) receptors, neurokinin-1 (NK-1) receptors, histamine and dopamine receptors. Available and effective antiemetic agents target these receptors, usually in combination, targeting more than one receptor at once. Chemotherapeutic agents and anesthetics stimulate the release of these neurotransmitters and induce nausea and vomiting. Chemotherapy induced nausea and vomiting (CINV) has five categories. Acute-onset CINV can be triggered a few hours (within 24 h) after chemotherapy initiation. Delayed CINV occurs after the acute phase after >24 h (peak in 2–3 days, and can last up to 1 week). Some CINV patients develop 'anticipatory nausea and vomiting', occurring prior to administration of a chemotherapeutic agent and attributed to the adverse memory of prior CINV. Breakthrough emesis is a type of vomiting that occurs within 5 days after the prophylactic or rescue use of an antiemetic agent. Refractory emesis is defined as vomiting which occurs after chemotherapy administration in subsequent chemotherapy sessions where antiemetic prophylaxis has failed in earlier sessions. Age, sex, dose and emetogenicity are risk factors for developing CINV; for example, females and younger patients are at a higher risk, and a higher chemotherapy dose is also a relevant factor. Neurokinin-1 (NK-1) receptor antagonists represent the newest class of antiemetic agents that are effective for the prevention of chemotherapy-induced nausea and vomiting (Aapro et al. 2016; Grunberg et al. 2011; Hesketh 2008) (Fig. 16.1).

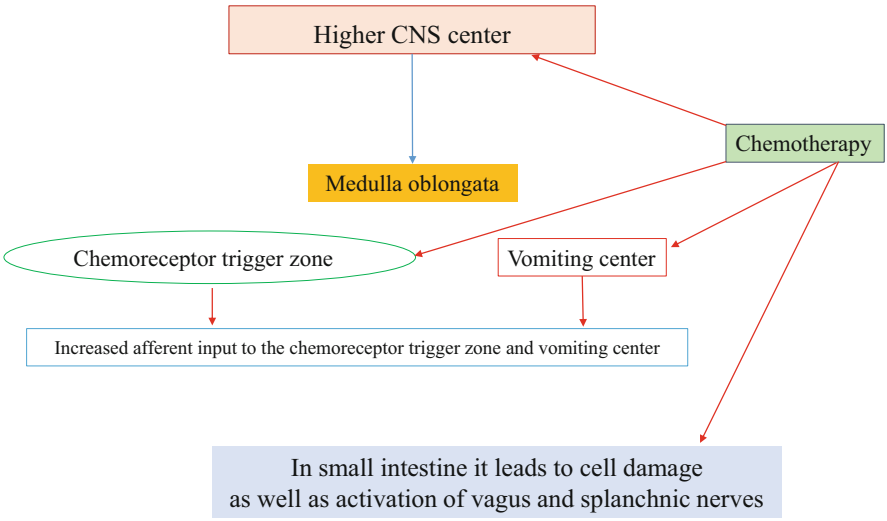


Fig. 16.1 Proposed pathway of chemotherapy-induced emesis

16.3.4 Neurokinin-1 Receptor Antagonists

16.3.4.1 Aprepitant/Fosaprepitant

Pharmacodynamics and Indications

Aprepitant is a highly selective and potent NK-1 receptor blocker, thus alleviating the emetic effects of substance P. It has a low affinity for NK-2 and NK-3 receptors and little or negligible affinity for 5-HT3 and dopamine receptors. Fosaprepitant is a phosphoryl prodrug of aprepitant, is water-soluble and is converted to its active form 30 min after intravenous administration via phosphatases. Aprepitant crosses the blood brain barrier (BBB) and occupies NK-1 receptors, thereby antagonizing the effects of substance P in the CNS and periphery. The drug has been approved for the prevention of nausea and vomiting in patients receiving moderately emetogenic chemotherapy (MEC) and highly emetogenic chemotherapy (HEC), for example cisplatin-containing, regimens. There have been significantly higher rates of response when given in combination with a 5-HT3 receptor antagonist (e.g. ondansetron) in addition to a corticosteroid (e.g. dexamethasone) for delayed-onset CINV (Zhang 2019; Aapro et al. 2016) (Fig. 16.2).

16.3.4.2 Pharmacokinetics

Administration and Dosing

Aprepitant is administered orally and intravenously, while fosaprepitant is available only intravenously. It is commercially available under the trade name Emend®, which contains a nanoparticle form of the drug with a diameter below 200 nm and

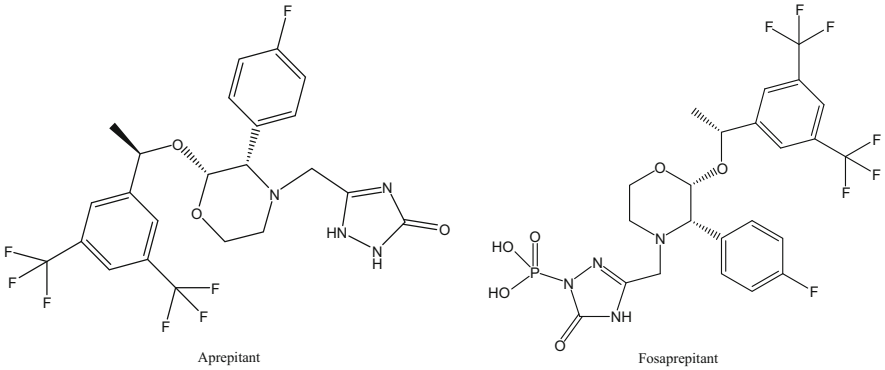


Fig. 16.2 Structures of aprepitant and fosaprepitant

is coated and encapsulated. A dose of 125 mg of aprepitant is given on the first day, followed by 80 mg once daily. Onset of action is 1 h when administered orally, with peak plasma level at 4 h. Aprepitant has a bioavailability of 60–70% after oral administration, indicating a low liver first pass extraction.

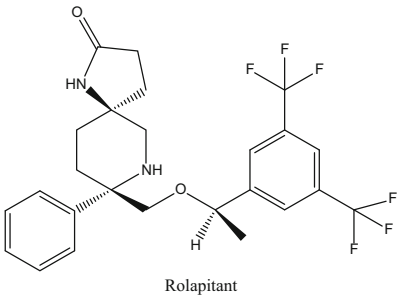
Metabolism and Excretion

Metabolism is mainly mediated by cytochrome 450 enzymes, especially CYP3A4, and less by CYP1A2 and CYP2C9. Therefore, aprepitant can reduce the plasma concentrations of drugs metabolized by these isoenzymes. The apparent biological half-life ($t_{1/2}$) is 9–13 h, with a total clearance of 60–85 ml/min (Roos et al. 2017).

16.3.5 Rolapitant

16.3.5.1 Pharmacodynamics and Indications

Rolapitant is a novel, orally active, selective, high-affinity, competitive NK-1 receptor antagonist, with a prolonged half-life of approximately 180 h. One advantage of rolapitant over aprepitant and other antiemetic agents is that it does not interact with (inhibit or induce) CYP3A4, which is involved in the metabolism of numerous drugs. Therefore, rolapitant is unlikely to interact with drugs metabolized through this enzyme. However, the drug has shown moderate activity on CYP2D6, acting as a reversible inhibitor (Wang et al. 2018). Rolapitant was globally approved for use in 2015 in combination with other antiemetics, for delayed-onset emesis associated with initial or repeat courses of emetogenic cancer chemotherapy. It is usually administered in combination with a 5-HT3 antagonist and a corticosteroid (Rapoport et al. 2016; Syed 2015).



Rolapitant

Fig. 16.3 Structure of rolapitant

16.3.5.2 Pharmacokinetics

Administration and Dosing

Rolapitant is well absorbed from the gut when taken orally, with or without the presence of food. The recommended dose of the drug is 180 mg, taken about 1–2 h before initiating chemotherapy. It can be detected in the plasma 30 min after oral intake. Rolapitant has an extended $t_{1/2}$, reaching 160–180 h, which is longer than the half-life of other NK-1 antagonists. Therefore, a single dose of the drug is sufficient to prevent the risk of developing delayed-onset phase nausea and vomiting and also improves the patient's compliance with treatment.

Metabolism and Excretion

Rolapitant is mainly metabolized by CYP3A4 to form its metabolite M19 (C4-pyrrolidine-hydroxylated rolapitant), and a number of insignificant inactive metabolites. However, in animal studies most of the drug is excreted unmetabolized, mainly by the liver (14% in urine, 73% in feces) over 6 weeks. The pharmacokinetic parameters of rolapitant are not significantly affected in patients with mild or moderate hepatic and renal impairment, and thus dosage adjustments are not required (Glass et al. 2019, Syed 2015, Wang et al. 2018) (Fig. 16.3).

16.4 Vasoactive Intestinal Peptide (VIP)

Vasoactive intestinal peptide (VIP) is the most abundant neuropeptide in the gut, controlling intestinal motility and water and electrolyte secretion. VIP belongs to the glucagon/secretin superfamily; it was isolated from porcine small intestine and shown to have a similar amino acid sequence than glucagon and secretin (Said and Mutt 1970; Mutt and Said 1974). VIP not only functions as a peptide neurotransmitter in the gastrointestinal tract but also in the central and peripheral nervous systems.

16.4.1 Physiology

16.4.1.1 Biosynthesis and Action

VIP contains 28-amino acid neuropeptide, sharing a 68% homology with the PACAP, that is, pituitary adenylate cyclase-activating polypeptide, which is also a neuropeptide (Vu et al. 2015). A common ancestral gene is responsible for the synthesis of both VIP and PACAP. Breakdown of a certain pre-pro-peptide precursor by the actions of peptidases (endoplasmic reticulum peptidase) obtains pro-VIP, which undergoes further post-translational modifications resulting in the production of two peptides, vasoactive intestinal peptide and peptide histidine methionine (PHM). VIP triggers biological responses through VPAC1, VPAC2, PAC1 and PAC2, that is, all are G protein-coupled receptors which are available in the central nervous system, the heart and other tissues. VPAC receptors were cloned in the last decade of the twentieth century, revealing the existence of a new G protein-coupled receptor subfamily named Class B or Class II GPCR. Members of this subfamily share the general structural scheme, having 7-transmembranous helices (TM I to TM VII), interconnected by intracellular and extracellular loops (Couvineau et al. 2013) (Fig. 16.4).

Other family members include glucagon, secretin, CGRP, glucagon-like peptide-1 (GLP-1), growth hormone-releasing factor and several others. Stimulation of VIP receptors subsequently leads to an increase in cytosolic concentrations of cAMP and calcium by coupling with adenylyl cyclase through G proteins. In addition, it has been reported that the neuropeptide also binds to non G protein receptors called 'accessory proteins or GPCR-interacting proteins (GIP)', which play an important role in various functions including control of targeting, trafficking and signaling of

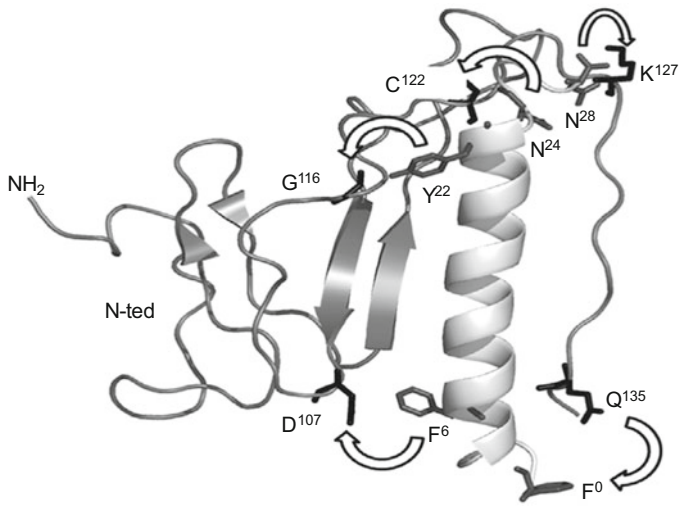


Fig. 16.4 3D structural model of VPAC1 receptor showing different binding pocket for VIP. (Reprinted with permission from Couvineau et al. 2013)

GPCRs (Bockaert et al. 2004). VIP participates in a variety of biological and pathological processes including metabolic processes, smooth muscle relaxation, acting as a cytokine-like peptide, and control of neuronal and endocrine cells, and also plays important roles in some cancers, modulation of immune responses and the circadian rhythm.

16.4.2 Pathophysiology

According to experimentation, certain disease conditions such as hypertension, obesity, diabetes and hypothyroidism are responsible for the reduction of VIP-elicited adenylyl cyclase activity (Said and Rattan 2004 & Said et al. 1996). The role of VIP as a vasodilator and free-radical scavenger in the reduction of myocardial ischemia and reperfusion injury has been established by several preclinical studies (Said 1967). Thus depletion of Myocardial VIP may lead to cardiomyopathy and myocardial fibrosis.

VIP is an important mediator for gut smooth muscle relaxation and also responsible for stimulation of water, electrolyte, enzyme and mucus secretion. It thus increases the propulsion of chyme in the gut (Furness et al. 1995; Lelievre et al. 2007). VIP and its receptors play an important role in the control of ovarian folliculogenesis (Bruno et al. 2011). VIP deficiency plays an important role in the pathogenesis of asthma, pulmonary arterial hypertension and cystic fibrosis (Said 1989; Szema et al. 2006 & Said et al. 2007).

16.4.3 Pharmacology

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) are important structurally related neuropeptides in the peripheral and central nervous systems. VIP and PACAP are involved in many pathophysiological processes related to the digestive tract, cardiovascular system, airways, reproductive system, immune system, endocrine glands and brain (Vaudry and Laburthe 2006; Sherwood et al. 2000). Even though VIP and/or PACAP used to play an important role in the treatment of inflammatory and neurodegenerative diseases, due to their short half-lives, they are no longer used for therapeutic purposes (Delgado et al. 2004; Gomariz et al. 2006; Gozes et al. 1999). PACAP and VIP have quite similar affinity (high affinity) for the classical VIP receptors. There are three different types of receptors for VIP and PACAP which were revealed by receptor cloning in 1990. VIP and PACAP both have high affinity for the two receptors, VPAC1 and VPAC2 (Harmar et al. 1998& Laburthe et al. 2002). Both VPAC1 and VPAC2 are G protein-coupled receptors (GPCRs) which stimulate both by either activation of adenylyl cyclase (AC) through Gs protein or activation of phospholipase C (PLC) through Gq and/or Gi/Go protein. The receptor activity-modifying proteins (RAMPs) determine the balance between coupling to AC versus PLC. These VPAC1 and VPAC2 receptors belong to the class B or Class II family of

Table 16.1 Agonists and antagonists of VPAC1 and VPAC2 receptors

	VPAC1	VPAC2
Agonist	[Ala11,22,28]VIP	Ro 25–1392
Antagonist	PG 97-269	Unavailable
References	Nicole et al. (2000); Gourlet et al. (1997)	Xia et al. (1997)

GPCRs. These class B or class II receptors for peptides bear low sequence homologies with other members of the superfamily of GPCRs. VPAC receptors show several common properties with other class II GPCRs such as a large N-terminal extracellular domain (>120 residues) with 10 highly conserved amino acids including six cysteines and several potential N-glycosylation sites. Secretin, helodermin, GRF and PHM (peptide histidine methionineamide), the other members of the VIP-secretin structural family, can also bind to VPAC1 or VPAC2 receptors with less affinity (Laburthe et al. 2002). Agonists and antagonists of VPAC1 and VPAC2 receptors are described in Table 16.1.

16.5 Neuropeptide Y

The neuropeptide Y family comprises three polypeptides activating four distinct receptors, including neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP). NPY is one of the most expressed neuropeptides in the central and peripheral nervous systems, whereas PYY and PP are considered neuroendocrine hormones. NPY was isolated and identified for the first time from porcine hypothalamus brain tissue in 1982 by using a novel chemical assay that allowed the detection of peptides with amidated C-terminals. Afterwards, human NPY was isolated from adrenal-medullary pheochromocytoma tissue. Neuropeptide Y is a 36-amino acid α-amidated peptide (Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH2) acting on G protein-coupled receptors. All three peptides have 36-amino acid residues and play vital roles in neural and humoral communication between body tissues. It was named neuropeptide Y because it contains many tyrosine (Y) residues in its structure and to distinguish it from PYY that possesses a very similar structure to NPY. The coexistence of NPY with classic neurotransmitters (e.g. GABA and glutamate) has been frequently observed (Tatemoto 1982).

16.5.1 Physiology

16.5.1.1 Biosynthesis and Receptors

Neuropeptide Y is synthesized in the central nervous system and is expressed preferentially in interneurons. Abundant quantities of NPY receptors can be found in numerous regions of the brain. The biological and physiological activities of NPY

are mediated by at least five types of receptors that have been identified, Y1, Y2, Y4, Y5 and Y6; however, Y6 has not been assigned to any biological function. Additional distinguished receptor subtypes exist in other species. The Y receptors belong to the G protein-coupled receptor family, exhibiting its effects through inhibiting cAMP-dependent kinase. NPY plays vital roles in the gut-brain axis communications, by being synthesized in the brain and acting locally in a paracrine fashion in the GIT through its receptors to regulate motility and electrolyte secretion. It thus regulates appetite and metabolism (Cox 2007).

16.5.2 Pathophysiology

The local action of NPY-like peptides after their exocytotic release depends on their concentration, receptor selectivity, the expression of Y receptors and the presence of specific peptidases influencing half-life (von Hörsten et al. 2004). The potential roles of NPY in the etiology and pathophysiology of mood and anxiety disorders, as well as related alcohol use disorders, have been extensively studied. Thus, modulation of NPY-ergic activity within the CNS, via ligands aimed at different receptor subtypes, may be an attractive target for treatment development for affective disorders, as well as for alcohol use disorders (Thorsell and Mathé 2017). NPY has been found to stimulate intake of food, preferably carbohydrate intake. It decreases latency to eat, increases the drive to eat and delays satiety by augmenting meal size (Beck 2006).

The interactions between the immune system, that is, immune cells and gut neurohormones, especially NPY, play an important role in producing inflammation in inflammatory bowel disease (IBD). Thus, NPY may be targeted to diminish the inflammation in IBD (El-Salhy and Hausken 2016). The significance of investigating the underlying pathophysiological mechanisms of arteriosclerotic cardiovascular disease and the role of NPY in this regard is great. It is involved in the pathogenesis of arteriosclerosis via aggravating endothelial dysfunction and growth of vascular smooth muscle cells, by the formation of foam cells and platelet aggregation (Zhu et al. 2016; Sun et al. 2017). NPY, a major peripheral vascular contractive neurotransmitter, interacts with its receptors and is thus associated with the pathology and development of diabetes. It further contributes to diabetes-induced cardiovascular disease by promoting the proliferation of endothelial cells and vascular fibrosis (Sun et al. 2017). The effects of NPY on different species are described in Table 16.2.

16.5.3 Pharmacology

NPY is one of the most effective orexigenic peptides that are mostly found in the brain (Beck 2006; Hofmann et al. 2019). NPY acts via four different types of functional G protein-coupled receptors (GPCRs) known as Y1, Y2, Y4 and Y5 (Table 16.3). All of these NPY receptors act via coupling to Gi and thus by inhibiting the synthesis of cAMP. Y1 is mainly distributed to the brain, that is, anterior

Table 16.2 Effect of NPY on different species

Species	NPY effects on different target	Reference
Human	1. Reduction of vasodilation and plasma Exudation. 2. Reduction of nasal airway resistance and mucus production. 3. Inhibition of cholinergic component airways.	Baraniuk et al. (1992); Lacroix et al. (1996); Fujiwara et al. (1993)
Dog	Vasoconstriction.	Laitinen et al. (1987)
Guinea pig	Inhibition of cholinergic component.	Stretton and Barnes (1988)

Table 16.3 NPY receptors and their possible role

Receptor	Y1	Y2	Y4	Y5
Agonist actions	Angiogenesis, anxiolysis, cellular proliferation, circadian rhythm regulation, endocrine regulation, increase feeding, sedative, vasoconstriction.	Angiogenesis, anticonvulsant, anxiogenesis, cellular proliferation, decrease feeding, gastrointestinal motility and secretion.	Decrease feeding, gastrointestinal regulation.	Anticonvulsant, circadian rhythm regulation, increase feeding.

thalamus, cerebral cortex, medial geniculate and amygdala; peripheral nervous system, that is, superior cervical and dorsal root ganglion; and in peripheral blood vessels, that is, intra-myocardial, colonic and renal blood vessels.

In contrast, the Y2 receptor is distributed in the central nervous system, that is, hypothalamus, lateral septum, hippocampus and amygdala, peripheral nervous system, intestine, certain blood vessels, hypothalamus, lateral septum, hippocampus and amygdala. Y2 agonists administered centrally can increase BP whereas stimulation of the central Y1 receptor decreases BP. NPY shows its effect on angiogenesis and circadian rhythms via Y2. In comparison to Y1 and Y2, Y4 has limited distribution in the brain. However, Y4 is present in skeletal muscle, thyroid gland, heart, stomach, small intestine, adrenal medulla and nasal mucosa. Y5 mRNA receptor is extensively distributed in the CNS and the periphery, and it is present in the intestine, ovary, testes, prostate, spleen, pancreas, kidney, skeletal muscle, liver, placenta and heart. NPY-inhibited LH release and regulation of seizures and brain excitability are mediated via the Y5 receptor (Gehlert 2009).

The effects on feeding are mediated through at least two receptors, the Y1 and Y5 receptors. The NPY system that regulates feeding in dietary-induced obesity is generally located in the hypothalamus (Beck 2006). In arteriosclerotic cardiovascular disease, increased peripheral NPY was found to be involved in the pathophysiological process of atherosclerosis by affecting the vascular endothelial dysfunction, the proliferation of vascular smooth muscle cells, the local inflammatory response of plaques, the formation of foam cells, and activation and aggregation of platelets.

The role of NPY in the manifestation of atherosclerotic cardiovascular disease through the central and/or peripheral nervous system was detected. Increased NPY was related to dyslipidemia, hypertension, obesity, diabetes, impaired glucose tolerance and smoking, which are all risk factors for arteriosclerotic cardiovascular disease (Zhu et al. 2016).

16.6 Other Neuropeptides

16.6.1 Opioid Neuropeptides

The endogenous opioids system includes numerous peptides that are widely distributed throughout the human body, all acting as ligands for opioid receptors including endorphins, enkephalins, dynorphins and, most recently discovered, endomorphins, all of which are biologically active products that are released at the synaptic terminals of opioidergic neurons. Classical opioid receptors are divided into three subtypes, the μ receptor (MOR), δ receptor (DOR) and κ receptor (KOR), all belonging to the GPCR family, and consisting of highly homologous 7-transmembranous helices linked with extracellular peptide loops (Li et al. 2012). Opioid neurotransmission appears to affect many CNS functions, such as nociception, cardiovascular regulation, respiratory rate, neuroendocrine activity, aggressive, locomotive, pleasure and sexual behaviours, and learning and memory.

16.6.2 Endorphins

Endorphins are natural endogenous opioid neuropeptides, are one of the major products of the precursor proopiomelanocortin (POMC) and are secreted by the anterior pituitary gland through the hypothalamus in response to certain physiological triggers such as strenuous physical exercise, stress and pain, and they resemble opiates in their ability to produce analgesic effects and inhibit transmission of pain signals. Other active products of this precursor include adrenocorticotrophic hormone (ACTH) and a-melanocyte-stimulating hormone (a-MSH). The word endorphin is contracted from the words *Endogenous* and *morphine*. Four types of endorphins are produced in the human body, α-, β-, γ- and σ- endorphins, each having different numbers and types of amino acids in their molecules, between 16 and 31 amino acid residues in each peptide molecule (Shrihari 2017; Li et al. 2012).

Endorphins are widely distributed in many parts of the body, mainly in the pituitary glands as well as in the brain. β-endorphin is the most potent and abundant, in terms of natural pain relief, and is present in the neurons of both the central and peripheral nervous system. They are released during pain or stress, and are associated with sexual and maternal behavior. Additionally, endorphins have been found to be associated with states of pleasure including emotions brought about by laughter, intercourse, love and even appetizing food (Sprouse-Blum et al. 2010).

Endorphins mediate their actions mainly through μ-opioid receptors (MOR), μ referring to morphine. μ-receptors are found presynaptically in various brain regions and act by inhibiting neurotransmitter release, for instance by inhibiting the release of the inhibitory neurotransmitter GABA that decreases the inhibition of dopamine pathways, and consequently leading to increased dopamine release. This process leads to deviant synaptic pliability, which causes addiction. Moreover, the aforementioned binding of endorphins to their receptors also triggers chemical processes that prevent the release of substance P, among other tachykinins, which is one of the substances that participate in the conveyance of pain. MORs not only modify transmission and perception of nociceptive stimuli, but are also associated with opioid-induced bowel dysfunction, reduction in the respiratory rate in response to high CO_2 levels and abuse liability due to the manifestation of tolerance and dependence (Li et al. 2012;Dalayeun et al. 1993). From a clinical standpoint, the ideal opioid-based drug would be one that provides rapid pain relief while producing minimal physiologic or psychologic side effects. Use of endogenous peptides as drugs has remained a challenge, since peptides do not cross the blood-brain barrier (BBB), and are quickly degraded in the bloodstream prior to delivery to their sites of action in the brain.

MORs are targeted in clinical use, and several μ-receptor agonists and antagonists have been developed. DAMGO is a pentapeptide derived from the endogenous δ-receptor's ligand enkephalin. It is a well-known compound, having its pharmacology assessed *in vitro* and *in vivo*, and is frequently used as a reference substance. It is highly selective for the μ-receptor with an affinity approximately 1000 times higher than for the δ-opioid receptor. Several studies have proven than DAMGO is 20 times more potent than morphine against nociception when administered intracerebroventricularly to mice, while it is comparable to morphine when administered subcutaneously or intravenously. Despite its effective pharmacological properties, DAMGO shows very limited CNS effects when administered systemically (Lindqvist et al. 2016). Another agonist, fentanyl, is a phenylpiperidine-related synthetic opioid with a very high affinity toward μ-receptors. It is fast acting and approximately 50 to 100 times more potent morphine, with a good solubility and a relatively low molecular weight. Sufentanil and hydromorphone are μ-opioid receptor agonists clinically used in anaesthesia and postoperative analgesia. Hydromorphone possesses a high affinity to both μ-opioid and δ-opioid receptors, while sufentanil is a highly μ-receptor-selective agonist (Yang et al. 2018). Although the μ-opioid receptor agonists induce strong analgesic effects and sedation, it is not undeniable that they also cause side effects such as respiratory depression, constipation and euphoria. Subsequent chronic MOR agonism promotes opioid tolerance and physical dependence.

Antagonists of the μ-opioid receptor are also available, such as naloxone and naltrexone that can suppress central endogenous opioid receptor systems. Naltrexone is a long-acting competitive antagonist, which is suitable for oral administration, and has been studied and used as an adjunctive in opioid addiction management programs. In morphine-dependent subjects, naltrexone was found to be more potent than nalorphine and twice as potent as naloxone. Naloxone is typically administered

in cases of opioid overdose to mitigate bodily response to the opioid (Glanz et al. 2018; Gonzalez and Brogden 1988). Another example is alvimopan, as it has a high affinity for MOR, but low systemic absorption. High concentrations of opioid receptors can be found throughout the gastrointestinal tract, and stimulation of these receptors by opioid analgesics has a direct local effect on bowel function and motility (Vaughan-Shaw et al. 2012). Reports have shown that alvimopan accelerates the gastrointestinal recovery period and therefore it is indicated for patients undergoing radical cystectomy for bladder cancer, which is associated with delayed gastrointestinal recovery that prolongs hospital stay (Cheryl T. Lee et al. 2014). Other MOR antagonists include levallorphan and nalmefene, the latter being clinically used to reduce alcohol dependence (Clément Palpacuer et al. 2015). In comparison to chronically morphine-treated mice, repeated peptidomimetic-treated mice developed less analgesic tolerance and/or physical dependence. Analysis of *in vitro* and *in vivo* data has permitted structure activity relationships to guide further discovery and chemical synthesis of opioid analgesics that suggest improved clinical use.

16.6.3 Enkephalins

Enkephalins are pentapeptides first discovered and isolated from porcine brain tissues in 1975, involved in the regulation of pain. There are two forms of enkephalins, Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) containing the amino acid leucine, and Met-enkephalin (Tyr-Gly-Gly-Phe-Met) containing methionine (Comb et al. 1982), both generated from a common precursor proenkephalin-A (PENK-A) by proteolytic enzymes. Leu-enkephalin and met-enkephalin are metabolized by several enzymes called enkephalinases that include endopeptidases, aminopeptidases and angiotensin-converting enzyme (Thanawala et al. 2008), among others. Both enkephalin forms exert their effects through the δ-opioid receptor (DOR) that belongs to the GPCR family. Met-enkephalin is found mainly in the adrenal medulla and the brain acting as a neurotransmitter/neuromodulator and as an active regulator of cell proliferation. Like μ-receptors, δ-opioid receptor signaling investigations have primarily focused on mechanisms of opioid analgesia (Al-Hasani and Bruchas 2011). Despite MOR-based analgesics being potent and efficient in alleviating acute severe pain, they are ineffective in treating chronic pain syndromes, which is why the attention has been driven to other receptors, particularly δ-receptors, showing to be potential targets for developing novel opiate analgesics for chronic pain management. Nonetheless, initiating tolerance is a limitation for their use as long-acting opioid analgesics (Charfi et al. 2015). DPDPE is one of the early synthetic DOR agonists developed, and is structurally similar to met-enkephalin (Bilsky et al. 1995). SNC80 is the first non-peptidic-selective DOR ligand developed and it successfully produced antidepressant, anxiolytic and analgesic effects, but its use was limited due to causing convulsions when administered in high doses (Dripps et al. 2018). Other agonists include BMS986187, a potent δ-opioid receptor-positive allosteric modulator that enhances the affinity of leu-enkephalin to the δ-opioid receptor, yet it has a low potency due to limited

receptor phosphorylation and ultimately low receptor internalization and a slower onset of desensitization (Stanczyk et al. 2019). Many other DOR agonists have been developed, but they have not been used clinically due to limited data or considerable side effects, but are currently being used in scientific research, such as BU48 (Broom et al. 2002), BW373U86, KNT127, DPI125 (Yi et al. 2017) and TAN67 (Min et al. 2017). Peptide compounds do not cross the blood-brain barrier, and therefore naltrindole, a non-peptide antagonist analogue of enkephalin, was developed. It is a very potent, selective δ-receptor antagonist, used for biomedical research (Granier et al. 2012; Werling et al. 1989). Another DOR antagonist is buprenorphine, an effective medication in the maintenance treatment of opioid dependence, particularly heroin, maintaining people in treatment at any dose above 2 mg. Buprenorphine was approved for medical use in the 1980s, and it is heavily prescribed by health care professionals. However, despite its potency and efficiency, it is associated with many adverse effects, such as those accompanying opioid use, including nausea and vomiting, dizziness, drowsiness, impairments in cognitive performance and withdrawal symptoms (Strand et al. 2019). It has been proposed that activation of MOR with concurrent antagonism of the δ-receptors could attenuate the development of opioid tolerance and dependence.

16.6.4 Dynorphins

Endoproteolytic cleavage of the precursor prodynorphin by pro-protein convertase-2 (PC2) enzyme produces multiple biologically active peptides including dynorphin A, dynorphin B, big dynorphin, α- and β-neo-endorphins and leumorphin, composed of high numbers of hydrophobic and basic amino acid residues, mainly lysine and arginine (Chavkin 2013; Seidah et al. 1998). Following formation, they are stored in dense-core vesicles in neuron endings, which need prolonged stimuli to release their contents into the synaptic cleft (Drake et al. 2007). Dynorphins bind preferentially to κ-receptors (KOR) with minimum affinity to MOR and DOR subtypes. κ-receptors belong to the G protein-coupled receptor (GPCR) superfamily and have two subtypes, K1 and K2; however, only one has been cloned. Stimulation of the receptor by kappa opioids causes conformational changes in the 7-transmembranous helices of the receptor, leading to Gα subunit dissociation from Gβγ dimer and inhibition of adenylyl cyclase, and thus downstream cAMP production. The Gβγ dimer modulates the conductance of Ca+2 and reduces voltage-gated channel openings, for example K+ channels. KOR agonists activate kinase cascades, for example GPCR kinases (GRK), as well as members of the mitogen-activated protein kinase (MAPK) family. Chronic KOR agonist activation results in an upregulation of adenylyl cyclase, and this may contribute to desensitization, tolerance and physical dependence (Bruchas and Chavkin 2010). Dynorphin and its κ-receptors are highly expressed and distributed throughout brain regions presynaptically and mainly in the hypothalamus, hippocampus, midbrain, medulla and spinal cord. Dynorphins act as modulators of pain response, maintain appetite and body weight and intervene in circadian rhythm control (Anderson et al. 2019).

16.7 Conclusion

This chapter describes some of the different classes of neuropeptides. The biosynthesis and metabolism with their physiology, pathophysiology and pharmacology have been described. Several agonists and antagonists of these peptide receptors continue to show great success in the development of therapeutic agents for the treatment of a variety of disorders, which include cardiovascular, epilepsy, immune, psychiatric, substance abuse and body weight disorder.

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Pharmacology of Gasotransmitters (Nitric Oxide and Carbon Monoxide) and Their Action

17

Rohitas Deshmukh, Ranjit K. Harwansh, Nabamita Bandyopadhyay, Shantanu Bandopadhyay, and Puneet Kumar

Abstract

In the last decades, gasotransmitters have gained attention for their crucial role in pathophysiological and cellular functions. Gasotransmitters are gaseous mediators of the cellular signaling and biological responses from one end to the other. The isoform of these gaseous signaling molecules includes NO (nitric oxide), CO (carbon monoxide), and H_2S (hydrogen sulphide), collectively called gasotransmitters. The diverse role of these gasotransmitters in cell and molecular biology as well as biochemical processing has been well validated through several scientific and clinical studies. The biosynthesis, interaction, and movement of gasotransmitters inside the cellular systems are critical especially in terms of their pharmacological response. These gaseous molecules are very toxic and hazardous to human health at higher concentrations but at lower levels they may be considered as therapeutic agents. They can easily diffuse through all the cell membrane and act on their targets for generating pharmacological responses. Due to its gaseous nature, the cellular interactions at the target sites are complex and make it a critical task for researchers to understand. The mode of action and molecular pathways are still under the exploratory phase. Apart from their toxic nature, there are several pharmacological activities such as cardioprotective,

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antihypertensive, smooth muscle relaxant, vasodilator, antithrombotic, antitumor, etc. that have been reported for these gaseous molecules. This gaseous family has become a promising area for multidisciplinary research in order to establish their importance in relation to disease and health. The perspective of these gasotransmitters is required to validate the clinical translational and future investigation following clear cut understanding of their pharmacological properties. This book chapter deals with the biosynthesis, pharmacological application and clinical utility of gaseous molecules mainly NO and CO.

Keywords

Natural gases · Novel bioactive molecules · ENOG · Signal transduction · Heme oxygenase · Biomarker

Abbreviations

AD	Alzheimer's Disease
ARDS	Acute respiratory distress syndrome
BP	Blood pressure
C/EBP	CCAATT-enhancer-binding protein
CaM	Calcium-binding messenger protein calmodulin
cGMP	3,5-Cyclic guanosine monophosphate
CO	Carbon monoxide
CO_2	Carbon dioxide
CoHb	Carboxyhemoglobin
COPD	Chronic obstructive pulmonary disease
CORMs	CO releasing molecules
CP	Cisplatin
CVD	Cardiovascular disease
CYT2E1	Cytochrome P450 2E
DCM	Dichloromethane
ED	Erectile dysfunction
EDHF	Endothelium-derived hyper polarizing factor
EDRF	Endothelium-derived relaxing factor
ENOG	European Network on Gasotransmitters
eNOS	Endothelial NOS
ER	Endoplasmic reticulum
GTN	Glyceryltrinitrate
GTP	Guanosine5-triphosphate
GTT	Glucose tolerance test
H_2S	Hydrogen sulfide
Hb	Hemoglobin
HIF	Hypoxia inducible factor
HO	Heme oxygenase

HO-1	Heme oxygenase-1
HO-2	Heme oxygenase-2
HUVECs	Human umbilical vein endothelial cells
iNOS	Inducible NOS
MAPK	Mitogen activated protein kinases
MI	Myocardial infarction
nNOS	Neuronal NOS
NO	Nitric oxide
NOS	Nitric oxide synthase
O_2^-	Peroxide radical
$OH^\bullet$	Hydroxyl radical
ONOO	Peroxynitrite
PAH	Pulmonary arterial hypertension
PI3K	Phosphatidyl inositol 3-kinase
PI3K	Phosphatidylinositol-3 kinase
PKC	Protein kinase C
PPARc	Peroxisome proliferator-activated receptor-c
RAS	Renin–angiotensin system
ROS	Reactive oxygen species
$S^\bullet$	Superoxide radical
sGC	Soluble guanylatecyclase enzyme
SiRNA	Small interfering RNA
TB	Tuberculosis
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cell

17.1 Introduction

Environmental gases like hydrogen sulfide (H_2S), carbon monoxide (CO), carbon dioxide (CO_2), nitric oxide (NO), etc. are treated as toxicants and health hazards. Apart from being hazardous to health, studies have shown these natural gases have some health benefits (through cellular and molecular mechanisms), following which they are considered gasotransmitters. The term gasotransmitter was postulated in the early twenty-first century after their biological importance in our body as gaseous signaling molecules was recognized. These signaling molecules became the thrust area of research to establish their molecular pathways at the cellular level during the last decade. The human health benefits of gasotransmitters have been explored through scientific investigation worldwide (Wang 2014; Sukmansky and Reutov 2016; Kolluru et al. 2017).

The fact about these gasotransmitters is that they are highly toxic at higher concentration but at low concentration they may work as a gaseous signaling molecule and produce specific biological activities (Andreadou et al. 2015). The biological role of NO was first discovered in the year 1987, after the identification of

vasodilation by acetylcholine mimic response through the vascular endothelium of mammals. Firstly, in biological systems NO is released endogenously as a signaling molecule (Lowenstein et al. 1994). After understanding the cellular interaction and signaling of NO, CO, H_2S, as well as ammonia have been included as new members of the gasotransmitters family. Moreover, in the near future methane could be the next analog of this family (Wang 2014).

Gasotransmitters are categorized as novel bioactive molecules on the basis of the following criteria:

- Tiny molecules of gaseous family
- Can easily diffuse through biological membrane or cell membrane
- Should not interact with receptors
- Production and regulation via enzymes only
- Its properties may be exogenously modulated
- Should have specific targets on the molecular as well as cellular level (signal transduction) but not induced by any chemical messengers

A major promising area of research on gasotransmitters concerns the following concepts:

- Collective interaction of different gaseous molecules
- Targeting of common cells or targets by various gaseous molecules
- Recognition of place of cellular targets and production place of gasotransmitters

These concepts definitely help understand the relevance of NO, CO, H_2S, and others in signal transduction in the human body. They could be novel therapeutic lead molecules for treatment of several human health problems through scientific validation. Reportedly, gasotransmitters can effortlessly move across biological membranes by interacting with a hydrophobic layer of the membrane. For example, in an animal study it has been observed that normally gaseous molecules move through biological membranes by using transporter proteins like aquaporin-1. It can help low molecular weight gases such as NO, CO_2 and NH_3 to cross cell membranes easily (Garcia-Mata and Lamattina 2013). The overview of biosynthesis of gasotransmitters along with their role on vascular system is schematically represented in Fig. 17.1.

This chapter discusses the role, pathophysiological consequences, biochemistry, and clinical application of gasotransmitters, particularly that of NO and CO. It will help us understand the importance of these gasotransmitters as therapeutic bioactive molecules or drug candidates in association with already established drugs for enhancing their efficacy against various diseases.

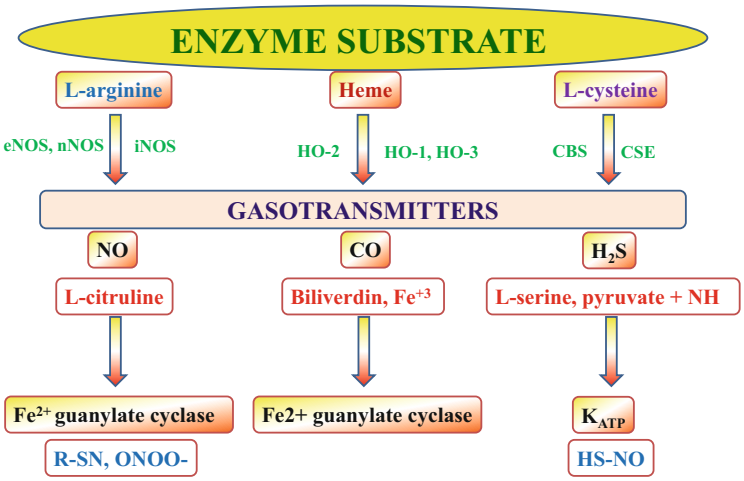


Fig. 17.1 Synthesis and major vascular effects of the gasotransmitters

17.2 Gaseous Signaling Molecules

In the recent past, the importance of different gaseous signaling molecules to their target sites and related biological responses have been clearly defined and validated through integrated/scientific approaches. There are various types of gaseous molecules like NO, CO, CO_2, H_2S, and so on, which have been proven as gaseous signaling molecules by transduction the information from one cell to other cells as biological responses which are endogenously produced (Szabo 2010; Ryter and Choi 2013; Paul and Snyder 2018a, b). The basic features of gasotransmitters and neurotransmitters have been explained in Table 17.1.

NO was recognized as the first endogenously produced gaseous molecule. In various mammal cells, NO was synthesized from L-arginine as substrate by different analogs of nitric oxide synthase (NOS). Discovery of NO as a novel biological

Table 17.1 Difference between gasotransmitters and neurotransmitters

Mode of action	Gasotransmitters	Neurotransmitters
Release	Cytoplasm release	Exocytotic vesicle
Reuptake	No	Yes
Removal mechanism	Nonenzymatic like oxidation, scavenging, methylation, etc.	Enzyme dependent
Revert direction	Bidirectional	Pre to postsynaptic membrane (single direction)
Membrane receptors	Not required	Required

gasotransmitter in mammals facilitated in better understanding of signal transduction in cells in order to modulate their functions. Apart from its diverse biological importance, NO is known as an endothelium-derived relaxing factor (EDRF) (Farrugia and Szurszewski 2014; Zhao et al. 2015).

Another isoform of the gaseous signaling molecule is CO. Actually, it is a heme metabolite, produced by an inducible enzyme, i.e., heme oxygenase-1 and -2 (HO-1 and HO-2), which is constitutively released. In most cases the biological features of CO are similar to NO; for example, it exhibits as a smooth muscle relaxant (vascular tissues), a vasodilator (slows down the blood pressure in case of hypertension) and cardioprotective (in case of ischemic or reperfusion heart disease) (Motterlini and Foresti 2017).

Another endogenous gasotransmitter is H_2S, also known as the rotten-egg gas, mainly expressed in mammalian cells. It is produced from L-cysteine and homocysteine after enzymatic reaction of various enzymes, including 3-mercaptopyruvate sulfurtransferase, cystathionine β-synthase, and cystathionine γ-lyase. It has numerous health benefits through transducion of signals (neurotransmission) and regulation of the various physiological tasks in the body (neuromodulation). H_2S is well known for its cognition improvement through enhancing memory, nociception, and learning function. It is considered an endothelium-derived hyper polarizing factor (EDHF) (Polhemus and Lefer 2014; Paul and Snyder 2018a; b).

Of lates, continuous research on these gaseous signaling molecules has established their diverse biological role in the body. Apart from their traditional function as signaling substance, they have been identified as cellular regulators. These molecules can produce different biological functions at a particular site through multifarious chemical interactions with cells, proteins, as well as metabolites. With smart technique and engineering technology, these gaseous molecules are being developed as therapeutic agents that can be administered inside the body and release gases in a sustained fashion (Cheng and Rong 2017).

17.3 European Network on Gasotransmitters (ENOG)

The ENOG has two major goals for research and regulation of gasotransmitters in European countries. The first goal is to enhance research on different gaseous molecules such as NO, CO, H_2S, etc. in order to improve their competitiveness, quality, and efficacy in the development of therapeutic agent against various diseases. The dissemination of information on gasotransmitters among the European groups has been through various modes like research problems, skills, knowledge sharing, expertise, and gasotransmitter biosynthesis. The interactions of gasotransmitters with their targets for producing cellular effects are also described by the ENOG among their team. ENOG also help to share the information in multidisciplinary research group through updating and providing the idea, knowledge and training about gasotransmitters through synthetic chemistry, computational drug design and pharmacology approaches for establishing gasotransmitters as drug candidates (Papapetropoulos et al. 2015).

Table 17.2 Function of ENOG working groups for promotion and development of gasotransmitters

ENOG groups	Functions
Group 1	Molecular control of gasotransmitter production and signaling
Group 2	Gasotransmitters in disease
Group 3	Chemistry and in vitro pharmacology of gasotransmitter-modifying molecules
Group 4	Evaluation of gasotransmitter-modifying agents in animal models of disease

Another important and integral goal of ENOG is as follows (Papapetropoulos et al. 2015):

- Dissemination of idea, knowledge, skill, and experience shared among different participating members, ENOG members, and global community.
- Knowledge sharing and application about novel drug (therapeutic agent) development against disease. In this context, the small-medium enterprises will benefit with expertise in NO and CO based advanced drug delivery systems.
- To assist and properly guide the young scientist to continue biomedical research in the current topic.

The different ENOG working groups for different purposes are mentioned in Table 17.2.

17.4 Nitric Oxide (NO) as Signaling Molecules

NO was invented in 1980 as a first gaseous signaling molecule. It has an important role in biological chemistry through understanding its cytotoxicity and the signal transduction process. Especially in the fields of immunology, neuroscience, and physiology, NO has been recognized as a physiological key element for several pharmacological functions of the human body. In the year 1992, Robert F. Furchgott and his group received the Nobel Prize for discovering NO as a physiological signaling molecule, particularly for the cardiovascular system. Different physiological pathways of NO have been identified such as neurotransmission, vascular permeability, synaptic plasticity, renal function, platelet aggregation and adhesion, senescence, and hepatic metabolism. The molecular level activity of NO has also been observed through tumor suppression and host immunity (Miller and Megson 2007; Nagpure and Bian 2016; Mir and Maurya 2018).

In the recent past, NO has become the most investigated and famous gasotransmitter throughout the world. NO is generally produced by conversion of L-arginine to L-citrulline through the enzymatic action of NOS. According to the functionality, NOS has been subcategorized as eNOS (endothelial NOS), iNOS (inducible NOS), and nNOS (neuronal NOS). eNOS is an active moiety generally found in platelets and endothelial cells. iNOS is a highly contributing enzyme for

generating NO in immune responses and inflammations, while nNOS as the name indicates, is mainly identified in neuronal cells (Luo et al. 2014; Shefa et al. 2017).

The diverse kinds of NO-associated biological activities have been reported via modulating and transducing the signals in neuronal cells, maintaining the tone of vessels, pro-angiogenic and immune response. In eNOS knockout mouse, several incidences like poor blood pressure, hypertension, and atherosclerosis have been accounted, although, their action on thrombotic conditions was not seen in the same animal. Moreover, endogenous NO has been reported to interfere with platelet action, but not to its response. NO can also inhibit the function of GPIIb/IIIa fibrinogen with platelets, P-selectin expression, and 5-HT secretion (Truss and Warner 2011a; b; Poulos and Li 2017).

Acute inflammatory response leads to maintenance of tissue homeostasis by a self-regulating process, while chronic inflammatory response involves long range of progression of diseases such as arthritis, neurodegenerative problems, atherosclerosis, cancers, etc. Alleviated level of endogenous NO can affect the aforementioned inflammatory responses. This endothelium produced gasotransmitter is accountable for the regulation of tonicity of the vascular system. Its action is not limited to this, but it has been associated with several pathological and physiological processes. Particularly, in case of normal physiology, nNOS and eNOS are responsible to exert the actions while in injury cases iNOS is responsible. These NOS groups are not only involved in NO generation but also in the production of different molecules like transition metals, peroxynitrite (ONOO) and S-nitrosothiols through the process of oxidation or reduction. Generally, NO exhibits its action through sGC (soluble guanylatecyclase enzyme) and resultant intermediate includes GTP (guanosine5-triphosphate) and cGMP (3,5-cyclic guanosine monophosphate). During platelet aggregation inhibition and inflammatory cell apoptosis regulation, NO can work through cGMP-independent pathway. Additionally, the translation or transcription is modulated by NO via obstructing the signaling pathways, including phosphatidylinositol-3 kinase (PI3K), G-proteins, mitogen-activated protein kinases (MAPK), glyceraldehyde dehydrogenase and RAS (Renin–angiotensin) system (Socco et al. 2017; Wang et al. 2019).

17.4.1 Biosynthesis of NO

Biologically, NO is synthesized from L-arginine by catalytic activities of NOS enzymes, heme containing proteins (Forstermann and Sessa 2012). The resultant intermediate product includes N^{G}hydroxy-L-arginine and L-citrulline during NO generation. The regulation of NO synthesis through macrophages has been understood clearly by L-arginine uptake by the pathway of inducible L-arginine-NO (Kovacevic et al. 2017). The generation of NOS enzymes based NO via peritoneal macrophages and monocyte-derived macrophages has been studied in knock out C57BL6 mice (Moncada and Higgs 1993; Neilly et al. 1994; Venketaraman et al. 2003). The detailed schematic of NO biosynthesis is shown in Fig. 17.2.

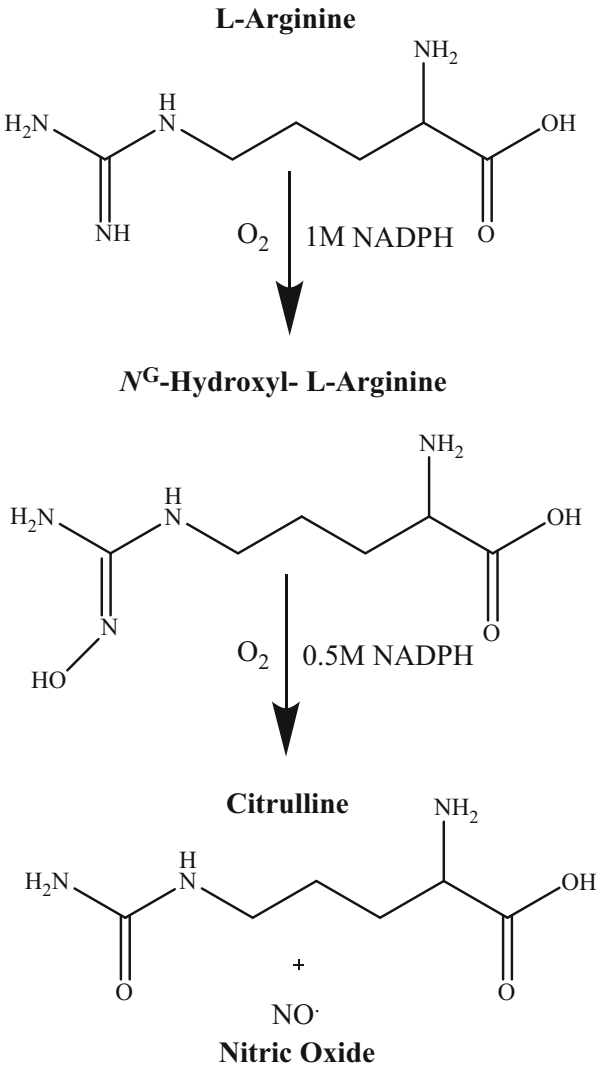


Fig. 17.2 Biosynthesis of nitric oxide (NO)

eNOS forms NO in the blood vessel lining that results in vasodilation of smooth muscle through activation of sGC and cGMP (Wen et al. 2018). It leads to the constant flow of blood and regulation of blood pressure. Moreover, NO has a significant role as an anticoagulant by inhibiting platelet and clot formation. But nNOS-mediated NO has a crucial role in neuronal cell signaling process. In case of iNOS, it is broadly distributed in the body and contributes in an intrinsic immune system of the body. During microbial infection/invasion, NOS enzymes are highly expressed in smooth muscle, macrophages and hepatocyets, resulting in the release of NO at the microgram level. NO is also found in infected cells along with ROS (reactive oxygen species) like peroxide (O_2^-), superoxide ($S^{\bullet}$), and hydroxyl ($OH^{\bullet}$)

radical. It has a certain role in reducing the microbial loads through natural immune response systems (Doroszko et al. 2018).

17.4.2 Activity at Receptor Levels

The eNOS is an endothelial constitutive enzyme involved in catalytic activity that regulates cell signaling through Ca^{2+} and calcium-binding messenger protein calmodulin (CaM). The NO is produced after interaction of eNOS with Ca^{2+}-CaM complex. NO has the ability to diffuse cell membranes and can interact with targeted intracellular molecular sites of the cells (Piazza et al. 2018). NO can react with the heme substance sGC causing conversion of GTP to cGMP, which regulates the various pharmacological activities (smooth muscle vasorelaxation through the signal transduction cascade) (Forstermann 2010).

It has been proved that sGC, i.e., the prosthetic heme group are responsible for NO generation. However, if this heme group is eliminated the NO is not formed. There are diverse kinds of biological activities reported for NO, which includes wound healing, host defense, immune response, penile erection, angiogenesis, tumor suppression, bronchodilation, vasodilation, platelet aggregation, regulation of vascular tone, stem cell proliferation, hormone secretion, hemoglobin (Hb) delivery of oxygen, inflammation regulation, pulmonary hypertension, gastrointestinal mobility, septic shock, gene and neuronal communication, glaucoma and neural degeneration, and smooth muscle cell replication neurotransmission (Fleissner and Thum 2011). These biological effects of NO mainly depend upon the sGC activation and cGMP formation via GTP. Although, other cGMP pathways of NO like nitrotyrosine, nitrite and nitrate have also been established (Omar et al. 2016).

17.4.3 Role in Biological Systems

Biological activities mainly depend on different NOS enzymes and their location. There are three subtypes of NOS in mammals: (1) eNOS is responsible for CVS, (2) nNOS is accountable for the brain or nervous system, and (3) iNOS is liable for innate immune response (Lee et al. 2017). The NO has a specific role in CVS through Ca^{2+}-dependent NOS. There are several chemical substances like acetylcholine, ATP, bradykinin, and other agents that can stimulate the flux of calcium known as NOS agonists and can modulate the NO synthesis in vascular endothelial cells. Likewise, physical factor/agonists, *viz.*, electrical current, light, electromagnetic fields, acupuncture, flow, and shear stress can stimulate the NO synthesis in vascular endothelial tissues (Arzumanian et al. 2003; Piazza et al. 2018).

About 90% consumption of NO is taken up by the blood, resulting in inhibition of platelet and thrombus formation. The rest of the NO diffuses to the vein and arteries (smooth muscle), and activates a cascade of effects like smooth muscle relaxation. These effects are directly proportional to the vasodilation that results in reduced blood pressure. The CVS sustains the NO level along with blood flow. The level of

NO is dependent upon the rate of blood flow. As the blood flow increases, endothelial tissues increasingly secrete NO to attain a constant level in the blood stream (Riddell and Owen 1999; Vanhoutte and Gao 2013).

In case of pathological events like atherosclerosis, the NO level is reduced due to blockade of cholesterol, resulting in vasoconstriction (Li et al. 2014). This abnormality of CVS leads to decreased blood flow and causes increased blood pressure (hypertension) (Forstermann et al. 2017).

In this context, nitroglycerin is generally used for cardiac disease like hypertension, which undergoes metabolic transformation and forms NO in CVS endothelial tissues where it produces vasorelaxation and increases the blood flow in smooth muscle including atherosclerotic arteries, thereby improving cardiovascular functions. The NO has a certain role as an anticoagulant by preventing platelet and blood clot formation in cardiac arteries. If absent there may be chances of coronary thrombosis, which is a main cause of heart stroke. Thus, NO has a potent gaseous signaling molecule with an important role in human life (Rochette et al. 2013; Zang et al. 2018).

17.4.4 Clinical Utility

The irregular NO generation via different NOS enzymes or signaling cells are related to several pathological conditions in the organs. In many disease states, NO metabolites like peroxyl nitrite are raised. The role of NO in various diseases is discussed in the following.

17.4.4.1 Cardiovascular Disease

NO has a crucial role in cardioprotection against the sequence of cardiovascular disease (CVD) like diabetes, hypertension, and hypercholesterolemia. The main cause of CVD is atherosclerosis, which leads to dysfunction of endothelial vascular system. NO provides protection to the heart from these uncomplicated pathological conditions via regulation of vascular tone and blood pressure (BP) as well as reduction in the proliferation of smooth muscle cell by inhibiting leukocyte adhesion and clotting formation (platelet aggregation) (Naseem 2005). In case of cardiovascular disease (CVD), especially in pulmonary hypertension and ischemic heart disease, the traditional treatment involves application of NO donors (Munzel and Daiber 2018).

17.4.4.2 Diabetes Mellitus

Type I diabetes is also associated with vascular problems of retinopathy, nephropathy, and hypertension. These factors are largely responsible for the high death rate in type I diabetic patient. NO is an endothelium-derived relaxing factor that plays an important role in the regulation of altered NO activity and vascular tone in insulin-dependent diabetic condition. However, certain mechanism of NO in diabetic vasculopathy remains challenging (Traub and Van Bibber 1995).

Adela et al. (2015) reported that the high level of NO was noted with increased concentration of glucose in serum, which may be due to the activation of endothelial cell and thereby enhanced NO production. Thus, hyperglycemia enhances NO generation in Type-2 diabetes (Adela et al. 2015). Moreover, NO exhibits an important role in the progression and regulation of vascular disease (diabetes and its complication), which is generally affected by the ROS and NOS (van den Born et al. 2016).

17.4.4.3 Erectile Dysfunction

Erectile dysfunction (ED) is a common problem in men due to altered relaxation of corpus cavernosum smooth muscle and thereby reduced blood supply to penis. ED is associated with several factors including age, socioeconomical condition, depression, hypertensions, diabetes, etc. Erectile function (flaccidity and erection) of the penis is performed by the smooth muscle tone. The flaccidity and erection is maintained by sympathetic-contractile factors and parasympathetic-based smooth muscle relaxation factors, respectively. NO has been recognized as the prime vasoactive chemical mediator, noncholinergic and nonadrenergic neurotransmitter responsible for penile erection and relaxation through smooth muscle. nNOS and eNOS mediated NO is released from the nerves (nonadrenergic and noncholinergic parasympathetic) and endothelial tissues in the corpus cavernosum and blood vessels of the penis (Cartledge et al. 2001).

The NO exhibits its mechanism of action through 3′,5′-cyclic guanosine monophosphate (cGMP) pathway, cGMP maintains the function of intracellular contractile proteins and calcium channels for relaxation and erection of penis (Burnett 2006; Dashwood et al. 2011). For ED treatment, sildenafil and analogues are generally used to increase the blood flow in nitrergic nerves of the penis by inhibiting the phosphodiesterase and cGMP-induced vasodilation (Al Omar et al. 2016).

17.4.4.4 Respiratory Disease

NO plays a crucial role in maintenance of the respiratory system including vascular smooth muscle, neurotransmission, lung cell, and airway. NO is highly exploited as therapeutic agent for regulation of respiratory disorder. Release of NO is variable and depends on the different enzymes and ROS, which can help in the generation of NO (Antosova et al. 2017).

In case of neonatal respiratory distress syndrome, persistent pulmonary hypertension, and broncho-pulmonary dysplasia, inhaled NO exhibits significant effect to the patients. Treatment may be improved in conjugation with NO simulators and drugs like prostaglandins to produce synergism therapeutic effect. Along with the treatment approach, potentiating the therapeutic effect, NO donor could be effective. It is most important to develop such a type of delivery technique for respiratory system that can modulate the release of NO in a controlled manner and reduce side effects by decreasing the formation of unwanted by-products (Akter et al. 2016).

In case of respiratory tract problems like asthma and chronic obstructive pulmonary disease (COPD), the exhaled NO is used as a biomarker for therapeutic

purposes, particularly during the treatment of acute respiratory distress syndrome (ARDS). Nowadays, anti-inflammatory and analgesic treatment involves using of NO-NSAIDs, isoxazolopyrazoles and nitroso derivatives based on hydroxyimoyl chloride) for potentiating therapeutic effect. This combination system reduces gastrointestinal irritation and bleeding by providing cytoprotection to the mucosal lining of the stomach. Thus, NO can be used as a clinical agent for treatment of diseases (Abdelall et al. 2017).

17.4.4.5 Angina Pectoris

The role of NO in regulation of various physiological functions including angina pectoris has been established as biological marker. Promising in gaseous signaling molecules, there is importance in the evolution of these markers for clinical uses. NO has good vasodilation activity, due to which it is used as a clinical agent in case of angina pectoris. NO exhibits biological activities through cell permeability, inflammation, vasodilation, vascular and platelet function. The underlying mechanism of action of NO is based on hemodynamic (vasoldilation of arteries) and non-hemodynamic activities (nitroglycerine treatment) in dual dependent and independent ways. For instance, glyceryltrinitrate (GTN), SNP, and molsidamine are used for the treatment of angina (Quillon et al. 2015; Divakaran and Loscalzo 2017). It can also be used in the treatment of cardiac problems like pulmonary hypertension and cardiac failure. The wide application of organic nitrate is limited due to development of tolerance (Divakaran and Loscalzo 2017).

17.4.4.6 Myocardial Infarction and Atherosclerosis

Heart problems like myocardial infarction (MI) and atherosclerosis (ASC) are due to inflammation and fat-deposited arterial blockade, respectively, which has become challenging throughout the globe. An inflammatory biomarker, C-reactive protein (CRP), has a significant role in prediction of MI, coronary artery disease (CAD), and other heart problems. CRP can exert its activity by inhibiting the endothelial cell NO generation through deactivating eNOS. Low level of NO can produce its effect by stimulating cell apoptosis and inhibiting (CRP based) angiogenesis in endothelial cell (Fordjour et al. 2015).

Blood vessel problems like restenosis (abnormal narrowing of an artery or valve) can also be treated through NO by inhibition of platelet and cell proliferation. It has been reported that dietary organic nitrates based NO may also have a role in inhibition of platelet aggregation after conversion into nitrites inside the body. Both types of function of NO, i.e., vasodilation of smooth muscle and inhibition of coagulants/platelets facilitate useful management of heart diseases including myocardial infarction (MI) and atherosclerosis. Thus, there is the future scope for development of newer kind of clinical agents that produce a prolonged release of NO for management of CVD (Bohlen 2015; Fadel 2017).

In case of atherosclerosis, the NO and its relation to inflammation is justified. It has been established that this disease is linked with inflammatory process and leads to the production of plaque. The inflammatory cells i.e., macrophages and monocytes inducing proliferation of plaque may be considered as a major factor for

producing disease(s). In this context, apoptotic cells are taken up by phagocytes without producing any unwanted conditions of pro-inflammatory response. With understating of this NO phenomenon in relation to inflammation, it has been observed that apoptosis can be a pathway to relapse of plaque. Thus, it can be predicted that NO has a specific role in controlling the problems associated with inflammatory response, but its function is limited to the concentration only at local milieu, the timing and administering route can also affect (Baran et al. 2004).

The main pathway behind MI and ASC is NO/cGMP. This pathway plays a crucial role in different pathophysiological conditions like MI and atherosclerosis. Apart from diverse kind of genes and enzymes which involve in NO production and cGMP, is linked with MI risk and CAD (Wobst et al. 2015).

17.4.4.7 Inflammation

There is a inherent relation between NO mediated apoptosis and inflammation which in turns depend upon the concentration level at the target site. iNOS produced higher concentrations of NO cause apoptosis (a programmed cell death) while eNOS and nNOS mediated lower level of NO can produce cytoprotective activity. For example ONOO species which may be considered for apoptotic determining process, although the specific function of ONOO in inflammatory cell apoptosis is still un-cleared. Hence the concept of NO-apoptosis is applicable in inflammatory conditions (Lee et al. 2017).

It has been studied that activated macrophages triggers the sensitive tumors (murine) and thus initiating NO based apoptosis in both activated anti-tumor T cells and normal tumor cells. Therefore, NO mediated macrophage induces apoptosis (death of nearby cells) that raises the elimination of apoptotic cells results in facilitating the resolution phase of inflammatory environment. These inflamed cells lead to cancerous due to oxidant enriched surroundings, after sometimes these phenomena cause relentless and self-responsive oxidative stress due to generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (MacMicking et al. 1997a, b; Baran et al. 2004).

17.4.4.8 Cancer

In negative perspective, some of the NO related activities is related to oxidative stress in various cancers including melanoma. At low level of NO ($<$100 nM), it can produce the metastasis, proliferation and angiogenesis while at higher levels of 400–500 nM it may cause cytotoxicty and cell death (apoptosis). Therefore, NO donors have been considered as a promising treatment approach of different cancers. NO-NSAIDs are good example of this approach that NO-NSAIDs have been reported to be effective in arresting the metastasis and tumor proliferation as compared to pure drug itself (Abdelall et al. 2017).

NO shows anticancer property against colon cancer (HCT116 cancer cell) by inhibiting cell proliferation and production (Olah et al. 2018). In case of melanoma, NO mediates its effect via enzymatic process and free radical production. Thereby, NO can help in formation and proliferation of melanoma by a bunch of mechanisms involving inhibition of apoptosis and immune cells, alteration of angiogenic

processes, activation of tumor-associated macrophages and stimulation of pro-tumorigenic cytokines (Yarlagadda et al. 2017).

NOS2-based NO has been identified as a key driver for progression of breast cancer that is pioneering in the finding of novel targets which may be therapeutically effective for the treatment of estrogen receptor-negative (ER-) disease (Basudhar et al. 2017). No has wide dual distinct role in tumor or cancer proliferation and progression but sometimes it prevents tumor initiation/proliferation and act as an anticancer agent. NO has diverse kind of cellular functions through different process involving regulation of metabolic, posttranslational, and transcriptional functions (Keshet and Erez 2018).

17.4.4.9 Tuberculosis

iNOS-based NO has a crucial role in host defense systems against *Mycobacterium tuberculosis* in tuberculosis (TB) infection. TB has an internal complex environments to combat the therapeutic agent, resist and counter the effects of NO, RNS and ROS in intracellular surrounding of bacteria. The bacterial proteosome in TB is responsible for resistance against host-mediated nitrosative and oxidative stress by deprivation of stress-induced protein damage. It has been reported that TB genes (noxR1 and noxR3) have protection ability against oxidative molecules (ROS, RNS, and NO) and their harmful events, i.e., apoptosis and necrosis (Bhat et al. 2017; Braverman and Stanley 2017a, b).

The TB bacteria can change nitrates to nitrites in case of hypoxia, which favors anaerobic environment and dormancy stage for TB. Recent reports suggest that NO is an important gas transporter for host defense mechanism and diversified pathophysiological consequences of *Mycobacterium tuberculosis* infections. NO could be explored along with anti-tubercular drugs for enhancement of their efficacy. The quest for developing highly effective anti-tubercular agents and vaccines are utmost required for prevention of TB (Chinta et al. 2016a, b).

17.4.4.10 Alzheimer's Disease

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the persistent loss of neuronal behavior with aging in people. In AD patients, memory and other mental function is destroyed progressively. AD is the common mental illness worldwide (Heneka et al. 2013). NO is considered as a gaseous messenger in the mind (CNS, central nervous system) and its effect in memory recognition is well explored. Particularly NOS inhibitors and NO donors are involved in recognition of object memory. The role of NO CNS is well studied and it can be considered as a potential agent for destruction of memory as well as cognition but its potential neurotoxicity cannot be neglected (Pitsikas 2015).

Cifuentes et al. (2017) reported that impaired NO synthesis aggravates the progression of AD in APPPS1 mice (Cifuentes et al. 2017). Austin and Katusic (2016) reported that the NO has a crucial role in neuronal tissue by promoting p25 production and tau phosphorylation in condition of improper functioning of eNOS. In this order, overexpressed condition of amyloid precursor protein (APP) and β-site APP-cleaving enzyme 1 (BACE1) are seen with loss of eNOS in brain tissue of

mice. Evidence suggests for establishing new concept for understanding of the molecular mechanism of NO with endothelial impairment in the context of cognitive decline and AD progression. Moreover, endothelial NO is recognized as a protector of healthy mind and key agent establishing activity between CNS and cerebrovascular system (Katusic and Austin 2014; Austin and Katusic 2016).

17.5 Carbon Monoxide (CO) as Signaling Molecules

CO is an atmospheric gas and was considered as an industrial chemical by-product. In industry, it is produced by the combustion of fossil fuels and the burning of tobacco (MacMicking et al. 1997a, b; Fadel 2017). It is considered an enemy to human health as it is known to cause death by asphyxiation. At a higher concentration it binds to hemoglobin 200 to 300 times stronger as compared to oxygen and forms carboxyhemoglobin (CoHb). CoHb cannot bind to oxygen, thus reducing the oxygen carrying capacity of Hb leading to toxicity and hypoxia in the human body resulting in the death. In addition, it can interact with many intracellular proteins which leads to toxicity in various body cells. Owing to this harmful effect, this gas was labeled a "silent killer" for over 100 years (Li et al. 2009; Truss and Warner 2011a, b).

In the 1960s it was discovered that the metabolism of heme by heme oxygenase (HO) can endogenously produce CO in the body. At that time, people were unable to realize the implication of this finding. As such, endogenous synthesis of CO was considered as a metabolic waste product of heme degradation (Chinta et al. 2016a, b; Braverman and Stanley 2017a, b). But the endogenous synthesis initiated the concept that normal CO levels can control various functions of the body like NO. It was in 1987, Brune and Ullrich discovered the similarity of CO with NO in activating the soluble guanylate cyclase in the mammalian system. Very soon in 1993, Synder and his associates explained the physiological function of CO, which was found to be similar to that of NO (Verma et al. 1993).

This observation finally paved the way for the subsequent finding of other functions of CO. Since then the physiological importance of CO as a signaling molecule has been established and it is presently counted among the three gasotransmitters along with NO and H_2S. The gaseous nature of CO allows it to pass freely through the plasma membrane without the aid of specialized transport systems toward the targeted cell, thus allowing the CO to quickly impact the cellular function and behavior, which is important for physiological regulation. Signaling of CO inside the cells can control various physiological functions, mainly in the nervous and cardiovascular systems. Till date, CO has been found to have effective anti-proliferative, anti-inflammatory, and anti-apoptotic properties (Wu and Wang 2005a, b; Wallace et al. 2015).

CO is gaseous in nature and has no taste, color, and odor. It is a diatomic oxide of carbon formed by partial oxidation of carbon-containing molecules. CO is chemically stable with a molecular weight of 28.01-Da with carbon and oxygen atoms attached by a triple bond (Wu and Wang 2005a; b; Heinemann et al. 2014). The

solubility of CO in water is 2.3 mL/100 ml at 20 °C, and without considerable energy input it does not react with water. The specific gravity of CO in gaseous form is 1.250 g/L at 0 °C. In order to calculate the molarity in the gas phase, 1 ppm = 1.25 mg/m^{-3} (44.6 nM) at 25 °C (Von Burg 1999). Owing to its lipophilicity, it is able to freely cross the lipidic bilayer of the plasma membrane. CO is a relatively stable neutral molecule because it does not contain free electrons and therefore, it is not as reactive as the other gasotransmitters.

17.5.1 Biosynthesis of CO

Before the discovery and establishment of the concept of gasotransmitters, the scientific community was aware of the fact that the human body can endogenously synthesize CO (Coburn et al. 1963a; b). In 1966, Tenhunen et al. reported that degradation of senescent RBC results in the formation of CO. Later on, after about two decades, it was reported that the enzyme HO is accountable for the formation of CO by breaking down toxic heme as a protective mechanism (Tenhunen et al. 1968). The biosynthesis of CO takes place in cells and tissues various through endogenous metabolic procedures and the rate of synthesis in humans is estimated to be 0.42 ml/h (Sjostrand 1952; Coburn et al. 1963a; b).

In blood, the level of COHb is accountable for the endogenous production of CO. It increases during environmental pollutions and in smokers (up to 10%). About 86% of endogenous CO synthesis occurs through heme metabolism, whereas the residual part arises from cytochrome P450-dependent metabolism of xenobiotics or lipid oxidation processes (Wu and Wang 2005a; b; Owens 2010).

17.5.1.1 Biosynthesis by Heme Degradation

The major pathway for the production of CO is through HO catalyzed heme metabolism. This catabolism primarily takes place in the reticulo-endothelial system constituting of spleen and liver. It was Sjostrand who first revealed the formation of endogenous CO via oxidation of the α-methene bridge carbon of heme. Tenhunen et al. reported the involvement of HO enzymes in the degradation of the heme to form CO (Tenhunen et al. 1968).

The biosynthesis requires heme as substrate and HO processes the heme through three oxidation cycles, each requiring molecular oxygen. HO along with cytochrome P450 reductase in the presence of NADPH reduces the iron center of the bound heme molecule, thus allowing the binding of oxygen to the reduced iron. The second electron from NADPH activates the oxygen to form a peroxo-intermediate that attacks the heme ring. Thus, α-*meso*-hydroxy-heme is formed from the first oxidation cycle. In the second cycle, CO is released before the formation of verdoheme intermediate. In the final cycle, biliverdin IXα (FeIII) is formed, which further dissociates to free biliverdin and Fe (II), with the involvement of additional NADPH. Thus, three reaction products are generated in heme degradation process: CO, ferrous iron, and biliverdin-IX (blue-green pigment). The iron is recycled and biliverdin reductase converts the biliverdin-IX into bilirubin-IX (yellow pigment),

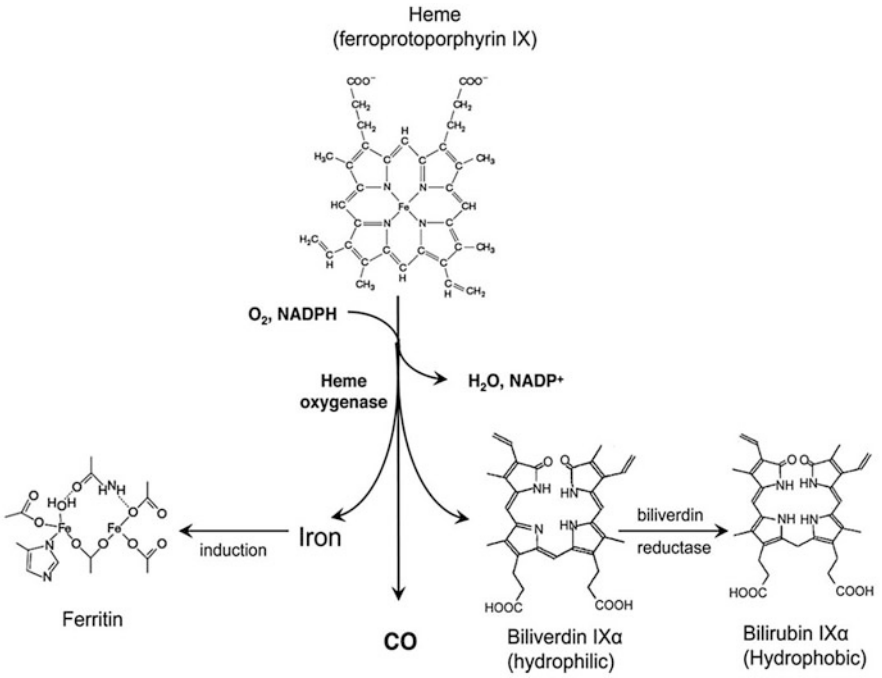


Fig. 17.3 Biosynthesis of CO by hemoglobin degradation mechanism of red blood cells catalyzed by heme oxygenase (HO) (Adapted from Fig. 2 of Wu and Wang 2005a, b)

which is excreted from the body via urinary pathway (Fig. 17.3). During injury, lysis of RBC takes place and deoxygenated Hb is formed, which is observed as a dark red/purple color formation. Later on a green tinge appears at injury site due to oxidation of heme to form biliverdin. This pigment is gradually converted to bilirubin causing a yellow coloration (Johnson et al. 2003).

HO is reported to have three isoforms, i.e., inducible HO-1 and two constitutive forms, namely, HO-2 and HO-3. The HO-1 has low levels of expression in most tissues under physiologic conditions except spleen. HO-1, known as the stress protein (i.e., hsp32) is a redox-sensitive response protein. HO-I activity is up-regulated in oxidative stress like inflammatory reaction or disease-like conditions. Furthermore, HO-1 is up-regulated in case of cellular stress and therefore found to be a vital antioxidant enzyme. Depending on the nature of the stimuli and cell types, the induction of HO-1 can be moderated by various signaling pathways like protein kinase C (PKC), cAMP-dependent mechanisms, $Ca2^{+}$-calmodulin-dependent protein kinase and the phosphoinositol pathways (Durante et al. 1997; Immenschuh et al. 1998; Terry et al. 1999; Wu and Wang 2006). In addition, it has been reported that mitogen-activated protein kinases (ERK and P38) and tyrosine phosphorylation induce HO-1 in some tissues (Alam et al. 2000; Chen and Maines 2000).

HO-2 is constitutively expressed under basal conditions in most human tissues and predominantly in the brain and testes. At a low level, it is also expressed in liver, myenteric plexus of the gut, distal nephron segments, and endothelium. HO-2 is mainly responsible for basal HO activity and thus CO production. It is activated by calcium-calmodulin and casein kinase 2 in neurons. Similar to NO and H_2S, CO is vastly concentrated in endothelial layer of blood vessels, which regulates the vascular tone (Wang 1998; Abraham et al. 2003; Boehning et al. 2003; Abraham and Kappas 2008).

Earlier, HO-3 was considered a pseudo gene, but following its cloning from the rat brain it was observed to be an inactive paralogue. The HO-3 protein has little HO activity and therefore its activity is uncertain and not much is known about the HO-3. It is found in the liver, heart, spleen, thymus, prostate, kidney, testis, and brain (McCoubrey Jr. et al. 1997; Hayashi et al. 2004).

17.5.1.2 Cytochrome P450

Cytochrome P450–dependent metabolism of certain xenobiotics sometimes leads to the formation of CO as a by-product. During the metabolism of dichloromethane (DCM), and methylene chloride, an increase in COHb levels in blood has been observed to result in the formation of CO. In addition, oxidative dechlorination of DCM by cytochrome P450 2E1 (CYT2E1) also results in the production of CO. The depletion of hepatic glutathione (GSH) from DCM metabolism also increased CO production. The CO that is formed by the above mechanism forms a complex with cytochrome P450 and inactivate them (D. Stewart et al. 1972; Takehito and Yoshifumi 1988; Amsel et al. 2001).

17.5.1.3 Lipid Peroxidation

Toxicological studies have revealed that inhalation of CO causes lipid peroxidation in the rat brain by neutrophil recruitment. Moreover, in vitro studies indicate that CO can be formed as a by-product of lipid peroxidation processes. Arachov et al. also demonstrated microsomal NADPH- and Fe(II)-dependent lipid oxidation also originates CO. This CO production occurred in the presence of HO inhibitors and was eliminated by inhibitors of cytochrome P450 2B4 (R Thom 1990; Archakov et al. 2002).

17.5.2 Catabolism of Endogenous Carbon Monoxide

The catabolism of the CO takes place by three mechanisms, i.e., expiration, scavenging, and oxidation, which are discussed below.

17.5.2.1 Expiration

CO is majorly expelled from the systemic production via lungs. CO diffuses through the alveolar-capillary membrane, which is mainly dependent on the amount of hemoglobin in the pulmonary capillaries and alveolar gas volume (Untereiner et al. 2012). The concentration of CO in the alveoli is determined by the partial

pressure of oxygen and CO as these two gases compete for binding of same iron site. Nowadays, CO is used to measure the heme metabolism and level of bilirubin. Moreover, the level of CO is also used clinically for the determination of jaundice in infants (Stevenson et al. 1994; Okuyama et al. 2001).

17.5.2.2 Scavenging

In normal physiological conditions, HO catalyzed heme degradation mechanism is the major source of endogenously generated CO in our body. The body store of CO is a combination of CO inhaled from the environment and xenobiotics metabolized by hepatocytes (Kubic and Anders 1978). About 80% of CO binds with Hb present in RBC and forms as COHb and other heme proteins which are accountable for the remaining CO load (Coburn 1970). The CO body stores are transferable. During hypoxia, it is reported that CO moves from blood to tissue where CO binds to heme proteins (Coburn and Mayers 1971).

17.5.2.3 Oxidation

It has been believed that oxidation of CO results in the formation of CO_2 involving cytochrome c oxidase of mitochondria. However, no studies have been reported in mammalian tissues related to its oxidation and that too under physiological conditions. The microbes that live in CO oxidize it to form CO_2 in the presence of CO dehydrogenase. Moreover, in chemistry the oxidation or of CO to CO_2 is also well established. Also the atmospheric CO reacts with hydroxyl radicals to yield HO_2 (hydroperoxyl radical) and CO_2 (Allen and Root 1957).

17.5.3 Role in Biological Systems

CO performs a diverse role in human physiology, described in the following.

17.5.3.1 CO-Mediated Vasorelaxation

Vasorelaxation by CO can be mediated by three main cellular mechanisms: (1) activation of sGC, (2) stimulation of big-conductance calcium-activated potassium channels (BKCa), and (3) NOS induction (Botros and Navar 2006; Fabiani et al. 2008; Li et al. 2008).

It has been reported that the CO releasing molecules (CORMs) dilate renal afferent arterioles to relax precontracted aortic rings, increase perfusion flow in cirrhotic liver, and decrease intra-hepatic vascular resistance (Botros and Navar 2006; Van Landeghem et al. 2009).

CO can block potent vasoconstrictors synthesis including endothelin-1 and indirectly reduce vascular resistance. Under the conditions of oxidative stress, CO act differentially, resulting in vasoconstriction. In 2009, Lamon et al. reported CO mediated constriction in the renal arteries of rats. They also demonstrated that the inhibition of pro-oxidant molecules like NADPH oxidase, NOS, and xanthine oxidase convert CO from constrictor to dilator. Therefore, it is the redox state of the cell that decides if CO acts as vasodilator or vasoconstrictor (Lamon et al. 2009).

17.5.3.2 CO-Mediated Cardiac Protection

CO is responsible for cytoprotection after vascular injury. This has been demonstrated by a number of experiments. During an ischemic injury, a 10–50 μM dose of CORM-3 results in considerable improvement in myocardial activity, leading to reduced infarct size and less cardiac muscle damage. This was further confirmed by CORM-3 inhibitor studies. In an isolated heart experiment, the cardioprotective effects mediated by CORM-3 were eliminated by adding mitochondrial ATP-dependent potassium channel inhibitor like 5-hydroxydecanoic acid (Clark et al. 2003; Cepinskas et al. 2008; Nakao et al. 2011). In an experiment in mice conducted by Clark et al. using a cardiac allograft model, CORM-3 substantially improved the survival rate of transplanted hearts. The CORM-2 can up-regulate the Bcl-2 (anti-apoptotic protein) expression and down-regulate caspase-3 activation and protect the liver from ischemia–reperfusion injury (Cepinskas et al. 2008).

17.5.3.3 Anti-Inflammatory Effects of CO

CO and CORMs have been reported to show anti-inflammatory activity. It has been reported that CO can inhibit or surmount the initiation of pro-inflammatory enzymes such as iNOS and cyclooxygenase-2 and inflammatory transcription factor NF-κB (Freitas et al. 2006; Chin et al. 2007). They also activate the signal transduction pathways like GC signaling, p38 MAPK, peroxisome proliferator-activated receptor-c (PPARc), HIF-1a, and CCAATT-enhancer-binding protein (C/EBP) -b/d to exhibit anti-inflammatory effect (Nizamutdinova et al. 2009; Lakkisto et al. 2010; Nakao et al. 2011). In addition, CO inhibits the production of prostaglandin-2, pro-inflammatory cytokines, IL-1b, -2, -6, -10, ICAM-1 and macrophage inflammatory protein-1 in macrophages, T cells, and colonic epithelial cells. Needless to say, the administration of CO or CORMs decreases cell adhesion, neutrophil movement to the site of inflammation, leukocyte rolling, and LPS (lipopolysaccharide) activated macrophagic cells (Urquhart et al. 2007; Nizamutdinova et al. 2009).

17.5.3.4 CO and Angiogenesis

It has been well established that angiogenesis process is stimulated by the vascular endothelial growth factor (VEGF). CO gas or CORMs exposure to VSMCs, human microvascular endothelial cells, rat primary cardiomyocytes, H9C2 myocytes, and human umbilical vein endothelial cells (HUVECs) increases the expression of VEGF. The mechanism of angiogenesis takes place through the involvement of p38 kinase-dependent pathway or phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin and MEK/ERK-dependent pathways. Additionally, uptake of CO-donor methylene chloride at single dose 500 mg/kg promotes blood vessel formation in the infarct heart. This process occurs via VEGF-B and hypoxia inducible factor (HIF)-1α that accelerates improvement in cardiac muscles and functions (Li et al. 2005; Lin et al. 2011).

17.5.3.5 Anti-Aggregatory Effects of CO

CO can also block platelet aggregation during vascular damage and preserve blood flow. Wagner et al. reported that monolayer of rat aortic VSMCs culture expresses increased level of inducible HO-1 when observed under high stress in a time-dependent manner. This results in increased intracellular cGMP levels in co-incubated platelets. This is further verified by inhibitor studies. The cells were treated with HO-1 inhibitor (30 μM snap-IX) that inhibits the stimulatory effect on the platelet cGMP level induced by sheared VSMCs. Likewise, under hypoxic conditions CO inhibits the production of platelet-derived growth factor-B in the endothelial cells (Wagner et al. 1997). Hence, it can be suggested that CO is a VSMC-generated messenger that can block the platelet aggregation. This aggregation is facilitated by hemodynamic forces enabling improved blood flow at damaged cardiac portions. Altogether, more experimentation is required to understand the basic mechanism of oxidative stress induced by HO-1 up-regulation as well as the role of CORMs in the inhibition of platelet aggregation under in vivo conditions.

17.5.3.6 Cell Proliferation and Apoptosis of CO

Anti-Apoptotic Effects

CO exhibits apoptosis in various cells and tissues of the body in a very selective and specific manner. For instance, it shows an anti-apoptotic to hepatocytes, cardiomyocytes, and endothelial cells, thus protecting the cells and tissues from injury. The anti-apoptotic activity of CO is dependent on p38 MAPK signaling, phosphorylation of protein kinase R (e.g., endoplasmic reticulum kinase), and/or through Akt activation (Choi et al. 2003; Clark et al. 2003; Kim et al. 2008). Besides, CO also inhibits tumor necrosis factor-(TNF) -α- and endoplasmic reticulum (ER) stress-induced cellular apoptosis. Interestingly, mitochondrial membrane permeabilization can be prevented by low CO concentrations (10–100 μM) in isolated mouse liver cells, thus stopping the formation of pro-apoptotic molecules. However, high level of CO (250–500 μM) can activate the swelling in mitochondria (Queiroga et al. 2011).

Pro-Apoptotic Effects

CO exhibits pro-apoptotic effect on fibroblasts and hyper-proliferative smooth muscle cells (Zheng et al. 2009). It also blocks the smooth muscle cells of the trachea in humans and propagation of vascular smooth muscle cell in rats both under hypoxic and normoxic states. Zhou et al. showed an inhibition of fibroblast proliferation with the decrease degree of fibrosis in mice when exposed to CO. They discovered that CO blocked the cell cycle in G0/G1 phase via a cGMP-dependent mechanism. CO at a concentration of 100–200 ppm inhibited the proliferation of VSMC at G(1)/S transition phase and obstructed the activation and production of cyclin A. Thus, the HO/CO signaling system offers an important function in the regulation of cell survival (Zhou et al. 2005).

17.5.4 Clinical Utility

This section discusses the pathophysiological implications of CO for the following diseases.

17.5.4.1 Diabetes

Diabetes is a metabolic disease distinguished by high of glucose in the systemic circulation. It can be classified as insulin-dependent (Type 1) or non-insulin-dependent (Type 2) diabetes mellitus. It has been observed that in diabetic patients there is an increase exhaled CO levels. The CO concentration in normal healthy persons is 2.9 ppm whereas it is observed to be at 4.0 and 5.0 ppm for Type 1 and Type 2, respectively. Thus, there exists a relationship between glycemia and exhaled CO levels. This observation was further validated through oral GTT (glucose tolerance test). In case of healthy humans, there is a connection between blood glucose concentrations (i.e., 3.9–5.5 mM) and amount of CO expired (i.e., 3.0–6.3 ppm). This abnormal level is restored to normal within 40 min of glucose intake (Paredi et al. 1999). This relationship was explained by Lundquist and associate in intact mouse islets wherein HO activity was initiated by glucose. In an experiment with Goto-Kakizaki rats model (i.e., defective pancreatic β-cell with HO-2 expression), low CO was observed with insulin deficiency. This study signified that HO-2 has an important function in insulin secretion and glucose metabolism (Henningsson et al. 1999).

17.5.4.2 Vascular Proliferative Diseases

VSMC hypertrophy and hyperplasia are the hallmarks of vascular proliferative diseases. The pathophysiology is due to disturbed redox signaling of cellular proliferation and apoptosis. CO arrests hyper-proliferative VSMCs, increases cell movement, and generation of bone-marrow-derived progenitor cells to shed vessels and prevent intimal hyperplasia (Raman et al. 2006; Ramlawi et al. 2007).

Hypertension

Hypertension is termed as consistent increased blood pressure distinguished by increased systolic pressure (i.e., above 150 mm Hg) and high diastolic pressure (i.e., above 90 mm Hg). It is characterized by vascular structural changes like atherosclerosis (hardening of blood vessels due to fat deposition) and co-arctation of aorta (narrowing of the aorta) which results in high resistance and elevated blood pressure. This hemodynamic stress causes initiation of HO-1 activity, which in turn stimulates the activity of sGC, and cGMP in VSMCs (Wegiel et al. 2010).

CO at a concentration of 60 ppm can decrease the hypertrophy of left ventricles and aorta in mouse model which results in the inhibition of angiotensin II-dependent hypertension. CO can reduce the phosphorylation NOX and Akt resulting in reducing ROS production, thereby protecting the cells (Ndisang et al. 2004).

Pulmonary arterial hypertension (PAH) is typified as high blood pressure of lungs. The obstruction in small arteries in the lung causes increased pressure in the vessels leading to right ventricle failure. CO has a capacity to reduce the PAH.

Kobayashi et al. demonstrated that in mice the PAH and right ventricular hypertrophy are reversed by inhalation of CO (250 ppm, 1 h/day for 2 or 3 weeks). Moreover, it also restores the pressure in the pulmonary artery and right ventricule along with pulmonary vascular structural design (Kobayashi et al. 2007).

The underlying principle in the inhibition of hypertension is the involvement of the NOS pathway. The HO-1/CO system can be up-regulated by hemin supplementation, which can be used in the treatment of hypertension. In a study in young hypertensive rats, hemin therapy was reported to decrease the blood pressure. A 21-days-based implanted hemin osmotic minipumps releasing 15 mg/kg/day provide prolonged safety against hypertension in 12-week-old SHRs. It is the accumulation of residual hemin within VSMCs that results in normalization of blood pressure of SHR. Thus, this new approach of hemin application at physiologically or therapeutic concentrations can be a novel alternative approach for the management of hypertension.

Atherosclerosis

Atherosclerosis is a disease characterized by the hardening of the arteries due to the deposition of plaque. It slowly and silently blocks arteries, obstructing the blood flow in the heart. It is one of the primary causes of strokes, heart attacks, and peripheral vascular disease. It involves inflammation, endothelial dysfunction, and vascular proliferation. It is evident that during atherosclerosis, HO-1 is up-regulated, which is important in the management of disease (Johnson et al. 2006).

CO acts as strong anti-atherosclerotic agent owing to VSMCs apoptotic properties, initiation of endothelial cell proliferation, and anti-inflammatory activity. Wang et al. reported that in balloon angioplasty-induced vessel injury in rats, CO at a concentration of 250 ppm blocked the development of atherosclerotic lesions. The CO blocks leukocyte infiltration and VSMC proliferation thereby resulting in protection of arteries from hyperplasia (Wang et al. 2001).

17.5.4.3 Myocardial Infarction

MI is also known as heart attack, which is due to necrosis of the myocardium caused by insufficient blood flow due to embolus, thrombus, or vascular spasm. In MI, cardiomyopathy occurs quickly, owing to ventricular fibrillation. CO can also give protection to heart from MI (Shahrbaf et al. 2018).

In hyperoxia-reoxygenation damage, cardiac cells model, CO reduces infarct size and protects cardiac muscle damage. Stein et al. observed that pre-exposure of CO gas (1000 ppm) in rat myocardial ischemia–reperfusion injury model decreases the affected part and also inhibits the movement of monocytes and macrophages in the infected part. The involvement of p38 MAPK and Akt–eNOS pathways, including cGMP production is responsible for the cardioprotective effects of CO on cardiac ischemia–reperfusion injury (Stein et al. 2005). In addition, inhalation of CO (250 ppm) protects the cardiac system during reperfusion after bypass and improved cardiac activity (Fujimoto et al. 2004).

17.5.4.4 Central Nervous System

It has been reported that CO at a low concentration exhibits a protective role in vascular and neuronal system of CNS. It shows its beneficial effect in cases like stroke, Alzheimer's Disease and traumatic brain injury. During various pathological conditions there is an increase level of ROS resulting in inflammation and injury to the brain cells. CO/CORMs helps to scavenge the ROS and protect the brain cells from oxidative injury and apoptosis. The neuroprotective mechanism of CO is due to either direct binding of heme protein or by indirect inducing of the HO-1. The increased level of CO up-regulates the synthesis of HO-1 which acts as a neuroprotective (Choi 2018).

Alzheimer's Disease is a form of mental deterioration characterized by beta-amyloid formation resulting in neuron death and brain tissue shrinkage. CORM also plays an important role in the management of Alzheimer's Disease. It was reported that in the human neuroblastoma SH-SY5Y, and in rat hippocampal neurons, 10 μM CORM-2 protects amyloid-β-induced toxicity (Hettiarachchi et al. 2014).

17.5.4.5 Renal Disorder

In the kidney, CO is generated by the enzymatic action of HO enzymes. The isoform HO-1 is mainly expressed in the kidney in response to various pathological and physiological stimuli. It has been demonstrated that CO and CORM inhalation therapy is beneficial in various renal injuries. It is believed that it protects the renal cells by limiting the oxidative stress and apoptosis. It increases the viability of cell by promoting the cell survival pathway. The renal vascular and tubular functions are regulated through CO in the kidney. It protects renal vessels against excessive vasoconstriction and limits the sodium reabsorption in the tubule cells, thus playing a crucial role in the regulation of blood pressure. In addition, during Cisplatin (CP) therapy of cancer, it accumulates in nephron and results in acute renal injury and nephrotoxicity. CO and CORM-3 has been reported to have anti-apoptotic effects and prevents nephrotoxicity (Tayem et al. 2006; Csongradi et al. 2012).

17.5.5 Carbon Monoxide and Various Ion Channels Interaction

Ion channels are the vital constituent of a cell, which is responsible for the activity of any pharmaceuticals. The activity of CO also depends on its interaction with various ion channels, such as K^+ channels. The K^+ channels superfamily is composed of calcium-activated KCa channels, voltage-dependent Kv, and ATP-sensitive K_{ATP}.

17.5.5.1 Carbon Monoxide and KCa Channels

In 1997, Wang et al. conducted a series of experiments in rat tail artery tissues and demonstrated evidence for a direct interaction of BKCa channels and CO. Their study claims that CO can relax artery tissues under in vitro conditions. This claim was further proved by inhibitor studies. They showed that the addition of pharmacological inhibition of BKCa channels blocks the CO-induced vasorelaxation. The

CO interacts with the histidine residue of BKCa to induce vasorelaxation by the opening of the channels. CO shows its stimulatory effects by interacting specifically with BKCa, α subunit (Wang et al. 1997).

In another set of experiments on newborn porcine cerebral arterial smooth muscle cells, CO at a sub-micromolar level increases the BKCa channel activity and this activity is independent of the cGMP pathway (Xi et al. 2004). Also, the CO activity was confirmed using BKCa channel blocking agents, e.g., TEA and iberiotoxin. They totally eliminate CO-induced vasodilation activity of the pial arterioles of newborn pigs (Leffler et al. 1999).

17.5.5.2 Carbon Monoxide and KATP Channels

Foresti et al. showed that CO released from CORM-3 (tricarbonylchloro-(glycinato) ruthenium (II)) induced a relaxation of phenylephrine-precontracted aorta tissues in a dose-dependent manner. In the above experiment, when Glibenclamide, a KATP channel blocker, was incubated for 15–30 min, it significantly reduced the vasodilator activity. But till date, no direct electro-physiology experiment has been done to investigate the direct interaction of KATP channels and CO (Foresti et al. 2004).

17.5.5.3 Carbon Monoxide and Calcium Channels (L-Type)

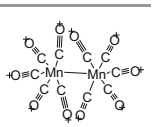
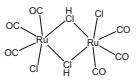
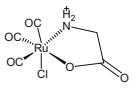
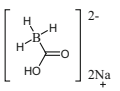
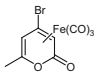
The regulatory effect of CO on L-type Ca^{2+} channels is quite controversial. CO exhibits an inhibitory activity against the cardiac L-type channel in HEK293 cells, H9c2 cells, and native rat cardiomyocytes. It is believed that CO increases the mitochondrial ROS formation and inhibits channel activation. As a result, there occurs a change in the C terminal end of L-type Ca^{2+} channel at three precise cysteine residues located at 1789, 1790, and 1810 (Hou et al. 2008). On the contrary, CO activated L-type Ca^{2+} channels in recombinant intestinal smooth muscle cells of human through NO-dependent mechanism. The variation in the cellular redox state or tissue-specific splice may be the reason behind these contradicting observations (Dallas et al. 2009).

17.5.6 Therapeutic Applications of Carbon Monoxide and CORMs

In the late 1990s, the concept of delivering low concentrations of CO gas had begun being investigated for alleviating various pathophysiological conditions characterized by oxidative stress and inflammatory states. This was a new and quite provocative proposal concept for management of pathology of disease. However, it is also evident that CO interferes in oxygen transport and its delivery. Therefore, its sustained inhalation can cause problems which are related to systemic effects and thus limiting the use of CO in therapeutics. Later on it was found that this drawback could be overcome by packing CO in a more stable chemical form called CORMs. The different types of CO and their properties are given in Table 17.3.

Initially, CO was considered a toxic gas and a “by-product” of heme metabolism in human physiology. Only recently, the pharmacology of CO has been explored in human physiology and recognized as a gasotransmitter. CO and CORMs are now

Table 17.3 Name, chemical structure, and properties of CORMs

Type of CORMs	Molecular Formula	Chemical Name	Structure
CORM-1	$[Mn_2(CO)_{10}]$	Manganese decacarbonyl	
CORM-2	$([Ru(CO)_3Cl_2]_2)$	Tricarbonyldichlororuthenium(II) dimer	
CORM-3	$(Ru[CO]_3Cl(glycinate))$	Tricarbonylchloro(glycinato)ruthenium II	
CORM-A1	$Na_2(H_3BCO_2)$	Disodium carboxylato(trihydrido)borate (1-)	
CORM-F3	$[C_9H_5BrFeO_5]$	η-4-(4-bromo-6-methyl-2-pyrone) tricarbonyl iron (0)	

considered as potential therapeutic agents exhibiting cytoprotective effects. It has been demonstrated by various preclinical models that CO and CORMs modulate inflammation, apoptosis, and cell proliferation to restore homeostasis of the body (Ling et al. 2017). Figure 17.4 illustrates the therapeutic and biological effects of CO and CORMs in different clinical indications.

17.5.6.1 Inflammation

CO and CORMs can be used in modulating immune-suppression and inflammation. They act as therapeutic agents in numerous models of inflammatory disease such as COPD, asthma, and airway hyper-responsiveness, and carrageenan-induced mesenteric inflammation. In addition, many phase II clinical trials have also validated the anti-inflammatory activity of these gasotransmitters. CORM-2 can be employed in the management of allergy as it significantly lowers the release of histamine and expression of CD203c in the mast cells of guinea pigs and human basophils (Vannacci et al. 2004).

The lipopolysaccharide (LPS)-activated murine J774 macrophagecells, stimulates the inflammatory response by the production of NO and TNF- α involving NF-κB. CORM-2 inhibits the translocation of NF-κB into the nucleus and completely eliminates the inflammatory response. Also, CORM-2 reduces the expression of inflammatory molecules like nitrite and iNOS in LPS stimulated macrophages (Lee et al. 2003). In a *Plasmodium* infected mouse experimental cerebral malaria model where the infection stimulated an inflammation, the inhalation of CO for 3 days after the infection considerably reduced the inflammation in

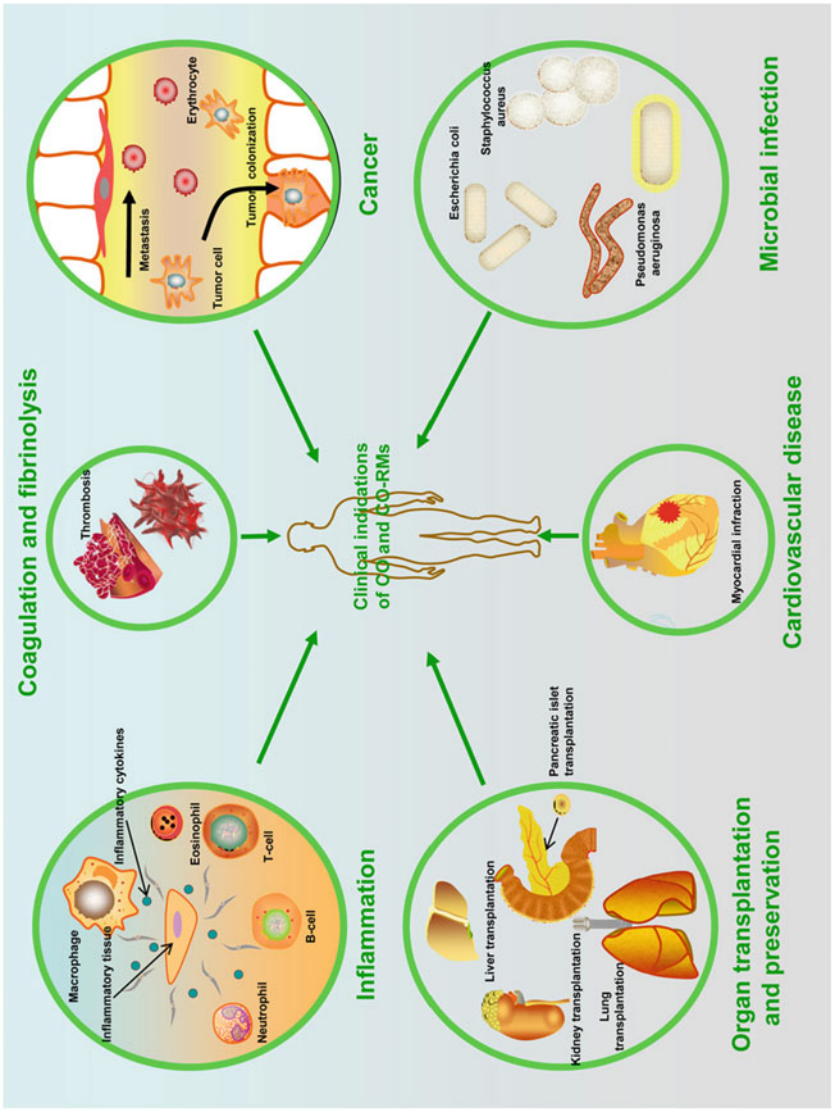


Fig. 17.4 Summary of Therapeutic Applications of CO and CORMs (Adapted from Fig. 2 of Ling et al. 2017)

mouse brain and improved the survival rate; in contrast there were heavy casualties in the control group (Pamplona et al. 2007).

17.5.6.2 Cardiovascular Disease

The HO-1/CO/CORMs have been extensively explored for the cure of CVDs. CORMs are essentially employed in the management of those diseases where the HO-1 system plays a useful and fundamental function. In HO-1 deficient mice with an abdominal aortic transplantation model HO-1/ CO/CORMs display a cardio protective role. In this study, the control mice died within 4 days of transplantation due to severe arterial thrombosis. In contrast, CORMs in mice considerably enhanced the survival rate by 62% with reduced platelet aggregation, confirming the anti-aggregatory properties (Chen et al. 2009).

The exogenous exposure of CO and intravenous infusion of CORM-3 in mouse coronary artery occlusion model presented a reduced myocardial infarct size and exhibited a substantial decrease in occurrence of reperfusion-induced tachycardia and ventricular fibrillation (VF) (Varadi et al. 2007).

In vascular proliferative diseases, CO/CORMs exhibit a notable homeostatic effect. They block the hyper-proliferation of vascular smooth muscle cells, suppress atherosclerotic lesions, weaken intimal hyperplasia, and enable re-endothelialization. In PAH, CO also exhibits its beneficial effect. In mouse models it restores pulmonary arterial, right ventricular pressure and pulmonary vascular architecture to near normal. These cardioprotective effects is due to the involvement of the BK channel, cGMP-dependent pathway, p38 MAPK, ion cardiac L-type Ca^{2+}channel, and inhibition of CYP450 (Fujimoto et al. 2004).

17.5.6.3 Organ Transplantation and Preservation

In addition to anti-inflammatory and cardiovascular properties mentioned earlier, CO and CORMs have been utilized in organ transplantations including heart, kidney, lungs, liver, and pancreatic islet. They are employed throughout the transplantation process and exhibit a beneficiary effect to the donor, organ, and recipient (Motterlini and Otterbein 2010).

In one of the heart transplantation studies in rodents, CO exposure and CORM-3 administration for 8 days prolonged the cardiac allograft survival. During organ transplantation CO and CORMs work as organ protectants by reducing ischemia/ reperfusion injury (Fujisaki et al. 2016).

In another experiment, porcine kidney was subjected to 10 min warm ischemia and 18 h of cold storage. Application of CORM-3 brought back renal blood flow and enhanced the renal function parameters such as creatinine clearance rate and glomerular filtration rate. A similar observation was also obtained in the transplantation of porcine kidney model where an excised pig kidney graft was transplanted back into the abdominal cavity after being cold flushed and stored in UW solution (an organ preservation solution) for 2 days. The CO gas bubbled kidneys had greater viability and less infiltrates (Bagul et al. 2008).

17.5.6.4 Microbial Infection

CO and CORMs exhibited antimicrobial action against varied bacteria, including gram-negative bacteria, e.g., *Escherichia coli* or *Pseudomonas aeruginosa* and gram-positive like, *Staphylococcus aureu*. CO inhibits the respiratory chain and cuts off adenosine triphosphate (ATP) supplies in bacteria, resulting in cell death. Additionally, CO activates host immune responses and promotes phagocytosis of bacteria by expression of Toll-like receptor 4. In CORMs, the inner metal core is responsible for the antibacterial activity. In one study, CORM-2 and tetraethyl ammonium molybdenum pent carbonyl bromide (ALF062) produced ROS inside the *E. coli*, causing DNA lesions and cell death. Apart from antibacterial activity, they also exhibit anti-leishmanial and anti-trypanosomal effects (Nobre et al. 2007).

17.5.6.5 Cancer

Intriguingly, in cancer therapy HO-1/CO has dichotomous function. At low concentrations, HO-1/CO, in cancer cells exerts cytoprotective, pro-proliferative and pro-angiogenic property. However, at high concentration, CO exhibits antitumor activity. HO-1 overexpression has been established in several tumor cells such as pancreatic cancer, prostate cancer, melanoma, and Kaposi sarcoma. Generally, CO at the physiological dose can be formed via tumor cells or tumor-infiltrating macrophages to produce a pro-tumor effect. Therefore, inhibition of HO-1 can be utilized for anticancer therapy. For example, in a mouse cancer model small interfering RNA (siRNA) mediated HO-1 silencing results in lower tumor growth rates and angiogenesis. Inhaled CO or CORMs at a higher concentration could be lethal to tumor cells (Simon et al. 2011).

In a CD1 athymic mouse model inhalation of 500 ppm CO for 1 h a day considerably lowers the cancer cell growth and the resultant angiogenesis. The anti-tumor activity might be due to mitochondrial ROS generation, acceleration of oxygen consumption, inhibition of cellular protein synthesis, and reduction of cellular antioxidants. The high level of CO is toxic to the normal tissue; therefore, these preclinical data may not be clinically valid (Wegiel et al. 2013).

17.6 Conclusions

The risk-to-benefit ratio of the gaseous molecules as transmitters is quite low if maintained at lower concentrations. Based on their specificity of molecular mechanism they can be widely utilized for various pathological conditions of the body. The cellular interactions are pivotal for understanding the signaling of these gaseous molecules comprising of NO, CO, and H2S. Besides, ammonia is being the latest addition and methane is under consideration as a likely candidate. The European Network on Gasotransmitters has set up basic regulations for advanced research in order to achieve enhanced therapeutic activity. The vital role played, especially, by NO and CO is at the basis of importance of these gasotransmitters.

NO being the first signaling molecule has provided the underlying concepts in signal transduction. NO has been recognized as a physiological key element for

several pharmacological functions of the human body. Diverse kinds of NO (nNOS, eNOS and iNOS) associated with varied biological activities have been reported for modulating and transducing the signals within the cells. NO is important for regulating various disorders in different organs like, brain, kidney, liver, heart, etc. Besides, NO is also crucial for modulating the tumor activity and immune system.

CO was initially considered an environmental hazard and harmful to human health. However, its physiological role became evident in the early 1990s in various pathophysiological conditions. In order to prevent high dose toxicity, a chemically stable form of CO is produced endogenously termed as CO-releasing molecules (CORMs). CO and CORMs are now considered as potential therapeutic agents that exhibit cytoprotective effects.

Further, it has been demonstrated by various preclinical models that CO and CORMs modulate inflammation, apoptosis, and cell proliferation to restore homeostasis of the body. Lately, it has been reported in the treatment of renal disorder, CNS regulation, cardiovascular disorder, cancer, and microbial infection. Moreover, the utilization of exhaled CO as a biomarker in metabolic syndrome like diabetes could be a newer approach to measure the disease severity and assess the drug therapeutic efficacy.

Thus, with the advancement of research on various gasotransmitters and exploring their mechanism of action, a new gateway will be opened for the treatment of various pathological conditions. In future, this will help in drug designing, prevention and treatment of different types of diseases.

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Sodium Channels: As an Eye of the Storm in Various Clinical Pathologies

18

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Abstract

Voltage-gated sodium ion channels are essential to maintain the excitability and activity of neurons and neuronal network. Several studies have been done to explore the basic properties of ion channels, their existence and physiological characteristics. Till date we know that 11 genes are responsible for encoding of 9 families of sodium channels (Nav 1.1 to Nav 1.9) and are classified according to varying degrees of sensitivity to Tetrodotoxin (TTX). These are localized in various sites such as skeletal muscles, central nervous system (CNS), cardiac muscles, and peripheral sensory neurons. Any aberration in its structure and function leads to various clinical pathologies such as channelopathies (where dysregulation in receptors are directly responsible for initiation and progression) and diseases contributing to dysregulation of expression of sodium channels cause various neurological disorders. In this chapter, we emphasize the composition, function, and regulation of sodium channels at the molecular level and the crucial role of sodium channels in the development and progression of various disease pathologies such as epilepsy, schizophrenia, familiar hemiplegic migraine and neuropathic pain.

Keywords

Voltage-gated sodium channels · Composition · Regulation · Channelopathies · Neurological disorders · Neuropathic pain

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Abbreviations

AIS	Axon initial segment
ARC	Activity-regulated cytoskeleton-associated protein
BFNIS	Benign familial neonatal-infantile seizures
ChIPs	Channel Interacting Proteins
CNS	Central nervous system
DRG	Dorsal root ganglia
EE	Epileptic encephalopathy
FGF	Fibroblast growth factor
FHF	Fibroblast homologous factor
FHM	Familiar hemiplegic migraine
IE	Erythromelalgia
IFMT	Isoleucine, Phenylalanine, Methionine, and Tryptophan
IHC	Immunohistochemistry
PEPD	Paroxymal Extreme Pain Disorder
PNS	Peripheral Nervous System
Nav	Voltage-gated sodium channel
NMDAR	N-methyl-D-aspartate receptor
TTX	Tetrodotoxin
VGSCs	Voltage-Gated Sodium Channels

18.1 Introduction

Voltage-gated sodium ion channels are required for excitation of cells. Their history can be traced back to 1941, when Kenneth S. Cole and Richard F. Baker confirmed the existence of voltage-gated membrane pores in giant squid axon (Cole and Curtis 1939). Alan Hodgkin and Andrew Huxley won the Nobel prize for their work on basic properties of ion channels and their mechanisms of excitation and inhibition in 1952. Later in 1970 and 1981, the existence and physiological characteristics of ion channels were explored by Bernard Katz, Ricardo, and Miledi et al. and Erwin Neher and Bert Sakmann et al., using noise analysis and patch clamp techniques, respectively (Cole and Baker 1941; Roger et al. 2015).

So far, 11 genes are responsible for the encoding of 9 families of sodium channels (Nav 1.1 to Nav 1.9). These genes are classified according to varying degrees of sensitivity to Tetrodotoxin (TTX) and can be traced to four paralogous locations in the chromosome segment (Ambrose et al. 1992; Wang et al. 1992; Burgess et al. 1995; Beckers et al. 1996; Kozak and Sangameswaran 1996; Plummer et al. 1998; Catterall et al. 2005; Bagal et al. 2015) (Table 18.1). More than 20 exons encode for 9 sodium channel α-subunits. Nav channels (1.1, 1.2, 1.3, and 1.7) are situated on the second chromosome in both humans as well as in the mouse whereas Nav channels 1.5 are situated at 2q24 and in case of mouse, present on chromosome 2. And the Nav 1.8 and 1.9 channels are present on 3p21–24 in case of humans and chromosome 9 in the mouse. Skeletal muscle contains the Nav 1.4 and the CNS contains the

Table 18.1 Classification of voltage-gated sodium channels based on location and tetrodotoxin sensitivity (in human and mouse)

Nav channel subtype	Location		Tetrodotoxin sensitivity	References
	Human	Mouse		
Nav 1.1	Chromosome 2	Chromosome 2	Sensitive	Catterall et al. (2005)
Nav 1.2	Chromosome 2	Chromosome 2	Sensitive	Catterall et al. (2005)
Nav 1.3	Chromosome 2	Chromosome 2	Sensitive	Catterall et al. (2005)
Nav 1.4	Chromosome 17	Chromosome 11	Sensitive	Ambrose et al. (1992); Catterall et al. (2005); Wang et al. (1992)
Nav 1.5	2q 24	Chromosome 2	Resistant	Catterall et al. (2005)
Nav 1.6	Chromosome 12	Chromosome 15	Sensitive	Burgess et al. (1995); Catterall et al. (2005); Plummer et al. (1998)
Nav 1.7	Chromosome 2	Chromosome 2	Sensitive	Beckers et al. (1996); Catterall et al. (2005); Kozak and Sangameswaran (1996)
Nav 1.8	3p 21–24	Chromosome 9	Resistant	Catterall et al. (2005)
Nav 1.9	3p 21–24	Chromosome 9	Resistant	Catterall et al. (2005)

Nav 1.6. The genes of Nav 1.4 are situated on the 17th chromosome (humans) and 11th chromosome (mouse) whereas genes of Nav 1.6 can be traced to the 12th chromosome (humans) and the 15th chromosome (mouse) (Catterall et al. 2005; Mantegazza and Catterall 2012). All the sodium channels structures are analogous to each other, but few amino acid replacements confer this resistance, for example, in Nav 1.5 which is predominantly localized in cardiac muscles, substitution of phenylalanine to cystine in pore area provides 200 times reduction in sensitivity to TTX (Yamagishi et al. 2001). A similar replacement is also observed in Nav 1.8 and Nav 1.9, which are localized in peripheral sensory neurons where phenylalanine is replaced by serine, which provides even greater resistance (Mantegazza and Catterall 2012). Channelopathies are the group of diseases characterized by any aberration in the structure or function arising from a mutation in the genes encoding for sodium channels. In this chapter, we will study the composition, function, and regulation at the molecular level of sodium channels and the crucial role of sodium channels in the development and progression of various disease pathologies (Roger et al. 2015).

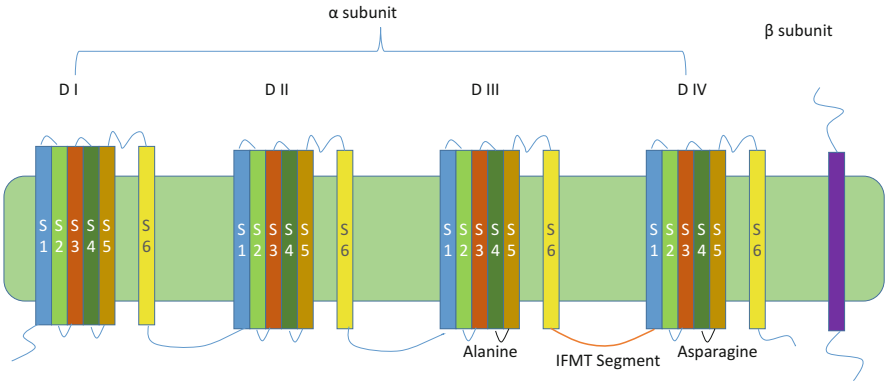

Fig. 18.1 Composition of sodium channel: α subunit (Domains- I, II, III & IV); β subunit and IFMT segment (Isoleucine, Phenylalanine, Methionine, and Tryptophan)

18.1.1 Composition of Sodium Channels

Sodium channels are heteromeric integral glycoproteins composed of two types of subunits, α (initiator) and β (regulatory). Each α subunit contains six segments, which houses four domains (DI-DIV) around the central pore. Arginine-rich segment 4 (S4) is also known as the "voltage sensor," and the hairpin segment (P loop) forms the "ion filter" between segment 5 (S5) and segment 6 (S6) responsible for ion permeability (King and Vetter 2014). There is a group of amino acids called IFMT, segment "I" Isoleucine, "F" Phenylalanine, "M" Methionine, "T" Tryptophan, present between segment 6 of DIII and Segment 1 of DIV (Fig.18.1). This IFMT segment has an affinity to bind to hydrophobic amino acids (like alanine and asparagine) between segment 4 and segment 5 of DIII and DIV; this binding is responsible for the refractive nature of the ion channel (Tata 2013). Pore consists of tubular vestibule that forms a cavity, ion-selective filter, and intracellular gate activator. There are two models that explain the organization of voltage-gated sodium channels: (1) Sliding helix and (2) Helical screw. Both models suggest the presence of positively charged residues in segment 4 that serves as gating (Frank and Catterall 2003). These positively charged residues are held together by corresponding residues having negative charge, of segments 1, 2, and/or 3 in active state. But in the inactive state, these residues are held inside due to the Coulomb force of negatively charged resting membrane potential. When depolarization takes place, changes in membrane polarity occur, which results in relieving of this electrostatic force. As a result, these positive, rich residues move out opening the pore, causing an influx of sodium ions, thus initiating the cell activation process. The outward movement of segment 4 is "voltage dependent." On the other hand, all the downstream mechanisms are "voltage independent."

β subunits are integral regulatory proteins found to be associated with the α subunit. It consists of three domains: extracellular, transmembrane, and intracellular domains. β subunits are served as cell adhesion molecules that are responsible for

control of the surface expression of voltage-gated sodium channels, cell-to-cell communication and cellular migration. β1 subunit in association with **Neurofascin 186** and **Contactin** provides increase in surface expression of voltage-gated sodium channels. β1 subunit knockout leads to disruption of neuronal–glial interaction and reduction in the number of Nodes of Ranvier in myelinated neurons which results in disrupted saltatory conduction (Marban et al. 1998).

18.1.2 Molecular Functioning of Sodium Channels

Voltage-gated sodium channels (VGSCs) occur in three forms: (1) active (2) refractory, (3) closed. From closed state to go on to open state requires depolarization, changes in membrane polarity to positive, which usually happens during an action potential, and within a few milli-seconds VGSCs progress to refractory state, also known as the inactive state, is a mechanism that protects cells from excessive stimulation. This is followed by the closed state when the cell membrane attains the normal repolarized potential. During closed state, the "voltage sensor" segment 4 is held inside due to the overall negative resting membrane potential of the cell. The depolarization relieves the electrostatic force on segment 4 pushing it to move out, thus opening the intracellular ion gate. This allows sodium ion conductions, leading to increased sodium concentration inside the cell. When the intracellular gate is opened, the IFMT segment binds to hydrophobic amino acids (like alanine and asparagine) between segment 4 and segments 5 of DIII and DIV, as there is no steric hindrance caused by intracellular ion gate due to conformational change. This state is called a refractory state/inactive state. In the inactive state, sodium ions cannot pass through causing prevention of the cell from overstimulation. From a refractory state to go to closed state again requires a change in membrane potential. Whenever cell resting membrane potential is restored, segment 4 domain is attracted toward the cell, augmenting its electrostatic influence on segment 4 resulting in a conformational change to the closed ion channel (Catterall 1992; Marban et al. 1998) (Fig. 18.2).

18.2 Regulation of Voltage-Gated Sodium Channels

Channel Interacting Proteins (ChIPs) are endogenous proteins that interact with voltage-gated Na^+ channels. Some examples of ChIPs are connexion 43, Caveolin 3, Calcium-calmodulin kinase 2, ankyrins, telethonin, plakophilin, neuronal precursor cell-expressed developmentally downregulated 4 (nedd4), fibroblast growth factor (FGF) and its homologous factors (FHFs). These endogenous proteins regulate the sodium channel expression and its functioning (Savio-Galimberti et al. 2012).

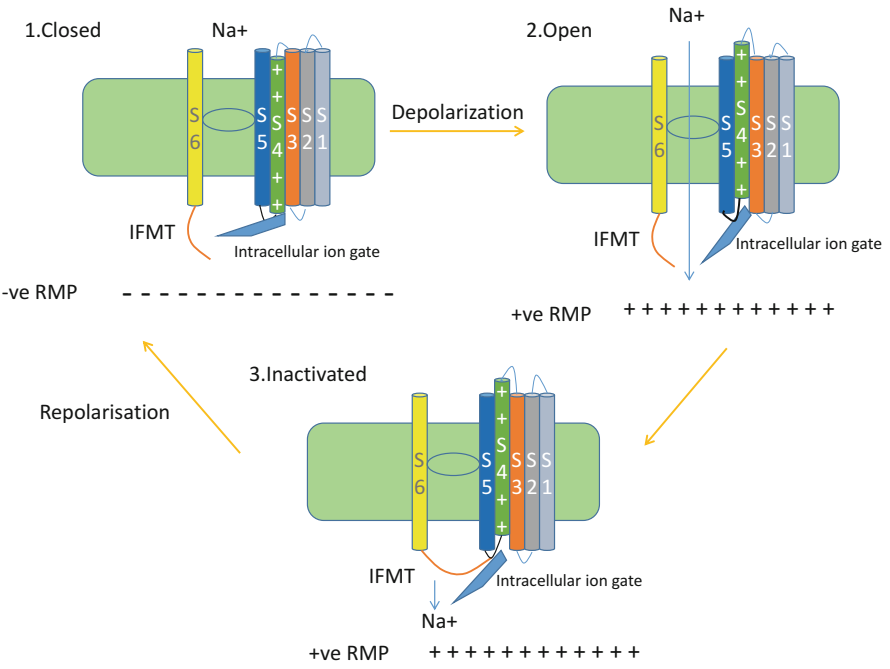

Fig. 18.2 Molecular functioning of sodium channels: Closed, opened and inactivated state; IFMT segment (Isoleucine, Phenylalanine, Methionine, and Tryptophan), RMP; Resting Membrane Potential

18.2.1 Cellular Trafficking and Surface Expression of Voltage-Gated Sodium Channels: Caveolae/Caveolin Interaction-Kinesin-VGSCs Interaction

Caveolae are membrane folding proteins predominantly found in muscle tissues, implicated for surface expression of number of receptors and Caveolins are linker proteins responsible for the rapid surface expression of sodium channels. These Caveolin proteins act as linker proteins and cause recruitment of voltage-gated ion channels from the intracellular pool following the cell stimulation. Kinesins are the molecular motor proteins responsible for the delivery of newly synthesized receptors (like VGSCs) vesicles from the nucleus, rough endoplasmic reticulum and Golgi body to the synapse, creating the intracellular pool of vesicles containing receptors. This intracellular pool of vesicles containing receptors is dynamic in nature. When a cell is stimulated, these caveolin proteins act as linker proteins causing mobilization of these vesicles and with the help of caveolae proteins, cause surface expression of voltage-gated ion channels receptors. Some recent studies show that kif 1B and kif 5A, kif 5B are kinesin members of kinesin 3 and kinesin 1 families respectively which are implicated in the cellular trafficking of voltage-gated ion channels like Nav 1.8. Mutation or overexpression of these genes and proteins leads to

neuropathic conditions like Charcot and Marie tooth disorder and neuropathic pain. Another study proved that β cell stimulation leads to increased surface expression of VGSCs. This is due to intracellular VGSCs pool mobilization by caveolin 3 and caveolae membrane proteins, VGSCs are surface expressed (Savio-Galimberti et al. 2012; Roger et al. 2015).

18.3 Sodium Channel and Related Clinical Pathologies

Sodium channel as discussed above is crucial for the functioning of any excitable cells, and any aberration in its structure or functioning leads to various clinical pathologies. There are two types of disorders that are classified based on the role played by the sodium channels on pathogenesis of diseases: (1) channelopathies (where dysfunctional receptors are directly responsible for initiation and progression) and (2) disease contributing to dysregulated expression of sodium channels (like cancer, neurological disorders) (Kaplan et al. 2016).

18.3.1 Function of Sodium Channels in the PNS and CNS

Sodium channels are important to maintain the excitable character as well as the activity of neurons and the neuronal network. They are ubiquitously expressed throughout the neurocyton and axons in neurons. Denseness of sodium channel is more at Nodes of Ranvier and axon initial segment (AIS), which is responsible for speeding nerve impulse conduction. This phenomenon is often referred to as saltatory conduction (Kaplan et al. 2016). The action potential is initiated from AIS and this characteristic is attributed to relatively high sodium channel receptor density at this site. Various IHC studies have validated this claim of determining distribution of sodium channel expression along AIS. In such studies Nav1.6 was densely expressed at distal AIS of retinal ganglion cell of rat, whereas Nav1.1 was aggregated at proximal AIS. Similar trend in expression was observed in cortical pyramidal neurons of rat showing increased Nav1.2 and Nav 1.6 at AIS. Hyperpolarization of NaV1.6 that depend on NaV 1.2 activity results in differential expression of sodium channel. When depolarlizing signals enter AIS, activation threshold of Nav 1.6 crosses more readily as compared to Nav1.2, which consequently generates action potential. This causes two types of currents: the first one propagates down the axon and the other results in backpropagating currents, which leads to activation of Nav1.2. This action potential is propagated from soma to axons and dendrites. The backpropagating currents are crucial for relating the neuronal output and regulating neuronal platicity. Dendritic VGSCs also contributes to action potential by amplifying the depolarlization response. Collectively, this data suggests the complex relationship of sodium channels distribution and neuronal activity. The data also hints at the susceptibility of brain networks to any aberrations in sodium channels function and its number by mutations, disorders, or action of drug (Kaplan et al. 2016).

18.3.2 Dysfunctional and Dysregulated Expressions of Sodium Channels as a Cause for Neurological Disorders

Channelopathies are the group of disorders arising from a mutation in genes encoding for voltage-sensitive ion channel or dysfunctional receptors arising from an autoimmune attack. This condition has been indicated for several diseases including epilepsy, Dravet Syndrome, complications associated with pain like congenital pain insensitivity, paroxysmal extreme pain disorder, primary erythromelalgia and cardiac arrhythmia (characterized by long QT syndrome), slow ventricular conduction and atrial standstill. Aggressive nature of breast (Nav1.5) and prostate cancer (Nav1.7) has been related with change in epigenetic regulation. Dysregulation of sodium channels has also been over-served in diabetes and chemical-induced neuropathies, neuromuscular disorders (like Eaton Lambert syndrome, Charcot Marie Tooth disorder) and various neurodegenerative disorders (like Alzheimer's, Parkinson's, and Schizophrenia). Sodium channels have been an important target due to their major role in these diseases.

18.3.3 Role of Sodium Channels in Epilepsy

Analysis of mutation in VGSCs in epilepsy has shown a complex data that results in a specific pattern showing connection between channel mutation and epilepsy. In VGSCs of interneuron, there are alterations in channel biological organization that are cell dependent in nature; for example, there is a loss of function in Nav1.1 or Nav1.6 due to depolarization in shifts in the voltage dependence of activation, hyperpolarization in shifts in the voltage dependence of inactivation, and haploinsufficiency (Hargus et al. 2013). Contrastingly, alteration in pyramidal cell channels in NaV1.2 or NaV1.6 leads to depolarization of shifts in the voltage dependence of inactivation and augmentation in persistent current (Ye et al. 2018). Haploinsufficiency in *SCNIA* triggers Dravet Syndrome (Bechi et al. 2012) and functional dropping in *SCN1B* (Patino et al. 2009). The function in *SCN2A* is associated with BFNIS (Misra et al. 2008). The functional gaining in *SCN8A* is related to epileptic encephalopathy and functional loss is related with its nonoccurrence (Martin et al. 2007). In patients of epilepsy, additional potential pathological mechanisms have been suggested, but not yet specifically identified. Mutations in the voltage sensor in votage-gated sodium channel result in exchange of the amino, acid, i.e., arginines (charged) with residues (neutral). As a result, voltage sensor leads to development of a leak current (omega/gating pore current) (Sokolov et al. 2005). In spite of the fact that it is not seen in epilepsy patients till now, in hypokalemic periodic paralysis, $Na_v1.4$ mutation is associated with an increase in omega current, proposing involvement in channelopathies (Sokolov et al. 2007). Resurgence of sodium current is proposed to be involved in epileptical conditions. In some subtypes of voltage-gated sodium channels, by membrane repolarization followed by prolonged depolarization pulse, a minor transient ingoing current can be attained (Raman and Bean 1997). Augmented resurgent current amplitude has

been related to augmentation in action potential firing frequency, proposing it as a possible pathological mechanism in epileptical conditions (Jarecki et al. 2010).

Some important advancement has been made in psychiatric disorders treatment and diagnosis. From data it has been supported that these disorders occur due to interplay of environmental and genetic risk factors. These disorders are caused by structural and functional damage in several brain areas such as the thalamus, amygdala, midbrain, and the prefrontal cortex. Since the early 1990s, gene mapping of ion channels have been done on human chromosomes, and identification of mutations in these genes has been performed. Many neurological disorders like epilepsy, migraine, and episodic ataxia are caused by dysfunction of brain electrical circuits, which has been attributed to ion channel mutations. Hence, it has been suggested that any change in activity of ion channel in the respective brain regions has been associated with psychiatric disorders etiopathology.

18.3.4 Sodium Channels Involved in Schizophrenia

Schizophrenia is a psychotic disorder with 1% prevalence worldwide. Symptoms involve negative symptoms (decrease in social interaction, anhedonia), positive symptoms (hallucination and delusion), and cognitive impairment. Many evidences have increasingly proposed that schizophrenia is a brain development and plasticity disorder, which involves strong alteration in activity and excitability of substantia nigra, hippocampus, ventral tegmental area, and prefrontal cortex. Recently, genetic linkage and association studies suggested risk genes for schizophrenia, including some ion channels genes (Imbrici et al. 2013).

By targeting the sequence of 10,198 samples, Rees et al. 2019 confirmed irregularities of activity of neuron and associated voltage-gated sodium channels in pathogenesis of schizophrenia. This is a sequence-based study that includes the schizophrenia of rare coding variants in neuronally expressed genes, including N-methyl-D-aspartate receptor (NMDAR) complexes and activity-regulated cytoskeleton-associated protein (ARC); but, bigger sample number is essential to disclose novel genes and particular biological mechanisms. In this study, they have sequenced 187 genes in a new dataset of 5207 cases and 4991 controls, which are selected on the basis of previous information related to schizophrenia. These genes were involved as members of ARC and NMDAR postsynaptic protein complexes, including voltage-gated sodium and calcium channels. A data for a total of 11,319 cases, 15,854 controls, and 1136 trios has been published sequentially and on the basis of this data, a rare variant meta-analysis was conducted. From this data, it was found that there is no single gene significantly involved in schizophrenia but exonic variants in the ARC ($p = 4.0 \times 10^{-4}$) and NMDAR ($p = 1.7 \times 10^{-5}$) synaptic complexes are a significantly involved risk factor for schizophrenia. Other than this, in this study it was also found that loss-of-function variants and missense variants at paralog-conserved sites were enhanced in voltage-gated sodium channels, specially the alpha subunits ($p = 8.6 \times 10^{-4}$) involved in schizophrenia. From this study it was evidenced that multiple voltage-gated sodium channels are involved in

schizophrenia pathogenesis and verify the involvement of ARC and NMDAR postsynaptic complexes (Rees et al. 2019).

18.3.5 Sodium Channels Involved in Familial Hemiplegic Migraine (FHM)

Familial hemiplegic migraine (FHM) can be autosomal-dominant in nature although its occurrence is rare (Watanabe et al. 1971). Manifestation of illness includes visual and speech hampering, aura and short duration of motor lethargy to some extent. Mutations of genes like CACNA1A, ATP1A2, and SCN1A through encoding of various ion transporting proteins are involved in occurrence of FHM. When compared with migraine arising due to periodic seizures of diseases associated with nervous system occurrence of FHM is atypical in nature caused due to alteration in a typical gene. Mutation of gene present in channel generated by calcium ion was the prime gene to be recognized and termed as CACNA1 α1-subunit and was primarily responsible for FHM1, which is most affected by the mutation where encoding gene of catalytic α2 subunit, present in Na+/K+-ATPase and termed as ATP1A2 gets mutated and around one-fifth of FHM2 families gets affected. Alteration of SCIN1A gene (Q1489K and L1649Q) has been found to cause FHM3. Formation of pores in α subunit of neuronal voltage-dependent gated sodium ion channel occurs through this gene (Dichgans et al. 2005; Carreño et al. 2013). As per current reports, atypical occurence of gene alteration leading to FHM3 was observed in a Chinese household on a 62-year-old woman (Shao et al. 2018). Earlier manifestation was transient ischemic attack which were ultimately identified as FHM having c.4495T>C alteration observed in SCN1A gene. The case study report ultimately deduced about role of SCN1A gene in FHM pathophysiology. Another study also reported occurrence of FHM3 through alteration in voltage-dependent sodium channel gates Nav 1.1 which is being encoded by SCNIA gene. This study was conducted to determine molecular flaws that are inheritable in nature and occur due to alteration related to FHM3 (L263V, Q1489K, and L1649Q). L1649Q is one of the mutations that failed to produce measurable current because it significantly reduced cell surface expression. Production of pronounced currents by two mutations occurs through co-expression of human beta1 and beta2 accessory subunits by tsA201 cells. Mutations when compared with WT-Nav1.1 showed principal depletion of functional phenotype that was additionally expressed by depletion of channel accessibility during consecutive stimulation. Data suggested that Q1489K mutation causes high current in continuation and decelerates the recovery from fast and slow inactivation as well as increased entry into slow inactivation. L263V, on the other hand, showed augmentation in functional characteristics which included detained entrance to slow inactivation, retarded entry but accelerated recovery in case of fast inactivation as well as high current in continuation. The two alternations (Q1489K and L1649Q) associated with typical FHM generated either full or partial loss in producing the outcome. On the other hand, L236V, which is an alteration, leads to

attainment of function and was associated with FHM as well as increased occurrence of generalized epilepsy (Kahlig et al. 2008).

18.3.6 Sodium Channels Involved in Neuropathic Pain

From the generation of action potential to its dissemination, voltage-dependent sodium ion channels perform a pivotal role. These channels are further identified as the genes that codes them from Nav1.1 to Nav1.9 with further subclassification based on function as genes that are sensitive to tetrodotoxin- (TTX-S) or genes that are resistant to tetrodotoxin (TTX-R). These are present on primary afferent sensory neurons and recent pharmacological and genetic data has provided evidence of the involvement of Nav1.3, 1.7, 1.8, and 1.9 in nociceptive transmission. Out of these, Nav1.8 is a TTX-R sodium channel that is highly localized on primary sensory afferent neurons.

18.3.6.1 Nav 1.3 Channel

$Na_v1.3$ can be termed as sodium channel sensitive to tetrodotoxin and is found in nerve cells of embryo but is also present in axotomized sensory nerve cells (Waxman et al. 2017). Nav1.3 channel produces fast stimulating and nonstimulating kinetics and quick retrieval from nonstimulating phase. Its presence has also been confirmed in distal tips of axon in experimental neuromas in rats and in human neuromas. Hyperresponsiveness and unpremeditated firing of neurons was observed with an increase in pain and altered electrophysiologal properties as manifestation when Na 1.3 was upregulated, whereas down-regulation of channel showed pain in trigeminal nerves in trigeminal ganglionic regions in ferret (Nassar et al. 2006). Protein and mRNA level of Nav1.3 was found to be diminished following administration of antisense oligodeoxynucleotides through intrathecal route resulting in reduction of hyperexcitability of DRG neurons and reduced pain in nerves after injury of spinal cord and sciatic nerve (Lindia et al. 2005).

18.3.6.2 Nav1.7 Channel

Nav1.7 channel is sodium channel sensitive to tetrodotoxin and present largely in sensory neurons and sympathetic ganglia providing fast stimulating and nonstimulating kinetics but in turn causes considerably slower recovery period from fast inactivation making it different from other tetrodotoxin sensitive channels. Down-regulation of mRNA and protein in Nav1.7 channels was done through tying of spinal nerve of neuropathic pain. Mutations that cause gain of function of SCN9A coding for sodium channel Nav1.7 are present in patients suffering from critical pain syndrome inherited erythromelalgia (IE) (Cregg et al. 2013) and paroxysmal extreme pain disorder (PEPD) (Dabby et al. 2011), whereas mutations that cause loss of function of SCN9A gene have been found in patients suffering from inherited insensitivity to pain and disabled sense of smell. Inherited erythromelalgia is specified by burning pain and hot skin flashes, which causes change in hyperpolarization of voltage, which in turn effects the activation. Paroxysmal extreme pain

disorder is manifested by extreme pain and burning sensation in rectal, ocular and submandibular region, which causes change in depolarizing voltage, which in turn effects steady-state inactivation.

18.3.6.3 Nav1.8 Channel

Nav1.8 channel is present mainly in sensory neurons of small diameter and trigeminal ganglionic neuron. This channel is tetrodotoxin resistant in nature and produces lower time period of depolarized stimulation and steady-state voltage-dependent nonstimulating kinetics but has high rate of retrieval from nonstimulation state. Depolarized stimulation of Nav1.8 sodium channel leads to upstroke in action potential by 80–90% of inward channels of sensory neurons from Nav1.8 null mice (Harty and Waxman 2007). Ongoing nociceptive unit can regulate both biophysical properties of Nav1.8 as well as its expression. Patients suffering from chronic pain due to dysfunction of nervous system or with increased sensitivity to chronic pain in local areas had increased content of Nav1.8 channels in regions having proximity to peripheral injury site. Manifestation of inflammatory mediators leads to altered level of tetrodotoxin channel with stimulating threshold shifted toward more negative potential through stimulation of protein kinase A. Increase in magnitude of Nav1.8 mediated channel is caused by prostaglandin E2, adenosine, and serotonin, which results in shift of relation between conductance of voltage toward negative spectrum and increasing time period of stimulating and nonstimulating of sodium channels in sensory neurons having small diameter (Black et al. 2004; Wood et al. 2004).

18.3.6.4 Nav1.9 Channel

$Na_v1.9$ channel is sodium channel resistant to tetrodotoxin and is present mainly in sensory nerve cells having smaller diameter. Nav1.9 channel provides voltage-dependent activation close to the potential of membrane present in the resting state (-70 mV). Steady-state nonstimulation occurs at comparatively positive potential (-45 mV) (Shah et al. 2010). These kinetic properties suggest that activation of Nav1.9 may prolong response to subthreshold depolarization and generate a persistence channel. Experiments on electrophysiology on sensory neurons of Nav1.9 null mice results in lowering of threshold for action potential electrogenesis and produces persistent channels leading to hyperexcitability. Inflammation leads to increased expression of Nav1.9 gene along with Nav1.9 mRNA, which ultimately results in substantial down-regulation of proteins in axotomized afferent neurons and several in vivo models that are suffering from pain in the somatosensory nervous system (Lolignier et al. 2011).

18.4 Conclusion

Normal functional and number of sodium channels are crucial for the functioning of any excitable cells. This, when mutations or disorder arise, which disturbs this balance, leads to various clinical pathologies. Channelopathies are disarrays arising

due to alternations in sodium channels. These disorders include manifestations like epilepsy, Dravet Syndrome, complications associated with pain, like loss of sensitivity to pain from birth, primary erythromelalgia and manifestation of paroxysmal extreme pain disorder, irregular heartbeat (signified by extreme QT syndrome), slow ventricular conduction, and atrial standstill. Aggressive nature of prostate (Nav1.7) and breast cancer (Nav1.5) has been associated with alteration in epigenetic regulation. Sodium channel upregulation has also been involved in diabetes and chemical induced neuropathies, presence of neuromuscular anomalies like Eaton Lambert Syndrome, Charcot Marie tooth disorder along with several disorders that are neurodegenerative in nature like Alzheimer's, Schizophrenia, and most importantly Parkinson's. Sodium channels are an important target due to their main role in these diseases. Dravet Syndrome (DS) also leads to haploinsufficiency of SCN1A along with functional loss of SCN1B. On the other hand, functional gain of SCN2A is associated with BFNIS. Likewise, functional gain of SCN8A is associated with epileptic encephalopathy (EE). Substitution of arginine I by uncharged amino acid residues will lead to alteration in VGSCs voltage sensor and hence lead to outflow in dispensing of current or alteration in sodium channel function resulting in resurgence of inward current, leading to increase in neuronal firing frequency. All these changes are some responsible factors for epilepsy. Sodium channels are also associated with development of psychotic diseases such as Schizophrenia, alteration in activity of hippocampus, substantia-nigra, ventral tegmental area, and prefrontal cortex, which in turn are manifestations associated with the development of Schizophrenia. Dysregulation in activity of neuron and voltage-gated sodium channels (VGSCs) was found to be involved in the development of Schizophrenia through targeted sequencing of 10,198 samples. Development of familial hemiplegic migraine (FHM) was also found to be related to sodium channels. FHM, which manifests by transient motor weakness to some extent and difficulty in having conversation along with optical disturbances is an autosomal dominant type disease although its occurrence is rare. Alterations in ATP1A2 that encodes for Na+/K + -ATPase dependent alpha2 subunit, which is catalytic in nature, and SCN1A (encodes for pore region of Nav1.1) are some of the etiological factors involved in the pathogenesis of FHM. Dysregulation of sodium channels (predominantly Nav1.5, Nav1.7, Nav1.8, and Nav1.9) leads to hypersensitivity of nervous system, which leads to hyperalgesia and allodynia observed during neuropathic pain. In another study, alternations that lead to functional gain of SCN9A gene, which in turn encrypts sodium channel Nav1.7, was found to be expressed in patients suffering from diseases associated with pain manifestations such as inherited erythromelalgia (IE) and paroxysmal extreme pain disorder (PEPD). On the other hand, functional loss of SCN9A is observed in patients with inborn insensitivity to pain and impaired sense of smell. Sodium channels targeting is an emerging concept that provides help in mitigation of various clinical disorders. However, there has been more than 70 years of research in understanding molecular dynamics and the functioning of sodium channels. The drugs that effectively target specific Nav channels and that are able to modulate its expression are yet to be discovered. Kinesins and Caveolae/Caveolin interaction plays an essential role in maintaining required number of functional VGSCs. Drugs

that are able to target these markers can be developed or repurposed as an alternative safe therapy for treatment of channelopathies.

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Pharmacology of Potassium Channels

19

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Abstract

Potassium channels constitute the largest and most ubiquitous and diverse ion channel family. They regulate several physiological processes including electrical signalling, hormone and neurotransmitter release, muscle contraction, cardiac function, cell proliferation and immune function, which are underscored by the association of K^+ channel mutations to numerous inherited diseases. Although many challenges remain unsolved, the availability of diverse expression systems, molecular cloning and genetic linkage analysis has led to the upgradation of available topological data, identification of disease-producing loci, and better understanding of mutation-linked channelopathies. Moreover, in 2016, a new nomenclature ($K_{Na}1.1$ for $K_{Ca}4.1$, and $K_{Na}1.2$ for $K_{Ca}4.2$) has been assigned and implemented in the IUPHAR database. These advances along with high-throughput screening are catalysing the discovery process of newer pharmacophores and modulators of K^+ channels. This chapter aims to provide a basic understanding of K^+ channels and offers an updated overview on the progress and opportunities of pharmacological approaches in the exploitation of these channels as therapeutic targets.

Keywords

Potassium channel · Ca^{2+}- and Na^+-activated K^+ channels · Inwardly rectifying K^+ channels · Two P domain K^+ channels · Voltage-gated K^+ channels

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Abbreviations

1-EBIO	1-Ethyl-2-benzimidazolinone
4-AP	4-Aminopyridine
AF	Atrial Fibrillation
AgTx	Agitoxins
ALS	Amyotropic Lateral Sclerosis
BK	Large conductance K^+ channels
CHF	Congestive Heart Failure
ChTx	Charybdotoxin
CLL	Chronic Lymphoid Leukaemia
CNS	Central Nervous System
DCT	Distal Convoluted Tubule
DRG	Dorsal Root Ganglia
DTx	Dendrotoxin
EA1	Episodic Ataxia type 1
EAG	*Ether-à-go-go*
EGTA	Ethylene Glycol Tetraacetic Acid
Elk	Eag-Like K^+ Channels
Erg	Eag-Related Genes
GABA	γ-Amino Butyric Acid
GAs	General Anaesthetics
GIRK	G-protein-activated Inward-Rectifier K^+ channels
HERG	Human *ether-à-go-go*
HMs	Hypoglossal Motoneurons
HTS	High-Throughput Screening
IbTx	Iberiotoxin
IK	Intermediate Conductance K^+ channels
IUPHAR	International Union of Basic and Clinical Pharmacology
K_{2P}	Two P domain K^+ channels
K_{ATP}	ATP-sensitive K^+ channels
KCO	Potassium Channel Opener
K_{ir}	Inwardly rectifying K^+ channels
KT	Kaliotoxin
mAb	Monoclonal Antibodies
MAC	Minimum Alveolar Concentration
MEM	Memantine
MgT	Margatoxin
MOR	μ-Opioid Receptor
mRNA	Messenger RNA
MTx	Maurotoxin
NBP	3-N-Butylphthalide
NMDAR	N-Methyl-D-aspartate receptor
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs

NxTx	Noxiustoxin
PHHI	Persistent Hyperinsulinaemic Hypoglycaemia of Infancy
PIP2	Phosphatidylinositol-4,5-bisphosphate
PTX	Pertussis toxin
RBCs	Red Blood Cells
SCLC	Small-Cell Lung Cancer cells
SeSAME	Seizures, Sensorineural deafness, Ataxia, Mental retardation, Electrolyte imbalance
SK	Small conductance K^+ channels
SSRIs	Selective Serotonin Reuptake Inhibitors
SU	Sulfonylureas
SUR	Sulfonylurea Receptors
T2-DM	Type II Diabetes Mellitus
TCAs	Tricyclic Antidepressants
TEA	Tetraethylammonium
TM	Transmembrane domains
TRAM-34	Triarylmethane-34

19.1 Introduction

Potassium channels form the largest and most diverse family among the ion channels encoded by about 78 genes (D'amico et al. 2013). The first atomic structure of a prokaryotic K^+ channel, KcsA, was determined from bacterium *Streptomyces lividans* (Kuang et al. 2015; Garcia et al. 2001; Luzhkov and Åqvist 2005; González et al. 2012). After this discovery, considerable efforts have been made to interpret the mechanism of the subtypes of the channel. The genomes of *Drosophila* and *Caenorhabditis elegans* were found to contain 30–100 genes for K^+ channels (Miller 2000). The subunit stoichiometry, topology, precise biophysical properties, and modulation by the ligands and second messengers have been addressed to a great extent for the K^+ channels. These studies have been conducted by using high-hroughput multiple assays, including $T1^+$ flux assay, ligand binding assay, voltage-sensitive dye-assay and $^{86}Rb^+$ flux assay (Yu et al. 2016; Longman and Hamilton 1992). Potassium channels switch the conformation between an open and a closed state to conduct K^+ ions down the electrochemical gradient of K^+ across the cell membrane (Mackinnon 2003). A typical K^+ channel consists of a tetramer with each monomer possessing one pore-forming (P) domain, which together comprises the pore for conduction. The potassium channels contain a highly conserved segment of amino acid sequences known as K^+ channel signature sequences (TVGYG) (Kuang et al. 2015; Miller 2000). These sequences form the selectivity filter for K^+ ions, which gives the channels more selectivity for K^+ (at least 10,000 times) over Na^+ ions. The specificity is due to the multiple binding sites of the selectivity filter that mimics a potassium ion hydrated shell. Moreover, the rate of conduction of these channels is very high (10^7 ions channel^{-1} s^{-1}) (Mackinnon 2003). The K^+ channels exist in three states, i.e. the channel is closed in the resting state, open in the activated

state and remains non-conductive in the inactivated state. The gating is formed by the bending of inner helix (intracellular) and the selectivity filter (extracellular) (Kuang et al. 2015). The wide tissue distribution allows potassium channels to influence various physiological functions, such as release of neurotransmitters and hormones, regulation of fluid secretion, controlling of heart rate, smooth and cardiac muscle contraction and clonal expansion of immune cells (Garcia et al. 1997; Garcia and Kaczorowski 2005). The activation of K^+ channels also regulates cellular excitability by influencing the action potential waveform. In excitable cells, the pharmacological activation of these channels reduces the excitability, albeit channel inhibition leads to excitability of the cell.

Several genetically linked and acquired diseases have been known to be associated with the alteration of the K^+ channel. Disruption of the K^+ channel gene underlies various pathologies involving epilepsy, neurodegeneration, schizophrenia, episodic ataxia, diabetes, deafness, cardiac arrhythmias, renal diseases, asthma and hypertension (Bergeron and Bingham 2012). This provides a basis to develop appropriate pharmacological interventions by targeting these channels. Most interestingly, the enthusiasm towards this arena was driven by the realization of the fact that the sulfonylurea class of antidiabetic drugs and class-III antiarrhythmic drugs act via regulating the K^+ channels. Not only this, many K^+ channel openers and blockers also offer therapeutic opportunities in a wide range of areas, including vascular or non-vascular muscle and immune, neuronal and cardiac systems.

Although continuous progress has been made in developing the underlying molecular pharmacology of the K^+ channels, still some of the subtypes remain unexplored and in most of the cases existing tools are not sufficient for probing these subfamilies in higher biological systems (Garcia and Kaczorowski 2016). Therefore, emphasis has been laid on the discovery of K^+ channel modulators in the last few decades. The basic K^+ channel modulators are mainly divided into two types: peptide venom toxins and small molecules. The peptide toxins exert their effect either by binding to the outer vestibule (e.g. toxins from snakes, sea anemones, scorpions and cone snails) or by interacting with the voltage sensor of the channel (e.g. hanatoxin obtained from spiders). However, the small molecules target the gating hinge, inner pore or α/β subunit interface. The small molecules either activate or block the channel. Depending on their selectivity towards target(s), they are also classified as specific target and multi-target molecules (Tian et al. 2014).

According to the classification of IUPHAR, four structural types of K^+ channels are (a) Ca^{2+}- and Na^+-activated K^+ channels, (b) inwardly rectifying K^+ channels, (c) two P domain K^+ channels and (d) voltage-gated K^+ channels (Alexander et al. 2017; Alexander et al. 2013). These individual classes are explained in detail in the later sections. Moreover, expression of the channel subtypes and their modulators are summarized in Table 19.1.

Table 19.1 Overview of potassium channels and their modulators

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
Ca^{2+}- and Na^{+}-activated (K_{Ca} and K_{Na})	Large conductance (BK)	$K_{Ca}1.1$	KCNMA1	Ubiquitous; brain, hair cells of cochlea, pancreatic β-cells, skeletal and smooth muscle, colon, kidney	NS004, NS1619, cromakalim, dehydrosoyasaponin-I, niflumic acid, MCI-154, Maxi-K diol, CGS-7181, NS-1608, BMS-20435211	Paxilline, TEA, ChTx, IbTx, slotoxin, EGTA, Ni^{+}, aflatrem, verruculogen, penitrem A	Kaczmarek et al. (2017); Wei et al. (2005); Coghlan et al. (2001); Kaczorowski et al. (1996); Sanchez and McManus (1996); Augustynek et al. (2016); Ledoux et al. (2006); Dogan et al. (2019); Faber and Sah (2007); Strøbæk et al. (2004); Hougaard et al. (2009); Weatherall et al. (2010); Syme et al. (2000); Castle et al. (2003)
		$K_{Ca}5.1$	KCNU1	Testis, spermatocytes	–	Ba^{2+}, quinine, quinidine	
		$K_{Na}1.1$	KCNT1	Brain, kidney, testis, hippocampus, olfactory bulb, frontal cortex	Bithionol, niclosamide, loxapine	Bepridil, quinidine	
		$K_{Na}1.2$	KCNT2	Brain, heart	Niflumic acid	Ba^{2+}, quinidine	
	Small conductance (SK)	$K_{Ca}2.1$	KCNN1	Brain, aorta, spinal cord, pituitary, oligodendroglioma, gastric tumour, glioblastoma	EBIO, NS309, GW542573X	UCL1684, apamin, tubocurarine, pancuroniumatracurium, TEA, lelurotoxin I, dequalinium, UCL2048, and UCL1530	
		$K_{Ca}2.2$	KCNN2	Brain, thalamus, pituitary, melanocyte, prostate, lung, liver, heart, skeletal muscle, myometrium, melanoma, oligodendroglioma	EBIO, NS309		

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
		$K_{Ca}2.3$	KCNN3	Brain, lymphocytes, skeletal muscle, prostate, kidney, vascular endothelium, heart, pancreas, colon, liver, head, neck, ovary	EBIO, NS309		
	Intermediate conductance (IK)	$K_{Ca}3.1$	KCNN4	RBCs, liver, pancreas, lymphocytes, placenta, prostate, colon, lung, colon, vascular endothelium	EBIO, NS309, SK-121, benzoxazoles (zoxazolamine and chlorzoxazone), isatin derivatives	TRAM-34, ChTx, IbTx, maurotoxin, clotrimazole, nitrendipine, oxime, malonate	
Inwardly rectifying (K_{ir})	Classical or strong inward rectifier	$K_{ir}2.1$	KCNJ2	Heart, brain, kidney, lung, placenta, skeletal and smooth muscle, macrophage	PIP_2	Spermine, spermidine, putrescine, intracellular Mg^{2+}, Ba^{2+}, Cs^{+}, Rb^{+}, memantine	González et al. (2012); Garcia and Kaczorowski (2005); Alexander et al. (2017); Shieh et al. (2000); Yamashita et al. (1996); Ishihara et al. (1996); Alagem et al. (2001); Hughes
		$K_{ir}2.2$	KCNJ12	Cerebellum, forebrain, heart, kidney, skeletal muscle		Intracellular Mg^{2+}, Ba^{2+}, Cs^{+}	
		$K_{ir}2.3$	KCNJ4	Brain, reactive astrocyte, kidney, heart, smooth muscle	Intracellular alkalization (pH 6.76), PIP_2, arachidonic acid, tenidap	Intracellular Mg^{2+}, Ba^{2+}, Cs^{+}, spermine, spermidine, putrescine	

	G-protein activated (GIRK)	$K_{ir}2.4$	KCNJ14	Brain, retina, neuronal cell in heart	Extracellular alkalization	Intracellular Mg^{2+}, Ba^{2+}, Cs^{+}	et al. (2000); Camerino et al. (2007)
		$K_{ir}3.1$	KCNJ3	Cerebellum, olfactory bulb, neocortex, thalamus, hippocampus, basal ganglia, brainstem, heart	PIP_2, ML297,	Tertiapin-Q, Ba^{2+}, Cs^{+}	
		$K_{ir}3.2$	KCNJ6	Cerebellum, pancreatic islets, brain	PIP_2	Tertiapin, halothane, bupivacaine, verapamil, MK-801, QX-314, antipsychotics such as haloperidol, thioridazine, pimozide, desipramine, clozapine, fluoxetine	
		$K_{ir}3.3$	KCNJ9	Brain	PIP_2		
		$K_{ir}3.4$	KCNJ5	Heart, pancreas, skeletal muscle, lungs, cerebellum, urinary bladder	PIP_2	Ba^{2+}, Cs^{+}, TEA, 4-AP, tertiapin-Q	
	ATP-sensitive (K_{ATP})	$K_{ir}6.1$	KCNJ8	Ubiquitous; pancreas, neurons, skeletal muscle, vascular smooth muscle	Cromakalim, diazoxide, minoxidil, nicorandil, iptakalim	Glibenclamide, tolbutamide	
		$K_{ir}6.2$	KCNJ11	Ubiquitous; pancreas, neurons, skeletal muscle, brain, heart	Cromakalim, diazoxide, minoxidil, nicorandil	Glibenclamide, tolbutamide	

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
	K^+-transport	$K_{ir}1.1$	KCNJ1	Kidney, pancreatic islets, skeletal muscle, pancreas, spleen, brain, liver, heart	VU590, VU591	Tertiapin-Q, δ-dendrotoxin, Ba^{2+}, Cs^+	
		$K_{ir}4.1$	KCNJ10	Glia, retina, ear, kidney	ATP, PIP_2	Ba^{2+}, Cs^+, imipramine, desipramine, nortryptyline, amitriptyline	
		$K_{ir}4.2$	KCNJ15	Brain, kidney, lung, pancreas, liver, testis	–	Ba^{2+}, Cs^+	
		$K_{ir}5.1$	KCNJ16	Brain, kidney, thyroid, spleen, liver, testis, retina	–	Ba^{2+}, Cs^+	
		$K_{ir}7.1$	KCNJ13	Cerebellum, hippocampus, kidney, thyroid, GI, stomach, small intestine, prostate, testis, lung, retina	–	Ba^{2+}, Cs^+	
Two P domain (K_{2P})	TWIK	$K_{2P}1.1$ or TWIK-1	KCNK1	Brain, heart, kidney, lung, liver, placenta	–		Garcia et al. (1997); Gada and Plant

		K_{2P}6.1 or TWIK-2	KCNK6	Pancreas, heart, placenta, lung, stomach, eyes, embryo	Arachidonic acid	Ba^{2+}, quinidine, volatile anaesthetics	(2019); Enyedi and Czirják (2010); Olschewski et al. (2017); Vivier et al. (2015); Lesage (2003); Wright et al. (2017); Tian et al. (2019); Hayashi and Novak (2013); Staudacher et al. (2018a, b); Kim (2005)
		K_{2P}7.1	KCNK7	Brain, spinal cord, retina			
	TREK	K_{2P}2.1 or TREK-1	KCNK2	Brain, lung, heart	Arachidonic acid, Chloroform, halothane, isoflurane, unsaturated fatty acid, lysophospholipids, fenamates, flufenamic acid, GI-530139	Ba^{2+}, PKA, PKC, sipatrigine, fluoxetine, chlorpromazine, haloperidol, loxapine, pimozide, fluphenazine, mexiletine, propafenone, amlodipine, nifedipine	
		K_{2P}4.1 or TRAAK	KCNK4	Brain, kidney, placenta, small intestine, prostate	Arachidonic acid, riluzole, unsaturated fatty acid, lysophospholipids	Gd^{+}, sipatrigine	
		K_{2P}10.1 or TREK-2	KCNK10	Kidney, liver, pancreas, prostate, thymus	Arachidonic acid, halothane, linoleic acid, doecosahexaenoic acid, riluzole, lysophosphatidylcholine, volatile anaesthetic halothane, isoflurane, GI-530139	Norfluoxetine, quinidine, chlorpromazine, haloperidol, loxapine, pimozide, fluphenazine	
	TASK	K_{2P}3.1 or TASK-1	KCNK3	Brain, heart, colon, small intestine, lung, pancreas, placenta, prostate, uterus, kidney	Halothane, isoflurane, volatile anaesthetics	Ba^{2+}, external pH (7.3), arachidonic acid, anandamide, *R*-(+)-methanandamide	

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
		K_{2P}9.1 or TASK-3	KCNK9	Brain	Halothane	External pH (6.5), ruthenium red, anandamide, *R*-(+)-methanandamide	
		K_{2P}15.1 or TASK-5	KCNK15	Brain, kidney, heart, lung, pancreas, liver, placenta, thyroid, adrenal gland, salivary gland	–	–	
	TALK	K_{2P}5.1 or TASK-2	KCNK5	Brain, kidney, liver, pancreas, placenta, small intestine	Halothane, volatile anaesthetics	External pH (6.5), LAs (lidocaine, bupivacaine, clofilium)	
		K_{2P}16.1 or TALK-1	KCNK16	Heart, liver, lung, pancreas, placenta	Isoflurane, NO, ROS	External pH, Ba^{2+}, quinidine, chloroform	
		K_{2P}17.1 or TALK-2	KCNK17	Heart, lung, liver, placenta, pancreas	NO, ROS, quinidine, propafenone, mexiletine, metoprolol, propranolol	External pH, Ba^{2+}, chloroform, sotalol, verapamil, amiodarone, ranolazine	
	THIK	K_{2P}12.1 or THIK-1	KCNK12	Brain, liver, lung, heart, kidney, colon, pancreas, spleen, ovary, placenta, prostate, thymus, small intestine	–	Propafenone, mexiletine, propranolol, lidocaine	

		K_{2P}13.1 or THIK-2	KCNK13	Brain, lung, heart, kidney, liver, spleen	Arachidonic acid	Ba^{2+}, halothane	
	TRESK	K_{2P}18.1	KCNK18	Cerebellum, cerebrum, brainstem, spinal cord, testis	Volatile anaesthetics (isoflurane, desflurane, sevoflurane, halothane), cloxyquin	External acidic pH, Ba^{2+}, quinine, quinidine, free fatty acid, lomotrigin, loratidine	
Voltage gated (Kv)	Kv1	Kv1.1	KCNA1	Pons, medulla, midbrain, cerebellum, hippocampus, auditory nuclei, node of Ranvier, kidney, retina, heart	–	α-Dendrotoxin, margatoxin, TEA, capsaicin, flecainide, nifedipine, diltiazem, resiniferatoxin	Gutman et al. (2005); Kaczorowski and Garcia (1999); Garcia et al. (1997); Felix et al. (1999); Coghlan et al. (2001); Hill et al. (1995); Kuzmenkov et al. (2015); Gutman et al. (2003); Alexander et al. (2017); Dalby-Brown et al. (2006); Humphries and Dart (2015);
		Kv1.2	KCNA2	Spinal cord, cerebral cortex, pons, medulla, cerebellum, thalamus, hippocampus, retina, Schwann cells	–	α-Dendrotoxin, margatoxin, nifedipine, flecainide, resiniferatoxin, anandamide	
		Kv1.3	KCNA3	CNS, T- and B-cells, platelets, microglia, macrophages, osteoclasts, testis, tonsils	–	Margatoxin, TEA, noxiustoxin, maurotoxin, correolide	

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
		Kv1.4	KCNA4	Corpus striatum, hippocampus, olfactory bulb, pancreatic islets, heart, skeletal and smooth muscle, lung carcinoid	–	Fampridine, UK78282, riluzole, quinidine, nicardipine	Vacher et al. (2007); Camerino et al. (2007); Alexander et al. (2013)
		Kv1.5	KCNA5	Hippocampus, cortex, pituitary, macrophages, microglia, cardiac myocytes, vascular smooth muscle, aorta, colon, stomach	–	Fampridine, verapamil, nifedipine, quinidine, propafenone, erythromycin, loratidine, terfenadine, ebastine	
		Kv1.6	KCNA6	Spinal cord, astrocytes, pulmonary artery smooth muscle, oligodendrocytes, testis, ovary, heart, lungs, colon	–	α-Dendrotoxin, TEA, ChTx, MgTx, hongotoxin, tumulustoxin	
		Kv1.7	KCNA7	Heart, liver, lung, skeletal muscle, placenta, CNS	–	Fampridine, noxiustoxin, 4-AP, nifedipine, amiodarone, quinidine, flecainide, tedisamil	

		Kv1.8	KCNA10	CNS, adrenal gland, kidney, heart, skeletal muscle	–	Fampridine, Ba^{2+}, ChTX, 4-AP, TEA, ketoconazole, pimozide, verapamil	
	Kv2	Kv2.1	KCNB1	Cerebral cortex, cerebellum, hippocampus, pancreatic β-cells, gastric cancer cells, insulinomas, lung, retina, cochlea, heart, skeletal muscle, germ cell	Linoleic acid	TEA, hanatoxin, halothane, tetrapentylammonium	
		Kv2.2	KCNB2	Cerebral cortex, cerebellum, hippocampus, pancreatic δ-cells, tongue, GI smooth muscle, sympathetic neurons	–	TEA, fampridine, quinine, phencyclidine	
	Kv3	Kv3.1	KCNC1	Cerebellum, cochlear and vestibular nuclei, substantia nigra, skeletal muscle, lung, testis, germ cell	–	4-AP, TEA, fampridine, cromakalim, diltiazem, nifedipine, flecainide, resiniferatoxin	
		Kv3.2	KCNC2	Pancreatic islets, Renshaw cells (spinal interneurons),	–	4-AP, TEA, fampridine, 8-bromo-cGMP, verapamil, D-NONOate,	

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
				fast-GABAergic interneurons, Schwann cells, mesenteric artery		3-isobutyl-1-methylxanthine	
		Kv3.3	KCNC3	Brainstem, forebrain, Purkinje cells, cerebellum, motoneurons, lens, corneal epithelium	–	4-AP, TEA	
		Kv3.4	KCNC4	Brainstem, hippocampus, skeletal muscle, parathyroid, prostate, pancreatic acinar cells	–	4-AP, TEA, sea anemone toxin BDS-I	
	Kv4	Kv4.1	KCND1	Heart, liver, kidney, pancreas, thyroid gland, lung, stomach, testis, pulmonary artery	–	Fampridine	
		Kv4.2	KCND2	Cerebellum, thalamus, basal ganglia, hippocampus, forebrain, cochlear nucleus, rodent heart	–	Hanatoxin, heteropodatoxins	

		Kv4.3	KCND3	Cerebral cortex, cerebellum, cardiac myocytes, smooth muscle	–	Niflumic acid, bupivacaine, DIDS, nicotine	
	Kv5	Kv5.1	KCNF1	Brain, liver, heart, skeletal muscle, kidney, pancreas	–		
	Kv6	Kv6.1	KCNG1	Brain, uterus, skeletal muscle, testis, ovary, prostate, germ cell, kidney, placenta, pancreas, bone, skin	–		
		Kv6.2	KCNG2	Fetal brain, myocardium, germinal centre, B cells	–		
		Kv6.3	KCNG3	Whole brain, spinal cord, thymus, adrenal gland, pituitary, small intestine	–		
		Kv6.4	KCNG4	Brain, liver, colon, small intestine	–		
	Kv7	Kv7.1	KCNQ1	Heart, liver, GI tract, kidney, lung, ear, rectum, pancreas, placenta	–	Clofilium, XE991, linopirdine, mefloquine, azimilide	
		Kv7.2	KCNQ2	Hippocampus, thalamus,	Retigabine	XE991, linopirdine, TEA, L-735821	

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
				cerebellum, brainstem, cerebral cortex, lung, testis, breast, eye, placenta, small intestine, DRG, sympathetic ganglia			
		Kv7.3	KCNQ3	Hippocampus, cerebral cortex, cerebellum, brainstem, thalamus, DRG, sympathetic ganglia, colon, eye, head, neck, retina	Retigabine	Linopirdine, 4-AP, clofilium, CTX, E4031	
		Kv7.4	KCNQ4	VSM, outer hair cells of ear and cochlea, placenta	Retigabine	XE991, linopirdine, TEA, bepridil	
		Kv7.5	KCNQ5	Cerebral cortex, thalamus, hippocampus, skeletal muscle, VSM, sympathetic ganglia	Retigabine, BMS204352	XE991, linopirdine	
	Kv8	Kv8.1	KCNV1	Kidney, brain	–	–	
		Kv8.2	KCNV2	Lung, kidney, liver, thymus, pancreas,	–	–	

				spleen, testis, ovary, colon, prostate			
	Kv9	Kv9.1	KCNS1	Lens, melanocytes, brain,	–	–	
		Kv9.2	KCNS2	Brain, spinal cord, retina, pulmonary artery	–	–	
		Kv9.3	KCNS3	Brain, breast, eye, colon, heart, kidney, muscle, skin, testis, stomach, uterus, lung	–	–	
	Kv10	Kv10.1	KCNH1	CNS, melanoma cells, tumour cells	–	Quinidine, intracellular calcium	
		Kv10.2	KCNH5	CNS, heart, lung, liver, kidney, pancreas, placenta, muscle	–	Quinidine, intracellular calcium	
	Kv11	Kv11.1	KCNH2	Heart, CNS, lymphocytes, endocrine cells, testis, ovary, prostate, tonsil, uterus, kidney, liver, lung, pancreas, microglia, blood cells, brain	RPR260243	Astemizole, terfenadine, disopyramide, E4031, dofetilide, ibutilide, MK-499, clofilium, cisapride, sertindole	
		Kv11.2	KCNH6	Brain, hippocampus,	–	Sipatrigine	

(continued)

Table 19.1 (continued)

Family	Subfamily	Members	Gene	Tissue expression	Modulators		References
					Activators	Inhibitors/Blockers	
				uterus, lactotrophs, rat pituitary			
		Kv11.3	KCNH7	Brain, sympathetic ganglia, lactotrophs, CA pyramidal neurons, rat pituitary	–	Sertindole, pimozide	
	Kv12	Kv12.1	KCNH8	Brain, sympathetic ganglia, lung, uterus, colon, testis	–	Ba^{2+}	
		Kv12.2	KCNH3	Infant brain, amygdala, hippocampus, eye (retinoblastoma), lung (small-cell carcinoma)	–	Ba^{2+}	
		Kv12.3	KCNH4	Telencephalon, lung, oesophagus, pituitary, cerebellum, neuroblastoma, primary B-cell neoplasia, oligodendroglioma	–	Ba^{2+}	

19.2 Ca^{2+}- and Na^{+}-activated K^{+} Channels

Studies demonstrated that chelation of Ca^{2+} reduces the K^{+} efflux in RBCs, while K^{+} conductance increases after intracellular injection of Ca^{2+} into neurons (Kaczmarek et al. 2017). Based on these observations, the first gene from a *Drosophila* mutant (slowpoke or slo) encoding a Ca^{2+}-activated K^{+} channel was cloned and identified (Kaczmarek et al. 2017; Atkinson et al. 1991). This channel was termed BK or MaxiK (later named $K_{Ca}1.1$ or Slo1) because of its large conductance capacity and sensitivity towards both Ca^{2+} and transmembrane voltage (Contreras et al. 2013; Greenwood and Leblanc 2007). Screening of cDNA libraries for similar sequences of a K^{+}-selective pore subsequently led to the discovery of different genes encoding for other Ca^{2+}-activated K^{+} channel subtypes. The next two identified classes, i.e. $K_{Ca}2$ or SK (small conductance) family ($K_{Ca}2.1$ or SK1, $K_{Ca}2.2$ or SK2, and $K_{Ca}2.3$ or SK3) and $K_{Ca}3$ or IK (intermediate conductance) family ($K_{Ca}3.1$ or SK4), are insensitive to the membrane voltage (Adelman et al. 2012). Based on structural resemblance, three other genes were identified and named $K_{Ca}4.1$ (Slack or Slo2.2), $K_{Ca}4.2$ (Slick or Slo2.1) and $K_{Ca}5.1$ (SLO3).

Later it was observed that $K_{Ca}4.1$ (Slack) and $K_{Ca}4.2$ (Slick) are not only activated by the intracellular Ca^{2+} but also regulated by the Na^{+} and Cl^{-} concentration in the cytoplasm (Kaczmarek 2013). Based on this, a new nomenclature for the two channels ($K_{Na}1.1$ for Slack or former $K_{Ca}4.1$, and $K_{Na}1.2$ for Slick or former $K_{Ca}4.2$) was proposed by Kaczmarek et al. (2017), and later it was accepted and implemented in the IUPHAR database (Alexander et al. 2017; Kaczmarek et al. 2017).

Cryo-electron microscopy and X-ray crystallography revealed that $K_{Ca}1.1$ consists of 7-TM domains distinguishing it from canonical 6-TM K^{+} channels. It has an additional S0 domain preceding S1 along with the common S1–S6 domain sequence with N-terminal lying outside rather than inside of the cell (as shown in Fig. 19.1) (Meera et al. 1997). The RCK1 and RCK2 domains in the cytoplasmic C-terminal act as binding sites for Ca^{2+}, and the "gating ring" is formed together by the eight RCK domains of each tetrameric $K_{Ca}1.1$ channel (Wu et al. 2010; Wei et al. 2005). Unlike the $K_{Ca}1.1$ channel, $K_{Ca}2$ channels contain six α-helical TM (S1–S6) with a K^{+}-selective pore sequence linking S5 and S6 (Kaczmarek et al. 2017). These channels are voltage independent, and conduction through the channels occurs only after Ca^{2+}–calmodulin complexation (Xia et al. 1998). Similarly, $K_{Ca}3.1$ closely resembles the $K_{Ca}2$ family; however, unitary conductance of this channel is greater compared to $K_{Ca}2$. Therefore, it is also named intermediate conductance for the K^{+} or IK channel. The sensitivity of $K_{Ca}3.1$ is also determined by the association of Ca^{2+} to calmodulin. $K_{Na}1.1$ and $K_{Na}1.2$ differ from $K_{Ca}1.1$ in transmembrane topology as they lack the S0-TM. Though these are voltage-sensitive channels, they lack the repeated motif of amino acids in S4 segments like $K_{Ca}1.1$. The two RCK domains form the gating ring of the pore similar to that of $K_{Ca}1.1$. The $K_{Ca}5.1$ has a similar topology as $K_{Ca}1.1$ (Kaczmarek et al. 2017). $K_{Ca}5.1$ is expressed mainly in sperm cells and activated primarily by voltage and internal alkalinization as in the case of sperm capacitation (Navarro et al. 2007; Zeng et al. 2015).

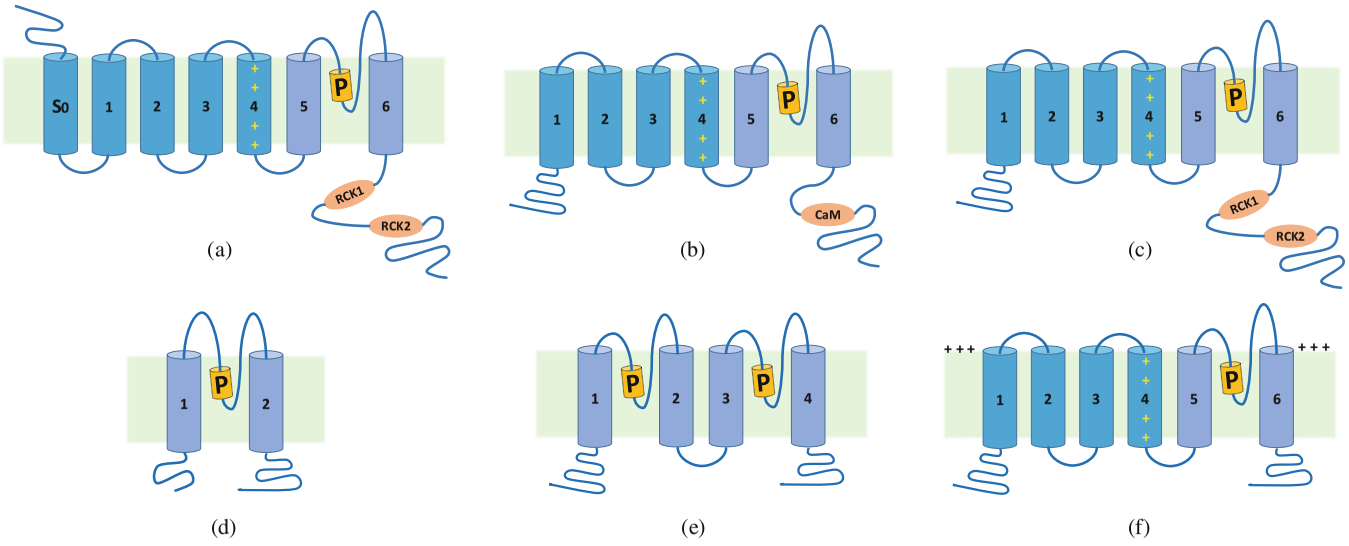

Fig. 19.1 Schematic diagram of the transmembrane topology of different potassium channels: (**a**) Large conductance $K_{Ca}1.1$ and $K_{Ca}5.1$ channels with additional S0 domain in the structure and Ca^{2+}-binding sites RCK1 and RCK2, (**b**) small and intermediate conductance $K_{Ca}2.1$, $K_{Ca}2.2$, $K_{Ca}2.3$ and $K_{Ca}3.1$ channels with calmodulin as the Ca^{2+}-binding site, (**c**) large conductance $K_{Na}1.1$ and $K_{Na}1.2$ (formerly known as $K_{Ca}4.1$ and $K_{Ca}4.2$) channels with RCK1 and RCK2 subunits (without S0 domain), (**d**) inwardly rectifying (K_{ir}) channels or a typical 2-TM domain family, (**e**) two P domain (K_{2P}) or 4-TM potassium channels with two P-loops forming a tandem and (**f**) voltage-gated K^+ channels (K_v) with 6-TM domain comprising homomeric and heteromeric tetramers

19.2.1 Pharmacology of Ca^{2+}- and Na^{+}-activated K^{+} Channels

Molecular cloning of Ca^{2+}- and Na^{+}-activated K^{+} channels is anticipated in the development of the modulators of these channels. These channels are known for their critical dependency of channel conduction on intracellular Ca^{2+} and activation by Na^{+} (newer concept) and, therefore, implicated in many physiological and pathological conditions. BK channels are involved mainly in hypertension, coronary artery spasm, stroke, urinary incontinence and several neurological disorders like psychoses, schizophrenia and epilepsy (Coghlan et al. 2001; Shieh et al. 2000; Humphries and Dart 2015). IK channel modulators have long been proposed for treatment strategies for diarrhoea, asthma, atherosclerosis, sickle cell anaemia and autoimmune diseases like rheumatoid arthritis (Shieh et al. 2000; Lam et al. 2013; Grunnet et al. 2011), while SK channel modulators are being investigated as potential therapy for ataxia, memory disorders, narcolepsy, epilepsy, atrial fibrillation and alcohol dependence (Kaczmarek et al. 2017; Camerino et al. 2007). Mutations in the newly classified $K_{Na}1.1$ result in a variety of early-onset epilepsies like malignant migrating partial seizures, Ohtahara syndrome and autosomal dominant frontal lobe epilepsy (Kim and Kaczmarek 2014). In contrast, $K_{Na}1.2$ and $K_{Ca}5.1$ are least studied as compared to the other subtypes. Because of the wide range of distribution, the Ca^{2+}- and Na^{+}-activated K^{+} channels are a good target in several physiological and pathological conditions (also refer to Table 19.1).

19.2.1.1 Modulators of BK Channels ($K_{Ca}1.1$, $K_{Na}1.1$, $K_{Na}1.2$ and $K_{Ca}5.1$)

The association of auxiliary subunits is the key factor for governing pharmacological properties *via* $K_{Ca}1.1$ channels. The earlier reported modulators include activators like glycosylated triterpenes (e.g. dehydrosoyasaponin-I) and blockers such as indole diterpenes (e.g. paxilline, aflatrem, verruculogen and penitrem A) (Kaczorowski et al. 1996; Sanchez and McManus 1996; Augustynek et al. 2016). Paxilline showed enhancement in the binding of ChTx to the BK channels in ligand binding studies. This effect is determined by the binding of paxilline to the alpha-subunits of BK channels (Sanchez and McManus 1996). $K_{Ca}1.1$ channels are activated by benzimidazolones like NS-004 and NS-1619 and induce membrane hyperpolarization. Other classes of drugs which activate these channels include benzopyrans (e.g. cromakalim), dihydropyridines (e.g. nitrendipine), biarylamines (e.g. niflumic acid, MCI-154), terpenoids (e.g. Maxi-K diol), biarylureas (e.g. CGS-7181, NS-1608) and 3-aryloxyindoles (e.g. BMS-204352) (also refer to Table 19.1) (Camerino et al. 2007; Calderone 2002; Ledoux et al. 2006). BMS-20435211 is a vasoactive molecule, being investigated in clinical trials for its potential as a treatment strategy for migraine (ClinicalTrials.gov Identifier: NCT03887325). Carbonic anhydrase (CA) inhibitors such as acetazolamide, bendroflumethiazide, ethoxzolamide and dichlorphenamide also exhibited BK activation properties. The majority of small-molecule $K_{Ca}1.1$ activators possess structural homology, i.e. two aromatic rings linked *via* either a heterocyclic or a urea spacer (Coghlan et al. 2001). Non-selective activation of BK channels by tamoxifen

(oestrogen receptor antagonist) at its therapeutic concentration may explain their role in tamoxifen-induced QT prolongation and arrhythmia.

The scorpion toxins iberiotoxin (IbTx), charybdotoxin (ChTx) and slotoxin are specific blockers of $K_{Ca}1.1$ with higher selectivity and potency (Garcia-Valdes et al. 2001). These channels are also blocked by tetraethylammonium (TEA), Ni^{+} and ethylene glycol tetraacetic acid (EGTA) (Dogan et al. 2019). Non-selective vascular BK blockers include gallopamil and verapamil. BK channels are also inhibited by ketamine (dissociative anaesthetic) and clotrimazole (antifungal) (also refer to Table 19.1) (Kaczmarek et al. 2017).

No specific pharmacological agents are known to selectively act on $K_{Na}1.1$, $K_{Na}1.2$ and $K_{Ca}5.1$. Drugs such as bithionol, niclosamide and loxapine can pharmacologically activate these channels. Loxapine is more selective towards $K_{Na}1.1$ unlike bithionol, which also activates $K_{Ca}1.1$. Quinidine is known to ameliorate the symptoms of malignant migrating partial seizures in infancy by blocking $K_{Na}1.1$ currents (Bearden et al. 2014; Yang et al. 2006; De Los Angeles Tejada et al. 2012). The mechanism of quinidine-mediated amelioration of these symptoms is not very clear as selectivity of quinidine is not limited to $K_{Na}1.1$ channels. Various non-specific $K_{Na}1.1$ blockers include Ba^{2+}, clofilium and bepredil (Bhattacharjee et al. 2003).

Similarly, $K_{Na}1.2$ is less explored and reportedly activated by the fenamate class of NSAIDs like niflumic acid, with low potency. These agents are known to uncouple the channels from Na^{+} or transmembrane voltage-regulated modulation causing greater conductance of current. As they affect other channels including $K_{Ca}1.1$, this action is non-specific in nature. Other pharmacological blockers of these channels include clofilium, isoflurane and quinidine (Bhattacharjee et al. 2003; Berg et al. 2007).

The selective pharmacological agents of $K_{Ca}5.1$ are not known yet. However, this channel is blocked by Ba^{2+}, quinine and quinidine up to some extent (Sánchez-Carranza et al. 2015; Tang et al. 2010).

19.2.1.2 Modulators of SK Channels ($K_{Ca}2$ Family)

SK channels are smaller conducting channels underlying the afterhyperpolarization (AHP) currents in the regulation of Ca^{2+} influx and electrical signalling in neurons. The functional elucidation of the SK channel has benefited from the discovery of the SK channel activators. 1-Ethyl-2-benzimidazolinone (1-EBIO) is the prototype of the activators (Pedarzani et al. 2005; Faber and Sah 2007). 6,7-Dichloro-1*H*-indole-2,3-dione-3-oxime (NS309) is also well known as a selective activator of SK including all the three subtypes (Strøbæk et al. 2004). The first selective $K_{Ca}2.1$ activator, GW542573X, is capable of activating the channel even in the absence of Ca^{2+} and is reported to influence the gating process by binding to Ser293 in S5 near the physical gate (Hougaard et al. 2009). CyPPA is a useful pharmacological tool to distinguish SK2/SK3 from SK1/IK as these channels have overlapping expression patterns (Hougaard et al. 2007).

The SK channels are inhibited by (i) natural peptide toxins like bee venom, apamin and scyllatoxin (leiurotoxin I), (ii) neuromuscular blockers like tubocurarine,

atracurium and pancuronium, (iii) antiseptics such as dequalinium and (iv) bis-quinolium analogues such as UCL1684, UCL1848 and UCL1530 (also refer to Table 19.1) (Wei et al. 2005; Ledoux et al. 2006; Weatherall et al. 2010; Campos Rosa et al. 2000). UCL1684 is an about 5000 times more selective blocker of SK compared to IK channels (Fanger et al. 2001; Rosa et al. 1998).

19.2.1.3 Modulators of IK Channels ($K_{Ca}3.1$)

IK channel activators may be of therapeutic interest in hypertension, peripheral vascular diseases and cystic fibrosis. Although not very specific, 1-EBIO and clinical benzoxazoles zoxazolamine and chlorzoxazone are described as potential activators of $K_{Ca}3$ or IK channels. Few isatin derivatives have been extensively studied as IK channel openers (Syme et al. 2000).

ChTx possesses higher affinity towards the IK channels in T-cells and inhibits them. IbTx, maurotoxin and their recombinant variants are effective blockers of IK in thymocytes and erythrocytes (Coghlan et al. 2001; Castle et al. 2003). The non-selective IK channel blocker clotrimazole reduces T-cell proliferation and secretion of IFN-γ in T-cells to produce anti-proliferative effects (Jensen et al. 1999). These blockers are believed to inhibit the dehydration of RBCs and, therefore, identified as effective therapy for sickle cell anaemia (Goodman et al. 1998). Clotrimazole is also considered as one of the pharmacological strateies for treatment of sickle cell anaemia due to its inhibitory effect on the IK channels of RBCs. Newer analogues of nitrendipine as well as oxime and malonate derivatives are claimed to possess a selective inhibitory effect on IK (Jensen et al. 2003). TRAM-34 (triarylmethane) is also a potent and selective IK blocker which exhibited *in vitro* immunosuppression.

19.3 Inwardly Rectifying K^+ Channels (K_{ir} Channels)

Sir Bernard Katz first described the membrane conductance attributed to K_{ir} channels. He discovered that in frog skeletal muscle fibres, K^+ ions move more readily into the inside compared to the outside (called inward rectification) (Doupnik 2017). These channels were so named because of this characteristic degree of inward rectification, i.e. prominent asymmetrical conduction of potassium in the inward direction compared to the outward current (Coghlan et al. 2001). The inward rectification is observed due to the blockade of depolarized potential (outward current) by intracellular Mg^{2+} ions, natural polyamines, putrecine, spermidine and spermine (Kurata et al. 2004; Köhling and Wolfart 2016; Jiménez-Vargas et al. 2017; Nichols and Lopatin 1997).

X-ray crystallography has detailed the 3D structure of K_{ir} channels to the atomic level. Structurally, K_{ir} channels are the simplest among the K^+ channel families, with four subunits formed of 2-TM domains separated by a segment of pore-forming (P) elements (as shown in Fig. 19.1) (Humphries and Dart 2015). The transmembrane domain regulates the gating and ion selectivity, whereas the cytoplasmic domain is involved in the control of gating by G-proteins, Na^+ ions and nucleotides.

K_{ir} channels conduct K^+ ions to a greater extent under hyperpolarization which decreases under depolarization, thereby maintaining the resting membrane potential and cellular excitability.

In humans, K_{ir} channels are classified into seven subfamilies encoded by 15 known genes (denoted KCNJx). These seven subfamilies are (1) $K_{ir}1.1$ or ROMK1 (KCNJ1), (2) $K_{ir}2.1$–2.4 (KCNJ2, 4, 12, 14), (3) $K_{ir}3.1$–3.4 (KCNJ3, 6, 9, 5), (4) $K_{ir}4.1$–4.2 (KCNJ10, 15), (5) $K_{ir}5.1$ (KCNJ16), (6) $K_{ir}6.1$–6.2 (KCNJ8, 11) and (7) $K_{ir}7.1$ (KCNJ13). These subfamilies differ in their properties and kinetics producing numerous physiological activities. The K_{ir} channel family has also been classified into four subsets depending on functional characteristics: strong inward-rectifier ($K_{ir}2.x$), G-protein-activated inward-rectifier ($K_{ir}3.x$), ATP-sensitive K^+ channels ($K_{ir}6.x$/SURx) and K^+-transport channels ($K_{ir}1.1$, $K_{ir}4.x$, $K_{ir}5.1$ and $K_{ir}7.1$) (Alexander et al. 2017). The physiological activities of K_{ir} channels are dependent on localization of channel and regulation of pore opening and ion flux by ions, lipids, nucleotides, polyamines or other intracellular proteins. Decrease in intracellular ATP opens up the ATP-sensitive K^+ channels (named so), exhibiting inward rectification. Similarly, G-protein-activated K_{ir} shows inward rectification via pertussis toxin (PTX)-sensitive G-proteins, orchestrating the cellular excitability and G-protein signalling. A negatively charged Asp residue in the TM2 helix causes strong inward rectification by increasing the affinity for Mg^{2+} as in the case of $K_{ir}2.x$ (Hibino et al. 2010).

19.3.1 Pharmacology of K_{ir} Channels

The K_{ir} channels are widely distributed in neurons, glial cells, cardiac myocytes, blood cells, osteoclasts, endothelium, epithelium and oocytes exhibiting distinct roles in both normal and pathophysiological conditions. Alteration in the functions of these channels may lead to several genetically linked and acquired diseases. Genetic studies have linked many rare human diseases with K_{ir} channel gene mutations. For example, recessive loss-of-function mutations in the KCNJ1 gene or $K_{ir}1.1$ cause type II Bartter syndrome (Doupnik 2017; Dworakowska and Dolowy 2000), and loss-of-function mutations in $K_{ir}2.1$ are associated with Andersen syndrome (LQT7) (Hibino et al. 2010; Giudicessi and Ackerman 2012). Acquired pathological conditions such as electric remodelling associated with atrial fibrillation (AF) can occur due to upregulation of $K_{ir}2.1$ and $K_{ir}3.1/3.4$ channel activity. K_{ATP} channels are implicated in glucose metabolism and contractility of heart. Generalized seizures and SeSAME syndrome are associated with $K_{ir}4.1$ channelopathy (Bhave et al. 2010; Seifert et al. 2018). Permanent neonatal diabetes occurs due to gain-of-function mutation in $K_{ir}6.2$/SUR1. Moreover, new therapeutic approaches, such as designing of K^+ sparing diuretics for CHF and HT by targeting renal $K_{ir}1.1$ channels and treating AF by selectively inhibiting atrial $K_{ir}3$ channels, have spurred research interest towards this family.

At physiological voltage, generation of PIP_2 by ATP-dependent kinases activates K_{ir} channels (Huang et al. 1998). Ba^{2+} and Cs^+ (classical blockers) are known for

effective blocking of majority of the K_{ir} channels and are used to explore their physiological roles (González et al. 2012). This inhibition is more prominent when the membrane is hyperpolarized. Despite limited number of K_{ir} channel blockers, physiological and pharmacological assays have explored compounds with selectivity towards particular types of K_{ir} channels. These compounds are discussed under specific subtypes.

19.3.1.1 Modulators of Classical or Strong Inward-Rectifier K^+ Channels ($K_{ir}2.x$)

Recent electrophysiological and pharmacological studies showed that several reagents possess K_{ir} current blocking properties, but their effects are not specific to the channels. Specific activators and blockers for classical K_{ir} channels are not well known. PIP_2 can act as an endogenous activator of the $K_{ir}2.1$ channel. The endogenous $K_{ir}2.1$ channel blockers include Mg^{2+}, spermine, spermidine and putrescine (also refer to Table 19.1) (Yamashita et al. 1996; Ishihara et al. 1996). The side effect of chloroquine, i.e. lethal ventricular arrhythmia, is also linked to the blockade of $K_{ir}2.1$ currents. Memantine (MEM), a derivative of amantadine, blocks $K_{ir}2.1$ currents and regulates the functional activities of microglia or macrophages (Tsai et al. 2013). The homomeric $K_{ir}2.x$ channels demonstrated a substantial difference in the sensitivity towards Ba^{2+} and Cs^+ in the heterologous expression system. The blockage of $K_{ir}2.1$ by Ba^{2+} is highly dependent on voltage (Alagem et al. 2001). Intracellular Mg^{2+} can act as an endogenous inhibitor of $K_{ir}2.2$ channels. The gating mechanism of $K_{ir}2.2$ can be inhibited by the classical blockers Ba^{2+} and Cs^+ (Takahashi et al. 1994). Intracellular Mg^{2+}, putrescine, spermine and spermidine are well-known endogenous blockers of $K_{ir}2.3$ currents (Alexander et al. 2017; Lopatin et al. 1994). $K_{ir}2.3$ channels are moderately inhibited by the first-generation H_1-antihistaminics (mepyramine and diphenhydramine) and partially inhibited by a protein tyrosine kinase inhibitor, genistein. Similarly, intracellular Mg^{2+} acts as an endogenous inhibitor of $K_{ir}2.4$ channels, while Cs^+ and Ba^{2+} act as blockers of the channels. The blockade of human $K_{ir}2.4$ channels by Cs^+ is more voltage dependent compared to the Ba^{2+}-mediated blockade (Hughes et al. 2000). Moreover, enhancement in tonic activity and increase in frequency of induced spike discharge in hypoglossal motoneurons (HMs) caused by Ba^{2+}-mediated blockade indicate that $K_{ir}2.4$ channels are the major regulators of excitability of motoneurons *in situ* (Töpert et al. 1998).

19.3.1.2 Modulators of G-Protein-Activated Inward-Rectifier K^+ Channels (GIRK or $K_{ir}3.x$)

$G_{\beta\gamma}$ protein-mediated PIP2 activation by lipid kinases causes endogenous activation of all subtypes of GIRK channels (Huang et al. 1998). ML297 is the first identified selective and potent molecule which can act as an activator of $K_{ir}3$ or GIRK. This molecule possesses good antiepileptic properties (Wydeven et al. 2014; Kaufmann et al. 2013). Tertiapin, a toxin obtained from honey bee venom, showed $K_{ir}3.1/3.4$ blocking property. A modified oxidation-resistant toxin, tertiapin Q, blocks I_{KACh} in isolated cardiac myocytes without affecting other currents. Also, cardiac I_{KACh}

channels are blocked by quinine, quinidine and verapamil. Antipsychotics such as haloperidol, clozapine, thioridazine and pimodine modulate the combination of subunits, i.e. $K_{ir}3.1/3.2$ and $K_{ir}3.1/3.4$, in heterologous expression in *Xenopus* oocytes (Kobayashi et al. 2004). Similarly, antipsychotics like imipramine, desipramine, amitriptyline, citalopram, fluoxetine and maprotiline act on $K_{ir}3.2$, $K_{ir}3.1/3.2$ and $K_{ir}3.1/3.4$ channels, which are also targeted by general anaesthetics (GAs) like halothane, isoflurane, enflurane and F3 (1-chloro-1,2,2-trifluorocyclobutane). Bupivacaine, a local anaesthetic, acts on $K_{ir}3.2$, $K_{ir}3.1/3.2$, $K_{ir}3.1/3.4$ and $K_{ir}3.4$ by binding with cytoplasmic regions of the channels without any subunit combination specificity. Other drugs targeting $K_{ir}3.x$ subunits include MK-801 (NMDAR antagonist), QX-314 (classical cation channel blocker), ifenprodil (NMDAR antagonist) and R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (dopamine D_1R antagonist). Though these compounds are known to act on $K_{ir}3.x$ subunits, the binding sites of most of them were not investigated. Montandon et al. (2016) reported that fentanyl regulates GIRK channel-mediated rhythmic breathing and contributes to the respiratory depression by μ-opioid receptors (MOR) (Montandon et al. 2016).

19.3.1.3 Modulators of ATP-Sensitive K^+ Channels (K_{ATP} or $K_{ir}6.x/SURx$)

K_{ATP} channels have the most therapeutic potential among other subtypes and are modulated by two major classes of drugs, sulfonylureas (SUs) and potassium channel openers (KCOs). SUs such as glipizide, glimepiride, glibenclamide, tolbutamide and acetohexamide are well known to stimulate insulin secretion in type II diabetes mellitus (T2-DM) patients (Garcia and Kaczorowski 2005; Sturgess et al. 1985; Challinor-Rogers and McPherson 1994). The resulting effect is due to binding of the drugs with sulphonylurea receptors (SURx) of K_{ATP} channels, causing membrane depolarization by inhibiting K^+ efflux in the pancreatic β-cells (Abraham et al. 1999). SUs confer more sensitivity towards $K_{ir}6.2/SUR1$ compared to $K_{ir}6.2/SUR2A$ (Rubaiy 2016).

K_{ATP} channel openers constitute the diverse class and the largest number of small molecules modulating the K^+ channels. Potassium channel openers (KCOs) such as nicorandil, pinacidil and diazoxide activate the K_{ATP} channels (Ackerman and Clapham 1997; Lawson 2000; Camerino et al. 2007). KCOs are effective in the therapeutic management of myocardial ischemia, CHF, urinary incontinence, bronchial asthma and certain skeletal muscle myopathies (Shieh et al. 2000; Ackerman and Clapham 1997; Lawson 2000; Camerino et al. 2007). The sensitivity of the KCOs towards native K_{ATP} channels is attributed to the expression of SUR subtypes in different tissues, i.e. pancreatic β-cell (SUR1), cardiac (SUR2A) and smooth muscle (SUR2B). The pancreatic β-cell K_{ATP} channels are readily opened by compounds such as diazoxide, which is clinically approved for treatment of insulinoma-associate hypersecretion of insulin and persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI) (Akopova 2018). In contrast, these channels are weakly activated by pinacidil and remain unaffected by nicorandil or cromakalim, whereas cardiac channels are activated by nicorandil, pinacidil and cromakalim but not affected by diazoxide, and the smooth muscle K_{ATP} channels are activated by all

the three compounds (Robertson and Steinberg 1990; Edwards and Weston 1995). The pharmacological properties are known to produce, only when there is co-expression of $K_{ir}6.2$ with an appropriate SUR subtype leading to K_{ATP} conductance (Lawson 2000; Quast 1992). The side effects of SUs are also related to their ability to cross-react with other K_{ATP} subtypes. Bimakalin (a selective KCO) exhibited a dose-dependent vasodilation but failed to show anti-ischemic benefits in patients with coronary artery disease (CAD) during exercise-induced angina pectoris (Chan et al. 2008). Levosimendan, a K_{ATP} channel opener, showed significant reduction in pulmonary capillary wedge pressure in severe low-output HF patients following cardiac surgery and peripartum cardiomyopathy. Levosimendan has entered into the clinical studies for heart failure, amyotrophic lateral sclerosis, ventricular dysfunction, myocardial infarction, hip fracture and cardiorenal syndrome (Rubaiy 2016). Another new antihypertensive drug, iptakalim, synthesized by Thadweik Academy of Medicine, China, also activates K_{ATP} channels in the endothelium of resistance blood vessels (Wang et al. 2015; Duan et al. 2011). Iptakalim (KCO) as well as fluoxetine (classical antidepressant) showed alleviation of chronic mild stress depressive behaviour in wild-type mice. However, these symptoms are partially ameliorated in $K_{ir}6.2^{-/-}$ mice (Fan et al. 2016).

5-Hydroxydecanoic acid (5-HD) is a selective mitochondrial and plasma membrane K_{ATP} channel blocker. It is highly used in the study of the physiological role of mito-K_{ATP} channels. Non-sulfonylurea K_{ATP} channel blockers such as meglitinide, nateglinide, repaglinide and mitiglinide are also used to treat T2-DM. These drugs act by inhibiting pancreatic β-cell K_{ATP} channels, i.e. blocking $K_{ir}6.2$/SUR1 channels. Many derivatives of P1075, a cyanoguanidine K^+ opener, have been known to antagonize the vascular KCOs. These new-generation drugs called PNU compounds include PNU-37883A, PNU-89692, PNU-97025E and PNU-99963 (Rubaiy 2016; Chowdhury et al. 2017).

19.3.1.4 Modulators of K^+-Transport Channels ($K_{ir}1.1$, $K_{ir}4.x$, $K_{ir}5.1$, $K_{ir}7.1$)

Unlike other subtypes, the advancement of molecular pharmacology of ROMK or $K_{ir}1.1$ is quite limited. Tertiapin (bee venom peptide) is a selective and potent blocker of the rat $K_{ir}1.1$ current but exhibits 100 times less potency in human isoforms. Tertiapin and δ-dendrotoxin bind to the external vestibule of the K^+ conduction pore to inhibit $K_{ir}1.1$ channels (González et al. 2012). Two synthetic compounds, VU590 and VU591, were identified and screened at Vanderbilt University, USA. These compounds inhibit rat $K_{ir}1.1$ with IC_{50} values 290nM and 240nM, respectively. VU591 contributes to basal K^+ secretion by inhibiting ROMK in DCT without affecting Na^+ transport (Bhave et al. 2011).

Tricyclic antidepressants (TCAs) such as imipramine, desipramine, nortryptyline and amitriptyline block $K_{ir}4.1$ currents. Voltage-independent inhibition of $K_{ir}4.1$ currents is observed in the case of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, fluvoxamine and sertraline. The therapeutic activity and adverse effects of TCAs and SSRIs are thought to be associated with the blockade of astroglial K_{ir} channels by interacting with T128 and E158 residues of the conduction

pore of $K_{ir}4.1$ channels. No effective blockers of $K_{ir}5.1$ are reported till date. While $K_{ir}7.1$ remains unusually insensitive to the typical blocking property of Ba^+ and Cs^+, interestingly, no other activators or blockers are known for this channel.

19.4 Two P Domain K^+ Channels (K_{2P} Channels)

The first potassium channel containing two P domain in tandem was the TOK-1 channel discovered from the yeast *Saccharomyces cerevisiae* (Goldstein et al. 2001; Ketchum et al. 1995). These channels are referred to as "twin pore" or "tandem pore" as they possess nonconventional topology, i.e. dimers of dimers: two subunits forming a mature channel containing two non-identical pore-forming P domains (P1 and P2) which are arranged in tandem (as shown in Fig. 19.1) (Tian et al. 2014; Humphries and Dart 2015; Schneider et al. 2014). Most of the K_{2P} channels conduct voltage-independent outward current as they lack the voltage-sensing S4-TM domain (Coghlan et al. 2001; Goldstein et al. 2001). These channels also act as the classical background "leak" K^+ channels or open rectifiers and maintain negative resting membrane potential (Köhling and Wolfart 2016; Piechotta et al. 2011).

Fifteen mammalian KCNK genes encode K_{2P} channels. These channels were distributed among six clades on the basis of sequence homology and pharmacological characteristics. These are as follows: (1) two-pore domain weak inward-rectifying K^+ channels or TWIK ($K_{2P}1$ or TWIK-1, $K_{2P}6$ or TWIK-2, and $K_{2P}7$) are weak inward rectifiers sensitive to pH; (2) TWIK-related K^+ channels or TREK ($K_{2P}2$ or TREK-1, $K_{2P}4$ or TRAAK, and $K_{2P}10$ or TREK-2) are mechano-gated channels regulated by several stimuli – pH, stretch, osmolarity, temperature, neuroprotective agents and volatile anaesthetics; (3) TWIK-related acid-sensitive K^+ channels or TASK ($K_{2P}3$ or TASK-1, $K_{2P}9$ or TASK-3, $K_{2P}15$ or TASK-5) are inhibited by extracellular acidification and hypoxia; (4) TWIK-related alkaline pH-activated K^+ channels or TALK ($K_{2P}5$ or TASK-2, $K_{2P}16$ or TALK-1, and $K_{2P}17$ or TALK-2) are sensitive to alkaline pH and oxygen concentration; (5) tandem pore domain halothane-inhibited K^+ channels or THIK ($K_{2P}12$ or THIK-2, $K_{2P}13$ or THIK-1) are sensitive to halothane and arachidonic acid; (6) TWIK-related spinal cord K^+ channels or TRESK ($K_{2P}18$) are sensitive to free acids, protons, heat, anaesthetics and increased membrane tension (Jiménez-Vargas et al. 2017; Feliciangeli et al. 2015; Renigunta et al. 2015; Talley et al. 2003; Lesage and Lazdunski 2000).

19.4.1 Pharmacology of Two P Domain K^+ Channels

Given their critical physiological roles, K_{2P} channels represent a major target for the modulation of the physiological changes and pathological states. For example, $K_{2P}1$ and $K_{2P}2$ channels play an important role in the regulation of atrial size and heart rate and are, therefore, a putative target for antiarrhythmic drugs. Similarly, $K_{2P}18$ channels are good candidates for pain management as the pain signalling pathway

is strongly controlled by the $K_{2P}18$ channels. Moreover, K_{2P} channels are implicated in arrhythmia ($K_{2P}1$), depression ($K_{2P}2$), atrial fibrillation ($K_{2P}3$), migraine ($K_{2P}18$), cancer ($K_{2P}1$, $K_{2P}5$, $K_{2P}9$) and inflammation ($K_{2P}12/13$) as well as in the neuropathic pain ($K_{2P}18$) (also refer to Table 19.1) (Feliciangeli et al. 2015; Gada and Plant 2019; Li and Toyoda 2015).

Considering the lead optimization and drug discovery process, several practical limitations were observed. The unique structure of the K_{2P} family restricted the easy extrapolation from the available data of other K^+ channel subfamilies. The lack of a structural basis of the K_{2P} channels, until the explanation of the crystal structure of $K_{2P}1$ and $K_{2P}4$ in 2012, had stymied the development of pharmacophores of these channels (Gada and Plant 2019). Moreover, the electrophysiological characterization for understanding the biological significance of K_{2P} channels has been impeded by the poor or absent heterologous expression in *Xenopus* oocytes or COS-7 cells (Enyedi and CzirjáK 2010). The ubiquitous distribution of many subunits also limits the therapeutic modulation of specific K_{2P} channels. To develop any pharmacological modulator of these subunits, the researchers must overcome these barriers with available HTS techniques and necessary measures. Nevertheless, the numbers of small molecules and drugs interacting with the K_{2P} channels are steadily increasing over time. These channels are weakly sensitive or insensitive to the classical K^+ channel blockers including 4-AP, TEA, Cs^+ and Ba^+ (Dogan et al. 2019). Some other potent modulators have already been identified and are discussed in the succeeding section (Tian et al. 2014; Es-Salah-Lamoureux et al. 2010; Kasap and Dwyer 2018; Gada and Plant 2019).

19.4.1.1 Modulators of the TREK Family

There is evidence that putative inhaled anaesthetics also target K_{2P} channels (Patel and HonorÉ 2001; Olschewski et al. 2017). The minimum alveolar concentration (MAC) of general anaesthetics such as chloroform, desflurane and halothane variably increased in the TREK-1 knockout model. Nitrous oxide (N_2O), cyclopropane and xenon also activate the $K_{2P}2$ channels (Hudson et al. 2019). Similarly, halogenated inhaled GAs such as sevoflurane and isoflurane exhibited selective activation of K_{2P} channels subtypes like TREK-1, TRAAK, TASK-3 and TRESK. The anaesthetic effect is probably due to the membrane hyperpolarization via the K_{2P} current in combination with $GABA_A$ receptor activation (González et al. 2012). The fenamate class of NSAIDs (e.g. flufenamic acid, mefenamic acid and niflumic acid) selectively activates K_{2P} channel by interacting with the N-terminals (Gada and Plant 2019). Flufenamic acid at 100 μM concentration produced a 250% enhancement of the TREK-1 current, while mefenamic acid and niflumic acid at 100 μM concentration exhibited 150–180% enhancement of the K^+ current (Vivier et al. 2015). Recently, a selective opener of TREK-1 and TREK-2, GI-530139, showed effectiveness in hyperpolarizing the dorsal root ganglia (DRG) neurons in rats (Gada and Plant 2019; Loucif et al. 2018). An anti-ischemic and anticonvulsant drug, riluzole, potentiates TREK-1 as well as TRAAK currents in a dose-dependent manner. Riluzole is currently used in the treatment of amyotrophic lateral sclerosis (ALS) (Vivier et al. 2015; Lesage 2003).

Sipatrigine, a neuronal Na^+ and Ca^{2+} channel inhibitor, reversibly inhibits both TREK-1 and TRAAK in a dose-dependent manner. This effect in combination with glutamate inhibition makes this drug a choice for treatment of depression (Lesage 2003; Meadows et al. 2001; Tsai 2008). 3-N-Butylphthalide (NBP) extracted from celery seed also reversibly inhibits TREK-1 current. This neuroprotective agent is used in China for treatment of ischemic stroke (Ji et al. 2011). SSRIs such as fluoxetine reversibly block TREK-1 and related TASK-3 channels in a voltage-independent manner. Similarly, norfluoxetine, active metabolite of fluoxetine, is a more potent blocker of TREK-1 current (Kennard et al. 2005). In addition to SSRIs, several other antipsychotics such as chlorpromazine, loxapine, haloperidol, pimozide and fluphenazine also exhibited dose-dependent and reversible inhibition of both TREK-1 as well as TREK-2 but not TRAAK channels (Thümmler et al. 2007). Other pharmacological classes of drug inhibiting TREK-1 current include antihypertensive dihydropyridines (e.g. amlodipine and nifedipine), calcium antagonists (e.g. flunarizine) and antiarrhythmic agents (e.g. mexiletine and propafenone) (also refer to Table 19.1) (Vivier et al. 2015).

19.4.1.2 Modulators of the TASK Family

TASK-1 and TASK-3 are insensitive to many drugs unless they are used at higher concentrations. Both the channels are opened by inhalational GAs at clinically relevant concentrations, targeting a short cytoplasmic region nearer to 4-TM (Patel et al. 1999). Classical blockers of K^+ including Ba^{2+}, TEA, ChTx, aminopyridines and apamin also produced modest effects on TASK-1 and TASK-3 channels. Drugs such as quinidine, bupivacaine and ethanol partially inhibit the TASK-1 and TASK-3 currents, while fluoxetine and norpropoxyphene block only TASK-1 current (also refer to Table 19.1). Ruthenium red dye is a useful tool to separate TASK-1 and TASK-3 channels as it nearly causes complete blockade of TASK-3 but has little effect on TASK-1. However, this cationic dye is not employed to study TASKs in native systems as it non-specifically blocks many other ion channels (Kim et al. 2000; HajdÚ et al. 2003; CzirjáK and Enyedi 2003).

Anandamide and synthetic cannabinoid receptor agonists such as WIN552122 and CP55940 are the potent blockers of the human TASK-1 current. Routine use of anandamide and methanandamide to identify TASK-1 channels in native tissues has been disproved as they also possess comparable potency for TASK-3 in different species (Enyedi and CzirjáK 2010). Two TASK channel antagonists, PK-THPP and A1899, showed effective breathing stimulant property in rats (Cotten 2013). Similarly, terbinafine was identified as a selective and potent activator of TASK-3 channels in the whole-cell patch clamp method (Wright et al. 2017). Tian et al. (2019) discovered a small molecule, NPBA (N-(2-((4-nitro-2-(trifluoromethyl)phenyl)amino)ethyl) benzamide), which possesses higher selectivity and potency as a novel TASK-3 activator (Tian et al. 2019). Modulators of TASK-5 channels are not yet known.

19.4.1.3 Modulators of TALK, THIK and TRESK Families

Similar to other K_{2P} channels, TASK family members (i.e. TASK-2, TALK-1 and TALK-2) are also least affected by the classical blockers (e.g. 4-AP, TEA, Cs^+).

Among volatile GAs, chloroform is the potent activator of TASK-2 (Patel and HonorÉ 2001; Gray et al. 2000; Yost 2000). Clofilium inhibits TASK-2, while drugs like quinidine, bupivacaine and lidocaine are strong inhibitors of TASK-2 and weak inhibitors of TALK-1 (Hayashi and Novak 2013; Girard et al. 2001; Gutman et al. 2005). Whole-cell patch clamp and two-electrode voltage clamp studies demonstrated the sensitivity of antiarrhythmic drugs to TALK-2 channels in the *Xenopus* oocytes expression system. Significant activation was exhibited by quinidine, propafenone, mexiletine, metoprolol and propranolol, while drugs such as sotalol, verapamil, amiodarone and ranolazine inhibit the TALK-2 current (also refer to Table 19.1). Human TALK-2 channels were reported to be sensitive towards multiple antiarrhythmic drugs (Staudacher et al. 2018a). Activation of the THIK-1 channel by arachidonic acid and inhibition by halothane allow this channel to be easily distinguished from TREK, which is activated by halothane (Kim 2005). Therapeutic modulation of THIK-1 channels can make them a suitable target of antiarrhythmic drugs. Drugs such as propafenone, mexiletine, propranolol and lidocaine are known to inhibit THIK-1 channels (Staudacher et al. 2018b). TRESK channels are activated by volatile anaesthetics including isoflurane, desflurane, sevoflurane and halothane within the clinically used concentrations. Cloxyquin activates the TRESK channel independent of Ca^{2+}/calcineurin association and is hence used for examining TRESK conductions in native cells (Lengyel et al. 2017). Additionally, TRESK ($K_{2P}18$) is insensitive to several classical K^+ channel blockers, such as 4-AP, apamin and CsCl, and K_{ATP} channel blockers, like tolazamide and glipizide (also refer to Table 19.1). Arachidonic acid, quinine and quinidine are potent blockers of this channel, while extracellular Ba^{2+}-mediated inhibition is observed at higher concentrations. Amide local anaesthetics such as bupivacaine, tetracaine, rupivacaine, chlorprocaine, mepivacaine and lidocaine are reported to influence mouse and human TRESK. About 240 substances were tested on mouse TRESK. Among these, zinc, mercuric ions and mibefradil (non-specific to K_{2P} channels) were found to efficiently inhibit the current. Antidepressants, fluoxetine and sipatrigine also block the TRESK current along with the TREK family members, while lomotrigin (an anticonvulsant) showed selectivity towards only TRESK (10^{-5}M). Loratadine (an antihistaminic) also exhibited TRESK inhibition with a micromolar IC_{50} (Enyedi and CzirjáK 2015).

19.5 Voltage-Gated K^+ Channels (K_v Channels)

The first ever cloned gene from *Drosophila* was the voltage-gated *Shaker* channel followed by the phenotyping of the other potassium channel subtypes in insects, rats and mammals including humans (Gutman et al. 2005). The *Drosophila Shaker* channel acts as a prototype of all voltage-gated channels, consisting of a tetramer of homologous α-subunits, each comprising 6-TM segments and a circumferentially arranged membrane re-entering P-loop (Yellen 2002; Jensen et al. 2012). In brief, the four S1–S4 segments act as voltage sensors, which contain arginine (positively charged) in the S4 segment, while the K^+ conduction pore is formed by the four

S5-P-S6 sequences. The gating of the pore is regulated by pulling the S4-S5 linker (Kuang et al. 2015; Pongs 1999; Sokolova et al. 2001; Kim and Nimigean 2016; Wulff et al. 2009; Jackson 2017). Both the amino and carboxy terminals are found to lie on the intracellular side of the membrane (as shown in Fig. 19.1). Membrane depolarization activates majority of the K_v channels, whereas hyperpolarization leads to closing of the channel. The K_v channels are encoded by about 40 genes constituting the largest K^+ family, which is categorized under 12 subfamilies. This wide diversification of the channel arises from several factors, such as heteromultimerization, modifier subunits, alternate mRNA splicing, post-translational modification and association of other proteins like calmodulin and minK (Gutman et al. 2005). The voltage-gated channels are mainly classified as follows: K_v1 ($K_v1.1$–1.8), K_v2 ($K_v2.1$–2.2), K_v3 (3.1–3.4), K_v4 ($K_v4.1$–4.3), K_v5 ($K_v5.1$), K_v6 ($K_v6.1$–6.4), K_v7 ($K_v7.1$–7.5), K_v8 ($K_v8.1$–8.2), K_v9 ($K_v9.1$–9.3), K_v10 ($K_v10.1$–10.2), K_v11 ($K_v11.1$–11.3) and K_v12 ($K_v12.1$–12.3) subfamilies.

19.5.1 Pharmacology of Voltage-Gated K^+ Channels

The K_v channels are often expressed together with voltage-gated sodium or calcium channels as they regulate the action potential firing. As the K_v current controls the excitability of the cell, the pharmacological modulation of these channels may regulate the cellular excitability. Therefore, K_v channels play a significant role in Ca^{2+} signalling, proliferation, volume regulation and secretion as well as migration (Wulff et al. 2009). Not only this, the K_v channel is also found to be involved in the regulation of cellular activation in lymphocyte and cancer cells; consequently, K_v blockers have showed effectiveness in inhibition of proliferation and cellular activation (Chandy et al. 2004; Pardo et al. 1999; Comes et al. 2013). Overall, mutations or any defects in K_v channel genes are associated with a number of diseases, ranging from autoimmune diseases to cancer to cardiovascular, neurological and metabolic disorders. For example, episodic ataxia type 1, or EA1, is associated with loss-of-function mutations of the *KCNA1* gene ($K_v1.1$) (Maljevic and Lerche 2013; Lehmann-Horn et al. 2003), whereas loss-of-function mutations of $K_v1.1$/ $K_v1.2$ lead to neuromyotonia associated with small-cell lung cancer (SCLC) cells and limbic encephalitis (Camerino et al. 2007). K_v channels are also implicated in neuronal apoptosis ($K_v1.1$, $K_v1.3$), ischemic cell death ($K_v1.5$), epilepsy ($K_v1.1$, $K_v1.2$, $K_v1.4$, $K_v3.2$, $K_v4.2$, $K_v4.3$), Alzheimer's disease ($K_v3.4$, $K_v4.2$), tinnitus ($K_v7.1$– $K_v7.5$) pain ($K_v7.1$–$K_v7.5$) and neuropsychiatric disorders ($K_v7.1$–$K_v7.5$) (also refer to Table 19.1) (Tsantoulas 2015; Shah and Aizenman 2014; Langguth et al. 2016).

The pharmacological modulation of K_v channels can be effective in the therapeutic management of various human channelopathies, which is further underlined by the transgenic mice models. Metal ions, peptide toxins and small molecules are well known to regulate K_v current conduction by either external or internal binding to the conducting pore or by altering the channel gating mechanism (Wulff and Zhorov

2008). However, the lack of well-defined crystal structures of physiologically important channels (e.g. $K_v1.5$, $K_v7.2$ and $K_v11.1$) is one of the major hurdles in the rational design of modulators. Importantly, impressive progress has been made in the structural study in the last decade. Beside this, antibodies and toxins have been continuously engineered to obtain better delivery of the drug to the channel-expressing cells (Wulff et al. 2009; Zhou et al. 1998). The early-stage development of K_v modulators presents many challenges, but the fact cannot be denied that there are considerable opportunities for forthcoming success. Tremendous work has been devoted to identify the agents that interact with the voltage-gated K^+ channels with higher affinity.

19.5.1.1 Modulators of the K_v1 Family

The K_v1 family is sensitive to the classical non-peptidyl blockers (TEA, 4-AP), but dramatic variation is observed between members of the families (Mathie et al. 1998). $K_v1.1$ channels are selectively blocked by the dendrotoxins (DTx) isolated from green and black mamba snakes. Among the different DTx, δ-DTx and dendrotoxin K are highly selective and currently used as a diagnostic tool to determine the presence of $K_v1.1$ channels in real cells (Vacher et al. 2007). Other well-known peptide toxins such as ChTx, maurotoxin (MTx), noxiustoxin (NxTx) and kaliotoxin (KTx) also block $K_v1.1$ channels (Kaczorowski and Garcia 1999). Many newer toxins have been isolated and identified as modulators of these channels, but the selectivity can only be achieved by protein engineering. Small molecules inhibiting this channel also include capsaicin, flecainide, nifedipine, diltiazem and resiniferatoxin (also refer to Table 19.1) (Gutman et al. 2005).

The $K_v1.2$ channel is the most abundant K_v1 subtype in the mammalian CNS. Due to the lack of a specific tyrosine residue for TEA binding, the $K_v1.2$ channel offers resistance to the higher concentration of external TEA. However, 4-AP shows sensitivity at the normal concentration range required to block the $K_v1.1$ channel. Tityustoxin-Kα from *Tityus serrulatus* (a Brazilian scorpion) blocks $K_v1.2$ subunits. These subunits are also inhibited by δ-DTx and dendrotoxin K. The elucidation of the functional roles of $K_v1.2$ channels in CNS can be done by using a 28-amino-acid peptide toxin, OsK2, isolated from *Orthochirus scrobiculosus* (Central Asian scorpion) (Dudina et al. 2001). Nifedipine, flecainide, resiniferatoxin and anandamide have also been reported to block $K_v1.2$ channel conduction.

Scorpion venom NxTx was the first identified peptidyl blocker of potassium channels. This venom inhibited the $K_v1.3$ channel obtained from heterologous expression in *Xenopus* and in Jurkat cells (Garcia et al. 1997; Swanson et al. 1990; Sands et al. 1989). ChTx has been known to inhibit $K_v1.3$ channels in human T-cells and neuronal tissue (Swanson et al. 1990). In search of selective toxins, margatoxin (MgTx) from scorpion *Centruroides margaritatus* was characterized as a high-affinity blocker against $K_v1.3$ channels (Augustynek et al. 2016). Importantly, MgTx does not show any effect on ChTx-sensitive K^+ channels (Garcia-Calvo et al. 1993). Other toxins from scorpion are KTx, agitoxins (AgTx), *Pandinus imperator* venom (Pi1, Pi2 and Pi3) and *Centruroides limbatus* venom (hongotoxin-1) which also inhibit $K_v1.3$ channels (Panyi 2005). Peptidyl toxin

from sea anemone (e.g. BgK, ShK and AsKS) and spider venom (e.g. hanatoxin 1 and hanatoxin 2) are also well-known blockers of the K_v channels (Garcia et al. 1997). A natural triterpene, correolide, is a potent blocker of $K_v1.3$ but showed affinity for other members of the K_v1 family (Felix et al. 1999). Pharmacological agents such as naltrexone, sulfamidbenzamidoindane, UK-78282, WIN-17317-3 and CP 339818 have been extensively studied for their inhibitory effect on $K_v1.3$ channels (Coghlan et al. 2001; Hill et al. 1995).

Fairly mild acidosis (pH = 6.5) can inhibit the $K_v1.4$ current due to the slow recovery of N-type inactivation, which is a useful diagnostic characteristic of this subtype (Claydon et al. 2000). $K_v1.4$ channels are blocked by 4-AP (0.7–13 mM) but exhibit resistance to external TEA, DTx or similar toxins. Few small molecules are known to inhibit the $K_v1.4$ current, including UK78282, riluzole, quinidine and nicardipine (also refer to Table 19.1) (Gutman et al. 2005; Kuzmenkov et al. 2015).

Abundant distribution of the $K_v1.5$ channel makes it undoubtedly one of the most important channels. They are insensitive to TEA while sensitive to 4-AP even at relatively lower concentrations. Channel inhibition is exhibited by several therapeutic classes including calcium channel blockers (e.g. verapamil and nifedipine), antiarrythmic drugs (e.g. quinidine, clofilium and propafenone), antibiotics (e.g. erythromycin) and antihistaminics (e.g. loratidine, terfenadine and ebastine) (also refer to Table 19.1) (Zhang et al. 1997; Grissmer et al. 1994; Malayev et al. 1995; Gutman et al. 2005; Gutman et al. 2003).

$K_v1.6$ is sensitive to both TEA and 4-AP. Venoms like ChTx, MgTx, hongotoxin and tumulustoxin were identified as the potent blockers of these channels. $K_v1.7$ channels are insensitive towards external TEA but blocked by 4-AP, nifedipine, amiodarone, quinidine, flecainide and tedisamil, whereas reported blockers of $K_v1.8$ include Ba^{2+}, ChTX, 4-AP, TEA, ketoconazole, pimozide and verapamil (Gutman et al. 2005; Kuzmenkov et al. 2015; Gutman et al. 2003).

19.5.1.2 Modulators of the K_v2 Family

The K_v2 family ($K_v2.1$ and $K_v2.2$) is activated at low thresholds, so-called delayed rectifier channels. Linoleic acid acts as an activator of the $K_v2.1$ channel, while no activator is reported for $K_v2.2$. The classical blockers 4-AP and TEA are insensitive to these channels. The isolated venom, hanatoxin, from Chilean tarantulas binds to the S3/S4 linker to inhibit channel opening (Swartz and Mackinnon 1997a, b). Hanatoxin is known to act as the gating inhibitor of the $K_v2.1$ channel. Several blockers of $K_v2.1$ and $K_v2.2$ channels are well known. Both the channels are sensitive to blockers like TEA and 4-AP. Specifically, the conduction of $K_v2.1$ is inhibited by internal and external Ba^{2+}, internal Mg^{2+}, halothane and tetrapentylammonium, whereas the $K_v2.2$ current is inhibited by quinine and phencyclidine (Gutman et al. 2005).

19.5.1.3 Modulators of the K_v3 Family

The K_v3 family plays many important physiological and pharmacological roles, especially in mammalian CNS. The unique feature of K_v3 includes channel activation at a depolarized potential which is more positive than −20 mV. $K_v3.1$ and $K_v3.2$ have showed little inactivation unlike $K_v3.4$, which exhibited rapid N-type

inactivation (Rudy and Mcbain 2001). K_v3 manifests extraordinarily high sensitivity towards 4-AP and TEA because of the presence of the tyrosine residue at the carboxyl terminal of P-loop. Drugs like cromakalim, diltiazem, nifedipine, flecainide and resiniferatoxin also inhibit $K_v3.1$ channels. The $K_v3.2$ current is blocked by 8-bromo-cGMP, verapamil, D-NONOate and 3-isobutyl-1-methylxanthine. $K_v3.3$ is reported to show variable inactivation kinetics in contrast to other K_v3 family members. The subunits of $K_v3.3$ are sensitive to hypoxia and hence implicated in oxygen sensing in pulmonary vasculature (Guo et al. 2008). Blood-depressing substance (BDS) from sea anemone, *Anemonia sulcata,* has two isoforms, BDS I and BDS II, which are selective and effective blockers of $K_v3.4$ (also refer to Table 19.1). Although the specificity of BDS I towards $K_v3.4$ has been questioned, these toxins at higher concentrations were earlier employed as selective tools for $K_v3.4$ (Riazanski et al. 2001; Yeung et al. 2005).

19.5.1.4 Modulators of the K_v4 Family

K_v4 ($K_v4.1$, $K_v4.2$ and $K_v4.3$) channels are strongly influenced by the accessory proteins like K_v4 channel-interacting proteins (KChIPs), DP66 and DPP10 to alter the channel gating (An et al. 2000). Absence of KChIPs in knockout mice shows increase in susceptibility of ventricular tachycardia and seizures (Gutman et al. 2005). These channels are relatively resistant to mM concentrations of TEA and provide moderate sensitivity to 4-AP. Fampridine is known to inhibit the $K_v4.1$ channel (Alexander et al. 2017). No other blocking agents are reported to block the $K_v4.1$ channel. The slower voltage-dependent activation and inactivation rates of $K_v4.2$ were blocked by hanatoxin and heteropodatoxins (HpTx1, HpTx2 and HpTx3) in *Xenopus* oocytes. Quinidine and flecainide are also potent blockers of the $K_v4.2$ current. Also, arachidonic acid modulates $K_v4.2$ by interacting with KChIPs (Holmqvist et al. 2001), whereas niflumic acid, bupivacaine, DIDS (Cl^- channel blocker) and nicotine (at concentrations seen during smoking) block the $K_v4.3$ conduction in oocytes (Gutman et al. 2005). It is reported that the level of $K_v4.3$ mRNA decreases in patients with paroxysmal AF (Gutman et al. 2005).

19.5.1.5 Modulators of the K_v7 Family

The $K_v7.1$ channel encoded by the KCNQ1 gene exhibits delayed rectifier current with varying activation kinetics between expression systems. Several pharmacological agents are already in consideration with regard to blocking of the $K_v7.1$ channel and its complexes. Clofilium and XE911 are potent inhibitors of the $K_v7.1$ channel. The novel benzodiazepine derivatives, L-735821 and L-364373, showed a distinct effect on the $K_v7.1$ channel. The former compound increases the ventricular action potential duration by inhibiting the $K_v7.1$ current, while the latter apparently activates the $K_v7.1$ conduction (Salata et al. 1996; Salata et al. 1998). Chromanol 293B has demonstrated inhibition of the KCNQ1/minK complex. XE991, mefloquine and azimilide are also reported as selective blockers of the $K_v7.1$ channel.

$K_v7.2$ and $K_v7.3$ constitute heteromultimers to produce larger currents (Langguth et al. 2016; Haick and Byron 2016). However, both the channels have slightly different gating mechanisms and sensitivities towards inhibitors (Robbins 2001).

K_v7.2 channels are resistant to 4-AP (2 mM) but are effectively blocked by TEA as they possess the tyrosine residue in P-loop. K_v7.2 channels are also blocked by XE991 and L-735821. In contrast, K_v7.3 channels are insensitive to external TEA (5 mM) and are inhibited by 4-AP, clofilium, ChTx and E4031 (Dalby-Brown et al. 2006). At present, little pharmacological data is available for K_v7.4 channels. This channel shows intermediate sensitivity to TEA. Retigabine or ezogabine, a K_v7.4 activator, was approved by FDA in 2011 for adjuvant therapy of partial-onset seizures in adults (Maljevic and Lerche 2013; Gribkoff 2003; Humphries and Dart 2015; Jenkinson 2006). The other compounds inhibiting this channel include bepridil and XE991 (Dalby-Brown et al. 2006; Augustynek et al. 2016). K_v7.5 channels are activated by BMS204352 and retigabine, and further promote conduction of the channels. TEA exerts little sensitivity towards K_v7.5 channels. XE991 also demonstrated selective inhibitory effect on K_v7.5 channels. Linopirdine is known to block all the members of the K_v7 family (Camerino et al. 2007; Kuzmenkov et al. 2015).

19.5.1.6 Modulators of K_v5, K_v6, K_v8 and K_v9 Families

The K_v5 family (K_v5.1), the K_v6 family (K_v6.1, K_v6.2, K_v6.3 and K_v6.4) and the K_v8 family (K_v8.1 and K_v8.2) have no function of their own but modulate the K_v2 channels' function. These channels along with K_v9 channels are referred to as modifiers.

19.5.1.7 Modulators of K_v10, K_v11 and K_v12 Families (EAG Family)

The EAG (*ether-à-go-go*) comprises the eag, eag-related genes (erg) and eag-like K^+ channels (elk) subfamilies. The eag 1 and 2 (K_v10.1, K_v10.2); erg 1, 2 and 3 (K_v11.1, K_v11.2, K_v11.3); and elk1 and 2 (K_v12.1, K_v12.2, K_v12.3) have been identified so far in mammalian brain (Alexander et al. 2017; Alexander et al. 2013; Dai and Zagotta 2017). Among the EAG family, K_v11.1 or HERG (human *ether-à-go-go*) is one of the notable members, as defect in the channel underlies LQT2 syndrome (Giudicessi and Ackerman 2012).

Quinidine and intracellular calcium are known to block both K_v10.1 and K_v10.2 channels. The monoclonal antibody, mAb62, an anti-K_v10.1, was studied *in vitro* on the human breast cell line (MDA-MB-435S) and *in vivo* using tumour model nude mice. It showed accumulation for at least 1 week in the tumour cells *in vivo* due to Cy5.5 fluorophore labelling (Napp et al. 2016). A monoclonal antibody, mAb56, antagonizes the K_v10.1 (hEag1) current and exhibits anti-proliferative and *in vivo* tumour cell growth inhibition in breast, ovarian, colon, melanoma, fibrosarcoma and pancreatic carcinoma (Gómez-Varela et al. 2007).

The HERG (K_v11.1) channels are effectively blocked by class III antiarrhythmic methanesulphonamides like dofetilide, MK-499 and E-4031. Several other compounds including antihistaminics (e.g. terfenadine), antibiotics (e.g. erythromycin), prokinetic agents (e.g. cisapride) and antipsychotics (e.g. sertindole) are known to block HERG channels (also refer to Table 19.1) (D'amico et al. 2013). Combination therapy of ketoconazole and terfenadine also prolonged the cardiac action potential synergistically by targeting HERG.

CD160130 preferentially blocks the $K_V11.1$ IB isoform expressed in leukaemic cells and induces apoptosis of primary cells of chronic lymphoid leukaemia (CLL) (Pillozzi et al. 2002; Pillozzi et al. 2007). Halofantrine and mefloquine block HERG channels, where the severe side effect of the former can be associated with HERG blockade. Moreover, ergotoxin specifically blocks HERG but showed no affinity to eag or elk channels. A drug like sipatrigine is reported to block $K_v11.2$, whereas $K_v11.3$ is blocked by pimozide and sertindole (Gutman et al. 2005). On the other hand, the pharmacology of the elk subfamilies is very limited. These channels are resistant to TEA (100 mM) but can be effectively blocked by Ba^{2+} (1 mM) (Trudeau et al. 1999).

19.6 Conclusion

Potassium channels have been extensively investigated for molecular diversity, subunit stoichiometry, channel assembly, precise biophysical properties and modulation by different pharmacological agents. The ubiquitous distribution and diversification of these channels allow them to play a pivotal role in the regulation of various cellular signalling processes that governs the action potential waveform, stimulus-secretion coupling, electrolyte transport and regulation of cellular volume. Concurrent with better insight into the channel topology and functions, the understanding of the pathophysiological regulation and genetic mutation of K^+ channels could lead to the identification and validation of novel modulators as a therapeutic strategy in disease conditions. Apart from peptide and non-peptide toxins, K^+ channels are the well-known targets of several pharmacological classes of drugs, including GAs; antiarrhythmic, antihypertensive, anti-ischemic, antidiabetic and antipsychotic drugs; and NSAIDs. Unlike the other subtypes, remarkable progress has been observed in the case of K_{ATP} channels. Compounds like diazoxide (KCO) and retigabine ($K_v7.4$ activator) are already approved for clinical use in PHHI and adjuvant therapy of partial-onset seizures in adults, respectively. Many other compounds such as levosimendan and BMS-204352 are under active clinical development. In addition to this, the recent introduction of new insight into $K_{Na}1.1$ (formerly $K_{Ca}4.1$) and $K_{Na}1.2$ (formerly $K_{Ca}4.2$) will definitely catalyse a transformation in the research and drug discovery process. Together with these progresses, the possibility of in-depth exploitation of other K^+ channel subtypes as potential pharmacological targets has also spurred the research thrive. In addition to the well-known venom toxins and small molecules, unravelling precise tissue-specific and targeted drug delivery has led to the introduction of gene therapy as a novel means for delivery of mAbs to target specific channel subtypes. Monoclonal antibodies such as mAb56 and mAb62, specific for inhibition of $K_v10.1$, have already been reported to possess higher therapeutic potential in targeting tumour cells *in vitro*. However, many challenges and hurdles in targeting these channel proteins remain to be resolved, including lack of proper structural data as well as defined molecular pharmacology of many K^+ channel subtypes. The promising high-throughput screening methods are expected to accelerate the identification and development of

novel pharmacological agents targeting these channels. It is to be anticipated that the improvised technology and newer delivery approaches could collectively enhance the in-depth knowledge of channelopathies and will disclose newer pharmacotherapeutic avenues in the upcoming years.

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Pharmacology of Calcium Channel

20

Santanu Mallik and Pratap Chandra Acharya

Abstract

Calcium channel plays a very crucial role in the regulation of various vital functions of the body. The effects and modes of action of various drugs on calcium channels have been outlined with every detail. The pharmacology of various subunits and subtypes of this channel has been discussed in view of drug development in the near future. The role of Ca_v1 channel in health problems such as Parkinson's disease, cardiovascular diseases, subtypes of Ca_v2 channels in terms of G-protein inhibition and synaptic vesicle release, Ca_v3 channels for peptide toxins, and $\alpha 2\delta$ ligands in amino acid transportation and synaptic transmission has been highlighted in the literature. Diseases like obesity, epilepsy, and anxiety can be treated easily by targeting the T-type calcium channel which is one of the best potential therapeutic targets for the aforesaid diseases. In epilepsy and neuropathic pain, $\alpha 2\delta$ subunit is the most prominent area of therapeutic target by the gabapentinoid drugs. Thus, scientific research on calcium channel pharmacology may become revolutionary in the management of various chronic diseases. In this chapter, all possible attention has been given to describe the super selectivity of different subtypes of calcium channels by focusing on isoforms of channels and biophysical properties in various target tissues.

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Abbreviations

AID	α-Interaction domain
CRMP-2	Collapsin response mediator protein-2
CYP450 3A4	Cytochrome P450 3A4
DUB	Deubiquitinating enzyme
GABA	γ-Aminobutyric acid
GPCR	G-protein-coupled receptors
IP_3	Inositol 1,4,5-trisphosphate
NAGly	N-Arachidonylglycine
pS	Picosiemens
TAT	Transactivator of transcription
US-FDA	United States Food and Drug Administration
USP5	Ubiquitin-specific peptidase 5
ω-TRTX-Hg1a	ω-Theraphotoxin-Hg1a

20.1 Introduction

Erwin Neher and Bert Sakmann, Nobel laureates (1970), invented the technique "patch clamp" (De la Peña and Gomis 2019) which confirmed the practical existence of ion channels in a physiological environment. Ion channels are transmembrane glycoprotein pores having the open and close properties like gates. These channels allow the movement of ions in a regulated manner. Electrochemical gradient governs the direction and rate of ion movement. The transportation is always down the gradient and the speed of ion transportation through the channels is very high which is approximately about 10^7 ions/s (Sanchez-Sandoval and Gomora 2019; Uzieliene et al. 2018). The types of calcium channels are shown in Tables 20.1 and 20.2 (Zamponi et al. 2015; Spedding and Paoletti 1992).

In the recent medical science, diseases like migraine, epilepsy, hypertension, and cerebral ischemia and rarer diseases like night blindness have been treated by targeting calcium channels. The channels allow calcium ions to flow out of the endoplasmic reticulum or to flow into the cell (Zamponi et al. 2010; Marchetti 2013). The opening of such ion channels is initiated due to the changes in voltage across the membrane, for example, signal propagation of nerve cells. One more factor which helps open the channel is the ligand molecule like inositol 1,4,5-triphosphate (IP_3) (Prakriya and Lewis 2015; Simms and Zamponi 2014). The pancreatic beta cells, neurons, myocytes, etc., have calcium channels. Such kind of channels forms most of the systematic molecular connection between biochemical signaling intracellularly

Table 20.1 Types and subtypes of calcium channels

1. Voltage-dependent calcium ion-selective channels L-type T-type N-type P-type
2. Calcium ion-selective channels Calcium ion release channels in the sarcoplasmic reticulum Receptor-operated calcium ion channels
3. Voltage-dependent ion channels (no calcium ion selectivity) (Na^+, K^+, etc.)

Table 20.2 Calcium channel currents and α1 subunits (Dolphin 2016; Hirano et al. 2017)

Ca^{2+} currents	Family	α1 subunit
L-type	$Ca_v1.1$ $Ca_v1.2$ $Ca_v1.3$ $Ca_v1.4$	Ca_v1
P-type N-type R-type	$Ca_v2.1$ $Ca_v2.2$ $Ca_v2.3$	Ca_v2
T-type	$Ca_v3.1$ $Ca_v3.2$ $Ca_v3.3$	Ca_v3

and is responsible for the depolarization of membranes. Calcium channels are responsible for the contraction of muscles, release of hormones, regulation of membrane excitements, regulation of gene expressions, etc. (Badou et al. 2013; Nanou and Catterall 2018; Nieto-Rostro et al. 2018).

Functioning of contraction–excitation coupling (Santulli et al. 2017), endocrine and exocrine gland secretion (Gambardella et al. 2017), secretion of hormones (DiMeglio and Imel 2019), platelet aggregation (Aggarwal et al. 2016), excitation–transcription coupling (Kim and Kim 2018), release of neurotransmitters (Bruckner et al. 2017), and chemotaxis (Larsch et al. 2015) are initiated by the calcium channels. Thus, calcium plays a critical role as an initiator and modulator of cellular functioning (Scott et al. 2016). This is the reason why calcium is considered as an important regulatory component in the human body for various cellular activities. It is important to focus on the biochemical and pharmacological depiction of calcium channels to understand its whole contribution and process mechanism (Raffaello et al. 2016).

Millions of calcium ions enter to the cells through calcium channels and help in contraction–excitation coupling and release of neurotransmitters in muscles and nerves. These channels are also available in neutrophils, lymphocytes, plant cells, and sperms which are also considered as non-excitable cells. Calcium channels are also found in intracellular membranes, but the type may vary with those present in the plasma membrane (Medrihan et al. 2013; Südhof 2013).

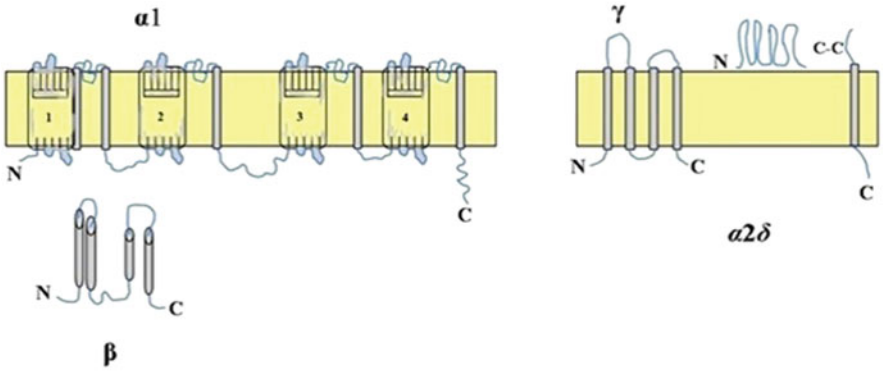

Fig. 20.1 Pictorial representation of $\alpha 1$, β, α-2δ and γ subunits of calcium channel. Four homologous domains of $\alpha 1$ subunit (1–4) with helical structures have been presented

The opening and closing of the calcium channels are the fundamental properties. Depending on the mechanism of these properties, calcium channels are categorized (Fig. 20.1) under two broad headings: (1) voltage dependent and (2) receptor operated. Voltage-dependent calcium channels are further subclassified depending on the pharmacological sensitivities, kinetic properties, and voltage sensitivities (Zamponi 2016).

1. Voltage-dependent calcium channels (Zamponi et al. 2015; Catterall and Swanson 2015):
   (a) L-type: Generally, this type of channel is activated by high voltages and has large conductance of ~25 pS (picosiemens).
   (b) T-type: This type of channel is activated by minimum voltages and is a transient current with comparably low conductance of ~9 pS.
   (c) N-type: It is an intermediate channel in between L- and T-types. This type of channel conducts an intermediate size of ~15 pS transient current and is usually activated with high voltages.

It has been reported that L- and T-type channels are present in skeletal and cardiac muscles where N-type channel is absent. In sympathetic neurons of ganglia, T-type is not present but both L- and N-types are available. The T-, L-, and N-types of receptors coexist in dorsal root ganglia sensory neurons. Furthermore, differences have been observed in the same type of channel located at different organs. For instance, L-type calcium channel present in the skeletal muscle and heart shows differences in electrophysiological and pharmacological properties (Kappel et al. 2013; Rose et al. 2013; Li et al. 2017a, b).

Receptor-operated calcium channels or calcium channels operated by receptor-dependent mechanism are usually opened after receiving signals from an activated associate receptor (Fig. 20.2). Chloride and ion channels are two major types

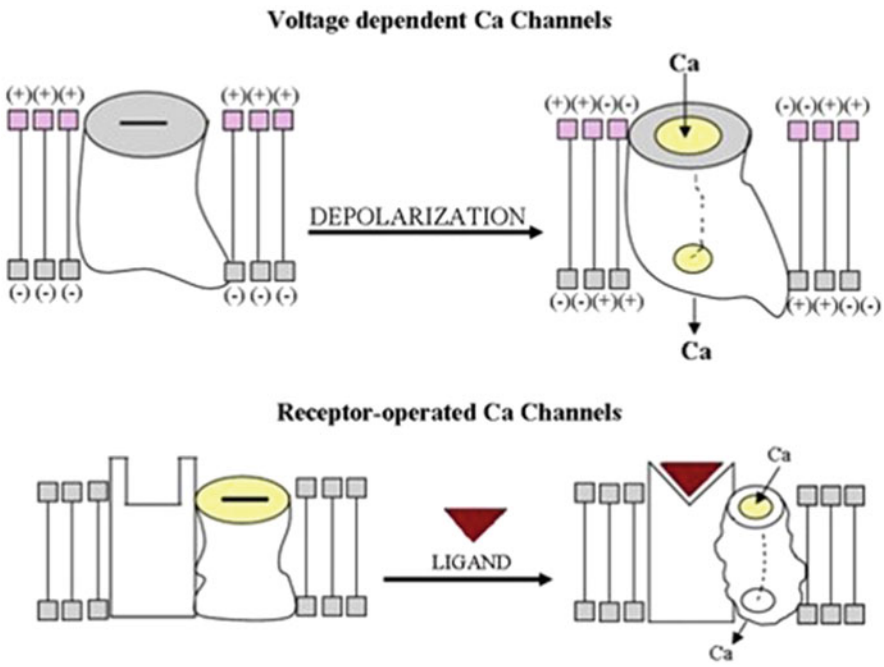

Fig. 20.2 Speculative representation of voltage-dependent and receptor-operated calcium channels and their mechanism of action

which come under receptor-dependent calcium channel. Chloride channels are generally associated with glycine and γ-aminobutyric acid (GABA) receptor, and nonspecific ion channels are found associated with nicotinic acetylcholine receptor (Szabó et al. 2014; Schwab et al. 2012; Yocum et al. 2018).

20.1.1 Pharmacology of Calcium Channels and Its State Dependency

Drug affinity entirely depends on the specific gating status of the channels for the drugs that target ion channels. State dependency is the reason behind the clinical utility of drug molecules. On the other hand, experimental use of a drug is an evidential proof of successful state-dependent inhibition. It is very important to contemplate hypothetical considerations of state-dependent inhibition, which includes drug-inactivation synergism, use-dependent block (the rate of nerve stimulation is proportional to the degree of block), guarded receptors (acyclic), and cyclic or allosteric receptor (Yoder et al. 2018).

The idea of drug binding was introduced by Armstrong in 1966 and Strichartz in 1973. Different strengths to resting closed (R), open (O), and closed inactivated (I) ion channels have been reported. Later on, the idea was accepted as the theoretical concept of a modulated drug receptor (Zaydman et al. 2014; Lenaeus et al. 2017).

Depending on this hypothesis, study on the biological phenomena with the help of physics and drug or medication action experiments on ion channels helped the medical science understand in a better way about the repression of signals of local anesthetics and sodium channel inhibitors such as the antiarrhythmic drugs, antiepileptics, and actions of other drugs (Borowicz and Banach 2014; Tikhonov and Zhorov 2017).

20.1.2 Drug Action and State Dependency

The three conformational states of calcium channels have been widely accepted: R state or resting, nonconducting; O state or open, conducting, or excited; and I state or inactivated, nonconducting. During the depolarization process, channels move to I through O from R and again back to the R state during the change in cell's membrane potential (hyperpolarization) (Alexander et al. 2015/16).

20.1.2.1 Drug Inhibition of the R State

The scientific findings are insufficient to understand the mechanism of R state-dependent inhibition. This interaction is realistic but suggested the transformation to O or I states which usually initiate drug dissociation from resting channels. Factually, for Ca^{2+} channels, no single evidence has been reported for a "use-dependent release" from the same block.

The difficulties with the interpretation of R state are represented in Fig. l. Peak current inhibition or the initial block by the drug mibefradil during the first test pulse may be elucidated as inhibition of resting channels (RB) with selectivity (Tang et al. 2016; Yang et al. 2014a, b).

20.1.2.2 State-Dependent Channel Inhibition – "O" and "I"

The binding of drug occurs only in the O state, and at this stage, the flow of the current gets interrupted which is depicted in Fig. 20.3b. Cumulative suppression of the current can be predicted from this model representation as shown in Fig. 20.3c. There are two exponential time courses which have been associated with these channels: recovery from inactivation ("fast phase") and dissociation of the drug channel complexes ("slow phase") (Fig. 20.3) (Striessnig et al. 2014; Laurent et al. 2016).

The last phase is almost undetectable as the concentration of drug is nearly similar to the saturation. The slow inactivation model as shown in Fig. 20.4a, b produces a very similar attribute of use-dependent current inhibition (Fig. 20.4c). However, among the two exponential time courses of the recovery processes, one depicts the recovery from slow inactivation and the other indicates recovery from the fast inactivated state.

20.1.2.3 Calcium Channel Inhibition – Molecular Studies

Recently, new important insights have been explored into the molecular events underlying calcium channel block by conducting pharmacological studies on

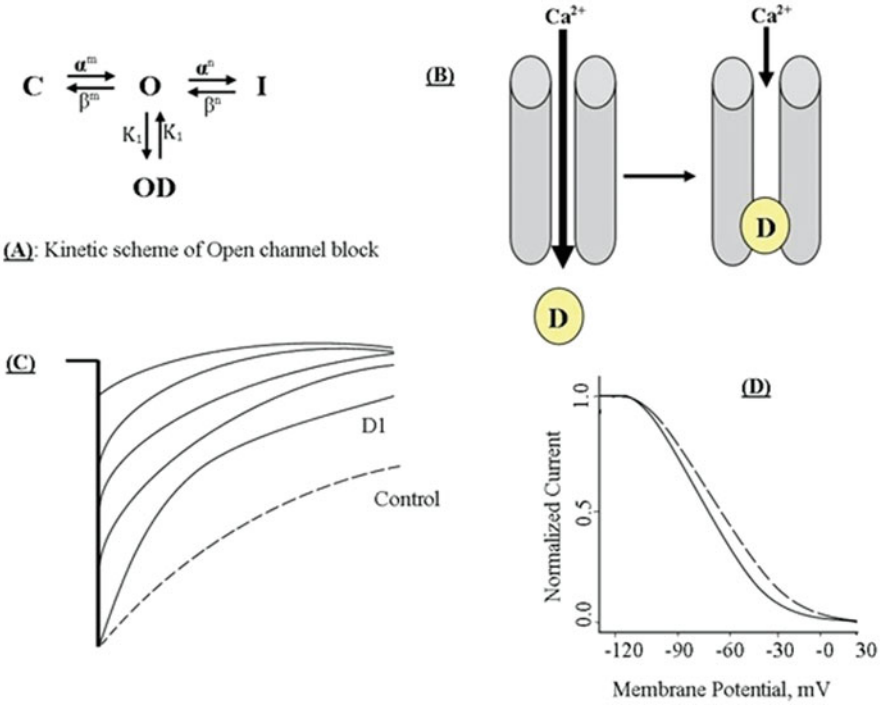

Fig. 20.3 Hypothetical open channel block model. (**a**) Open state model depicting kinetic scheme of calcium channel, (**b**) molecular mechanism involved in an open state, (**c**) changes in kinetics of use-dependent and open channel inhibition in reference to control, and (**d**) model graphical representation of normalized current versus membrane potential in open state kinetics

recombinant and mutant calcium channels of different subunit compositions. The subunit compositions strongly regulate the properties of high-voltage-activated calcium channels. Particularly, the four subunits strongly modulate the inactivation of high-voltage-activated channels (Prakriya and Lewis 2015; Zhang et al. 2017).

20.1.2.4 Mechanisms of Drug Action – At Molecular Level

The molecular level drug action can be easily understood by the actions of diltiazem, phenylalkylamines, and mibefradil.

The pore-forming transmembrane segments IIIS5, IIIS6, and IVS6 contain very important determinative of the putative receptor sites for phenylalkylamines, diltiazem, and dihydropyridines (Vega-Vela et al. 2017). Various studies reveal that a change in inactivation kinetics helps many of the mutation processes within the putative drug-receptor region (Laurent 2017). Moreover, it is evident that the affinity of phenylalkylamines, diltiazem, and mibefradil has been impaired by the mutation mechanism because it can also influence the channel activation. Various studies suggest that the process of channel inactivation is closely linked to state-dependent inhibition of Ca^{2+} channels (Zheng 2013; Santos et al. 2017). It is difficult to explain

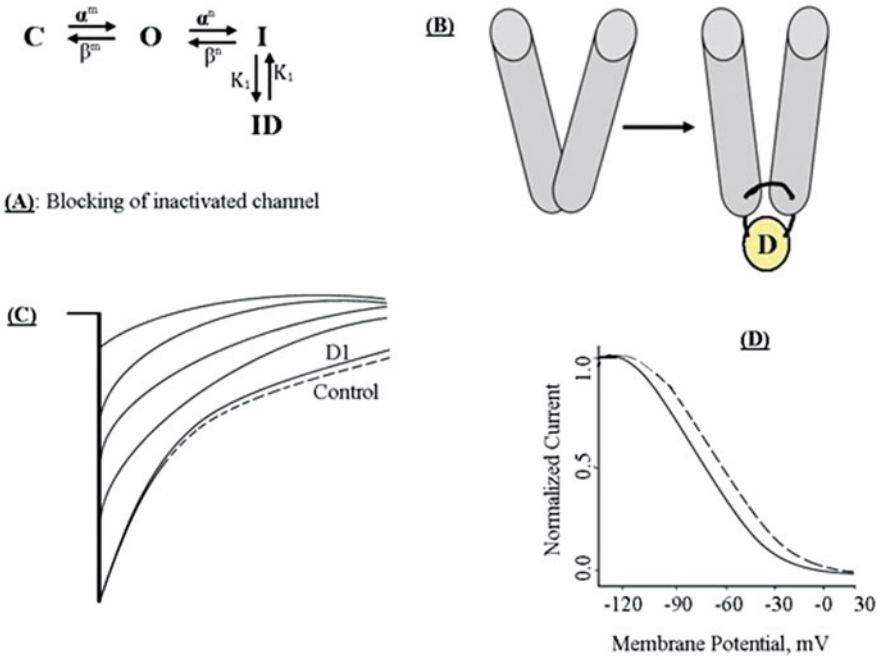

Fig. 20.4 Hypothetical model of inactivated channel block. (**a**) Inactivated state model depicting selective state transition kinetic scheme of calcium channel, (**b**) selective inhibition mechanism involved in an inactivated state, (**c**) current kinetics of use-dependent and inactivated channel inhibition in reference to control where D1 represents the initial impact of current on drugs in comparison to control, and (**d**) model graphical representation of normalized current versus membrane potential in inactivated state channel block kinetics

in depth about all recent findings on recombinant channels in terms of state-dependent pharmacology. For example, mibefradil represents a benchmark of an open channel blocker, showing insignificant changes in channel availability. However, these channels are expected to be more efficiently inhibited by "open channel blockers" (Krouse et al. 2015).

Mibefradil-sensitive co-expression of the 0.12.1 subunit does not increase but usually decelerating by nature. This result is inconsistent with respect to channel block model opening. The same observation was found for $Ca_v1.2$ inhibition by phenylalkylamines (Matsunami et al. 2012; Woo et al. 2019).

20.2 Ca_v1 Channels – Pharmacology

20.2.1 Clinical Pharmacology

For decades, treatment of hypertension and myocardial ischemia has been based on the L-type calcium channel blockers. They are the first-line choice for the above health issues and first choice for the physicians. The arterial vasodilators

dihydropyridines generally reduce arterial muscle tone, resistance of peripheral vascular tissues, and coronary and peripheral artery vasospasm. Dihydropyridines also reduce cardiac oxygen demand by lowering the arterial blood pressure. With the properties of spasmolytic effect, antianginal actions of dihydropyridines can easily be established. Dihydropyridines show negative inotropic actions and remain ineffective to sinoatrial node and atrioventricular node function at its precise therapeutic doses. Furthermore, verapamil and diltiazem are also negative dromotropic, chronotropic, and inotropic to their antihypertensive, vasodilating, and spasmolytic properties and thus inhibit myocardial oxygen consumption and increased heart rate due to exercise. In the treatment of hypertensive patients with angina pectoris, these drugs are suitable due to their direct cardiodepressant effects (Katzung 2017; Jaisser and Farman 2016).

The vasodilating effects of Ca^{2+} channel blockers result in unwanted effects like flushing, headache, dizziness, and hypotension at any therapeutic dose. Long-term use of dihydropyridines has therapy-limiting side effects of peripheral edema associated with ankle swelling. One of the major side effects of verapamil is it causes constipation and can be explained by L-type calcium channel inhibition in intestinal smooth muscles. Life-threatening health issues like reduced left ventricular function, cardiac arrhythmia, or atrioventricular block (AV block) can also be caused by verapamil. This problem becomes more prominent for the patients who administered β-adrenoceptor blockers or have previous medical history of heart-related diseases (Katzung 2017; Fardal and Lygre 2015).

No evidence has been reported for muscle weakness in skeletal muscles from the unit of $Ca_v1.1$ channels or increased hearing thresholds from inhibition of $Ca_v1.3$ in inner hair cells of the cochlea. At the same time, loss of vision from the unit of $Ca_v1.4$ in photoreceptor of the retina or disturbances in the central nervous system from the block of $Ca_v1.2$ and/or $Ca_v1.3$ in the brain have not yet been reported. L-type calcium channels always serve important functions where Ca^{2+} channel blockers cause no significant side effects at therapeutic doses in other tissues. Ca^{2+} channel blocker overdose may suppress insulin secretion, and as a result, hyperglycemia may occur only when plasma attains toxic level (Vallejo-Illarramendi et al. 2014).

20.2.2 Molecular Pharmacology

Ca^{2+} channel blockers can be categorized under different chemical classes depending on their clinical use. Nifedipine, felodipine, or amlodipine which falls under dihydropyridine group is most widely used. Benzothiazepine (e.g., diltiazem) and phenylalkylamine (eg., verapamil) usually interact with drug binding domains with high-affinity and activation gate of L-type calcium channel $\alpha 1$ subunits near to the pore (Fig. 20.4) (Bladen et al. 2014a, b; Chaugai et al. 2018; Ataei et al. 2019). Binding is stereoselective and reversible in nature. The binding takes place with dissociation constants in nanomolar range (0.1–50 nM) (Glossmann and Striessnig 1990). Cycling of the channel with normal voltage dependency through its open,

resting, and inactivated conditions which is also known as modulated receptor model usually interferes with bound drugs (Bain 2019).

Dihydropyridines, uncharged, stabilize primarily and prevail upon inactivated channel states. Inactivated channel conformation shows higher affinity and thus decreases with increased availability of inactivated channel states at voltage-dependent block or more depolarized membrane potentials (Zhao et al. 2019) which is favored by the open channel state and always favors the access of benzothiazepines and phenylalkylamines. Blocking of pore directly together with slowed recovery from inactivation with stabilization of inactivated channel state results in pronounced frequency or use-dependent inhibition.

Dihydropyridines which are Ca^{2+} channel activators also exist. For different reasons, the sensitivity of L-type calcium channels for dihydropyridine Ca^{2+} channel blockers varies in different tissues (Surmeier et al. 2019). Variable contribution of these L-type calcium channels to total L-type current is one of the prominent explanations. $Ca_v1.4$ and $Ca_v1.3$ exhibit about 5- to 10-fold down to dihydropyridines than $Ca_v1.2$, which is demonstrated as negative membrane potentials in heterologous expression systems (Ortner et al. 2014; Kang et al. 2012). Sinoatrial node is usually dominated by $Ca_v1.3$ which can explain the relatively weak inhibition of L-type pacemaker currents (Mesirca et al. 2015).

L-type current in substitute splicing of $\alpha 1$ subunits is another important factor affecting dihydropyridine sensitivity. It has been expositional that dihydropyridines, at lower concentrations, inhibit currents in arterial smooth muscle than in the functional myocardium for $Ca_v1.2$. The presence of dihydropyridine-sensitive splice variants predominantly expressed in arterial smooth muscle has been established by rigorous analysis of $Ca_v1.2$ $\alpha 1$ splice variants present in heart and smooth muscles. Moderate negative voltage activates some of these splice variants and brings out a steady-state Ca^{2+} window or inward current nearer to the resting potential of cardiac muscles (Li et al. 2017a, b; Fan et al. 2005). More amount of depolarized resting membrane potential available in smooth muscle usually favors inactivated channels obstructed by dihydropyridines in comparison to cardiomyocytes (Liao and Soong 2010).

Another way to enhance dihydropyridine sensitivity is that few of such splice variants are liable to more promised inactivation of steady state. There is also evidence that the drug binding domain 5s is affected by alternative splicing of $Ca_v1.2$ $\alpha 1$ and therefore the dihydropyridines can access the inactivated channels. Dihydropyridine sensitivity of $Ca_v1.3$ is slightly affected by alternative splicing in the C-terminus (Jang et al. 2013).

20.2.3 L-Type Calcium Channel as Potential Targets for Various Diseases

The pharmacotherapeutic potential of L-type calcium channel blocking in other tissues is a serious point of consideration. The physiological and pathophysiological role of L-type calcium channels other than the cardiovascular system has raised

importance to draw the attention of researchers. Efficient inhibition of L-type calcium channels in the brain is particularly the prime matter of concern.

Therapeutically relevant pharmacological effects can be theorized from findings in human mutations and mutant mice to consider Parkinson's disease and its neuroprotection as well as treatment of neuropsychiatric disorders like autism spectrum disorders and febrile seizures. Although clinical use of calcium channel blockers in cardiovascular diseases are well established, they may be used for other neurodegenerative indications (Bidaud et al. 2006; Matta et al. 2015).

20.2.3.1 Parkinson's Disease

A clinical trial of phase III has been started off to study the neuroprotective potential of the dihydropyridine isradipine in the early stage of Parkinson's disease (Lotia and Jankovic 2016). This phase III study has been initiated depending on the robust preclinical outcomes regarding the key role of L-type calcium channel arbitrated Ca^{2+} load in substantia nigra pars compacta neurons. For instance, isradipine is currently licensed to treat high blood pressure. Presently, the findings from preclinical in vivo neurotoxin-influenced Parkinson's disease models are not sufficient to predict whether $Ca_v1.2$, $Ca_v1.3$, or both isoforms are responsible for the proposed toxicity of Ca^{2+} ion. Calcium-mediated side effects like peripheral edema and/or hypotension, in clinical trials, limit long-term treatment of Parkinson's disease with large doses of dihydropyridines providing a strong support for efforts to discover calcium channel selective inhibitors (Surmeier et al. 2017; Yang et al. 2014a, b; Caricati-Neto and Bergantin 2016).

20.2.3.2 Neuropsychiatric Disease

Calcium voltage-gated channel α1 gene belongs to the family of genes which provides instructions for generating calcium channels. Genome-wide calcium channels have revealed a strong group of intronic single nucleotide polymorphisms in calcium voltage-gated channel α1 and the responsiveness for psychiatric disorders, including schizophrenia, manic-depressive illness, and severe depression in individuals. As reported in psychiatric genetics, this is one of the most consistent associations (Nanou and Catterall 2018; Quach et al. 2015).

The recent scientific findings indicate that the single nucleotide polymorphisms can increase mutations in voltage-gated α1 channel leading to autism in Timothy syndrome. These facts strongly advocate the re-evaluation of calcium channel blockers for the treatment of schizophrenia, bipolar disease, and major depression in certain individuals (Gershon et al. 2014; Lee et al. 2016).

20.2.3.3 Cardiovascular Disease

Recently discovered $Ca_v1.3$ plays a key role in aldosterone secretion. It may be one of the reasons why therapeutic doses of the dihydropyridine class of calcium channel blockers show no robust inhibitory effects on aldosterone secretion in humans. In the near future, potent $Ca_v1.3$ selective inhibitors might be discovered (Striessnig et al. 2014). Such inhibitors may not affect cardiac inotropy due to the unavailability of $Ca_v1.3$ channel in ventricular myocardium. The merged mechanism of action

seems to be clinically fruitful in patients with heart failure, in which elevated heart rate has been noticed may be due to increased sympathetic drive along with aldosterone which may be caused by secondary aldosteronism (Gordan et al. 2015). Heart rate at high level is always a risk factor in heart failure. But lowering of heart rate with specificity may be improved by cardiovascular outcomes with the hyperpolarization-activated cyclic nucleotide-gated channel blocking bradycardia agent ivabradine (Tse et al. 2017).

Physiological functions controlled by different L-type calcium channel isoforms identify these types of channels as new drug targets. It is a matter of high concern because the nonselective channel blockers are in clinical use for a long time. Furthermore, $Ca_v1.3$ selective Ca^{2+} channel blockers have high therapeutic potential for many indications including neuropsychiatric disorders (Lu et al. 2015).

20.3 Ca_v2 Channels – Pharmacology

20.3.1 $Ca_v2.1$ and $Ca_v2.3$ Channels and Their Prospective Roles

Peptide toxins that are collected from the venoms of organisms like fish-hunting molluscs, spiders, and scorpions can inhibit voltage-gated calcium channels. For example, polypeptide ώ-agatoxin, subtype IVA, having a molecular weight of 5210 kDa, collected from the venom of the North American funnel web spider *Agelenopsis aperta*, inhibits $Ca_v2.1$ channels (Lian et al. 2014). If they are carefully regulated with compounds that normalize deviant gain of function, $Ca_v2.1$ channels are not considered as good pharmacological targets in large extent where $Ca_v2.3$ channel inhibitors could potentially have an influential effect in pain (Duda et al. 2016) and seizure disorders (Kim et al. 2015).

These channels lack specific small organic inhibitors. The inhibitor of $Ca_v2.3$ channels, SNX-482 spider toxin, isolated from the venom of the spider *Hysterocrates gigas* also targets $Ca_v1.2$ L-type calcium channels with A-type K^+ currents. SNX-482 is also known as ω-theraphotoxin-Hg1a, or ω-TRTX-Hg1a (Zamponi 2016; Moutal et al. 2017; Rousset et al. 2015).

20.3.2 $Ca_v2.2$ Channels and Their Roles

In the context of $Ca_v2.1$ and $Ca_v2.3$ channels, there is extensive information available to the researchers related to N-type calcium channel inhibitors. The $Ca_v2.2$ channel-mediated cellular activities can be easily controlled for therapeutic purposes by four significant principles (Chai et al. 2017).

20.3.2.1 Direct Block of $Ca_v2.2$ Channel by Small Organic Molecules and Peptides

The peptides present in the venoms of a variety of raptorial organisms significantly retard $Ca_v2.2$ channels. Peptide toxin, ω-conotoxin GVIA, extracted from the fish-hunting cone snail *Conus geographus* selectively and potently inhibits $Ca_v2.2$ channels (Lewis et al. 2000; Zhang et al. 2015) (Fig. 20.6, pathway 2). To distinguish between P/Q-type and N-type currents in varying neurons, ω-conotoxin GVIA and ω-agatoxin IVA have been widely used. On the other hand, the above toxins also typify significant modes of action, namely, gating modification and pore block. Small 27-amino-acid peptide $\acute{\omega}$-conotoxin GVIA is made up of a backbone that is contrived by three disulfide bonds. The $\acute{\omega}$-conotoxin GVIA physically lodges into the permeation pathway and blocks the channels (Ramírez et al. 2017; Thapa et al. 2014).

The virtually irreversible blocking occurs if the unblocking rate constant remains low. The ω-agatoxin IVA is a much larger peptide (83 amino acids) than the $\acute{\omega}$-conotoxin GVIA and acts by blocking voltage sensor movement. If any membrane does not repetitively depolarize, it can produce poorly reversible inhibition. Repetitive depolarization allows the voltage sensors of the channel to remove the bound toxin (Kuwahara and Kimura 2015; Mir et al. 2016). Spider toxins like α-grammotoxin SIA which is a peptide by nature and isolated from the venom of the tarantula *Grammostola spatulata* act as gating inhibitors and inhibit both $Ca_v2.1$ and $Ca_v2.2$ channels. Another example is SNX-482 which is obtained from the tarantula *Hysterocrates gigas* and is a $Ca_v2.3$ channel blocker (Xu et al. 2018; Osteen et al. 2016; Bourinet et al. 2001).

It has been reported that fish-hunting molluscs produce enriched palette of calcium channel blockers having significant selectivity for $Ca_v2.2$ channels. The 25-amino-acid $Ca_v2.2$ channel pore-blocking toxin called ω-conotoxin MVIIA has been isolated from the venom of the snail *Conus magus* and can mediate potent analgesia in cancer patients with pain of cancer (Thompson et al. 2006) and rodents (Gardezi et al. 2016). The above context fits with the significant role of $Ca_v2.2$ channels in neurotransmitter released from afferent terminals. The $Ca_v2.2$ channel inhibitors such as ω-conotoxins MVIIB, MVIIC, and MVIID have been obtained from *C. magus*. In the same way, snails like *Conus striatus*, *Conus fulmen,* and *Conus catus* produce various Ca^{2+} channel blocking peptides. Such peptides usually have similar disulfide bridge arrangements and act as pore blockers. Among them, most of the peptides act selectively on $Ca_v2.2$ channels, for example, ω-conotoxins SIA, FVIA, CVID, etc. (Motin et al. 2007). Few other peptides like ω-conotoxins SIB and MVIIC have the role to block $Ca_v2.1$ channel. It has been reported that ω-conotoxin CVID has undergone analgesic tests in clinical trials (Bajaj and Han 2019; Carstens et al. 2011; Bourinet and Zamponi 2017).

The construction of chimeric channel and site-directed mutagenesis helps in investigating the blocking site for ω-conotoxins GVIA and MIIVA in the $Ca_v2.2$ subunit (Page et al. 2016). The above study indicates that the large extracellular domain IIIS5–S6 region is a key factor to determine ω-conotoxin GVIA block. Moreover, the dramatic reversibility of ω-conotoxins GVIA and MVIIA block

occurs due to proline and mutagens of a single glycine residue in the 1326-amino-acid position of the channel. An ensuing study reveals that *ώ*-conotoxins MVIIA and CVID cause changes in both the extent of inhibition and kinetics of the co-expression of $Ca_v2.2$ δ subunit channels (Wang et al. 2016; Mollica et al. 2015).

Various peptide toxins are highly selective and are high-affinity blockers to several Ca^{2+} channel subtypes, but they cannot cross the blood–brain barrier. A lot of pore-blocking conotoxins do not perform effectively as state-dependent blockers, namely, local anesthetics and anticonvulsants (Norton 2017; Bourinet and Zamponi 2017; Duggan and Tuck 2015). But the above issue can be overcome by increasing affinity and selectivity of developing small organic blocking agents. The peptidylamines are designed to mimic the pore-blocking activity of the larger ω-conotoxin molecules. This is executed by linking acid group of *N,N*-disubstituted leucine to amine group of tyrosine. The high-affinity block of $Ca_v2.2$ channels has been evidenced in various literatures. Moreover, phenylalanine and benzyloxyaniline derivatives have strong affinity toward $Ca_v2.2$ channel blockers, and management of pain has been reported with scientific evidences (Jin et al. 2013; Robinson et al. 2017; Brady et al. 2013).

Antipsychotics with D2 dopamine receptor-blocking activity are examples of few important $Ca_v2.2$ channel blockers (Siafis et al. 2018) (Fig. 20.6, pathway 2), which contain a core morpholine, piperidine, and/or piperazine structure and usually linked to 1 or 2 diphenyl moieties through alkyl chains. Such agents are renowned for their blocking property of N-type channels (Brimblecombe et al. 2015). Furthermore, several major compounds falling under this section have been confirmed for the management of pain in animal models. The dependency and intoxicating narcotic properties of ethanol are also abated, and the derivatives like pyrazole piperidines and amino-piperidine sulfonamide showed mixed action on $Ca_v2.2$ and $Ca_v2.3$ channels (François et al. 2014; Striessnig et al. 2015; Striessnig et al. 2014).

Cilnidipine is a drug which comes under dihydropyridines and acts as a blocker of L-type calcium channel. At the same time, it is also reported that some other drugs of the same class can also block N-type calcium channels with high accord. These drugs have shown analgesic properties in rats and kidney protective as well as antihypertensive properties in human volunteers. Such favorable effects of drugs may be imputable to their action on the sympathetic nervous system of N-type channels (Shetty et al. 2013; Adake et al. 2015; Manthri et al. 2015).

Moreover, other examples of $Ca_v2.2$ channel blockers found in the literature include (3*R*)-5-(3-chloro-4-fluorophenyl)-3-methyl-3-(pyrimidin-5-ylmethyl)-1-(1*H*-1,2,4-triazol-3-yl)-1,3-dihydro-2*H*-indol-2-one (TROX-1) which is an oxindole compound. This compound has analgesic properties and is a state-dependent inhibitor. Further, long-chain aliphatic monoamines (Beedle and Zamponi 2000) and farnesol also block (Roullet et al. 1999) $Ca_v2.2$ channels with stronger accord and reveal favored blockade of inactivated channels (Nimmrich and Eckert 2013; Page et al. 2016; Ripsch et al. 2012).

The inhibitions of $Ca_v2.2$ channels are also done by other classes of pharmacophores indicating that these channels have promising pharmacotherapeutic implications. Left side shift has been observed in the steady-state inactivation curve

of state-dependent blockage of $Ca_v2.2$ channels along with frequency-dependent inhibition of activity. However, small molecule $Ca_v2.2$ channel blockers are unknown in comparison to the peptide toxins. The physical interaction site of $Ca_v\beta$ subunit and mutation affecting only few nucleotides in the domain I–II region of $Ca_v2.1$ has no evidence for the effect of piperidine block (Adams and Berecki 2013; Schroeder et al. 2006; M'Dahoma et al. 2016).

20.3.2.2 $Ca_v2.2$ Channel and G-Protein Inhibition

Various G-protein-coupled receptors are practically connected to $Ca_v2.2$ channels (Fig. 20.5, pathway 1). Nucleotide exchange in the associated Gα subunit initiates the activation of these receptors and produces active signaling molecules like Gα-guanosine triphosphate and G$\beta\gamma$ subunit. The physical combination of $Ca_v2.2$ channel and G$\beta\gamma$ subunit regulates the potent voltage-dependent inhibition of channel response (Adams et al. 2012; Jurkovicova-Tarabova and Lacinova 2019).

The regulation of $Ca_v2.1$ channel occurs in parallel manner and goes through in a much smaller degree of inhibition. A large majority of drugs act through several G-protein-coupled receptors, and such receptors are linked with other downstream responder systems. The objective activity of a receptor agonist is niggardly linked to $Ca_v2.2$ in the case of opioid receptors. Morphine, a μ-opioid receptor agonist, is a potent analgesic which interacts with opioid receptors (Wright et al. 2015; Taylor 2009). The drug impedes $Ca_v2.2$ channels in dorsal horn synapses and activates G-protein-coupled potassium channels. It is believed that the receptor-induced inhibition of $Ca_v2.2$ channel reduces presynaptic calcium levels. As a result, it reduces synaptic communication between afferent nerve terminals. Morphine acts at μ-opioid receptors in the central nervous system, but it is very difficult to establish a clear relation between physiological outcome and moderation of $Ca_v2.2$ channels (Montandon et al. 2016; Baloh 2019).

Morphine is considered to be very selective for the μ-opioid receptors and discriminating agonists of the rest three other receptors, namely, delta-, kappa-, and nociceptin receptors of the extended opioid receptor family. The selective blocking of the μ-opioid receptors occurs through inhibition of $Ca_v2.2$ channels. The activation of μ-opioid receptors induces analgesia in numerous animal models. But clinically, there are no evidences of targeting analgesia by δ-opioid and nociceptin receptor. Only pentazocine, which is a *k*-opioid receptor agonist, is used as an analgesic (Turnaturi et al. 2016; Jokinen 2017; Winters et al. 2019).

Gamma-aminobutyric acid B ($GABA_B$) receptors or metabolic receptors are another type of receptors that interfere with $Ca_v2.2$ calcium channels in dorsal horn synapses. Although systemic GABA agonists such as baclofen is associated with CNS side effects, it is used intrathecally to treat spasticity and central pain arising due to spinal cord or in brain injury. The most interesting part is that the *α-conotoxin* Vc1.1, a disulfide-bonded peptide, and the structurally related peptide Rg1A have been evidenced to trigger outlying $GABA_B$ and resulting in $Ca_v2.2$ channel inhibition along with analgesia when delivered through intramuscular or intrathecal route. Improved oral bioavailability of acyclic version of the Vc1.1 has

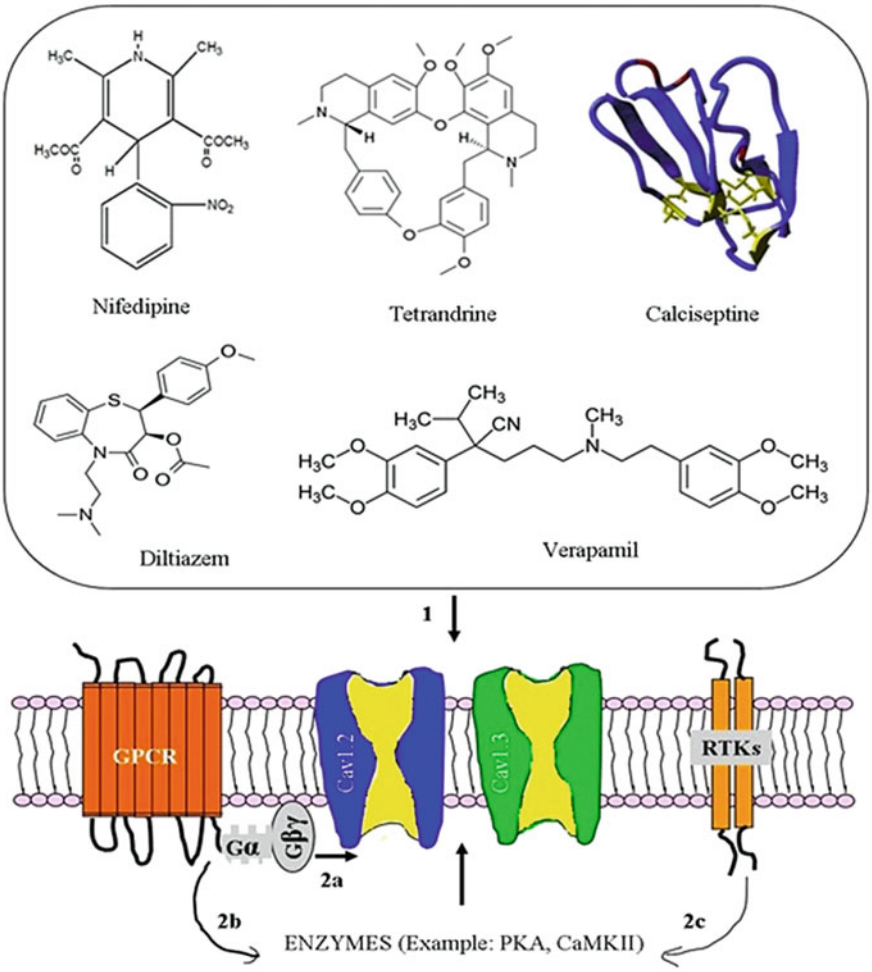

Fig. 20.5 Pathway 1 represents the modulation of drugs, toxins, and signals. L-type calcium channel active drugs are shown here. The potency of L-type channel blockers depends on membrane potential as shown in pathway 2. Different signaling pathways like G-protein (pathway 2a) or enzymes activated by G-protein-coupled receptor (GPCR) (pathway 2b) or receptor tyrosine kinases (RTKs) (pathway 2c)

been designed for better therapeutic response (Cai et al. 2018; Ren et al. 2019; Liu et al. 2018; Bowery 2016).

Overall, $Ca_v2.2$ channels are very important effectors of 7-transmembrane helix receptors. The physiological importance of this regulation can be clearly exemplified in the primary afferent pain pathway. The GPCR agonists can also regulate their downstream effects in many other physiological processes via Ca^{2+} channels. This process is connected to the entry of calcium that plays crucial role in cellular

physiology – the modulation and triggering of neurotransmitter release (Morrill et al. 2015; Patel et al. 2018).

20.3.2.3 $Ca_v2.2$ Channel Trafficking and Inhibition

CRMP-2, or collapsin response mediator protein-2, and $Ca_v2.2$-type calcium channels are associated with each other (Fig. 20.6, pathway 3), and the interactivity supports the channels in the plasma membrane. It is assumed that the rate of channel internalization has been slowed down and reversibly facilitates $Ca_v2.2$ channel-mediated release of, for example, calcitonin and peptide types of neurotransmitters (François-Moutal et al. 2015; Buchta et al. 2019).

In an opposite way, unsettling of $Ca_v2.2$ channel reciprocity with collapsin response mediator protein-2 can be attained by using peptides. TAT (transactivator of transcription) peptides or cell-penetrating peptides reduce the density of $Ca_v2.2$ channel in the plasma membrane and arbitrate analgesic effects in various pain managements. Thus, without blocking the function of a channel, calcium entry can be controlled by targeting the mechanism which controls channel density in the plasma membrane as arbitrated by $Ca_v2.2$. The other association mechanism of these channels with the ancillary $Ca_v2.2$ δ subunit is very censorious for $Ca_v2.2$ channel trafficking (Chew and Khanna 2018; Cassidy et al. 2014).

20.3.2.4 Synaptic Vesicle Release Machinery and $Ca_v2.2$ Coupling

The proteins which are involved in fast synaptic transmission are physically associated with $Ca_v2.2$ channels. Synthetic synprint peptides block $Ca_v2.2$ channel-mediated synaptic transmission and initiate the competitive interference of $Ca_v2.2$ interaction with syntaxin 1A. From the above illustration, it can be concluded that without changing the function or density of $Ca_v2.2$ channel, the pharmacological manipulation of $Ca_v2.2$ channel-mediated physiological processes can be executed easily (Wong et al. 2014; Ferron et al. 2014).

It is possible to identify small organic factitious of these synprint peptides with the support of $Ca_v2.2$ channel trafficking regulators. However, for management and treatment of conditions like pain, such facilities may be utilized to target $Ca_v2.2$ channel-mediated synaptic transmission as a better futuristic consideration.

20.4 Ca_v3 Channels – Pharmacology

20.4.1 Peptide Toxins

As the peptide toxins are unsuccessful in crossing the blood–brain barrier, they are not administered orally as therapeutic agents. Kurtoxin has high affinity to inhibit $Ca_v3.1$ calcium channels. This peptide is extracted from the venom of the scorpion species *Parabuthus transvaalicus*. The above compound functions as a gating modifier in a manner comparable to that described for the $\acute{\omega}$-agatoxin IVA, the P-type channel blocker. Again, kurtoxin targets both N- and L-type calcium channel

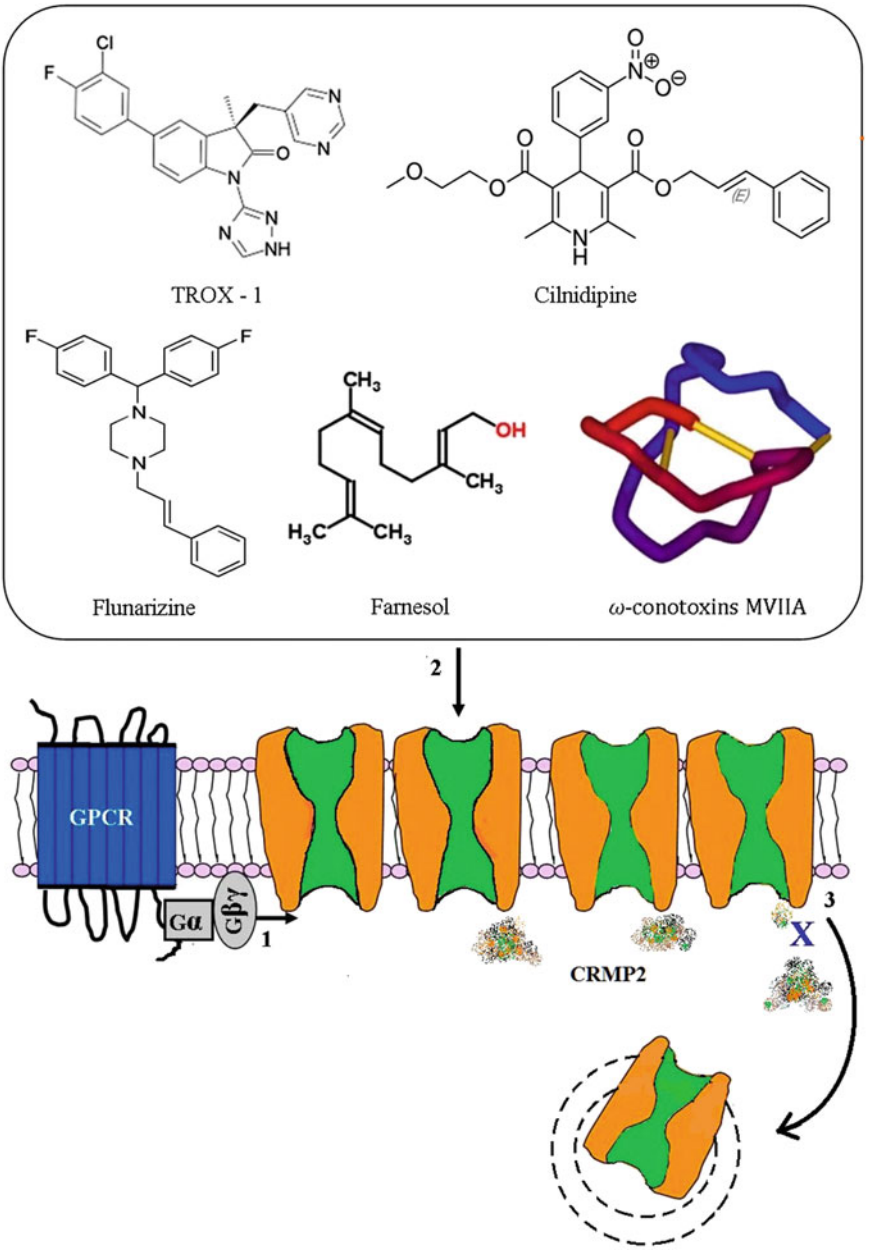

Fig. 20.6 N-type channels regulated by toxins, drugs, and various signals. Pathway 2 indicates the most pertinent group of active drugs. A different type of signaling pathway has been shown in pathway 3 representing the CRMP-2 (scaffolding of proteins)

isoforms and also shows its effects on sodium channel (Bourinet and Zamponi 2017; King et al. 2008; McDonough 2007; Mary et al. 2018).

The structure of kurtoxin shows similarities to other scorpion toxins. However, the unique surface properties of kurtoxin are the main reason for its action on T-type channels. Two additional *Parabuthus transvaalicus* scorpion toxins (KLI and KLII) also exert blocking effects on T-type calcium channels. Both toxins have weak impact on $Ca_v3.3$ channels but can easily block sodium channels and T-type channels (Antal and Martin-Caraballo 2019; Nanclares et al. 2018; Housley et al. 2017).

The most prominent sodium channel inhibitors are protoxins I and II which are peptides collected from the *Thrixopelma pruriens* tarantula. Subtype-dependent Ca_v3 channels are blocked by both peptides. Protoxin I advantageously blocks $Ca_v3.1$ channels over $Ca_v3.3$ and $Ca_v3.2$ channels. Having the best accord for $Ca_v3.2$ channels, protoxin II favors to act as a gating modifier. PsPTx3 is another peptide spider toxin, extracted from Theraphosidae tarantula, which has properties to block T-type calcium channels and perceptible selectivity for $Ca_v3.2$ channels (Kyle et al. 2017; Bladen et al. 2014a, b; Klint et al. 2012).

In overall comparison with N-type calcium channels, peptide toxin impedes Ca_v3 channels which remain relatively limited and derived mostly from arachnids. Most importantly, peptide blockers of T-type calcium channels should not be restricted to those derived from venomous species. For instance, an intrinsic agonist of the chemokine receptor CCR-2 (C-C motif chemokine receptor 2), monocyte chemoattractant protein-1, can directly and actively inhibit $Ca_v3.2$ T-type calcium channels (Ellinor et al. 1994; Silva et al. 2018; Bellampalli et al. 2019).

20.4.2 Inorganic Ions

The responsiveness to extracellularly applied nickel ions is a crucial feature of differentiating features of T-type calcium channels. With a minimum difference of one order of magnitude, $Ca_v3.2$ channels display a greater accord for nickel ions when compared to channels of $Ca_v3.1$ and $Ca_v3.3$. Generally, $Ca_v3.2$ channels show a distinctive histidine remnant inside the domain S3–S4 loop at position 191 which is the key reason behind such affinity (Antal and Martin-Caraballo 2019; Kang et al. 2006).

In the channels, the same remnant acts as a prime redox injunction site. This proceeds to hindrance of channel functionality by ascorbate, and in the presence of L-cysteine, the upregulation of channel function has been observed. During the presence of metal zinc ion, the distinctive regulation of Ca_v3 isoforms also takes place. The zinc ion strongly inhibits $Ca_v3.2$ channels in comparison to two other Ca_v3 isoforms. But under certain conditions, zinc ions also act as agonists of $Ca_v3.3$ channels by reducing the rate of deactivation (Voisin et al. 2016; Wagner et al. 2013; Bogeski et al. 2011).

On the other hand, magnesium ions also influence to moderate the activity of T-type channel. It has been reported that blocking affinity of distinctive magnesium blocking in solutions containing external calcium and barium underlies the

perceptible differentiations in the barium and calcium magnitude of $Ca_v3.1$ (Srebro et al. 2017; Barua et al. 2019).

Like the divalent metal ions like Mg^{2+}, Ca^{2+}, etc., T-type channels are also blocked by trivalent metal ions like Al^{3+}, Fe^{3+}, etc. Specially, one of the most potent lanthanides, yttrium, has been used for cloned human $Ca_v3.1$ channels, having an affinity of around 30 nM. Upon increasing the concentration of permeations, the block was greatly attenuated which indicates that the trivalent ions usually act by physically obstructing the pore of the channels (Himeno and Fujishiro 2019; Wang et al. 2018).

In a nutshell, for the selectivity and activity of T-type calcium channel, the metal ions are considered as very potent inhibitors. However, such ions are not recommended for therapeutic approaches but can be used as research tools for further discovery of various alternatives.

20.4.3 Organic Molecules

There are no shortfalls of T-type organic calcium channel blockers when compared with peptide toxins. Various categories of T-type calcium channel blockers were brought to light in different time frame (Fig. 20.7). The drug amiloride is one of the very first accepted T-type calcium channel blockers which acts as diuretics, blocking the $Ca_v3.2$ channels of about a single degree of magnitude with higher accord compared to $Ca_v3.3$ and $Ca_v3.1$ channels. But it is a fact that amiloride is not a distinct T-type calcium channel inhibitor. The antiepileptic agent ethosuximide comes under the succinimide group. This compound displays state-dependent inhibition and is a low-affinity blocker of all three categories of Ca_v3 channel isoforms (Zamponi and Diaz 2017; Striessnig et al. 2015; Seo et al. 2007).

Due to the pretended selective inhibition of mibefradil, a T-type calcium channel, initially it generated a significant excitement in this field. The United States Food and Drug Administration (US-FDA) had approved the drug earlier for the treatment of hypertension. But later on, it was reported across the world that the drug is metabolized by cytochrome P450 and had severe drug–drug interactions. Hence, mibefradil was withdrawn from the market. Few years later, a derivative of mibefradil (NNC-55-0396) was developed which has very less interaction with cytochrome P450 3A4 (CYP3A4) (Oshima et al. 2005; Wang et al. 2017; Okuyama et al. 2018).

T-type calcium channels react with synthetic cannabinoid and endocannabinoid receptor ligands. Anandamide, a neurotransmitter, and its derivative NAGly (N-arachidonylglycine) arbitrate mighty inhibition of Ca_v3 calcium channels. A synthetic carbazole derivative, NMP-7, acts as an agonist of cannabinoid receptors. The aforesaid compound and its derivatives usually involve in blocking of T-type Ca channels (Nam 2018; Qian et al. 2017).

Neuroleptic drugs like diphenylbutylpiperidines are familiar for their actions as D2 dopamine receptor antagonists. Numerous members of this class of compounds like penfluridol and pimozide significantly inhibit Ca_v3 channels. Major drug

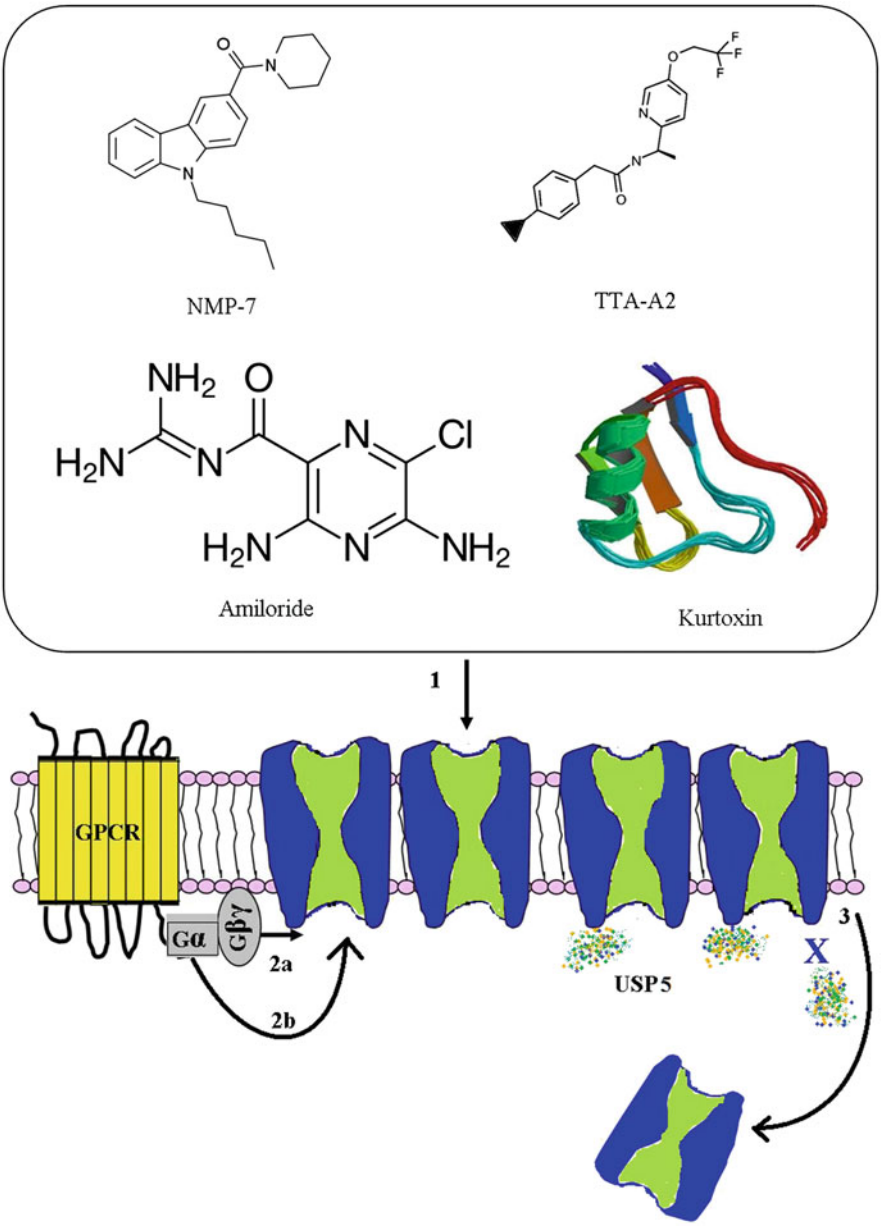

Fig. 20.7 Pictorial representation of different modulatory inhibitions of T-type calcium channels like kurtoxin. The figure shows that inhibitors may block the passage physically or by binding the gating mechanism (pathway 1). On the other hand, pathway 2a represents the direct regulation of calcium channel by activation of GPCRs or indirect regulation by CaMKII protein kinase (pathway 2b). The channel degradation may occur due to interference of isopeptidase T or also known as USP5 (ubiquitin-specific peptidase 5) (pathway 3) (Zamponi et al. 2015; Watanabe et al. 2015)

discovery initiatives confined to the piperidine core pharmacophore have helped in the synthesis of a number of T-type channel inhibitors with great selectivity and potency. For example, it includes a compound which is termed as Z944 and usually mediates potent Ca_v3 channel inhibition in several in vivo models of pain (Yang et al. 2016; Roebuck et al. 2018). Moreover both Z944 and TTA-A2, another Ca_v3 inhibitor, mediates state-dependent preferential inhibition of $Ca_v3.2$ T-type currents. It is also reported that increasing the dose of TTA-A2 leads to the sleep and high-fat diet-induced weight gain (Tomita et al. 2019; Snutch and Zamponi 2018; Santi et al. 2002).

It is reported that the T-type calcium channels have the aptness to interrelate with certain categories of dihydropyridines. Nimodipine and niguldipine, the L-type calcium channel blocking agents, also potently block T-type channels. Several scientific evidences depict that few types of dihydropyridine show better blocking activity on T-type channels rather than L-type channels. On the other hand, ST101 compound evinces a channel-activating response which is useful to further disentangle the role of T-type channels (Gadotti et al. 2015; Kuo et al. 2014; Zamponi et al. 2015).

Various classes of compounds as mentioned above have been tested for inflammatory and neuropathic pain in rodent models to arbitrate analgesia. It has been reported that two T-type channel blockers, namely, Z944 and ABT-639, have been tested in humans for efficacy and safety in pain management (Jarvis et al. 2014). Thus, the importance of $Ca_v3.2$ T-type calcium channels has been revealed in the foremost dorsal root pain pathway. Contrarily, ethosuximide is one of the prime T-type channel blocking antiepileptic drugs used in the absence seizures by targeting T-type calcium channels. In the same way, T-type calcium channels include zonisamide and valproic acid that are used clinically for the treatment of epilepsy (Powell et al. 2014; Snutch and Zamponi 2018).

20.4.4 Intervention with Ca_v3 Channel Regulation

An extracellular signaling molecule controls T-type calcium channels. This can be potent to be utilized for therapeutic uses. It has been reported that the T-type $Ca_v3.2$ channels are regulated by oxidation-reduction modulation. The inhibition of $Ca_v3.2$ channel activity is initiated by ascorbates via catalyzed oxidization of metals. The oxidation-reduction activity regulates the increase of $Ca_v3.2$ current amplitudes by L-cysteine (Guse 2015; Loperena and Harrison 2017). Such oxidation-reduction regulation takes place at a definite remnant, which is also involved in blocking Ni^{2+} of such channels, resulting in hyperalgesia. Hydrogen sulfide induces abnormally heightened sensitivity to pain or hyperalgesia through the actions of $Ca_v3.2$ channels in the same way. Again, administration of polaprezinc can arbitrate analgesia through its antioxidant activity in a model for interstitial cystitis (Latham et al. 2009; Watanabe et al. 2015).

By regulating intracellular messenger, Ca_v3 channel activity can be altered in a different way too. Such activity includes effects of direct binding of G-proteins,

protein kinases, and phosphatases. Thus, the "kiss of death" process for a protein, that is, ubiquitinating and deubiquitinating enzyme (DUB), namely, isopeptidases, deubiquitinases, and ubiquitin proteases, controls $Ca_v3.2$ channels, and the T-type channel regulation can possibly be used as a therapeutic approach for the management of pain (François et al. 2015; Joksimovic et al. 2018).

20.5 α2δ Ligands – Pharmacology

20.5.1 Mechanism of Action – α2δ Ligands

The α2δ subunit's molecular mechanism of action studies indicate that pregabalin and gabapentin produce very little or zero acute retardation of calcium currents in neuronal cell bodies transfected cells (Offord and Isom 2016). But there are few evidences of small acute inhibitory effects. The research shows that gabapentin is unable to inhibit calcium currents in model mouse dorsal root ganglion neurons. The current in dorsal root ganglions from $\alpha 2\delta$-1 is overexpressed in mice and inhibited by gabapentin (Tano et al. 2019).

It is also found that persistent application of the above drug markedly reduces calcium currents generated by several different $\alpha 1/\beta/\alpha 2\delta$ subunit combinations. During the use of $\alpha 2\delta$-1 or $\alpha 2\delta$-2, the effect has been observed (Kazim 2017). However, in the mutant $\alpha 2\delta$, $\alpha 2\delta$, and $\alpha 2\delta$-3 subunits the effect does not occur and also equally true for the subunits which bind very poorly with gabapentin. This type of evidences strongly indicates that effects of gabapentin are in fact occurring through binding to $\alpha 2\delta$ subunits (Freynhagen et al. 2016; Ikeda et al. 2018; Heyes et al. 2015).

20.5.2 α2δ-1 Splice Variants and Binding of Gabapentin

The alternative splice variant of $\alpha 2\delta$-1 (ΔA+BΔC) shows a reduced accord for gabapentin, whose ion is increased after nerve injury in dorsal root ganglion neurons. This indicates the possibility of variation in the expression extent of splice variants in neuropathic pain of individual patients (Patel and Dickenson 2016; Brockhaus et al. 2018).

20.5.3 α2δ Subunits and Ligand Binding Sites

The binding site for the antiepileptic drugs like gabapentin and pregabalin is considered to be at $\alpha 2\delta$-1 and $\alpha 2\delta$-2 subunits. These compounds are intended to increase the activation of GABA 2 receptor and synthesized to be immutable lipophilic analogs. Factually gabapentin does not affect GABA levels or GABA receptors. However, gabapentin is effective in few cases of epilepsy that are characterized by loss of consciousness and convulsions in human and other

experimental animal models (Donaldson and Beazley-Long 2016). A key step to untangle the function of gabapentin is to refine and point out the binding sites in the cerebrum by utilizing proteomic approaches and radiolabeled gabapentin. Investigational results indicate about the primary binding site which is $\alpha 2\delta$-1. The ^{3}H-gabapentin has been observed to refuse to bind with $\alpha 2\delta$-3, but easily accepts bonding with $\alpha 2\delta$-2. Accessory proteins of voltage-gated calcium channels, the $\alpha 2\delta$ subunits, are not considered as drug targets (Celli et al. 2017; Kadurin et al. 2016).

It is considered that the $\alpha 2\delta$ protein does not possess any binding site to ligands without having δ-1 in gabapentin. The Hill coefficient data near to 1 indicates lack of binding cooperativity for single affinity binding site. Initially, it was considered $\alpha 2\delta$-1 subunit as the binding site for gabapentin. The perceptible accord for gabapentin binding indicated a certain sequential elevation at various steps of decontamination of $\alpha 2\delta$ from model pig cerebrum (Zvejniece et al. 2015, Faria et al. 2017).

The affinity of ^{3}H-gabapentin towards $\alpha 2\delta$-1 is found to be increased threefold times during dialysis of brain membranes. Such results indicate the presence of endogenic ligand that occupies such sites. But its activity remains dubious and the nature is unknown. Howbeit, there are many endogenic amino acids like L-leucine which binds with $\alpha 2\delta$ subunits. The structure and function studies indicate that C-terminal lop of $\alpha 2\delta$-1 repudiated binding to ^{3}H-gabapentin (Arsene et al. 2018). Eventually, residues like the third arginine (R) in RRR motif are essential for gabapentin binding, as identified in $\alpha 2\delta$-1. The evolution of RRR to RRA in forms of calcium current enhancement and calcium channel trafficking in both $\alpha 2\delta$-1 and $\alpha 2\delta$-2 has typically reduced the activity of $\alpha 2\delta$-1 and $\alpha 2\delta$ subunits (Weiss and Zamponi 2017).

It has been observed that various endogenous small molecules bind to $\alpha 2\delta$-1 and $\alpha 2\delta$-2. The RRR motif is situated at the upstream of the von Willebrand factor A domain, and it is however anticipated to fit constructionally at the base of the von Willebrand factor A. The transformations of RRR to RRA lessen the accord of gabapentin binding for both $\alpha 2\delta$-2 and $\alpha 2\delta$-1. Anxiolytic influences of pregabalin has been observed in mice model by binding to $\alpha 2\delta$-1 rather than $\alpha 2\delta$-2. Both gabapentin and pregabalin show similar interests for $\alpha 2\delta$-1 and $\alpha 2\delta$-2 subunits, and therefore improved side effect profile of the $\alpha 2\delta$-1 selective ligands might be observed (Tano et al. 2019; Song et al. 2015).

By using ligand binding assays, it is easy to identify the compounds that displace ^{3}H-pregabalin or ^{3}H-gabapentin. Such displacement occurs with improved pharmacokinetics and impactful affinity for $\alpha 2\delta$-1 or with selectivity toward $\alpha 2\delta$-1. The major side effect of gabapentin, ataxia, is mediated via binding to $\alpha 2\delta$-2. There are other similar compounds like gabapentin which has also been found to bind to $\alpha 2\delta$ -1 (Chen et al. 2019; Dolphin 2016).

20.5.4 α2δ Ligand Drugs, Synaptic Transmission, and Transmitter Release

The impact of $\alpha 2\delta$ ligand drugs on transmission of synapse and release of transmitter is a very important subject of discussion in the context of pharmacology. The presynaptic terminals are strongly expressed by $\alpha 2\delta$-1 subunits. However, pain pathways can be explained by the observation of chronic effects of gabapentinoid drugs, and the drastic effects of gabapentinoid drugs to impede release of transmitter and synaptic transmission have been noticed in few in vitro systems (Uchitel et al. 2010; Patel et al. 2000). Deficiency of gabapentinoid drugs has acute effect on somatic calcium currents in various systems. But such drugs are believed to have differential effects on presynaptic terminal calcium currents in comparison to cell bodies. As a result, more rapid inhibition of ion occurs at that stage.

Therefore, calcium channel trafficking has been noticed prominently due to the higher turnover of calcium channel in presynaptic terminals than in somata. Depending on the reciprocity between these different processes and presynaptic sensitization, gabapentinoid drugs might act rapidly or more slowly. Activation of protein kinase C is an accurate example of this phenomenon (Rogawski and Bazil 2008; Gong et al. 2018).

20.5.5 Role of Amino Acid Transporters

The transport of gamma-aminobutyric acid in vitro is not facilitated with the presence of pregabalin and gabapentin. However, at neutral pH, both the drugs act as zwitterions. They use neutral amino acid transporter system L for uptaking cell membranes (Akanuma et al. 2018; Takahashi et al. 2018).

20.6 Caβ Subunits – Pharmacological Roles

20.6.1 $Ca_v\beta$ Subunit Biochemistry

The very first source of Caβ subunits was identified in the purified skeletal muscle voltage-gated calcium channel complex, and the gene for $Ca_v\beta 1$ was cloned later on. As the time proceeded, $Ca_v\beta 2$, Caβ3, and Caβ4, three more calcium subunit genes, were identified by similar studies. Cytoplasmic proteins or the $Ca_v\beta$ subunits participate in the binding process with high accord to the intracellular loop in between domains I and II of the Ca_v1 and Ca_v2 $\alpha 1$ subunits. The above binding motif is termed as the α-interaction territory which is actually an 18-amino-acid region in the proximal part of the I–II linker (Miriyala et al. 2008; Jangsangthong et al. 2011; Findeisen et al. 2017).

$Ca_v\beta$ subunits having a protected *src* homology-3 domain (or SH3 domain) were found by homology modeling processes. This subunit is also found in guanylate kinase like domain which is interconnected by a flexible loop. Due to the mutations

in the active site, the kinase activity is zero in the domain of guanylate kinase. There are three studies which have resolved the crystal structure of the conserved domains in various β subunits (Newman and Prehoda 2009; Stölting et al. 2015). The study showed that the AID (α-interaction domain) peptide interaction site is in a groove in the domain like guanylate kinase. The α-helical structure of the α-interaction domain in the intact I–II loop is inflicted by binding to the Caβ subunit. This is predicted to continue till the end of S6 in transmembrane domain I. In this way, β subunits are considered as safeguards to induce folding of the $\alpha 1$ subunit with accuracy (Hofmann et al. 2015; Kazim 2017; Hidalgo et al. 2019).

20.6.2 Pathophysiology and Potential Pharmacology Involving $Ca_v\beta$ Subunits

Most common and rare diseases like epilepsy, cardiac dysfunction, and others are connected to $Ca_v\beta$ subunit. Hypothetical consideration about designing of a drug targeting the indentation within $Ca_v\beta$ into which the α-interaction domain peptide is incorporated could impede the interaction between the β subunits and $Ca_v\alpha 1$. In this way, it reduces the function of calcium channel, which on the other hand is beneficial in certain medical conditions such as hypertension and chronic pain (Dolphin 2016; Felix and Weiss 2017).

However, the interactivity between $Ca_v\beta$ and the alpha-interacting domain region is of very high accord and includes a number of remnants. It is difficult to extract out the process through which small molecules compete for conjugation and the selectivity of different $Ca_v\alpha 1$ and β subunits. The use of cell-penetrating peptides describes the interference between the interaction between $Ca_v2.2$ and collapsin response mediator protein (Angelini 2015; Butcher et al. 2006; Korkosh et al. 2019).

To study the specific roles for different calcium channel isoforms, a number of splice variants and supplementary subunits have been identified, aided by existence of human mutations and knockout mouse models of these channels and their supplementary subunits. Physiological activities that are controlled by $Ca_v1.2$ and $Ca_v1.3$ are identified through different procedures. Both channels act as potentially novel drug targets if selectivity can be achieved (Dolphin 2016; Thalhammer et al. 2017). For the treatment of hypertension, nonselective blockers of these channels have been used and their profiles of adverse effect have been studied very closely. Depolarized potentials have been found in vascular $Ca_v1.2$, and due to this, the selectivity of dihydropyridines is nevertheless attained in vivo for aiming the tissue. It is intended to bind with greater accord to inactivated channels. Few specific drugs have promising therapeutic efficacy for selective $Ca_v1.3$ blockers with various indications like neuroprotection, neuropsychiatric diseases, Parkinson's disease, and resistant hypertension associated with hyperaldosteronism (Ross 2018; Torres et al. 2015).

Ensuring novel classes of $Ca_v2.2$ channel blockers with enormous selectivity, accord, and use dependence is a challenge for the researchers. Ziconotide (peptide $\acute{\omega}$-

conotoxin MVIIA) has been administered intrathecally for use in intractable pain. The $Ca_v2.2$ channel blockers which act as analgesics might also be effective in conditions such as drug anxiety and dependence. The Ca_v3 (T-type) channels are important regulators of pacemaker activity and neuronal firing and play crucial roles in the cardiovascular system (Elies et al. 2016; Woon and Balijepalli 2015). Their dysfunction contributes to a number of chronic complications such as epilepsy and pain. In this way, they are both potential and actual drug targets for such medical conditions, namely, ethosuximide. But due to the protection of absence seizures by ethosuximide, the present-day clinical use of many types of such promising T-type channel blocking small organic molecules has been largely restricted (Bourinet et al. 2016; Lee et al. 2019).

The Ca_v3 auxiliary subunit $\alpha 2\delta 1$ is a well promising drug target for pregabalin and gabapentin and has been used in chronic neuropathic pain. When combined with other drugs in several forms, it is useful in the treatment of epilepsy. The drug also shows binding interest with $\alpha 2\delta 2$, but not $\alpha 2\delta 3$. A selective or more potent $\alpha 2\delta 1$ ligand would have less side effects, and whether a similar ligand targeting $\alpha 2\delta 3$ might be of therapeutic use is still under investigation (Choi et al. 2016; Rui et al. 2016; Chew and Khanna 2018).

But few specific calcium channel blockers are likely to hold eminent promises and hope for therapeutic involvement in coming future.

20.7 Conclusions

Different types of calcium ion channels like L-type, T-type, and N-type found in muscle cells, neurons, or endocrine cells can be easily distinguished by their pharmacological responses. All the three types of ion channels are available in dorsal root ganglion with different activation threshold and identical selectivity.

Animal studies reveal that these ion channels can regulate the use of targeted drug delivery effectively. The $Ca_v1.2$ and $Ca_v1.3$ subunits have strong indications of enhancing selectivity of novel drugs, whereas nonselective dihydropyridines and other types of calcium channel blockers have been used for the treatment of elevated blood pressure for decades. The drug ziconotide, a peptide ώ-conotoxin MVIIA, is popularly used in unmanageable pain of individuals through intrathecal route and acts by blocking of $Ca_v2.2$ channel.

In modern times, the management of epilepsy and chronic neuropathic pain has been widely controlled by targeting $\alpha_2\delta$-1 subunit with minimal side effects. Drugs like pregabalin and gabapentin usually bind to the ligand binding site to exert therapeutic activity.

The Ca_v3 or T-type channels are connected with the cardiovascular system by regulating the action of pacemaker. Furthermore, it is essential to understand their interaction with anandamide, arachidonic acid, redox agents, and G-proteins. The randomized clinical investigation further indicates the promising use of Ca_v3 ligands in various disease management related to pain, heart, hypertension. In a nutshell, calcium channel blocking agents, depending on the selectivity and mechanism of their

pharmacological action, are going to hold great commitment for therapeutic arbitration in the coming days.

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