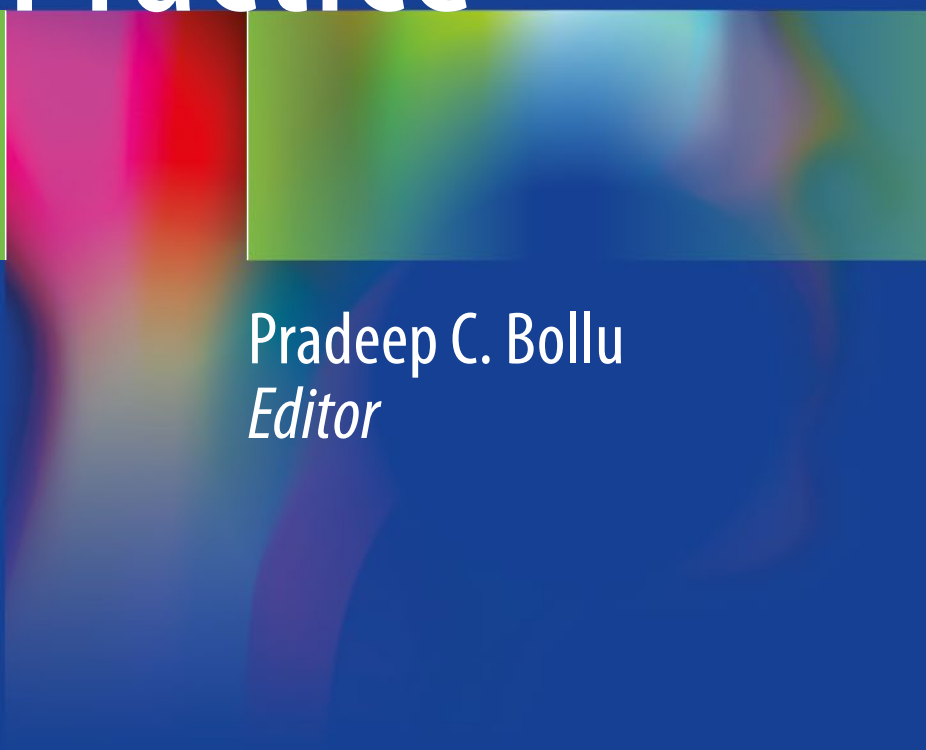
Neurochemistry in Clinical Practice



Pradeep C. Bollu
Editor

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I want to dedicate this book to three of my mentors who played a significant role in my professional and personal life—Dr. Irving Asher, Dr. Terry Rolan, and Dr. Mahesh Thakkar.

Preface

The human brain is a fascinating organ due to its structural complexity and organization. Billions of neurons and their trillions of synapses lay the foundation for the brain's operation. Yet, despite the innumerable brain cells, only a few neurochemicals mediate neuronal communications. As one would expect, the same neurochemical may be excitatory in one pathway while being inhibitory in another. Neurochemical imbalances underlie many disease states of the brain. I was always fascinated by how the manipulation of a neurochemical tone affects the functional state of the brain.

As clinical medicine continues to expand, it is becoming increasingly difficult for physicians to keep up with the understanding of how certain medications work at the fundamental level, altering the neurochemical balance in the brain to the desired effect. I always believe that a better understanding of the background neurochemistry would help us identify the appropriate pharmacological intervention for our patients. Accordingly, I put a lot of emphasis in my formal and informal teaching on understanding the neurochemical alterations in disease states and the rationale behind choosing the appropriate agent based on how it brings back the balance of these alterations.

This book is an attempt to make it easy for students of neuroscience to understand why these neurochemicals are relevant. I hope to make it enjoyable for the reader to understand the importance of these neurochemicals and help them make better decisions in their clinical practice. The book is broken down into chapters dedicated to these essential neurochemicals. Each chapter begins with a brief history of the neurochemical, the biochemical profile, metabolism, physiological functions, and the clinical aspects. Using this format, I am hopeful that the reader now has a chance to understand the clinical relevance of these neurochemicals.

Columbia, SC, USA — Pradeep C. Bollu

Acknowledgments

I want to thank Dr. Sireesha Murala for helping me organize the chapters, draw the tables and diagrams, and put together the content for some chapters. I also want to thank Dr. Elanagan Nagarajan and Dr. Tejas R. Mehta for their help in compiling the book and taking part in writing the content.

I want to thank my wife, Bhavana, for her incredible support during the process that helped me to put together this book. I am forever indebted to my mother, Parvathy, without whom I would not have become a physician.

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Chapter 1
Dopamine

Tejas R. Mehta, Sireesha Murala, and Junaid Siddiqui

Introduction

The nervous system of humans is a critical component of the body. It processes sensory information, controls motor activities, and dictates behavior through interactions between neurons that are spread over the body in an intricate web that works in different patterns, permutations, and combinations to process all the complex tasks that are a part of our routine. These tasks are processed in a fraction of a second in an orchestrated manner, sending information between neurons via connections called synapses. With close to 100 trillion synaptic connections in the brain alone, this relay of information is rapid, involving both electrical and chemical stimuli [1]. The electrical impulses represent the transfer of information within the cell body, while the chemical stimuli facilitate the transmission between two distinct cell bodies. These chemical stimuli are passed on from one neuron to the other via chemicals referred to as neurotransmitters [2]. For example, acetylcholine was the first neurotransmitter to be identified at the vagus nerve terminals on the heart in

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1926 when Otto Loewi observed that it slowed the heart rate. Several other neurotransmitters have since been identified that are associated with a variety of changes in the body. Neurotransmitters may be grouped into amino acids (glycine, gamma-aminobutyric acid, glutamate), monoamines (dopamine, epinephrine, norepinephrine, and serotonin), and neuropeptides (oxytocin, vasopressin, and orexins). Other lesser studied atypical neurotransmitters include adenosine triphosphate (ATP), nitric oxide (NO), and carbon monoxide (CO). These neurotransmitters have various functions throughout the body depending upon their chemical structure, physiological effects, location, and their receptor distribution.

One such neurotransmitter, first synthesized in 1910 by George Barger and James Ewens at Welcome labs in London as reported by Hornykiewicz, was 3,4-di hydroxyphenethylamine or 3,4-dihydroxy-tyramine, now called dopamine [3]. Later, Henry Dale from the same laboratory reported its weak sympathomimetic property and similarities to epinephrine [4]. In the subsequent years, more interest was drawn to this neurotransmitter, with Casimir Funk synthesizing dopamine chemically and naming it as D, L-dopa [5]. Later, Peter Holtz et al. discovered the enzyme aromatic-L-amino acid decarboxylase, which converted L-dopa to dopamine [6]. Holtz and Blaschko from Cambridge independently hypothesized that dopamine and L-dopa were intermediates in the biosynthetic pathway of catecholamines, epinephrine, and norepinephrine [7, 8]. Raab and Gigee discovered a catecholamine-like compound in the striatum that increased significantly after the administration of dopa [3, 9]. It was not until 6 years later that Kathleen Montagu with Weil Malherbe and Bone showed for the first time in history that dopamine was present in the human brain [10, 11]. While studying postmortem brains of patients with Parkinson's disease (PD), Huntington's disease, and other extrapyramidal disorders, Hornykiewicz found brain dopamine content in putamen and caudate to be lower [12]. This was a substantial discovery in the field of neurology and changed the way neurologists and physicians approached PD and other movement disorders.

Physiology and Functions

Owing to the different distribution and the unique characteristics that dopamine offers, the physiology and the functions associated with dopamine are vast and pivotal in the functioning of the human central nervous system (CNS), and, consequentially, the body.

Metabolism: Synthesis and Degradation

Dopamine in the brain is present in many different areas and mediate several different functions (see Table 1.1). The synthesis of dopamine occurs primarily in the neurons and the cells of the zona medulla of the adrenal glands [13]. Centrally,

Table 1.1 Functions of dopamine and associated neuroanatomical location

Functions of dopamine in the CNS	Location
Precursor to other catecholamines	Neurons of the brain
Movement	Basal ganglia
Motivation and pleasure	Nucleus accumbens and striatum
Cognition	Frontal lobe and prefrontal area
Reward system	Ventral tegmental area

dopamine synthesis occurs in the midbrain. CNS relies on the local neuronal biosynthesis of dopamine, as it cannot cross the blood–brain barrier by itself. L-Dihydroxyphenylalanine (L-dopa) produced from L-tyrosine is transported across the blood–brain barrier into a dopaminergic cell. Conversion of L-tyrosine to L-Dopa is the rate-limiting step and is catalyzed by tyrosine hydroxylase in the cytosol of catecholamine containing cells in the brain. Endogenous L-Dopa is then converted to dopamine by the enzyme L-aminoacid-decarboxylase or dopa decarboxylase. In dopamine-containing neurons, this is the final step of the biosynthetic pathway. Dopamine is then taken up into vesicles by a transporter protein, and an action potential triggers its release from axons of dopaminergic neurons. Another dopamine release site is from the neuronal dendrites of substantia nigra pars compacta and ventral tegmental area.

The enzymatic breakdown of dopamine is controlled by catechol-o-methyl transferase (COMT) and monoamine oxidase (MAO). COMT is predominantly expressed by glial cells and minimally in neurons. MAO has two isoforms—MAO-A and MAO-B are present in catecholaminergic neurons (e.g., cells in substantia nigra) and astrocytes, respectively [14]. Degradation via the COMT pathway involves the breakdown of dopamine into 3-methoxy-tyramine (3-MT). MAO further converts 3-MT into homovanillic acid (HVA), which is filtered through the kidneys. The MAO pathway breaks down involve in 3,4-dihydroxy-phenylacetaldehyde (DOPAL). DOPAL, under the action of the enzyme, aldehyde dehydrogenase gets broken down to 3,4 dihydroxy-phenylacetic acid (DOPAC) [14]. The DOPAC is also converted to HVA and is eliminated through the kidney. The dopamine biosynthesis is shown in Fig. 1.1.

Dopamine Receptors

Dopamine works by binding to specific receptors that are located in specific anatomical locations, which bring about the desired function of the system that is involved. Until 1990, dopamine receptors were classified into only two types [15]. Kebiban [16] discovered receptors, which stimulated adenylyl cyclase activity, leading to the increased cAMP formation (D1 receptors) and those that inhibited adenylyl cyclase activity (D2 receptors) [17]. In 1990, a D2 like receptor was isolated in the limbic region, but it did not couple with adenylyl cyclase. This was

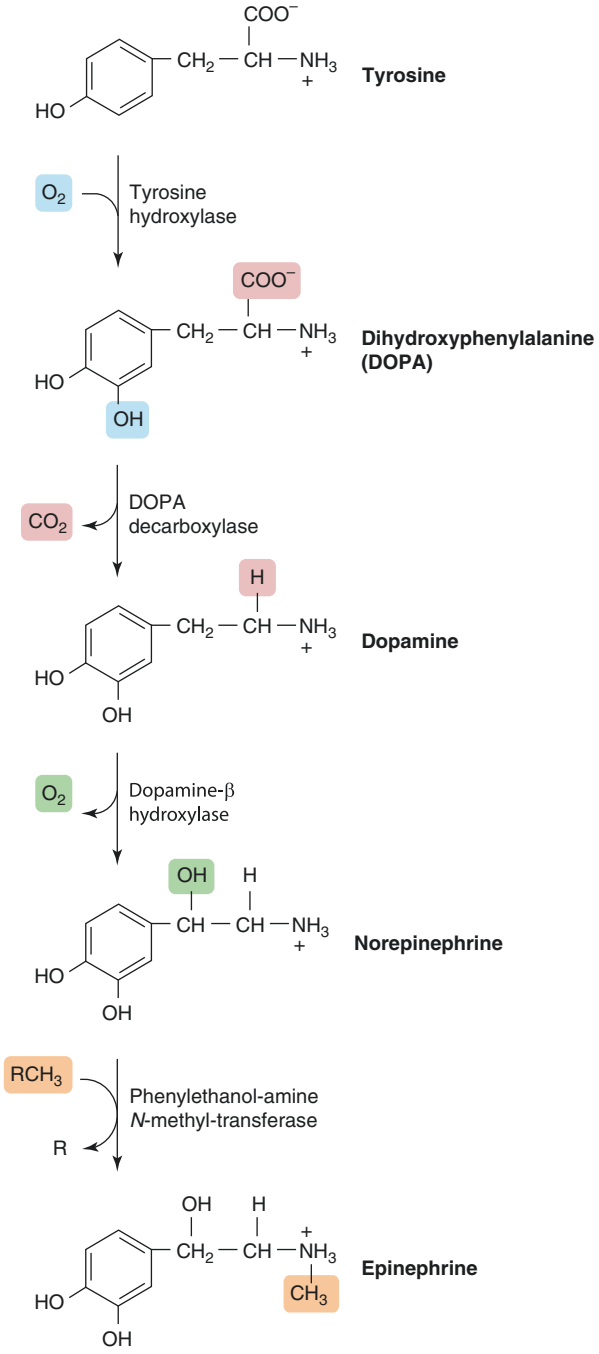

Fig. 1.1 Synthesis of dopamine

Table 1.2 Type of dopamine receptors and their neuroanatomical locations

Dopamine receptor	Location
D1 like	
D1	The caudate nucleus, hippocampus, dentate gyrus, substantia nigra, prefrontal, premotor, cingulate, and entorhinal cortices
D5	Thalamus, amygdala, frontal cortex, cerebellum, and midbrain
D2 like	
D2	The striatum, olfactory tubercle, nucleus accumbens, hypothalamus, substantia nigra compacta, ventral tegmental area (VTA)
D3	Nucleus accumbens, islands of Calleja, VTA, substantia nigra, internal globus pallidus, ventral pallidum
D4	Amygdala, frontal cortex, hippocampus, hypothalamus, mesencephalon

termed D3 receptor. The D5 receptor was the last to be discovered, found to be similar to D1 but was localized to the lateral mammillary nucleus, hippocampus, and peri-fascicular nucleus of the thalamus [18]. Currently, owing to the properties of these receptors, they are broadly categorized into two sets—D1-like receptors (D1 and D5) and D-2-like receptors (D2, D3, and D4). D1 receptors are the most widely distributed receptors in the human nervous system with followed by D2 receptors [19]. The D1-like receptors are either excitatory (via the opening of sodium channels) or inhibitory (via the opening of potassium channels). The D2-like receptors, however, act by inhibiting the target neuron [19]. Table 1.2 summarizes the location of dopamine receptors and their sites.

The D1 receptors are expressed primarily in the hippocampus, putamen, caudate, nucleus accumbens, hypothalamus, substantia nigra, olfactory tubercle, frontal and temporal cortex [20, 21]. These receptors are implicated in the control of cognitive functions of the brain, including memory and attention, whereas the D2 receptor is expressed in basal ganglia, ventral tegmental area, nucleus accumbens [22]. D3 receptors are located primarily in the olfactory tubercle, hypothalamus, and mesolimbic pathway and are associated with positive regulatory influences of dopamine and the production of neurotensin. D4 receptors are localized mainly in the limbic pathway [22]. D3 and D4 receptors mediate the actions of antipsychotics and play a vital role in the therapeutic management of several psychiatric disorders [23]. D5 receptors, on the other hand, are localized predominantly in the thalamus, frontal cortex, amygdala, cerebellum, and midbrain [13, 20].

Dopaminergic Pathways

There are four major pathways for the dopaminergic system in the brain, named according to the anatomical locations that are involved. They are called nigrostriatal, tuberoinfundibular, mesolimbic and mesocortical. Table 1.3 denotes the pathway and the function it serves.

Table 1.3 Dopaminergic pathways and their clinical significance

Dopaminergic pathway	Significance
Nigrostriatal	Coordination of movement
Tuberoinfundibular	Secretion of prolactin
Mesolimbic	Pleasure, reward, and goal-directed behavior
Mesocortical	The motivational and emotional response

The nigrostriatal pathway, which originates from the substantia nigra pars compacta and projects rostrally to the basal ganglia, plays a critical role in the control of motor function and learning of new motor skills [24]. Dopamine neurons in the pars compacta region send ascending projections to the dorsal striatum, modulating motor control. The degeneration of this system leads to Parkinson's disease, which is characterized by tremor, bradykinesia, rigidity, and shuffling gait [25, 26]. The blockade of this pathway is implicated in the effect of antipsychotics on the human body [26].

The tuberoinfundibular pathway originates in the arcuate nucleus of the hypothalamus, projects to the pituitary gland, and dopamine released from this inhibits the release of prolactin by lactotroph cells [27], earning the name prolactin inhibiting hormone (PIH)) [27].

The mesolimbic pathway where the dopaminergic projections originate from the ventral tegmental area and project to the pyriform cortex, amygdala, lateral septal nuclei, and the nucleus accumbens is implicated in dopamine's function related to reward systems and emotions [28]. Food, sex, drugs of abuse, and several other factors thus also act as stimulants of dopamine release in areas of the brain such as the prefrontal cortex and nucleus accumbens. Medications that are known to decrease positive symptoms of schizophrenia are known to block dopamine receptors in the mesolimbic pathway [28].

The mesocortical pathway is a critical pathway that is associated with the emotional and cognitive functions of the brain, and the dopaminergic fibers associated with these functions arise from the ventral tegmental area and project to the frontal cortex and septohippocampal area of the brain [29]. The various dopaminergic pathways are shown in Fig. 1.2 while Table 1.3 outlines the significance of each of these pathways.

Dopamine, Parkinsonism, and DAT Scan

Parkinsonism is a clinical syndrome characterized by the four cardinal features of tremor, rigidity, bradykinesia, and postural instability. They were later recognized and described in other disease entities such as Parkinson's disease (PD), Lewy body dementia (LBD), drug-induced parkinsonism, progressive supranuclear palsy

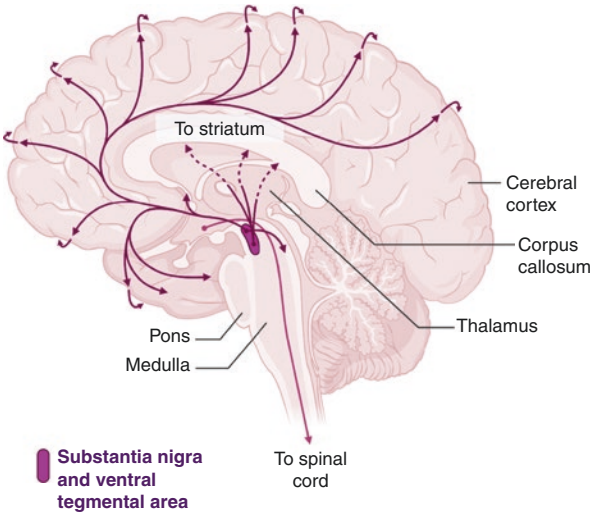

Fig. 1.2 Distribution of dopaminergic pathways in brain

Table 1.4 Stages of Parkinson's disease and the neuroanatomical locations that get involved as the disease progresses

Stage of PD	Structures affected
Stage 1	Dorsal motor nucleus of the vagus nerve, anterior olfactory nucleus
Stage 2	Raphe gigantocellular reticular nucleus, locus coeruleus
Stage 3	The basal nucleus of Maynert, basal forebrain
Stage 4	Amygdala and sub-nuclei of the thalamus
Stage 5	Temporal, parietal and frontal lobes
Stage 6	Motor and sensory areas of the brain

(PSP), corticobasal degeneration (CBD), vascular parkinsonism, and multiple system atrophy (MSA).

Parkinson's disease (PD) is one of the most common causes of parkinsonism that was first described by James Parkinson in his essay titled "Shaking palsy" in 1817. German pathologist Frederick Lewy reported neuronal cytoplasmic inclusions in various brain regions in 1912. It was early in 1919 when Tertiakoff reported the loss of neurons in substantia nigra pars compacta (SNc) of the midbrain, which was thought to be the cause of PD [30]. Dopamine depletion from the basal ganglia circuitry was recognized to lead to disruption in connections to the motor cortex and thalamus, which led to the manifestation of the cardinal signs of PD. Heiko Braak, in 2003, developed a staging system that characterizes disease progression in PD and is divided into a total of six stages, with each step attributed to abnormal pathology in a specific structure of the brain. The type of symptoms and its severity can be by the stage of PD as well. The six stages are summarized in Table 1.4, and the progression of the disease in the substantia nigra is illustrated in Fig. 1.3.

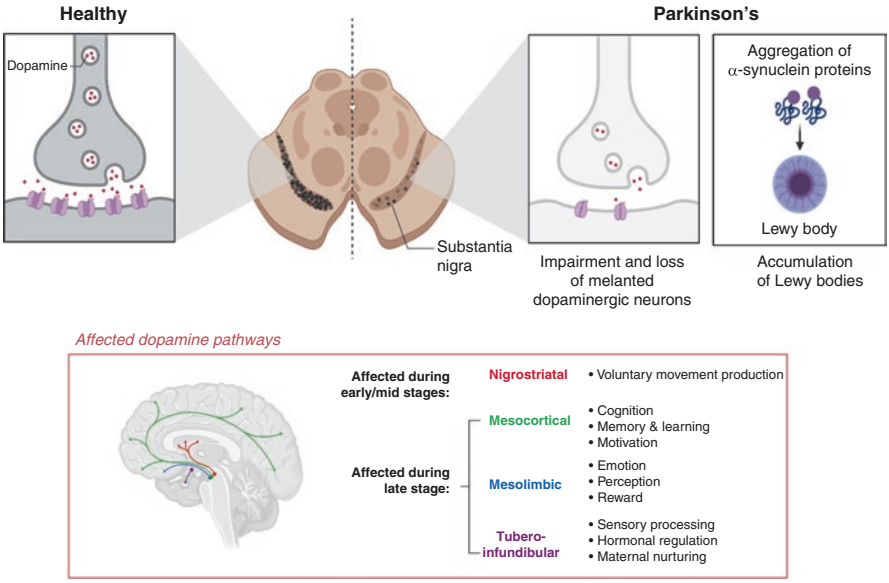

Fig. 1.3 Progression of Parkinson's disease in the Substantia Nigra

The basal ganglia are a group of subcortical nuclei that include the globus pallidus (Globus pallidus interna-GPi and Globus pallidus externa-Gpe), subthalamic nuclei (STN), caudate nucleus, substantia nigra pars reticulata—SNr, and pars compacta—SNc). These structures are components of networks that are associated with processing sensorimotor information, which aids in action selection, action planning, execution, and orientation of locomotion [31]. This network has received the most attention in the basal ganglia, and it is centered on the somatosensory, motor, and premotor cortices that send projection to the striatum. Two major basal ganglia projections to the striatum have been recognized—the direct and the indirect pathway, both of which are modulated by dopamine. Dopaminergic neurons from the SNc and VTA play a vital role in the motor-related activities of BG. A relatively recently described third, hyperdirect pathway is a glutamatergic projection from the cortex through the subthalamic nucleus and stimulates the inhibitory GPe [32]. The direct, indirect, and hyperdirect pathways are summarized in Fig. 1.4. Cells in the SNc have a relative deficiency of neuroprotective factors such as glutathione and may be exposed to high levels of stress due to dopaminergic metabolism. This may make them vulnerable to genetic and environmental insults leading to damage and eventual death. Another exciting aspect of the pathophysiology of PD is the ability of the cell to remove damage and mutated proteins through the ubiquitin-proteasome pathway [33].

Owing to the overlap of clinical symptoms of PD in other disorders falling under the umbrella term of "Parkinsonism," the need for an aid to diagnose PD and differentiate it from other disorders is essential to avoid misdiagnosis and delay in

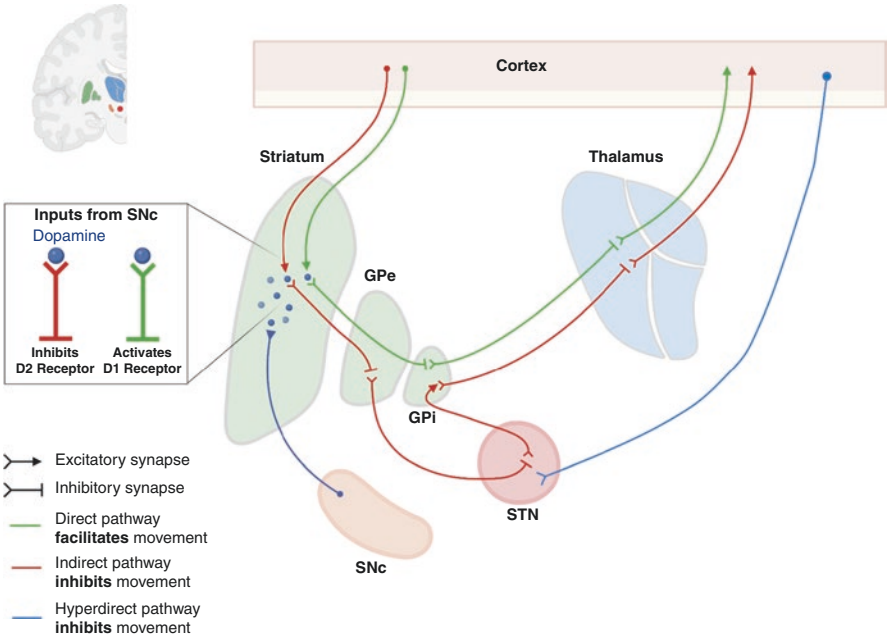

Fig. 1.4 Direct, indirect, and hyperdirect pathways of the basal ganglia. *Gpe* globus pallidus externa, *GPi* globus pallidus interna, *STN* subthalamic nuclei, *SNc* substantia nigra pars compacta

treatment. Dopamine transporter (DaT) imaging with a single-photon emission computed tomography (SPECT) is a neuroimaging technique that can detect loss of dopaminergic neurons in the striatum with very high sensitivity [34]. The use of several radiotracers such as as^{123}I-FP-CIT, ^{123}I-IBZM, ^{99}Tc^{m}-TRODAT-1, and FP-CIT, has made the diagnosis of PD more reliable by assessing the dopaminergic and non-dopaminergic systems in the BG. DAT SPECT scan is a handy tool for in vivo investigation of the pathogenesis of PD [35]. Patients with PD show markedly DAT levels in the striatum, which also correlates with the severity of the disease and gives an insight into disease progression [36] with an upregulation of post-synaptic striatal D2 receptors [37]. Another reported finding is the higher values of ^{123}I-IBZM striatal-occipital ratio of binding contralateral to the clinically most affected side that suggests D2 receptor upregulation and the reverse was seen using 123I-FP-CIT SPECT [38]. Dual isotope imaging using ^{123}I-IBZM and ^{99}Tc^{m}-TRODAT-1 is a useful method to evaluate changes of pre-and post-synaptic dopamine system in animal models of parkinsonism [39].

Differentiating PD from other disease entities has also become much more objective with the use of DAT SPECT imaging with a thorough clinical examination. ^{123}I-FP-CIT SPECT images with DAT ligands are helpful to determine whether parkinsonism is entirely drug-induced [40] with high levels of accuracy [41]. FP-CIT imaging demonstrates lower uptake in caudate and putamen in PD patients than patients with drug-induced parkinsonism and essential tremor [42]. SERT-dependent

^{123}I-FP-CIT imaging shows a mild decrease in SERT levels in PD compared to ET. At the same time, it is undetectable in PSP and DLB patients [43]. Voxel-wise analysis of ^{123}I-β-CIT SPECT shows a diffuse decline of monoaminergic transporter availability in multiple system atrophy compared with idiopathic Parkinson's Disease (IPD) [44].

Dopamine and Psychiatric Disease

Several psychiatric disorders have been found to be associated with dysfunction in the dopaminergic system. Schizophrenia is one of the most common psychiatric disorders affecting approximately 24 million people worldwide and is understood to occur due to the dysregulation of dopamine. Dopamine synthesis is elevated in the prodromal phases of the disease, with rates corresponding to the severity of the prodromal and cognitive deficits [45]. The prodromal phase is also known to be associated with elevated cerebral blood volume in the hippocampus, which is an index of increased neural activity [46]. The striatal dopamine is modulated in three major regions—the ventral limbic area (processing of emotional memory), associative areas (processing of cognitive information), and other regions processing sensorimotor information. There is increased synthesis and release of dopamine in patients with schizophrenia, along with the increased density of D2 receptors in these regions [47–53]. Increased synaptic dopamine in the dorsal striatum and abnormal post-synaptic dopamine sensitivity are related to the positive symptoms of psychosis, including hallucinations, delusions, and thought or movement disorders associated with the disease [51, 54]. Thus, the optimal modulation of striatal dopamine via the D2 receptor blockade shows improvement in positive symptoms in patients with schizophrenia [51]. Dopamine levels in the ventral limbic striatum are elevated in schizophrenia. In contrast, dopamine levels in the associative striatum are inversely proportional to the severity of negative symptoms in patients with this disease [52].

Drug addiction is a condition defined as a progressive loss of control over drug-seeking and taking that becomes compulsive and persists over time despite the adverse consequence [54, 55]. The property of dopaminergic neurons that plays a vital role in this condition is that dopamine modulates the reinforcing effects of a "reward" which can either be natural food and sex or reward associated with the use of drugs [56–59]. Other interesting properties associated with dopamine is the mediation of "incentive salience," defined as the extent to which the reward or associated cues are wanted [60], and impulsivity, expressed as a tendency to execute prematurely, poorly planned, and unduly risky actions is a behavioral feature of several psychiatric disorders as well as substance abuse [61–63].

Greater impulsivity correlates with lower D2 receptors in the striatum of patients abusing methamphetamine [64]. Reduced dopamine release predicts the choice for a dose of cocaine with minimal positive subjective effect over a monetary reward [65]. Another exciting aspect of dopamine associated with addiction is its role in

motivation. Motivation involves the resting of an impulsive behavior to obtain a more valuable reward, which requires more effort. Animal models have shown that increasing D2 receptor levels in the ventral striatum of rodents facilitate motivation, whereas D2 receptor antagonism decreases the willingness to exert effort for a preferred reward [65]. Higher dopamine transmission and D2 dependent dopamine signaling in the nucleus accumbens are associated with greater willingness to work for more substantial but effortful rewards and lower dopamine, and D2 receptor activity shifts the choice to a less effortful and smaller immediate reward [58, 66–68].

Tardive Dyskinesia

Tardive dyskinesia (TD) is abnormal involuntary movements of the orofacial region or dyskinetic movements of the extremities or the trunk [69] from the use of antipsychotic medications. The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) classifies TD as a movement disorder occurring on exposure to antipsychotics that persists for at least a month after discontinuing the culprit drug. However, this disorder is not exclusive in patients on long-term antipsychotics and may be seen in drug naïve patients after first-time exposure during an episode of psychosis [70–72].

Clinical data suggests that TD occurs from exposure to DA blocking agents, which leads to D2 receptor upregulation and DA dysregulation, and DA hypersensitivity [73, 74]. This has been confirmed in several animal studies showing reversible D2 receptor upregulation after exposure to DA receptor blockers, causing orofacial dyskinesia [75]. Another theory where DA may play a vital role is that of oxidative stress causing increasing DA synthesis and metabolism that results in increased production of free radicals.

Serotonergic and GABAergic systems may also contribute to the pathophysiology of TD. Blocking of serotonergic receptors would increase DA release at the raphe nucleus and inhibit DA production in the basal ganglia causing TD. However, this has been challenged by other theories implicating the role of D3 alleles in the pathophysiology of TD [76]. Another theory implicated the imbalance between GABA and DA to cause TD [77].

Dopamine and Punding

Punding is a stereotyped behavior characterized by an intense fascination with a sophisticated, non-goal oriented, repetitive activity that may be seen in PD patients on dopamine replacement therapy or patients with psychostimulant addiction [78–81]. It is also reported in patients on dopaminergic agonists used in conditions other than PD [82, 83] and diseases such as bipolar disorder, brainstem stroke, and dementia. With prevalence rates ranging from 0.34% to 4.2%, the patient's

awareness of this phenomenon might be reduced owing to its occurrence without subjective distress [84, 85]. Although the pathology behind punding remains unclear, it may be related to changes in the dorsal and ventral striatal structures, including the nucleus accumbens [86], and linked to reward mechanisms [87, 88].

Clinical studies also strengthen the role of D1 and D2 receptors in punding and back the theory of a common pathophysiological pathway shared by dyskinesias, addiction, and stereotypies [89–91]. Atypical antipsychotics and deep brain stimulation (DBS) can result in the improvement of punding in PD patients [78].

Dopamine and Restless Leg Syndrome

Restless leg syndrome (RLS), also referred to as Willis Ekbom disease, is a chronic sensorimotor disorder that predominantly but not exclusively affects the legs where the patient would complain of an unrelenting urge to move legs at night leading to discomfort and reduced sleep. RLS is the cause of significant distress and discomfort in patients with moderate and severe forms of the disease [92]. With its prevalence ranging from 5% to 10% in adults of Northern America and Europe, it is one of the most common sleep disorders [92, 93]. The diagnosis of RLS can be established by the RLS study group criteria using the mnemonic URGED, which is summarized in Table 1.5.

The pathophysiology of RLS, although not fully understood, emphasizes two central systems: iron and dopamine. Iron exists in the brain as an essential cofactor in monoamine neurotransmitter synthesis [94, 95]. Neurons of substantia nigra, which contain neuromelanin, have weak acid cation exchange properties that lead to its binding with iron and other metals [96]. These neurons are also responsible for the production of dopamine, and iron is an essential cofactor of tyrosine hydroxylase, which is a rate-limiting enzyme of L-DOPA synthesis. Animal studies demonstrate the development of poor sleep, increased locomotor activity, and increased physical pain on feeding rodents a diet low in iron [97, 98]. In addition, extracellular dopamine levels are high from decreased extraction of dopamine transporter whose levels were also low [99, 100]. In animal models, D1 and D2 receptor density in the caudate and the putamen decrease with iron deficiency compared to the usual diet [101–103].

Despite iron being a rate-limiting substance in L-DOPA synthesis, and the apparent expectation that iron deficiency would result in decreased tyrosine hydroxylase,

Table 1.5 Mnemonic used for the diagnosis of RLS

U—Urge to move
R—Rest induced
G—Gets better with activity
E—Evening and night worsening
D—Differential diagnosis does not sufficiently explain symptoms

human studies show that tyrosine hydroxylase levels were increased in substantia nigra of RLS patients [104]. In RLS, the increased tyrosine hydroxylase activity, producing more L-DOPA, followed by downregulation of dopamine receptors. Human CSF dopamine metabolite sampling shows higher levels of free dopamine, homovanillic acid, and 3-ortho-methyldopa (3-OMD) [105–107]. 3-OMD is a product of a minor alternative pathway for the metabolism of L-DOPA. Decreased activity of amino acid decarboxylase and increased activity of COMT and MAT result in increased DA levels [107, 108]. All of these suggest that RLS is a hyperdopaminergic state with an increase in extracellular dopamine.

The management of RLS relies on the use of dopamine agonists, which alleviate the symptoms of the disease. How does a hyperdopaminergic condition like RLS be managed by using dopamine agonists? In RLS, there is more dopaminergic activity in the setting of reduced dopamine receptors. This downregulation of dopaminergic receptors, coupled with low dopaminergic activity at night, creates a state of dopamine deficiency resulting in symptoms of RLS. The administration of dopamine agonists thus helps in managing and treating the symptoms of RLS.

Another interesting implication of dopamine-related to RLS is augmentation, which is marked by worsening RLS symptoms occurring earlier during the day with greater severity. Augmentation can be explained by increasing doses of dopaminergic agonists resulting in the downregulation of dopaminergic receptors with increased endogenous dopamine levels.

Dopamine, Sleep, and Wakefulness

Sleep is a vital behavior in animals and is essential for survival. Chronic loss of sleep may lead to adverse changes in the behavior, including altered food intake, skin lesions, compromised thermoregulation, weight loss or gain, and even death [109, 110].

Ventral tegmental area dopamine is known to play a vital role in emotional and motivational behaviors. It also plays a crucial role in the control and regulation of wakefulness. The ventral tegmental area and substantia nigra dopaminergic neurons have the same average firing rate across the sleep-wake cycle. However, the neurons of the ventral tegmental area have increased burst activity during waking and REM sleep that results in increased release of dopamine in vital target areas such as the prefrontal cortex and nucleus accumbens [111]. This burst activity is particularly prominent in the presence of rewarding or aversive stimuli requiring an alerting response [111]. Dopaminergic neurons of the ventral periaqueductal gray (vPAG) show increased activity during waking hours but not sleep, demonstrated by Fos studies [112]. Selective lesioning of these neurons leads to a reduction in 24 h amounts of wakefulness [112]. Dopaminergic neurons of the vPAG project to other parts of the ascending reticular activating system, including basal forebrain and thalamus, and receive inputs from the sleep-active VLPO neurons [112].

Dopamine and Hypothalamus

Dopaminergic neurons in the hypothalamus account for 10% of total dopaminergic neurons in the brain, which is approximately the same as that in substantia nigra [113]. Dopamine-containing neurons are distributed widely in the hypothalamus, especially in the medial part, which regulates the pituitary endocrine axis [113, 114], and they play a pivotal role in the inhibition of hormone release and formation of prolactin [115, 116]. These dopaminergic neurons also possess steroid receptors for estrogen and progesterone [117, 118].

Both dopamine receptor types—D1 like (D1 and D5) and D2 like (D2, D3, and D4) are widely distributed in the hypothalamus and play a critical role in the regulation of the hormones [119–121]. Dopaminergic agonists and antagonists affect several functions related to the hypothalamus, such as the response of pituitary to the vagus nerve, feeding behavior, water regulation, and regulation of plasma oncotic balance [116, 122–124]. It is also interesting to note that hypothalamic dopamine plays a role in psychiatric and behavioral disorders [125, 126].

Drugs Associated with Dopamine

Dopamine Precursor

Levodopa

Levodopa is the immediate precursor of dopamine and increases the synaptic dopamine in the brain. By doing so, it provides symptomatic relief to a patient with PD. It is administered along with carbidopa to decrease its peripheral breakdown and adverse effects peripherally. They are marketed together as Sinemet with different dose ratios. Titration of the dose can be made depending upon the severity of the disease and the adverse effects. Mechanism of action of various drugs used in Parkinson's disease are shown in Fig. 1.5.

Dopamine Agonists

Dopamine agonists have been used for a long time in the treatment of Parkinson's disease and restless legs syndrome. Table 1.6 summarizes the different dopamine agonists used and their properties.

Of the seven mentioned agonists, bromocriptine, pergolide, pramipexole, and ropinirole have been used in the management of PD.

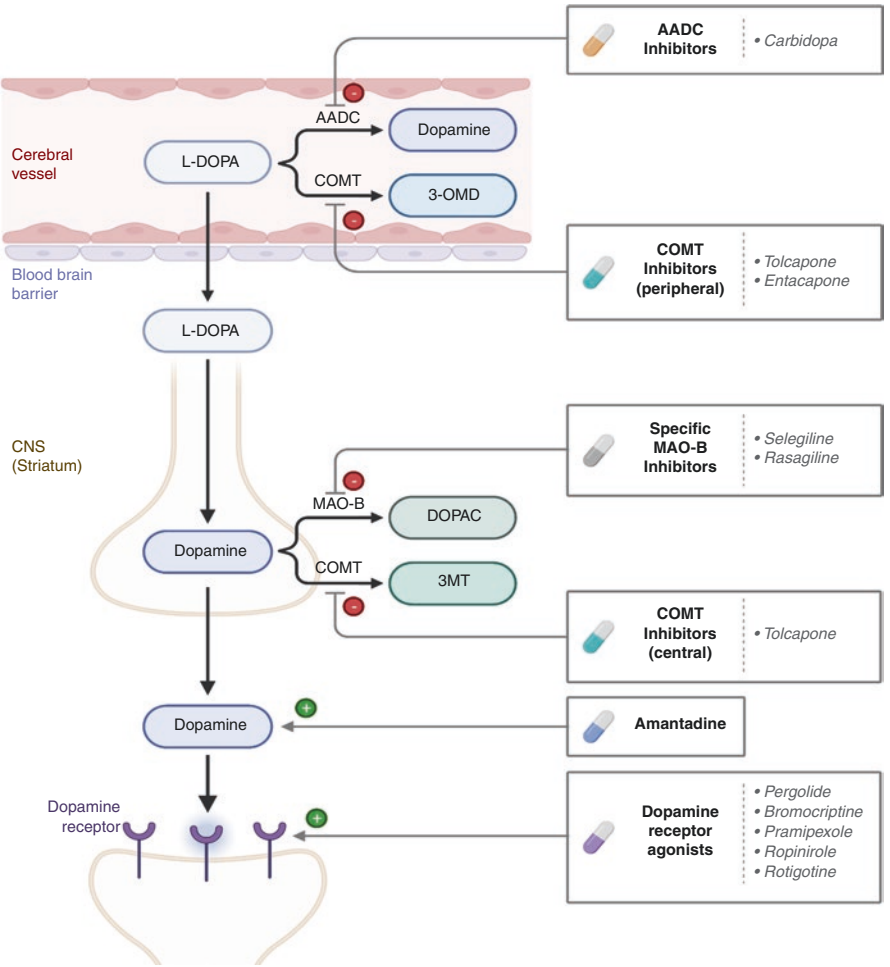

Fig. 1.5 Mechanism of action of various drugs against Parkinson's disease

Table 1.6 Dopamine agonists, their class, the receptor on which they act, and their half-lives

Agonist	Ergot or non-ergot derivative	Receptor where it acts	Plasma half-life
Bromocriptine	Ergot derivative	D2 agonist; D1 antagonist	3–6 h
Lisuride	Ergot derivative	D2 and D1 agonist	1–2 h
Pergolide	Ergot derivative	D2 and D1 agonist	15–42 h
Cabergoline	Ergot derivative	D2 agonist	65 h
Apomorphine	Morphine derivative	D2 and D1 agonist	10–100 h
Pramipexole	Aminobenzothiazole derivative	D2 and D3 agonist	3 h
Ropinirole	Non-ergoline derivative	D2 and D3 agonist	3–10 h

Table 1.7 Adverse effects due to dopamine agonists

Nausea
Dizziness
Somnolence
Hallucinations
Dyskinesias
Sleep attacks (associated with pramipexole and ropinirole) Compulsive behaviors Orthostatic hypotension

Bromocriptine was the first DA receptor agonist, introduced in 1974, approved for the management of PD [127–130]. It has an ergot structure and was used as adjunctive therapy to levodopa.

Pergolide was another ergot alkaloid approved for PD in 1988 [131–133]. Minimal data exist on using these ergot derivatives as initial monotherapy in early PD. The data reflects its benefit in most patients for a minimal duration with an increased frequency of adverse effects [134].

The non-ergot derivatives which have shown efficacy to manage patients with PD include Pramipexole and Ropinirole. Pramipexole was first used for the treatment of PD in 1997 and has shown to be good adjunctive therapy for advanced PD [135, 136]. Ropinirole was approved for use in PD in 1997 and was more effective than bromocriptine with or without selegiline [137].

Several adverse effects have been reported with dopamine agonist use (see Table 1.7). Older adults tend to be more sensitive to these adverse effects because of coexisting cognitive impairment and comorbid conditions [134]. These factors include underlying dementia, orthostatic hypotension, history of hallucinations, and dyskinesias. Hence, the statement "start low, go slow" holds much importance when prescribing these drugs to patients more than 70 years old. Utmost care is prudent when increasing the dose in this population.

Vesicular Monoamine Transporter (VMAT) Depletors

VMAT is a transporter located in synaptic vesicles and serves by storing neurotransmitters such as DA, norepinephrine, serotonin, and histamine in the neurons until they are needed [138–140]. VMATs are categorized into two: VMAT1, which is located in the peripheral nervous system and the CNS, and VMAT 2, found exclusively in the CNS [138–140].

There are two known VMAT2 inhibitors—reserpine, which irreversibly inhibits VMAT1&2, and tetrabenazine, which reversibly inhibits VMAT2. Although VMAT2 transports a variety of neurotransmitters, tetrabenazine exclusively affects dopamine in clinical doses. Tetrabenazine blocks DA transport into presynaptic vesicles leading to a rapid depletion of presynaptic dopamine proportionate to VMAT2 inhibition [138]. Tetrabenazine is rapidly absorbed and broken down into

Table 1.8 Adverse effects associated with VMAT2 inhibitors

Drowsiness
Sedation
Depression
Extrapyramidal effects
Insomnia
Akathisia
Anxiety
Nausea

its active metabolites—alpha and beta hydroxy-tetrabenazine [141]. Deutetrabenazine, on the other hand, is metabolized similarly but slower, resulting in more availability of the drug.

VMAT 2 inhibition reduces dopamine stimulation without the D2 receptor blockade and leads to decreased overstimulation of D2 receptors in the indirect pathway leading to less inhibition of the stop signal. At the same time, the direct pathway is inhibited, where the "go" signal is amplified by dopamine at D1 receptors [139, 140]. The adverse effects associated with VMAT2 inhibitors are summarized in Table 1.8. Figure 1.5 demonstrates the mechanism of action of dopamine agonists, VMAT2 inhibitors, and L-dopa on the CNS.

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 2
Serotonin

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History of Serotonin

In 1912, O'Connor suggested a substance present in the serum was responsible for the vasoconstriction, which was different from adrenaline. O'Connor also concluded that this chemical from platelets mimicked the actions of adrenaline in inducing vasoconstriction [1]. In 1937, Vittorio Erspamer et al. demonstrated that an extract from the enterochromaffin (EC) cells caused the intestines to contract and named it enteramine [2].

In 1948, Rapport et al. first isolated the vasoconstrictor substance from serum and coined the term serotonin [3]. In 1952, Erspamer showed that both enteramine and serotonin have the same chemical structure [4]. In 1953, Twarog and Page identified the presence of serotonin in the central nervous system (CNS) [5]. In 1957, Gaddum et al. introduced the first classification of serotonin receptors, 8 years after the discovery of serotonin [6].

In 1964, Dahlstrom and Fuxe identified serotonergic cell bodies in the raphe areas of the pons and midbrain and categorized them into B1–B9. Rostral cell bodies project their axons to the forebrain, medulla, and spinal cord [7].

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Neurochemical Profile

Serotonin in the Brain

Serotonin (5-hydroxytryptamine or5-HT) is a local hormone (autacoid) and a principal neurotransmitter in CNS and peripheral nervous system (PNS) [8]. In CNS, <1 in a million neurons produce serotonin, but most of the body's serotonin is produced in the periphery [9]. Serotonin does not cross the blood-brain barrier [10]. Serotonin modulates both the behavioral and neuropsychological processes such as anger, aggression, mood, reward, perception, attention, memory, appetite, and sexuality. Serotonin behavioral effects and other CNS functions are given in Table 2.1 [11].

Sundstorm et al. identified that the serotonergic neurons are first seen around 5 weeks of gestation [12]. Serotonin plays a crucial role in the embryonic developmental processes. Serotonin plays a vital role in building critical pathways for cognition, learning, and stress reactivity. The various physiological roles of serotonin are shown in Fig. 2.1 [13].

Serotonin in the Periphery

In the periphery serotonin is found mainly in the gastrointestinal (GI) tract, liver, and platelets [11]. About 95% of the serotonin in the body is produced by the enterochromaffin (EC) cells in the gastrointestinal tract, shown in Fig. 2.2 [9].

Table 2.1 Serotonin behavioral effects and other CNS functions [11]

Behavioral effects	Other CNS functions
Addiction	Body temperature
Aggression	Cerebellar regulation
Anger	CNS vascular tone
Appetite	Descending regulation of multiple organ systems
Fear	Emesis
Memory	Motor control
Mood	Respiratory drive
Perception	Sleep/circadian rhythms
Sexuality	
Stress responses	

CNS central nervous system

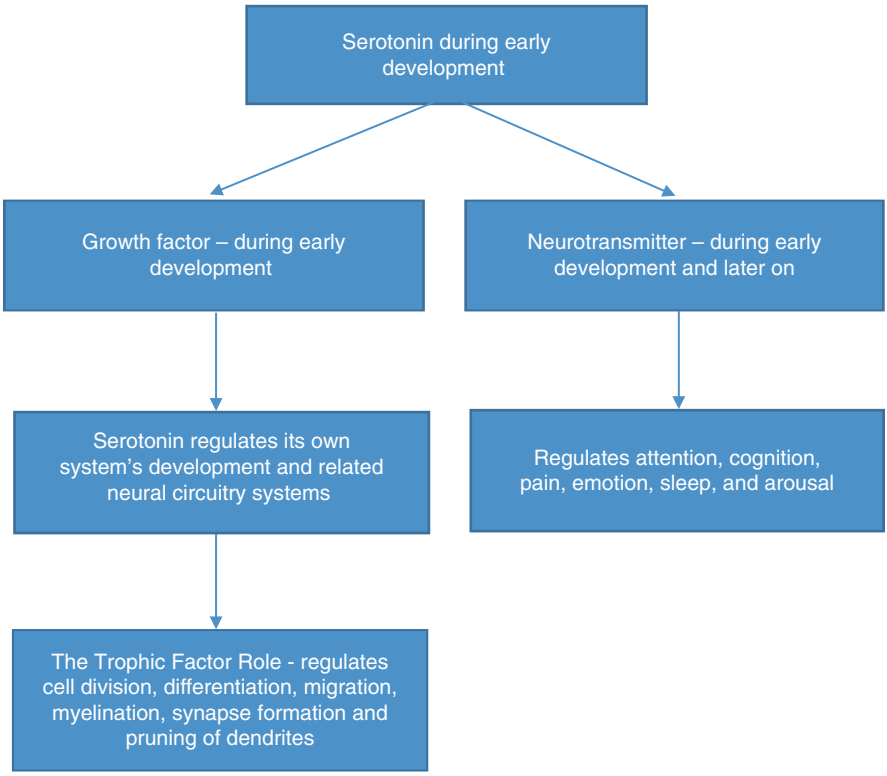


Fig. 2.1 Serotonin physiological roles [13]

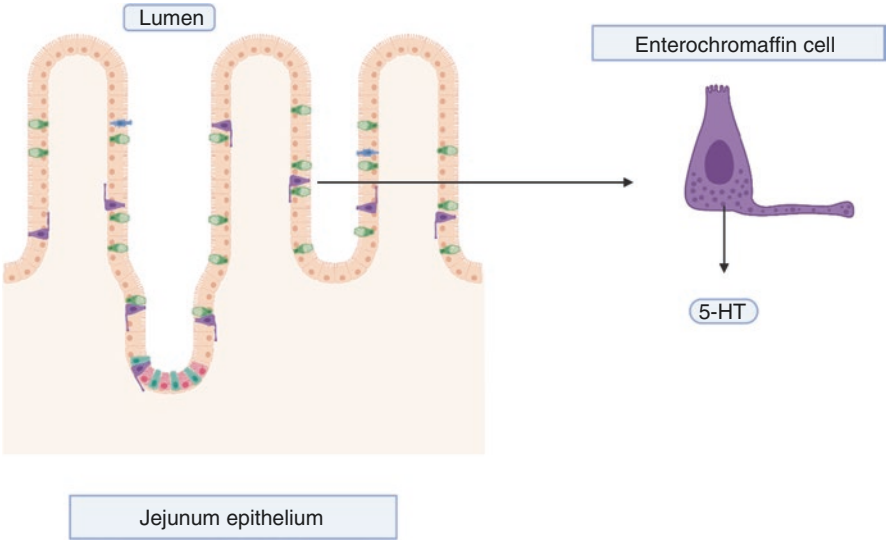


Fig. 2.2 Jejunum epithelium with enterochromaffin cells. *5-HT* serotonin

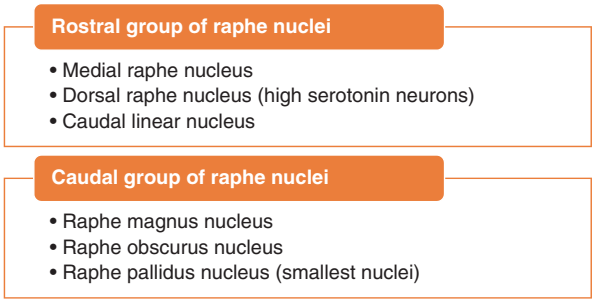
Serotonin is one of the key players in sending taste signals to the brain, in regulating pancreatic enzyme functioning, and in the peristaltic process [14]. Activation of taste bud cells by food causes serotonin release onto the sensory afferent nerves to conduct the taste information to the CNS [11, 15]. Alteration in the serotonin signaling pathways is involved in multiple functional bowel disorders like irritable bowel syndrome (IBS) [9].

The peripheral serotonin originates from the EC cells and is released into the bloodstream and is taken by platelets to store in the dense granules. Serotonin stored in the platelets is released if there is an injury or damaged endothelium. Serotonin acts as a chemotactic agent for multiple immune cells such as dendritic cells, eosinophils, and mast cells (MC). MCs exhibit the highest number of Tph1 mRNA expression and can synthesize, store, and secrete serotonin. Other immune cells such as macrophages, lymphocytes, basophils, and monocytes also secrete serotonin in response to an infection or injury [11].

Raphe Nuclear System

Within the CNS, serotonin is primarily produced from the neurons in the raphe nuclei found in the midline of the brainstem. The serotonergic neurons form the most complex and largest efferent system in the CNS [11]. The raphe nuclei express 5-HT1 receptors and function as auto-receptors in the brain to regulate the release of serotonin. Raphe nuclei also have some non-serotonin neurons [16].

The raphe nuclei are divided into a rostral group and a caudal group. The rostral group nuclei comprise 85% of the serotonergic neurons in the brain. Among the rostral group, dorsal nuclei have the largest number of serotonin neurons. The caudal group has fewer serotonergic neurons than the rostral group [17].



Cortico-limbic projections from the rostral group to the hippocampus, amygdala, thalamus and basal ganglia regulate cognition, mood, emotion, and stress response. Other roles of the raphe nuclei comprise regulation of movement, appetite, sleep cycles, and sexual function [18].

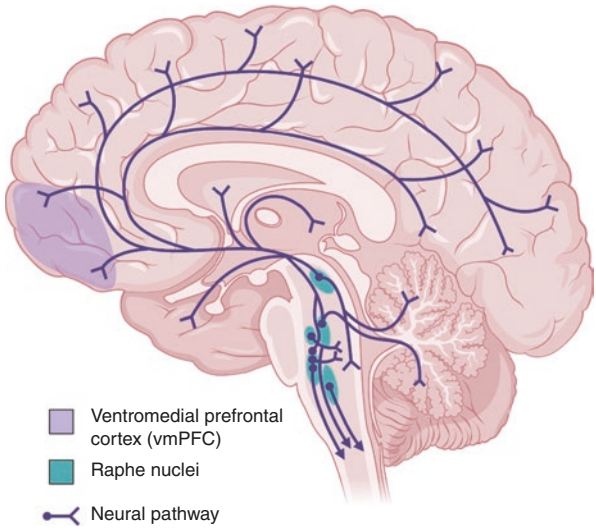


Fig. 2.3 Serotonergic projections from the raphe nuclei [11, 13]

Each neuronal cell in the brain is proximal to a serotonergic fiber, and almost all the behaviors and brain functions are controlled by serotonin [11]. Serotonergic projections from the raphe nucleus are shown in Fig. 2.3 [11, 13].

Brainstem serotonergic neurons transmit descending projections into the spinal cord to regulate the approaching nociceptive data [19]. Brainstem raphe serotonergic neurons transmit ascending projections to both limbic and cortical areas, which regulate the psychological pain perception [20]. Serotonergic input to pain-sensitive areas of the hypothalamic arcuate nucleus and thalamus leads to an analgesic effect [21].

Serotonin Receptors

Serotonin exerts its physiological functions through 14 distinct receptor subtypes, which are categorized into seven receptor families 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7. These receptors are categorized based on structural, functional, and signal transduction properties [10]. Serotonin receptor types, subtypes, their mechanism of actions, and functions are given in Table 2.2 [22].

The 5-HT receptor family comprises guanine nucleotide triphosphate (GTP) binding proteins (G-protein)-coupled receptors (GPCRs) except the 5-HT_3 family, which is a ligand-gated ion channel. These receptors are associated with their respective signal transduction pathways via G-proteins [10, 16].

Serotonin receptor gets activated either by 5-HT or medications by employing two intracellular mechanisms, which are plasma membrane depolarization or G-protein-mediated modifications of intracellular messengers such as cAMP, diacylglycerol, and inositol triphosphate [23]. Serotonin receptor subtypes 5-HT_1,

Table 2.2 Serotonin receptors, mechanisms, and actions [10, 22]

Receptors and subtypes	Mechanism	Potential and pathway	Actions
5-HT_1 – A, B, D, E, F	G_i/G_o-protein-coupled transduction - ↓ The cellular levels of cAMP	Inhibitory; CNS, blood, vessels	Addiction, aggression, anxiety, appetite, autoreceptor, blood pressure, cardiovascular function, emesis, heart rate, impulsivity, learning, locomotion, memory, mood, nausea, nociception, penile erection, pupil dilation, respiration, sexual behavior, sleep, sociability, thermoregulation, vasoconstriction
5-HT_2 – A, B, C	G_q/G_{11} - protein ↑ cellular levels of IP_3 and DAG	Excitatory; CNS, blood, vessels, GI tract, smooth muscle, PNS, platelets	Addiction, anxiety, appetite, cardiovascular function, cognition, GI motility, imagination, learning, locomotion, memory, mood, penile erection, perception, sexual behavior, sleep, thermoregulation, vasoconstriction
5-HT_3 – A, B, C, D, E	Depolarizing plasma membrane	Excitatory; CNS, PNS, GI tract	Addiction, anxiety, emesis, GI motility, learning, memory, nausea
5-HT_4	G_s-protein - ↑ cellular levels of cAMP	Excitatory; CNS, PNS, GI tract	Addiction, anxiety, GI motility, learning, memory, mood, respiration
5-HT_5 – A, B	G_i/G_o-protein-coupled transduction - ↓ the cellular levels of cAMP	Inhibitory; CNS	Autoreceptor, locomotion, sleep
5-HT_6	G_s-protein - ↑ cellular levels of cAMP	Excitatory; CNS	Anxiety, cognition, learning, memory, mood
5-HT_7	G_s-protein - ↑ cellular levels of cAMP	Excitatory; CNS, blood, vessels, GI tract	Anxiety, autoreceptor, memory, mood, respiration, sleep, thermoregulation, vasoconstriction

cAMP cyclic adenosine monophosphate, *CNS* central nervous system, *DAG* diacylglycerol, *GI* gastrointestinal, *IP3* inositol trisphosphate, *PNS* peripheral nervous system

5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 act through the modulation of adenylate cyclase. Serotonin receptor subtype 5-HT_2 (2a, 2b, and 2c) act by activating diacylglycerol and inositol triphosphate [10, 11].

The serotonin receptors regulate the release of numerous neurotransmitters such as dopamine, epinephrine or norepinephrine, GABA, glutamate, and acetylcholine. These receptors also regulate the release of various hormones such as oxytocin, cortisol, prolactin, vasopressin, and substance P. The serotonin receptors are reported to be involved in several biological and neurological processes such as anger, aggression, mood, reward, perception, attention, memory, appetite, and sexuality [11, 24, 25].

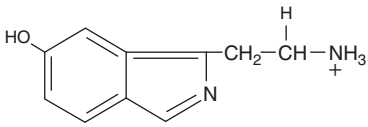


Fig. 2.4 Chemical structure of serotonin [16]

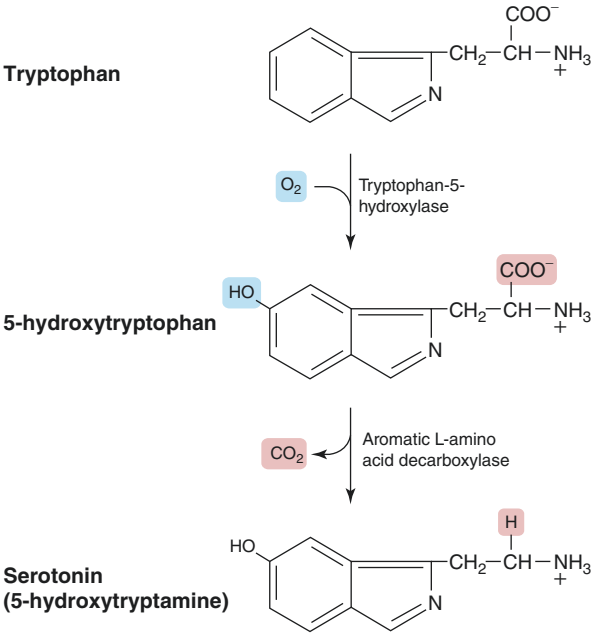


Fig. 2.5 Summary of serotonin synthesis [10]

Serotonin receptor families and their distinct subtypes are expressed in tissues of both central and peripheral systems that allow serotonin to exert its functions that are outlined in Table 2.2 [10, 22].

Serotonin Metabolism

Chemical Structure and Biosynthesis

The chemical structure of serotonin is shown in Fig. 2.4 [16]. Serotonin is synthesized from the essential amino acid L-tryptophan. Tryptophan is hydrolyzed by tryptophan hydroxylase (TPH) into 5-hydroxytryptophan (5-HTP), which uses oxygen, NADPH2, tetrahydrobiopterin, and either iron or copper. 5-HTP is decarboxylated by the enzyme L-aromatic amino acid decarboxylase with pyridoxal-phosphate as a coenzyme to form serotonin [26]. The summary of serotonin synthesis is shown in Fig. 2.5 [10].

The rate-limiting enzyme in the synthesis of serotonin is TPH; it has two isoforms. TPH1 is predominantly found in the periphery, while TPH2 is seen in the raphe nuclei of the brainstem [27, 28]. As serotonin does not cross the blood–brain barrier, central and peripheral pools of serotonin control the serotonin-dependent functions in both the brain and periphery [10].

Serotonin reuptake into the presynaptic vesicles for storage is transmitted through the vesicular monoamine transporter. The serotonin is transported into the synaptic vesicles through a proton gradient across the vesicular membrane [29]. Peripherally, serotonin is synthesized mainly in the EC cells of the gastrointestinal tract [30]. Carbohydrates increase the colonic and duodenal EC cell serotonin secretion [31].

Serotonin Metabolism

Serotonin is metabolized through various pathways. Serotonin is mainly metabolized by monoamine oxidase (MAO-A and MAO-B). MAO-A has a high affinity for serotonin than MAO-B; it metabolizes serotonin into 5-hydroxyindoleacetic acid (5-HIAA) [32].

Serotonin is metabolized into N-acetyl serotonin by arylalkylamine N-acetyltransferase (AAAT) enzyme, which in turn is metabolized to melatonin by the enzyme hydroxyindole-O-methyl transferase (HIMT) [33].

Serotonin is also metabolized through indoleamine 2,3-dioxygenase (IDO) to initiate the kynurenine pathway and form the end-products of kynurenic acid and quinolinic acid. In neurons, kynurenine is metabolized into picolinic acid to protect against neurotoxicity. The kynurenine pathway plays an important role in regulating serotonin synthesis and availability [10, 34].

IDO acts as a rate-limiting enzyme in the conversion of tryptophan to kynurenine, which is present ubiquitously, excluding the liver. IDO activation decreases the serotonin and increases the kynurenine level, commonly seen in depressive patients [35, 36].

A fourth catabolic pathway is the generation of N-methyl-serotonin from serotonin by the enzyme N-methyl transferase [37]. A summary of serotonin metabolism is shown in Fig. 2.6 [10].

Medications Acting on Serotonin System

Although serotonin has a basic chemical structure, the pharmacological effects of serotonin are more diverse, complex, and broad. Serotonin receptors and transporters play a vital role in the development of CNS medications and multiple current medications to regulate serotonin neurotransmission. Centrally acting serotonergic drugs are shown in Table 2.3 [11].

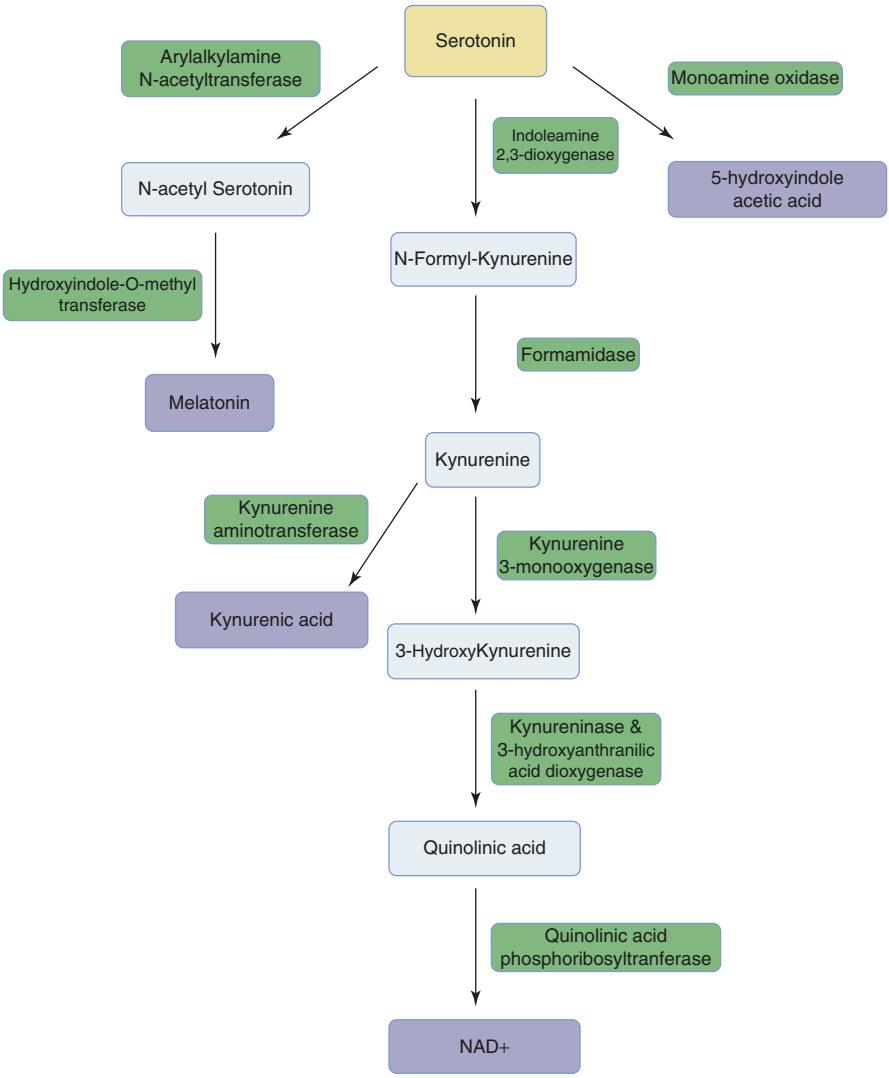


Fig. 2.6 Summary of serotonin metabolism [10]. *NAD* nicotinamide adenine dinucleotide

Serotonin levels can be regulated by various environmental factors and pharmacological agents. Dietary restriction of tryptophan or chemical inhibitors of TPH decreases the levels of serotonin in the brain, whereas selective serotonin reuptake inhibitors (SSRIs) like escitalopram and fluoxetine cause an increase in the synaptic serotonin levels [38].

Various 5-HT receptor agonists or antagonists are used as anti-emetic drugs, for the treatment of migraine, IBS, and neuropsychiatric disorders. 5-HT3 receptor antagonists are widely used in the treatment of chemotherapy and radiation-induced

Table 2.3 Centrally acting serotonergic drugs [11]

• 5-HT_3 receptor antagonists (ondansetron).
• Atypical antipsychotics.
• Buspirone.
• Ergotamine/methysergide.
• Fenfluramine.
• Hallucinogens.
• MAOIs.
• Other antidepressants.
• SSRIs.
• Tricyclic antidepressants.
• Triptans.

5-HT serotonin, *MAOI* monoamine oxidase inhibitor, *SSRI* selective serotonin reuptake inhibitor

nausea and vomiting [39]. Anti-emetic drugs (5-HT3 receptor antagonists) include ondansetron (Zofran), dolasetron (Anzemet), granisetron (Kytril), alosetron (Lotronex), and palonosetron [39, 40]. Drugs used in the treatment of irritable bowel syndrome include alosetron (a 5-HT3 antagonist) and tegaserod (Zelnorm) (a 5-HT4 agonist) [41].

Drugs used for the treatment of migraine (5-HT1D/1F receptor agonists) include rizatriptan (Maxalt), almotriptan, donatriptan, and frovatriptan. Most of these are 5HT-1D selective agonists, including the prototypical triptan, Sumatriptan [42]. 5-HT1D receptor agonists that are effective for the treatment of migraine are called triptans because the first agent introduced was sumatriptan. Even though the triptans are effective, whether they act only in the periphery or in the CNS has not been completely understood [43]. Other drugs for the treatment of migraine that act through 5-HT receptors include zolmitriptan (Zomig), naratriptan (Amerge), rizatriptan (Maxalt), and alniditan [42].

5-HT1A receptors have therapeutic potential for the treatment of depression and anxiety. Buspirone (Buspar) was the first long-chain arylpiperazine (LCAP) to become clinically available for psychiatric disorders [44]. The atypical antipsychotics include Quetiapine, Risperidone, Clozapine, Olanzapine, Ziprasidone, and Zotepine, that bind at both 5-HT2A and dopamine D2 receptors [45, 46].

The 5-HT transporter has a key role in affective disorders. Agents that can block the transporter thus augment the synaptic levels of 5-HT and are beneficial in the treatment of obsessive-compulsive behavior, depression, and panic disorders. Tricyclic antidepressants like imipramine and desipramine can block the 5-HT transporter and the norepinephrine reuptake transporter (NET) to different extents [47].

Drugs such as fluoxetine, paroxetine, sertraline, and fluvoxamine have been beneficial for the treatment of the obsessive-compulsive disorder, depression, anxiety, and panic disorder. The antidepressant trazodone, a weak SSRI, binds at 5-HT2 receptors and is also a 5-HT2 antagonist. SSRI mechanism of action and commonly used SSRIs are shown in Fig. 2.7 [47].

Hallucinogenic agents such as lysergic acid diethylamide (LSD), 2,5-dimethyl-4-bromoamphetamine (DOB), and 2,5-dimethoxy-4-iodoamphetamine (DOI) also act through the serotonin system for the treatment of hallucinations [48].

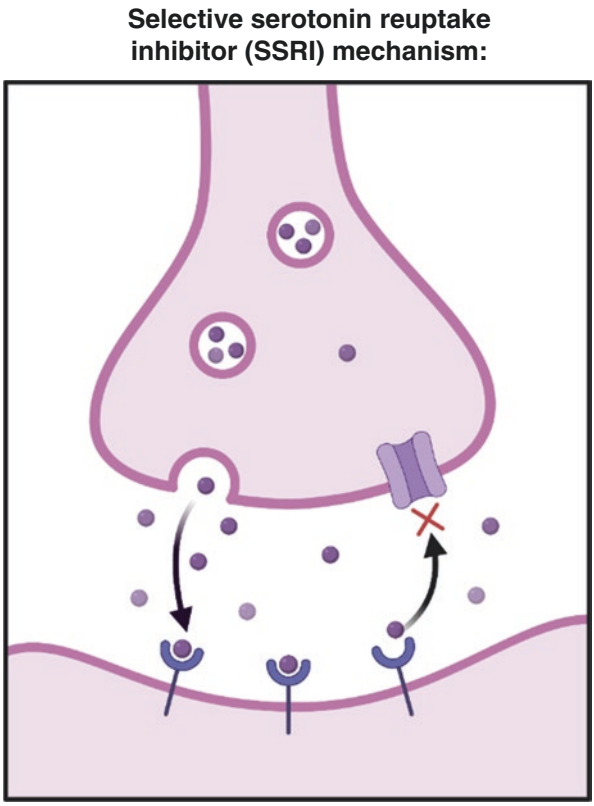


Fig. 2.7 Selective serotonin reuptake inhibitors (SSRIs) mechanism of action

Other Clinical Aspects

Serotonin in Migraine

Numerous clinical studies have implicated the crucial role of serotonin in the pathogenesis of migraines [49, 50]. Serotonin can induce vasoconstriction of the blood vessels at nerve endings, thus causing nociceptive pain [51]. Vomiting causes stimulation of intestinal motility and increases blood serotonin levels. Within the CNS,

the normal endogenous serotonin levels prevent migraines. Most neurons present in the dorsal raphe (site of emergence of the trigeminal nerve) and many in the trigeminal ganglia are serotonergic [49, 52, 53].

Various genetic studies have reported that serotonin is involved in migraine and distinctly that the migraine is a polygenic syndrome. Gormley et al. detected 38 susceptibility loci for migraine, in a meta-analysis of 375,000 subjects [54]. Thompson et al. reported a positive association at the 5-HT1D receptor locus in 64 extended families with migraine with aura [55].

An association between migraine and a functional polymorphism rs3813929 was localized in the gene 5-HT2C promoter region for the serotonin receptor [56]. Gasparini et al. determined the involvement of serotonin during a migraine and reported an increase in 5-HIAA, a metabolite of serotonin in the urine of 15 patients [57].

Serotonin Effects on Heart

Serotonin has various effects on the heart leading to its title as an amphibaric molecule [58]. Serotonin's vascular effects are variable such as it can cause diverse contractile responses in different fragments of the same coronary artery [59].

The 5-HT1 receptor regulates the vasodilator activity of serotonin, whereas the 5-HT2 receptor regulates the vasoconstrictive effect. The varied distribution of these two receptor subtypes is responsible for different responses of Serotonin in different vascular beds [60]. Serotonin leads to constriction in precapillary vessels and larger arteries, whereas it leads to vasodilation in large veins and arterioles [59, 60].

Serotonin, acting through the 5-HT2 receptor, augments the discharge and increases the actions of numerous vasoconstrictors such as angiotensin II, histamine, prostaglandin F2α, and norepinephrine [58, 60]. Elderly and hypertensive patients have augmented serotonin sensitivity, a risk factor for cardiac events and coronary artery disease [60, 61].

In atherosclerotic patients, loss of vascular endothelial barrier with 5-HT1 receptors leads to unopposed 5-HT2 receptor-mediated vasoconstriction [60]. In hypertensive patients, it was reported that decreased ability of platelets to bind serotonin leads to a reduced platelet survival time [62].

Serotonin in Psychosis

The cerebral cortex receives innervation from the serotonergic neurons, and 5-HT2A receptors have a major play in the regulation of neuronal circuitry in both the hippocampus and medial prefrontal cortex [63]. The role of serotonin in the CNS as a regulator of mood and behavior has received substantial attention [11].

5-HT2A receptor also has an involvement in attentional and cognitive deficits, which might also serve as a pre-psychotic basis for disintegration into psychotic thinking. Furthermore, neurocognitive and information processing deficits are recognized as vulnerability markers for schizophrenia patients who report difficulty maintaining concentration and memory [64]. Numerous information processing and cognition measures have been assessed as vulnerability markers for schizophrenia [65].

It has also been demonstrated that the 5-HT2A receptor has a vital role in deficits in pre-pulse inhibition of the startle response, which is an effective measure of sensorimotor gating that gets diminished in schizophrenia patients [45, 66, 67].

Serotonin Modulation in Obesity (Lorcaserin)

Serotonin is known to be a key regulator for energy balance and has a complex role in appetite and subsequent nutrient intake [68]. The inhibitory effects of serotonin on appetite were the main reason for the approval of receptor agonists for the treatment of obesity [69, 70]. The dorsal raphe (B7) of the midbrain nucleus has a significant percentage of the total brain's serotonin and has projections to hypothalamic nuclei and other feeding-associated forebrain areas [71].

It has been established that obesity can alter 5HT dorsal raphe neurons and 5HT terminal regions [72]. Lorcaserin is a selective serotonin receptor (5HT2C) agonist and decreases food intake principally by modulating the hypothalamic pathways involved in appetite. Lorcaserin (ADP356) acts on the central serotonergic system to reduce food consumption and body weight [73]. FDA approved Lorcaserin in 2012 for the treatment of obesity [74].

A double-blind placebo-controlled randomized clinical trial for lorcaserin investigated the efficacy of three different doses (10 mg or 15 mg once daily, 10 mg twice daily) in obese subjects ($n = 469$) for 12 weeks. The study reported that Lorcaserin caused a dosage-dependent weight reduction [75]. In phase III clinical trial that involved 3182 overweight or obese subjects [BLOOM (Behavioral modification and Lorcaserin for Overweight and Obesity Management)], lorcaserin (10 mg twice daily) caused 4% weight loss at 52 weeks [76].

Serotonin in Nausea/Vomiting

Nearly 80% of total body serotonin is found in the GI tract, whereas the rest is divided between the CNS and platelets [77]. These mucosal EC cells are the sensory transducers that release 5-HT to stimulate intrinsic (5-HT4 and 5HT1P) and extrinsic (5HT3) primary afferent nerves [78].

Serotonin is released into the gut by chemotherapeutic agents, radiation, and other medical interventions. The released serotonin binds to 5-HT3R to cause nausea and vomiting. 5-HT3R antagonists are used as anti-emetics for numerous conditions like chemotherapy-induced nausea/vomiting, radiation-induced emesis, and postoperative nausea/vomiting [78].

Within CNS, solitary tract nucleus (STN) and chemoreceptor trigger zone (CTZ) have the highest concentration of 5-HT3 receptors, and 5-HT3R antagonists control nausea and vomiting by acting at these regions. The first generation of anti-emetics includes dolasetron, granisetron, ondansetron, and tropisetron [78, 79]. Palonosetron, a second-generation 5-HT3R antagonist, acts at both central and GI sites [80].

Serotonin in Gut Motility

5-HT3 and 5-HT4 receptors are majorly involved in gut motility [9]. Both 5-HT3 and 5-HT4 receptors are present on neurons within the submucosal and myenteric plexuses, intrinsic and extrinsic sensory neurons, and EC cells. Serotonin is localized on both mucosa and neurons of the motor circuits, which regulate gut motility. 5-HT3R antagonists, used to treat nausea and vomiting, act on 5-HT3 receptors on intrinsic neurons that initiate propulsive motility, and extrinsic sensory neurons mitigate comfort and pain [81, 82].

5HT4 receptor agonists are used in the treatment of constipation, which acts by augmenting the release of 5-HT from mucosal EC cells, and by activation of both peristaltic reflex pathways and secretion [81, 83]. Prucalopride a dihydrobenzofurancarboxamide derivative is a high affinity, selective 5-HT4 receptor agonist that augments colonic motility [84]. Prucalopride significantly improves bowel movement frequency and satisfaction with bowel function [85].

Serotonin Syndrome

Serotonin syndrome and its various symptoms occur when a high level of serotonin causes the hyperactivation of both the central and peripheral serotonin receptors. Various studies have reported that 5-HT2A receptors play a vital role in the development of serotonin syndrome [86, 87].

Patients with mild serotonin syndrome are often afebrile. Moderate serotonin syndrome patients usually present with hyperthermia (40.8 °C), hypervigilance, mild agitation, horizontal ocular clonus, pressured speech, and hyperactive bowel sounds. Severe serotonin syndrome patients typically report hyperthermia (>41.18 °C), extreme changes in pulse rate and blood pressure, hallucination, and muscle rigidity. Few patients might also develop myoglobinuria, rhabdomyolysis, metabolic acidosis, kidney failure, seizures, respiratory failure, diffuse intravascular clotting, acute respiratory distress syndrome, coma, and death [86, 87].

Serotonin syndrome management should include discontinuation of all serotonergic agents. The next step involves providing supportive care to correct vital signs, maintain oxygen saturation greater than 93%, administer intravenous fluids, and administer serotonin antagonists. Adequate management helps in resolving the serotonin syndrome, typically within 24 h. In mild serotonin syndrome, management should include discontinuation of the serotonergic drugs, sedation with benzodiazepines, and careful clinical monitoring. Moderate serotonin syndrome requires additional monitoring of cardiac function, and using serotonin antagonist might be needed. In severe cases, invasive or non-invasive ventilation may have to be employed [86, 87].

Serotonin in RLS

Restless leg syndrome (RLS) is a complex neuropsychological syndrome that causes abnormalities in arousal, sensation, motor activity, and psychology. RLS is associated with an irresistible impulse to move, frequently along with sensory discomfort. RLS symptoms might deteriorate during the night due to inactivity and are relieved after some movement, disturbing the patient's sleep [88].

RLS is associated with a dopaminergic dysfunction in the CNS. The symptoms of RLS can be improved with dopamine agonists and worsened by dopamine antagonists. Abnormalities have also been reported in brain images of patients with RLS [89, 90].

Most antidepressants used in clinical practice worsen the symptoms of RLS [91]. Ohayen et al. demonstrated that SSRIs lead to a threefold increased risk of RLS [92]. Jhoo et al. reported that usage of magnetic resonance spectroscopy showed comparable availability of serotonin transporter protein in pons and medulla in subjects with RLS and control subjects, the severity of RLS increased due to decreased transporter availability. These outcomes supported that the dysregulation of serotonergic neurotransmission might worsen RLS [93].

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Chapter 3
Adenosine

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Introduction

Adenosine is an organic compound and a ubiquitous homeostatic substance that occurs in nature in the form of several derivatives. Adenine, which is one of the four bases that is the backbone of the DNA and RNA, combines with ribose or deoxyribose group and one or more phosphate groups to form Adenosine. Adenosine also plays a vital role as an extracellular signaling molecule. Adenosine was first reported in 1927 by Szent-Gyorgyi and Druy from the University of Cambridge, United Kingdom, where they observed slowing of the heart rate after injecting extracts from cardiac tissues into an animal [1]. It was after several steps of purification that they realized that the compound responsible for the slowing of the heart rate was an adenine-based compound [1].

Retrospectively, this compound which was responsible for the slowing of the heart rate, was in fact Adenosine [1]. It was in 1981 when excreted Adenosine was identified as a cell density signal that was able to induce the formation of fruiting bodies following starvation in Myxococcus xanthus [2]. This report was the first to

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establish the role of Adenosine in metabolism and prompted the investigators to use another term to describe this molecule—"retaliatory metabolite" [3]. It was around this time when Adenosine was utilized to manage patients with supraventricular tachycardia and has since then remained the mainstay for managing patients with this arrhythmia making it an indispensable drug in cardiology, anesthesia, and critical care medicine.

Metabolism

Chemical structure of Adenosine is shown in Fig. 3.1. The production of Adenosine relies on two systems—cytoplasmic S-adenosylhomocysteine hydrolase (SAHH) and intra- or extracellularly localized 5′ nucleotidases. Adenosine removal from the cell is catalyzed by two key enzymes—adenosine deaminase and adenosine kinase [4]. The metabolism of Adenosine is illustrated in Fig. 3.2.

Extracellular adenosine generation involves a two-step enzymatic reaction involving its precursor molecules—5′-adenosine triphosphate (ATP) and 5′-adenosine monophosphate (AMP). Extracellular ATP that is released by inflammatory cells, epithelia and platelet endothelia is acted upon by ecto-apyrase (CD39) which converts it to AMP. This is followed by conversion of AMP to 5′-ecto nucleotidase (CD73) to Adenosine making it available on cell surface to activate its receptors.

Intracellular adenosine generation involves the breakdown of S adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH) in the presence of the enzyme methyltransferase. SAHH then breaks SAH into Adenosine.

While in the cell, Adenosine is then broken down to Inosine in the presence of the enzyme Adenosine deaminase. Purine nucleoside phosphorylase facilitates the

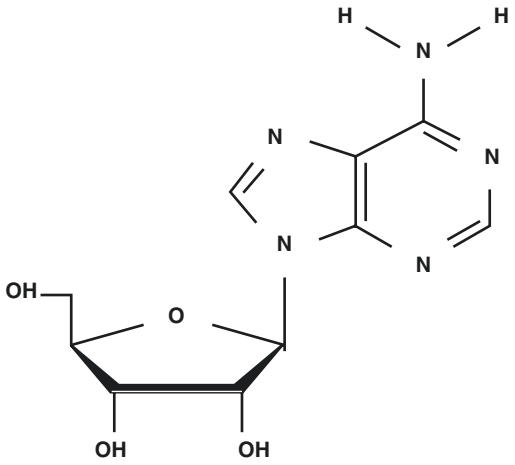

Fig. 3.1 Chemical structure of adenosine

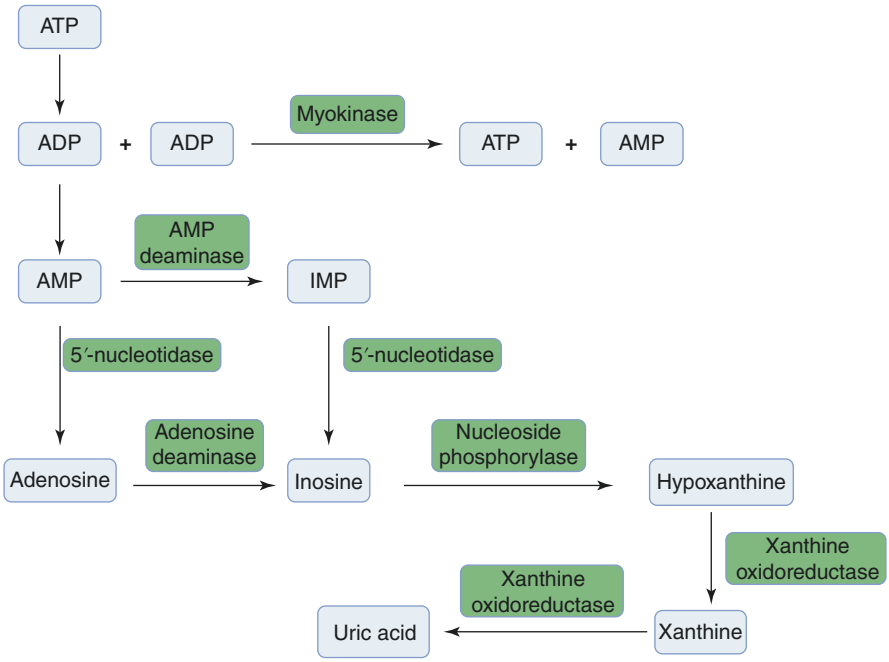

Fig. 3.2 Metabolism of adenosine [4]

conversion of Inosine to Hypoxanthine, which is further degraded to Xanthine in the presence of Xanthine oxidase. Xanthine oxidase further converts Xanthine to Uric acid, which is excreted out of the cell.

Receptors

Adenosine mediates its effect via its interaction of with four receptors, which are categorized by their high (A1, A2A, and A3) or low (A2B) affinity for the parent molecule. Each of these four receptors consists of a core domain that crosses the plasma membrane seven times, with each helix having 20–27 amino acids and are linked by three intracellular and three extracellular loops [5]. There is an extracellular NH2 terminus with one or more glycosylation sites and an intracellular COOH terminus that has sites for phosphorylation and palmitoylation. Each of these receptors differs in the number of amino acids they have as a part of their chain. Figure 3.2 demonstrates the structure of the receptors. These receptors are found throughout the body, including the cardiovascular, gastrointestinal, urogenital, nervous, and immune systems signifying the control of cardiac, metabolic, renal, and neurological activities. Table 3.1 summarizes the locations of various receptors where they have maximum density.

Table 3.1 Anatomical locations of various receptors and their densities

Receptor type	Anatomical location with highest density
A1	Cortex, hippocampus, cerebellum, eye, adrenal gland, heart
A2A	Thymus, spleen, leucocytes, striatum and olfactory bulb
A2B	Bladder, colon
A3	Mast cells, testis (in animal models)

The A1 receptor is widely expressed throughout the body, with the highest levels in the excitatory nerve findings of the brain [6]. The activation of the A1 receptor leads to inhibition of adenylyl cyclase, activation of potassium channels, blockade of N-type calcium channels leading to increased calcium inside the cell and inositol-1,4,5-triphosphate levels by the activation of phospholipase C. Activation of these receptors reduces the firing rate of neurons resulting in a reduction in neurotransmitter release [6].

The A2A receptors are distributed widely through the body, including the basal ganglia, the spleen, thymus, platelets, and leucocytes [7, 8]. The stimulation of these receptors leads to stimulation of cyclic AMP protein kinase pathway by coupling with G_s protein in the brain [5]. These receptors interact with several neurotransmitters to regulate vital functions associated with sleep, motor activity, behavior, and neuronal death. Peripherally, it regulates myocardial oxygen consumption, inflammation, and pathogenesis of cancer [9].

The A2B receptors are distributed widely but less in number as compared to the other adenosine receptors. Of the four receptors, this receptor is the most adenosine-insensitive receptor requiring high concentrations, which are rarely achieved under normal physiological conditions. A2B receptor signaling has been described in adverse conditions where adenosine levels elevate significantly. These conditions include increased tolerance to ischemia, acute inflammation, and the tissue's adaptation to hypoxia [10–16]. It works by stimulating the mitogen-activated protein kinase activity with an affinity that is the same as A2A receptors [17].

The A3 receptors work by the upregulation of nuclear factor kB signaling and the phosphoinositide 3 kinase (PI3K)-PKB-AKT pathway. The A3 receptors expression in humans is low but has been found to be upregulated only in patients with Crohn's disease, colon cancer, and rheumatoid arthritis [18, 19].

Alzheimer's Disease and Autism

Alzheimer's disease (AD) is the most common neurodegenerative disorder affecting close to 30% of people aged 85 years and older [20, 21]. Characterized by a progressive decline in memory and impaired cognitive skills leading to dementia, the pathophysiological hallmarks for this disease includes the loss of synapses and

neurons in the cerebral cortex and hippocampus, neurofibrillary tangles, and senile plaques composed of amyloid ß (Aß) plaques [22–24].

Adenosine exerts its action on cognition and memory via A1 and A2A receptors which are located mainly in the synapses, and control the release of glutamate and acetylcholine, which play a vital role in the processing of cognitive- and memory-based activities [25, 26]. Current evidence suggests that adenosine receptors change their localization and density in the regions of the brain affected by AD and post mortem studies that analyzed the frontal cortex of AD patients showed increased levels and total number of A1 and A2A receptors in early, as well as, in advanced stages of this disease [26]. Positron emission tomography (PET) studies support these findings by showing a reduction in A1 receptor binding potential in the thalamus and temporal cortex of AD patients compared to normal subjects [27]. Although the mechanism of how Adenosine contributes to the preservation of neurons remains unknown, it has been established that A2A receptor antagonism prevents synaptic loss and neuronal death due to Aß [28, 29]. Other contributing mechanisms that relate A2A receptors to prevent neuronal loss include the control of glutamate excitotoxicity [25], its effect on neuronal metabolism [30, 31], and keeping a check on the production of free radicals [32, 33]. Caffeine is a non-selective A1/A2 receptor antagonist and is a widely consumed psychoactive drug throughout the world [34]. It has been studied in association with AD where studies have found that caffeine utilization has an inverse relationship with age-related cognitive decline, thus backing the theory of Adenosine being associated with AD [35–38]. The use of caffeine or selective A2A receptor antagonists also prevents delayed memory deficits caused due to intracerebral administration of Aß plaques [28, 39]. Propentofylline, a mixed blocker of Adenosine and nucleoside transporters, has shown improvements in cognition in patients of vascular dementia [40].

Autism is a behavioral disorder of the developing brain affecting approximately 1 in 110 children, with a steady increase noted over time [41]. Although the cause of autism remains unknown, several genetic and environmental factors have been known to contribute to the pathophysiology of autism [42, 43]. Theories include the dysfunction of neurotransmitter systems in the central nervous system (CNS), immunological anomalies, developmental anomalies in specific brain structures, and autoimmune processes, which may be etiological causes or modulating factors associated with the development of this disease [44–46]. Metabolic abnormalities associated with purine synthesis and degradation, such as adenylosuccinase deficiency and Lesch–Nyhan syndrome, have also been associated with autistic patients making enzyme abnormalities a common denominator in this population of patients [47, 48]. The deficiency of Adenosine deaminase (ADA), the enzyme that catalyzes the irreversible deamination of Adenosine to inosine, is associated with a congenital immunodeficiency referred to as severe combined immunodeficiency (SCID) [49]. ADA enhances adenosine binding at A1 receptors [50].

Individuals with deletion of chromosome 22q11 display increased autistic symptoms, and about 20–50% of this population meet the diagnostic criteria of autistic spectrum disorder (ASD) [51]. The adenosine 2A receptor gene (ADORA2A), located on chromosome 22q11.23, is known to be associated with caffeine-induced

anxiety and the distinct differences in sleep patterns amongst humans [52, 53]. As described above, A2A receptors are widely distributed in the basal ganglia with the highest expression in the caudate nucleus, which is a structure associated with ASD [54]. Significantly higher concentrations of plasma adenosine levels amongst other biomarkers have also been seen in children with autism compared to controls in a recent study suggesting an increased vulnerability to oxidative stress and decreased ability to methylation as a contributing factor to developing autism [55].

Sleep and Restless Leg Syndrome

Sleep is an integral part of a human's life cycle and is critical for the proper functioning of the human body. It is divided into two distinct stages referred to as the non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The NREM sleep is characterized by the dominance of the parasympathetic system and remains vital to the body [56].

One of the key modulators of NREM sleep is Adenosine. The first studies of Adenosine's hypnogenic effects were conducted as animal experiments by Feldberg [57] and Haulica [58]. The administration of adenosine or A1 receptor agonists induces sleepiness by the inhibition of wake active neurons [59–61], while the excitation of sleep active neurons is mediated by the activation of A2A receptors [62–64]. Adenosine dampens the neuronal activity, and promotes sleep via inhibitory effects of glutamatergic inputs to cortical glutamatergic neurons, wake active cholinergic neurons and orexin neurons [65–67]. Of the widespread distribution of the receptors in the brain, those in the basal forebrain (BF), lateral hypothalamus, and the neocortex are considered to be the most important in mediating the effects of Adenosine in the regulation of sleep and wakefulness. Infusion of A1 receptor agonists in the BF, lateral hypothalamus and prefrontal cortex promotes sleep, whereas administration of A1 receptor antagonists in the same areas promotes wakefulness [66, 68]. Local perfusion of the A1 receptor antagonist in the BF leads to activation of wake active neurons and localized suppression of A1 receptor expression leading to reduced sleep time [69, 70]. Prolonged sleep deprivation also leads to upregulation of A1 receptor mRNA in BF and cortex, further implying the vital role that adenosine receptors in BF play in relation to sleep [71, 72]. Blockade of A1 receptors in the orexinergic lateral hypothalamus also produces a significant increase in wakefulness with a concomitant reduction in sleep during spontaneous bouts of sleep-wakefulness [73]. Antagonists of A1 and A2A receptors such as caffeine and theophylline effectively counteract Adenosine's sleep-inducing effects [34, 74].

Restless legs syndrome (RLS), also known as Willis-Ekbom disease, is a sensorimotor disorder characterized by an urge to move the legs at night, leading to difficulty in initiating sleep. It is one of the most common sleep disorders affecting 5–10% of adults in North America and Western Europe [75, 76]. Although the pathophysiology of this disease remains unknown, there are several stipulated theories, including a brain iron-deficient state [77], hyperdopaminergic state [78], and

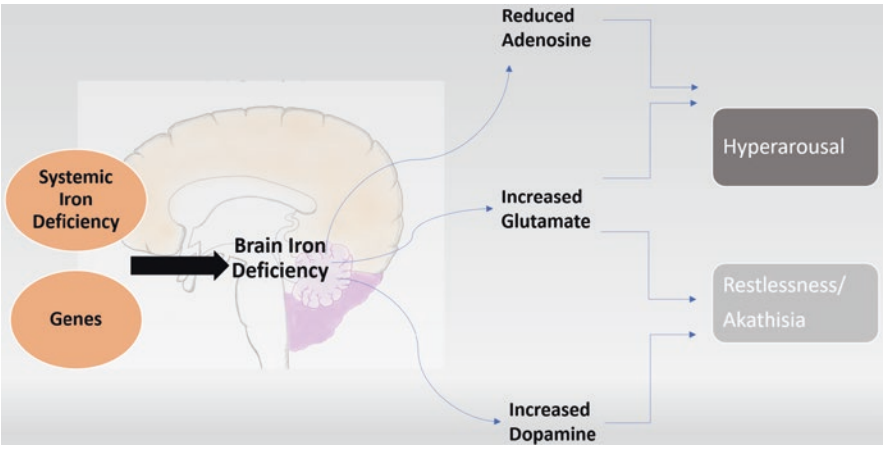

Fig. 3.3 Pathophysiology of RLS. Brain iron deficiency is the fundamental neurochemical abnormality that sets in a downstream effect with lower levels of adenosine and increased levels of glutamate and dopamine

several others. Figure 3.3 summarizes the different factors involved in the pathophysiology of RLS. A critical denominator linking all these theories—brain iron deficiency, hyperdopaminergic and hyperglutamatergic states is a hypoadenosinergic state [79]. Adenosine regulates the function of the dopaminergic system by its interaction between subtypes of adenosine and dopamine receptors [80]. This is accomplished by the formation of heteromers between adenosinergic (A1 and A2A receptors) with dopaminergic receptors (D1 and D2 receptors), leading to the formation of A1R-D1R and A2AR-D2R heteromers that form a part of macromolecular complexes including G protein and adenylyl cyclase enzyme [81]. These units are segregated into the two most populated types of striatal neurons: the striatonigral and the striatopallidal neurons and are vital in the fine-tuned inhibitory modulation of dopamine signals by Adenosine [79, 81]. As mentioned above, Adenosine exerts its somnogenic effect by inhibiting the cells of the interconnected arousal systems as well as the neurons of the targeted areas of the cortex [82, 83]. With decreased levels of Adenosine, there is an activation of arousal systems leading to hyperarousal which is an essential component of RLS.

Psychiatric Conditions

Schizophrenia is a mental disorder affecting close to 1% of the global population and has been listed by the World Health Organization as one of the top ten illnesses contributing to the global burden of the disease [84]. Table 3.2 summarizes the DSM 5 criteria for the diagnosis of schizophrenia, and Table 3.3 outlines the symptoms associated with the disease. Although the exact mechanism of this disease

Table 3.2 This table summarizes the DSM V criteria for the diagnosis of schizophrenia

DSM V criteria for the diagnosis of schizophrenia
Two or more of the following symptoms present for at least 1 month. At least one of these must be 1, 2 or 3. 1. Delusions. 2. Hallucinations. 3. Disorganized speech. 4. Disorganized or catatonic behavior. 5. Negative symptoms.
Social or occupational dysfunction or both for a significant period of time

Table 3.3 This table classifies the different types of symptoms seen in a patient suffering from schizophrenia

Positive symptoms	Negative symptoms	Cognitive deficits
Visual hallucinations	Anhedonia	Memory deficits
Auditory hallucinations	Flat affect	Problem with planning
Delusions	Isolation	Problem with decision making
Disorganized thinking	Resemblance to clinical depression	Inability to stay attentive

remains unknown, current pharmacotherapy of schizophrenia is based on the hypothesis that there is dopaminergic hyperactivity and NMDA receptor hypoactivity. Adenosine acts as a homeostatic bioenergetic regulator and modulates both dopaminergic and glutamatergic neurotransmission. This idea led to using allopurinol, a purine derivative in patients with schizophrenia, which showed moderate efficacy in decreasing aggressive behavior [85–87].

The effects of Allopurinol in patients with Schizophrenia led Lara and colleagues to propose a theory stating that purinergic system dysfunction may be the reason for neurotransmitter dysregulation leading to the pathogenesis of the disease [85, 88]. Increased density of A2A receptors is also found in the striatum of patients [89]. This could be due to a compensatory response triggered by decreased adenosinergic activity, which could, in turn, lead to a hypodopaminergic state [85, 88]. A Japanese study also suggested an involvement of A1 receptor polymorphism in the pathophysiology of the disease, further strengthening this association [90].

Depression is a state of low mood which is present across all situations which is often accompanied by low self-esteem, aversion to activity, and low energy. Depressive disorders are a common form of psychiatric disease and are believed to be the second leading cause of disability by the end of 2020 [91]. The association between depressive symptoms and Adenosine is based entirely on the effect of caffeine on mood. Caffeine, as explained above, in non-toxic doses is a potent antagonist of A1 and A2A receptors [34, 92]. Due to caffeine being consumed globally at different time schedules and varying amounts, it is hard to gauge its effects on mood accurately. The consumption of low to moderate doses of caffeine (<6 cups a day) is known to increase energy, improve attention, and decrease depressive symptoms and risk of suicide [37, 93]. In large amounts, caffeine is known to be a trigger of psychiatric symptoms ranging from mood fluctuation to full-blown psychosis in

vulnerable subjects [94]. It has also been noted that when subjects withdraw from regular caffeine consumption, they may have mood-related symptoms, including but not limited to sleepiness, irritability, restlessness, dysphoria, and nervousness [95–99]. The current therapeutic strategies used to manage depressive symptoms include tricyclic antidepressants such as nortriptyline, which are also known to reduce the action of ecto-nucleotides and Adenosine in a dose-dependent fashion [100]. Sleep deprivation and electroconvulsive therapy show an increase in the levels of Adenosine and the activation of A1 receptors in mouse models [101]. Preclinical models have also suggested that A2A receptor antagonists will be novel antidepressants considering how genetic depletion of A2A receptors results in an antidepressant-like phenotype in animal studies [102, 103]. This idea is also backed up by studies showing that A2A receptor antagonism relieves early hippocampal modifications induced by stress, which is the major environmental factor involved in the triggering of depressive states [100, 104].

Bipolar disorder is a psychiatric disorder that is characterized by periods of depression alternating with elevated moods. Kreplin, in 1921, first explored the association between manic symptoms and purinergic metabolism [105]. This was followed by studies that showed an increased purinergic turnover leading to increased excretion of uric acid during mania [106]. In the past, lithium was used to manage disorders associated with the metabolism of purines, such as gout [107]. Allopurinol, a xanthine oxidase inhibitor, was reported to improve symptoms with mania and led to the formation of a hypothesis that purinergic system might be affected in people with this disease [108, 109]. Caffeine, a non-selective adenosine receptor antagonist, is known to precipitate stimulant effects, can lead to a persistent state of arousal and exacerbate manic symptoms [110–112]. Heavy intake of caffeine contributes to the worsening of seasonal bipolar disorder [113].

Epilepsy

Epilepsy is a group of neurological disorders characterized by recurrent episodes of seizures. Adenosine is known to play a key role in the pathophysiology of this disease. A2A receptor polymorphisms are associated with encephalopathy in febrile status epilepticus, and an increased receptor expression and second messenger output are seen in these cases [114]. Polymorphisms in A1 receptors are also known to be associated with traumatic brain injury-related seizures [115]. Duplications of the chromosomal region associated with adenosine deaminase, an extracellular enzyme converting Adenosine to inosine, are associated with childhood epilepsy [116]. Genetic inactivation of A1 receptors leads to leads to spontaneous electrographic seizures in the cerebral cortex and promotes status epilepticus after traumatic brain injury [117, 118].

Systemic administration of A1 receptor agonists decreases seizures in several types of epileptic disorders and experimental models [119–122]. The activation of A1 receptors also increases the effects of standard antiepileptic drugs [123, 124].

Conversely, systemic administration of A1 receptor antagonists worsens seizures [125] and dampens the effects of antiepileptic drugs [126, 127]. Adenosine release is increased during epileptiform activity and is auto inhibitory due to the actions of A1 receptors, which was demonstrated by the use of selective A1 receptor antagonists in animal models [128–130]. Animal studies have suggested that genetic inactivation of A2A receptors is associated with the animal being seizure resistant. This confirms that A2A receptors are proconvulsant in nature [131]. A3 receptor activation leads to the promotion of seizure-like activity in the hippocampal slices [132, 133], whereas other studies have also demonstrated its anticonvulsant effect on systemic administration [134, 135]. This suggests A3 receptors have a region-specific impact on epileptiform activity. More human-based studies are required to understand in detail the effects of Adenosine and its receptors in cases of epilepsy.

Ischemia

Owing to its modulatory effect with cellular metabolism and its potent actions as a vasodilator, Adenosine was proposed as a regulator that would couple cerebral blood flow and metabolism [136, 137]. Early studies showed that extraluminal application of Adenosine led to dose-dependent dilations of isolated cerebral arteries in vitro [138, 139]. Infusion of Adenosine in the carotids leads to an increase in cerebral blood flow in humans [140]. Subsequent studies suggested that levels of extracellular Adenosine elevate dramatically in times of ischemia, which further piqued the interest of Adenosine and its analogs in the field of stroke [141–144].

It is speculated that the increase in Adenosine during ischemia may be a neuroprotective response [144]. This is supported by the fact that in times of hypoxia and/or ischemia, cellular activation of A1 receptors leads to inhibition of excitatory synaptic transmission [145–147]. This occurs by the reduced influx of calcium, decreasing the presynaptic release of excitatory neurotransmitters, including glutamate, which overstimulates the NMDA receptors and leads to an excitotoxic response [148–150]. In vitro models of ischemia and hypoxia have also demonstrated that adenosine and A1 receptor agonists decrease neuronal damage following hypoxia and/or oxygen-glucose deprivation in cortical or hippocampal cell cultures as well as brain slices [151–154]. Another exciting aspect of A1 receptor agonists is that it decreases reactive oxygen species production and increase survival in anoxia. Conversely, A1 receptor blockade leads to increased reactive oxygen species release and increased cell death [155]. Astrocytes prepared from A1 receptor knockout mice show more severe hypoxic cytotoxicity as compared to control [156].

For the first time in 1994, Gao and Phillis demonstrated that a non-selective A2A receptor antagonist decreased cerebral ischemic injury in animal models following global forebrain ischemia [157] and demonstrated its neuroprotective effects. These effects were then explained better with a study in 1999 reporting that there is an increased outflow of excitatory amino acids immediately after ischemia mediated by A2A receptors which regulate an excitotoxic cascade leading to cell death [158].

Administration of selective A2A receptor antagonist—ZM241385 reduces hippocampal injury and memory-based tasks as compared to others after four-vessel occlusions in rats [159].

This data backed the theory that the neuroprotective properties of A2A receptor antagonists is due to the control of excitotoxicity. Further studies also showed that A2A receptors that are located on glial cells stimulate glutamate outflow and inhibit the glutamate uptake transporter GLT-1 [160, 161]. Reduced glutamate outflow from neurons after brain ischemia is reasoned to be another cause of the neuroprotective effects of A2A receptor antagonists. Animal models have also demonstrated that systemic administration of A2A receptor agonists is protective in the global ischemia model [162]. While the cause of neuroprotective effects of A2A receptor antagonists is the mediation of central excitotoxicity, the A2A receptor agonists exert this effect due to its peripheral effects. Jones et al. in 1998 demonstrated that the peripheral administration of an A2A receptor agonist, CGS2180 protected the hippocampus against kainite-induced excitotoxicity [163] while the direct injection of this agonist failed to show the same result [164]. It was later discovered that A2A receptors in the bone marrow-derived cells were responsible for this effect by exerting their antithrombotic, antioxidant, and anti-inflammatory properties via reduction of cell adhesion factors, neutrophil activation, and platelet aggregation [165].

A2B receptors, on the other hand, are involved in the inflammatory response to ischemia. A selective A2B receptor antagonist—MRS1706 prevents elongation of astrocytic processes, which is a hallmark of astrogliosis induced by the selective stimulation of the A2B receptor [166]. Later on, the same group also concluded that A2B receptor functional impairment is a cell defense mechanism that counteracts the A2B receptor-mediated effects during the acute phase of brain damage, implying A2B receptors to be a target for modulation of early inflammatory responses [167].

Contrary to the clear-cut functions of the above-mentioned receptors and their contribution to regulating ischemia, the role of A3 receptors in this domain is a matter of intense debate. This is due to the conflicting results that have been seen in several studies. Animal studies have shown that administration of a selective agonist of A3 receptor agonist 15 min prior to global ischemia increased the loss of hippocampal neurons and mortality [168]. On the other hand, invitro selective activation of A3 receptors by the application of agonists inhibited the excitatory neurotransmission on cortical neurons [169]. The role of A3 receptors thus remains controversial and more studies should be conducted to understand the effects of this receptor on ischemic models and stroke.

Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disease of the central nervous system characterized by the features of tremor, rigidity, bradykinesia, and gait instability. Dopamine depletion leads to attenuation of the control striatal circuits

and reduces the inhibitory tonus to medium spiny neurons (MSNs) selectively expressing A2A receptors which become overactive [170]. The control of motor activity by Adenosine relies on the formation of heteromers, formed by co-localization of adenosinergic and dopaminergic receptors resulting in the coupling of intracellular signaling and pharmacological properties of each other [171, 172]. Hence, dopaminergic depletion in the striatum leads to A2A receptor over-signaling that causes bradykinetic symptoms of PD. A2A receptor blockade may prove to be adjunctive to the present-day therapeutic approaches.

This probably is reflected in the fact that adenosine receptor antagonists exert similar effects as dopamine agonists [173]. A2A receptor antagonists in particular improve motor functions in animal models of PD when given by themselves or in combination with dopaminergic agonists or levodopa [174–176]. After the onset of dyskinesia resulting from levodopa administration, the administration of A2A receptor antagonist leads to improvement of motor function without worsening of dyskinesia [175, 177–179]. The effect of Adenosine in managing PD symptoms is based on its ability to increase glutamate release, the abnormal transmission of which is an implicated pathogenic cause in PD [179–181]. Another proposed mechanism of neuroprotection may be the ability of the A2A receptor ability to inhibit glial activation [182].

Clinical trials testing istradefylline, an A2A receptor antagonist in PD patients, showed that this drug alone has no effect on patients [183]. However, when co-administered with low doses of levodopa, it promotes motor improvement without any deleterious effect on the dyskinesias [184].

Huntington's Disease

Huntington's disease (HD) is a neurodegenerative disease characterized by wild flailing movements of the limbs, which eventually leads to death due to the mutation of a gene that encodes for a protein called huntingtin [185]. This protein is associated with microtubules and several other proteins that are involved in synaptic function [186]. A mutation in the gene encoding for this protein also leads to an alteration in the synaptic transmission in patients suffering from HD [187]. Mutated huntingtin, on the other hand, leads to glutamatergic dysfunction by increasing expression and activation of NMDA receptors [188], inducing changes in the NMDA receptors [189], and increasing glutamate release along with a decrease in astrocytic glutamate clearance [190]. These changes affect the cortical thalamic afferent glutamatergic input leading to the degeneration of glutamatergic MSNs, which make up the majority of the striatal neuron population. The striatum is rich in A1 and A2A receptors, with the highest density of A2A receptors in the brain [180]. This makes the role of A2A receptors relevant in the case of motor disorders such as HD. Several animal studies associate a change in this receptor's density, expression, or signaling as a contributing factor in the pathophysiology of HD [191, 192].

Polymorphism of the ADOR2A gene, which encodes for A2A receptors, has also been reported to influence the age of onset of HD patients [193]. The MSNs

expressing A2A receptors receiving glutamatergic inputs from the cortex are highly vulnerable to the excitotoxic damage from continued exposure to glutamate, thereby supporting the role of A2A receptors in the pathophysiology of this disease [194]. A2A receptors are neuroprotective in nature owing to their ability to prevent the release of glutamate [195], decrease its levels [196], and by enhancing its uptake [197].

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease affecting close to 1.3 million people worldwide [198, 199]. This chronic neuroinflammatory demyelinating disease is characterized by the activation of the neutrophils, lymphocytes, and macrophages, leading to widespread inflammation, causing various clinical symptoms, including but not limited to chronic pain, depression, and cognitive symptoms, sensory disturbances, and ataxia [200, 201].

Adenosine plays a vital role in the regulation of all cells involved in the neuroinflammatory pathway that plays a key role in the pathophysiology of MS. It is an important modulator of neutrophils which express all four types of adenosine receptors and are affected by inflammatory mediators [202]. Adenosine is released from neutrophils after activation during inflammation and one of the key functions of Adenosine is the stimulation of neutrophils to bind to vascular endothelium [202]. A1 receptors are critical in the binding of neutrophils to endothelial cells as A1R increases the expression of p-selectin on endothelial cells on neutrophils [202]. Adenosine is also a vital signaling molecule involved in the proper functioning of the T lymphocytes. T cells express A2A, A2B, and A3 receptors with the A2A receptors expressed on peripheral T lymphocytes backing the theory that A2A receptors may have a significant effect on the T cell response [202]. Via activation of the A2B receptors, Adenosine inhibits IL-2 production, whereas NF-KB signaling pathway is stimulated by A2B receptor signaling in the activated T lymphocytes [202, 203]. A2A receptor signaling inhibits IFNγ secretion by the T cells [204].

With respect to monocytes and macrophages, Adenosine plays a vital role by inhibiting their differentiation as well as ceasing the production of active nitrogen and oxygen intermediates in these cells [205]. The enhanced release of anti-inflammatory IL-10 by macrophages is also regulated by adenosine [206].

Experimental autoimmune encephalomyelitis (EAE) are animal models for MS which play a vital role in studying this clinical entity in animal models. CD73, a cell surface enzyme that catalyzes the breakdown of AMP to Adenosine, is found in brain endothelial cells in choroid plexus epithelium and modulates the surveillance by lymphocytes in the blood and cerebrospinal fluid (CSF) [207]. Studies have suggested that adenosine receptors and CD73 are essential for the entry of lymphocytes into the CNS to induce EAE [208]. The expression of A1 receptors in macrophages is seen to be decreased in patients with MS, which implies its role in macrophage activation as well as CNS inflammation [209]. A1 receptors also regulate the

severity of EAE related pathological outcomes and reduces the inflammatory response in the CNS [209]. This was evidenced by the production of pro-inflammatory cytokines such as IL-6 was reduced in patients with a relapsing remitting form of MS compared to control groups when A1 receptors were stimulated [210, 211]. To further prove the role of A1 receptors in the pathophysiology of the disease, A1 receptor null mice show more severe forms of EAE as compared to wild type mice [212]. Chronic treatment with caffeine attenuates the pathological symptoms caused by EAE due to over expression of A1 receptor and downregulation of IFNγ mRNA [213]. However, the administration of ScH58261, an A2A receptor antagonist, restricts the entry of lymphocytes during the development of EAE, thereby making the mic resistant to EAE [207].

The manipulation of Adenosine and its receptors shows a promising future in the management of MS. Larger studies, including human trials, should be conducted to explore the venues of managing this neuroinflammatory and neurodegenerative disease with a neuromodulator that affects all the factors playing a role in its pathophysiology.

Adenosine Agonists

Drugs acting on agonist receptors and promoting the effects of Adenosine have been a subject of preclinical and clinical research for a long time. This section aims to explore the adenosine receptor agonists that have been tested based on their functions. For the sake of simplicity, the section will only cover agonists that have promising applications in the field of neurology.

In patients with epilepsy, there is an overexpression of adenosine kinase in the astrocytes which decreases level of Adenosine both inside and outside the cell. Adenosine 1, which was the earliest adenosine receptor agonist works as an antiseizure agent by inhibiting DNA methyltransferases, thereby reducing DNA methylation [214]. Owing to its rapid clearance, having a drug delivery system in place to ensure consistent and elevated concentrations are needed. One such notable system included the use of Adenosine releasing silk brain implants to manage refractory epilepsy model in animal models where Adenosine was encapsulated in the microspheres embedded in the silk fibrin scaffolds and was introduced surgically into the intrahippocampal cleft. The animal was then monitored for 10 days and showed a lack of seizures [215].

NNC-21-0136 15, an A1A receptor-selective agonist was an early agonist which was studied with respect to stroke. Although this drug never entered human testing, it showed a neuroprotective effect and also showed minimal hemodynamic effects [216, 217].

Another A1A receptor agonist like the above, GW493838 9 is an analog that has been modified at N^6 and 5′ positions and has shown promise and efficacy in animal models to reduce pain [218]. The drug, however, when administered orally in patients with chronic pain due to diabetes, showed no significant effect and also showed no analgesic effect in patients with post-operative pain [219].

As described in the above section, A1A receptors are distributed extensively throughout all excitatory nerve terminals in the brain, especially in areas such as basal ganglia, hippocampus, thalamus, and neocortex. In vivo imaging of these receptors holds promise in the diagnosing several conditions, including PD, AD, epilepsy, and stroke. Several such A1A receptor agonists have thus been used for PET brain imaging [220]. Of the agonists studied, the partial agonist at A1A receptor—MMPD 20 is considered to be highly selective for this receptor type with 16% maximal human A1A receptor activation [221]. As compared to other A1A receptor agonists who have a low degree of uptake in the brain from the periphery, MMPD 20 crosses the blood–brain barrier relatively easily owing to its lower molecular weight and polar surface [222].

First approved as a pharmacologic stress agent in 2008, Regadenson 21 is a moderately selective A2A receptor agonist which has been used for myocardial perfusion imaging [223]. This drug has also been tested in the niche of neuro-oncology to increase the concentration of temozolomide in the brain interstitium in patients with glioblastoma, and the concentration was measured using microdialysis [224].

Spongosine, a 2-methoxy derivative of Adenosine, was one of the first A2A receptor agonists that was studied in clinical trials [225]. This adenosine receptor agonist was studied as a part of a clinical trial assessing its role in managing diabetic neuropathic pain, but the clinical trial was terminated since the company discontinued small molecule research [226].

Adenosine Antagonist: Caffeine

Caffeine (1,3,7-trimethylxanthine) is a non-selective adenosine receptor antagonist and is the most widely used psychostimulant throughout the western world [34]. After consumption, it is absorbed quickly through the gut and reaches its peak concentration in the body within 30–60 min of intake [34]. Caffeine usage has shown promising results in several neurological disorders.

A 21-year-old follow-up study with a total of 1409 participants all over the age of 50 years showed that there was a substantial reduction in the incidence of Alzheimer's disease (AD) and dementia when caffeine was consumed in moderate amounts (3–5 cups) as compared to low coffee consumers (0–2 cups) [227]. Similar findings were seen in another study where coffee consumers had a 31% lower risk of AD (OR = 0.69, CI = 0.5–0.96) [228]. Animal models have replicated this effect as evidenced by a significant improvement in mice's memory administered caffeine compared to controls [229]. Another animal study also found caffeine's influence globally and its protective effect on spatial learning, working memory, and recognition [230].

Caffeine consumption has also been studied in association with Parkinson's disease (PD). A randomized control trial of 61 patients reported an improvement in the total unified Parkinson's disease rating scale by 4.7 points and motor manifestation by 3.2 points [231]. A 12.9 year follow-up study reported a lower hazard ratio for people who drank more than five cups of coffee (0.41, CI = 0.19–0.88) as compared to those who drank 1–4 cups (0.41, CI = 0.26–1.15) and 0 cups of coffee (1.00,

CI = 0.26–1.15) [232]. It has also been associated with decreasing the damage due to MPTP administration and improving motor function in animal models [233]. Pretreatment with caffeine attenuated MPTP induced dopamine loss in a dose-dependent manner in young male mice [234].

Caffeine has also been studied in association with HD, and human studies have shown that consuming more than 190 mg/day of caffeine in the last 10 years was associated with 1.6 years earlier onset of HD [235]. Animal studies reported that caffeine restored motor functions in male Sprague Dawley rats with HD model and at 21 days completely restored body weight [236].

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 4
GABA

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History of GABA

ϒ-Aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the central nervous system (CNS), with almost 40% of the synaptic inhibitory potentials mediated by GABA [1]. In 1952, Brock et al. described the postsynaptic inhibitory potentials in a cat's spinal motor neurons [2]. Kuffler (1954) and Florey (1954) demonstrated the presence of synaptic inhibitory and excitatory mechanisms in crustaceans [1].

In 1955, Florey and McLennan published that when factor I is applied topically to the exposed spinal cord, it inhibits the monosynaptic knee-jerk reflex in decerebrate cats [2]. In 1959, Florey and McLennan extracted the factor I (inhibitory action on the neurons) from the human brain, which showed the presence of a natural neurotransmitter GABA [1]. Kravitz et al., in 1963, and Otsuka et al., 1966, described the presence of an endogenous inhibitor at the neuromuscular junctions of lobsters [3, 4].

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Curtis, in 1959, narrated that the action of GABA in the CNS is because of a depressant role but not of a neurotransmitter role as its characteristics are not of a typical transmitter [5]. In 1968, Iversen and Neal reported that rapid intake of GABA into the cells plays a crucial role in the inactivation of the neurotransmitter. Bicuculline, a natural alkaloid, blocks the action of GABA and also exhibits postsynaptic inhibition in the cerebral cortex [1].

Olsen and Tobin reported the structure of ionotropic receptors ($GABA_A$) in the late 1980s [6]. GABA's action is modulated by certain substances like general anesthetics, benzodiazepines, and neurosteroids [1]. In 1955, Sternbach accidentally found the first benzodiazepine, chlordiazepoxide, which was marketed as Librium in 1960 [7].

In the mid-1970s, Roberts and colleagues reported the distribution of the enzyme glutamic acid decarboxylase (GAD), which is necessary for the formation of GABA [1]. Bowery, in 1980, demonstrated that GABA and Baclofen inhibit the K+ evoked release of the neurotransmitter from the brain samples in a bicuculline-insensitive manner [8]. Hill and Bowery, in 1981, reported this novel receptor as $GABA_B$ and the ionotropic receptor as $GABA_A$ [9].

When GABA is applied in lower concentrations (less than required to cause inhibition), it depolarizes the membrane to increase the resistance of the membrane eventually. The excitatory action of GABA is pH-dependent [2].

Neurochemical Profile of GABA

GABA in Sleep

GABA is the non-proteinogenic amino acid, which exhibits inhibitory properties in the CNS. The activation of $GABA_A$ receptors induces sleep [10], and GABAergic neurons of the hypothalamus in the ventrolateral preoptic nucleus promote sleep via the suppression of the arousal systems [11].

GABAergic pathway, which originates from the ventral medulla (vM), significantly promotes rapid eye movement (REM) sleep which is a vital part of the normal sleep cycle. vM GABAergic neurons initiate REM sleep and prolong their duration, while inactivation of these neurons has the opposite effect. The activation of rostral GABAergic neurons is adequate for both initiation and maintenance of REM sleep, which are partly mediated by the inhibition of REM-suppressing GABAergic neurons in ventrolateral periaqueductal gray matter [12].

In CNS, increasing GABAergic transmission via positive allosteric modulators (PAMs) of $GABA_A$ receptors augments the inhibition to produce sedation. GABA increases the duration of postsynaptic inhibitory potentials via $GABA_A$ receptors, while PAMs intensify the ongoing GABAergic neuronal transmission [13].

As $GABA_A$ is the most important receptor which promotes sleep, it is also the main target in developing sedatives and other natural anxiolytics [10, 14]. When GABA or other agonists bind to the $GABA_A$ receptor, an influx of chloride ions is triggered into the neuronal cells causing a negative membrane potential to inhibit the action potential firing [15].

Insomnia is characterized by difficulty in sleep quality, initiating, or maintaining sleep, in addition to substantial distress and impaired daytime functioning. Insomnia is seen in about 33–50% of the population, with higher rates reported in females [16]. Sleep disruptions are commonly caused by many conditions such as caffeine intake, stress, sleep disorders, and diseases; there is an increasing scope of research and investigations on the effect of GABA inhibitory neurotransmission. Sleep deprivation causes many chronic illnesses such as cardiovascular diseases, diabetes, and cancer [10].

Kim et al. found that when GABA and L-theanine are given as a combination, they act synergistically to increase the sleep duration and decrease sleep latency [10]. Winkelman et al. reported that a global decrease in GABA levels in primary insomnia (PI) patient brains and found that GABA levels are inversely correlated with the time awake after sleep onset in these patients [17]. However, Morgan et al. reported higher occipital GABA levels in people with primary insomnia than in the control group. They also hypothesize that the hyperarousal syndrome of PI may increase the GABA or an ineffective GABA signaling might have increased the cortical GABA levels to induce PI [11].

GABA in Neuronal Inhibition

Proper regulation of the CNS neural activity is achieved through distinct populations of GABA-releasing neurons [18]. $GABA_A$ receptors mediate both phasic synaptic inhibition and a persistent tonic form of inhibition signaling [19].

$GABA_A$ receptors increase the membrane permeability to both chloride and bicarbonate ions, which leads to an inward flux of anions and causes a hyperpolarizing postsynaptic response called inhibitory postsynaptic potential (IPSP). The increase in membrane conductance is “phasic” inhibition [18].

A low concentration of GABA in the extracellular space and GABA eluding from the synaptic cleft activates the receptors on presynaptic terminals of the adjoining neurons resulting in the persistent or “tonic” inhibition of $GABA_A$ receptors, which is distinct from phasic inhibition [18].

The distinct feature of phasic receptor activation is the shorter duration of GABA to which the postsynaptic receptors are exposed. The short stay of GABA in the synaptic cleft is because of rapid diffusion from the release area [20]. During steady state, the peak concentration of GABA is higher than necessary to elicit the receptor activation. However, because of less exposure duration, not all the postsynaptic receptors are fully engaged. As the GABA concentration duration is brief, deactivation of IPSP is achieved through the ion channel closure, which follows the ligand removal. A distinct feature of the phasic $GABA_A$ receptor inhibition is the rapid,

simultaneous opening of a small number of channels, which are clustered at the synaptic junction. In contrast, tonic inhibition is achieved through irregular, temporally dissipated activation of the receptors over the neuronal surface [18]. Both phasic and tonic $GABA_A$ receptor inhibition signals are received by cerebellar granule cells [21].

Phasic inhibition function is to prevent over-excitation of the neurons and thus avoid pathological state development, which is a crucial component of $GABA_A$ receptors and GABA-releasing interneurons in the CNS [18, 22]. Interneurons have a crucial role in phasic inhibition and concurring the pyramidal cells activity along with initiating and maintaining theta and gamma frequency network oscillations [18, 23].

Tonic activation of the $GABA_A$ receptor has more definite capabilities and is less liable to modulation than phasic $GABA_A$ activation. Tonic inhibition function is to increase the cell's input conductance [18] persistently. The excitatory input nature determines the kind of impact on the tonic inhibitory conductance [24].

Phasic inhibition of the neuron is achieved by the activity, number, and variety of the presynaptic GABA-releasing neurons, but determinants of tonic inhibition in the neurons are less clear [18].

GABA Receptors

GABA is a widely found neurotransmitter throughout the CNS exhibiting inhibitory properties [25, 26]. GABA exerts its actions through three major receptors, which are $GABA_A$, $GABA_B$, and $GABA_C$ receptors. $GABA_A$ and $GABA_C$ are ionotropic receptors, and $GABA_B$ are metabotropic receptors. Because of the similarity in pharmacology, few authors consider that $GABA_C$ as a subclass of $GABA_A$ receptors with subunits having different stoichiometry [27]. GABA receptor subunits are given in Table 4.1 [28]. $GABA_A$ and $GABA_B$ receptors and GABA transporter are shown in Fig. 4.1.

$GABA_A$ Receptor

$GABA_A$ receptors are the key inhibitory neurotransmitter receptors in the CNS [26]. $GABA_A$ receptors are ionotropic and bind to ligand-gated chloride (Cl^-) ion channels [30]; these receptors are members of the transmitter-gated ion channel superfamily, which include nicotinic acetylcholine receptors, glycine receptors, ionotropic 5-HT_3 receptors, and Zn^{2+}-activated ion channels [26, 31].

$GABA_A$ receptors have pentameric hetero-oligomers with multisubunit proteins, and 19 different subunits are known so far [28, 30, 32]. Three different subunits have 30 possibilities to form a pentameric receptor [26]. The most common $GABA_A$ receptors in the brain are composed of α_1, β_2, and Υ_2 subunits [30]. The schematic

Table 4.1 GABA receptor subunits [28, 29]

Receptor subtype	Subunits
GABA-A (19 subunits)	α_1 α_2 α_3 α_4 α_5 α_6 β_1 β_2 β_3 Υ_1 Υ_2 Υ_3 ε θ δ π ρ_1 ρ_2 ρ_3
GABA-B (2 subunits)	$GABA_{B1}$ ($GABA_{B1a}$ and $GABA_{B1b}$ isoforms) $GABA_{B2}$

GABA ϒ-aminobutyric acid

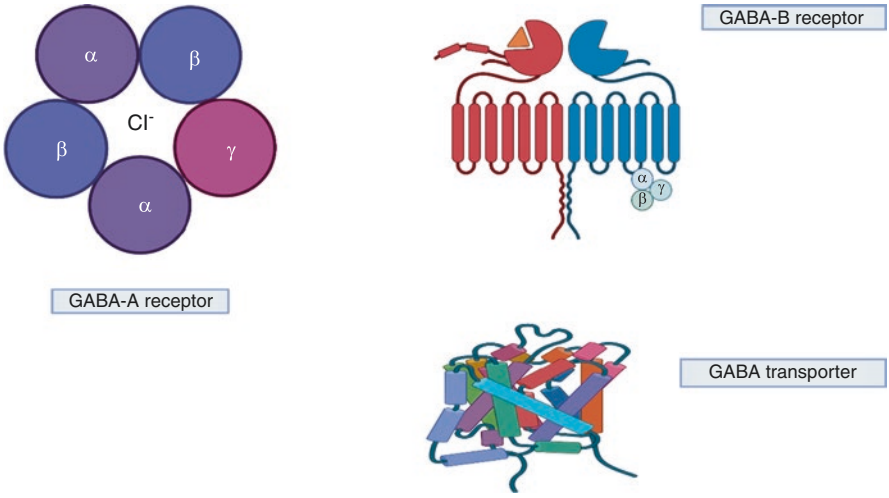

Fig. 4.1 GABA receptors and transporter. *GABA* ϒ-aminobutyric acid

representation of the $GABA_A$ receptor is shown in Fig. 4.2. This complex has five key binding domains in or around the chloride (Cl^-) channel for GABA, benzodiazepines, barbiturates, neurosteroids, and picrotoxin [25, 33].

Some units of $GABA_A$ receptors are broadly expressed in the CNS. Although subunit α_6 is only expressed in cerebellar granule cells and ρ subunit is expressed

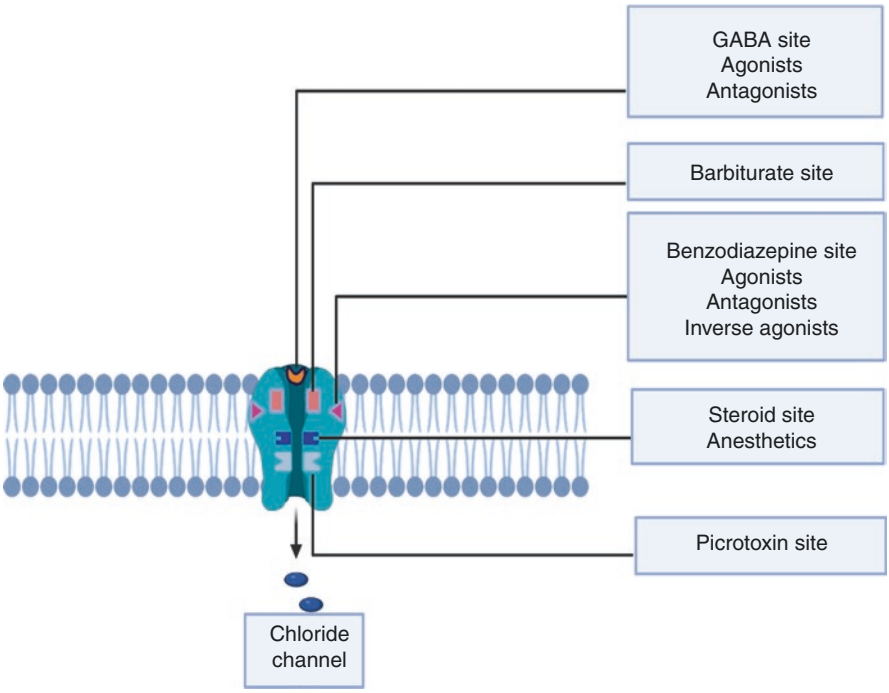

Fig. 4.2 Schematic representation of the $GABA_A$ receptor. The vertical cut-view of the receptor represents targets for various compounds, which represent the receptor complex. (None of the drug-receptor sites are implied). [25, 33]. *GABA* ϒ-aminobutyric acid, Cl^- chloride channel

mainly in the retina. Peripherally, $GABA_A$ receptors are found in smooth airway muscles of the lung, in the liver, and in various kinds of immune cells [26].

Modulation of $GABA_A$ receptors occurs through post-translational modification; various kinds of protein kinases phosphorylate the amino acid sites on the receptor subunits to modulate the channel activity and by interacting with receptor-associated proteins. $GABA_A$ receptors are also modulated by two endogenous molecules and by various exogenous small molecules [26]. $GABA_A$ receptors cause fast inhibitory postsynaptic potentials on the neuronal cell surfaces in the hippocampus and cortex, which leads to depolarization of primary afferent terminals [27].

$GABA_B$ Receptor

$GABA_B$ receptors are guanine (G) nucleotide protein-coupled receptors (GPCR), which modulate calcium (Ca^{2+}) and potassium (K^+) channels and inhibit the adenylyl cyclase. $GABA_B$ receptors have a central core domain consisting of seven transmembrane helices that are helpful in G-protein coupling, just like other GPCR

receptors. $GABA_B$ receptors are found presynaptically, postsynaptically, and on extrasynaptic membranes in the brain [29].

$GABA_B$ receptor is formed of two subunits, $GABA_{B1}$ and $GABA_{B2}$, and the conformational changes of these two subunits lead to the activation of the $GABA_B$ receptors [30]. The binding of GABA to the extracellular $GABA_{B1}$ subunit produces the movement of both $GABA_{B1}$ and $GABA_{B2}$, which causes a conformational change of the transmembrane helix to activate the G-protein. $GABA_B$ receptors are modulated both by receptor phosphorylation and interaction with receptor-associated proteins. The presence of $GABA_B$ receptors in the CNS and PNS are shown below [29].

$GABA_B$ receptors in CNS
- Cerebral cortex, thalamus nuclei, cerebellum, amygdala, hippocampus, habenula, substantia nigra, ventral tegmuntum, nucleus accumbens, globus pallidus, hypothalamus, ventral and dorsal horns of spinal cord.

$GABA_B$ receptors in PNS
- Autonomic ganglia, in the visceral tissues of stomach, intestine, heart and spleen.

$GABA_{B1}$ subunit has two isoforms, which are $GABA_{B1a}$ and $GABA_{B1b}$. $GABA_{B1a}$ has a tandem pair of extracellular domains (sushi domains) on the amino (N)-terminal [29]. $GABA_{B1a}$ is mainly located presynaptically, and $GABA_{B1b}$ subunit is found postsynaptically [27].

GABA receptors, which are present presynaptically, inhibit the neurotransmitter release through downregulating voltage-gated calcium channels, and those present postsynaptically decrease the neuronal excitability via activation of potassium conductance which causes late inhibitory postsynaptic potentials [27].

GABA Metabolism

GABA is regulated through multiple molecular mechanisms to mediate the transport, sequestration, synthesis, and degradation of GABA [34]. The chemical structure of GABA is shown in Fig. 4.3 [28].

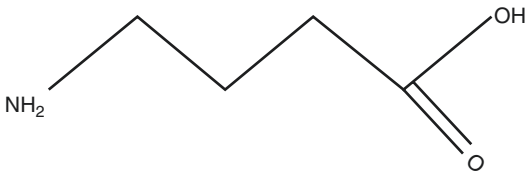

Fig. 4.3 Chemical structure of GABA [28]

Glutamate is converted to transmitter glutamate and GABA within the neurons [35]. In presynaptic neurons, glutamate acts as a precursor for the synthesis of GABA through the action of glutamic acid decarboxylase (GAD). GAD controls the synthesis of GABA along with the cofactor pyridoxal phosphate [27]. Different pathways for the synthesis, conversion, and metabolism of GABA are shown in Fig. 4.4 [36].

Neurons synthesize glutamate from glutamine, which is taken up by specialized transporters. In GABAergic neurons' presynaptic terminals, GABA is enhanced in the vesicles through vesicular inhibitory amino acid transporter (VGAT). VGAT is

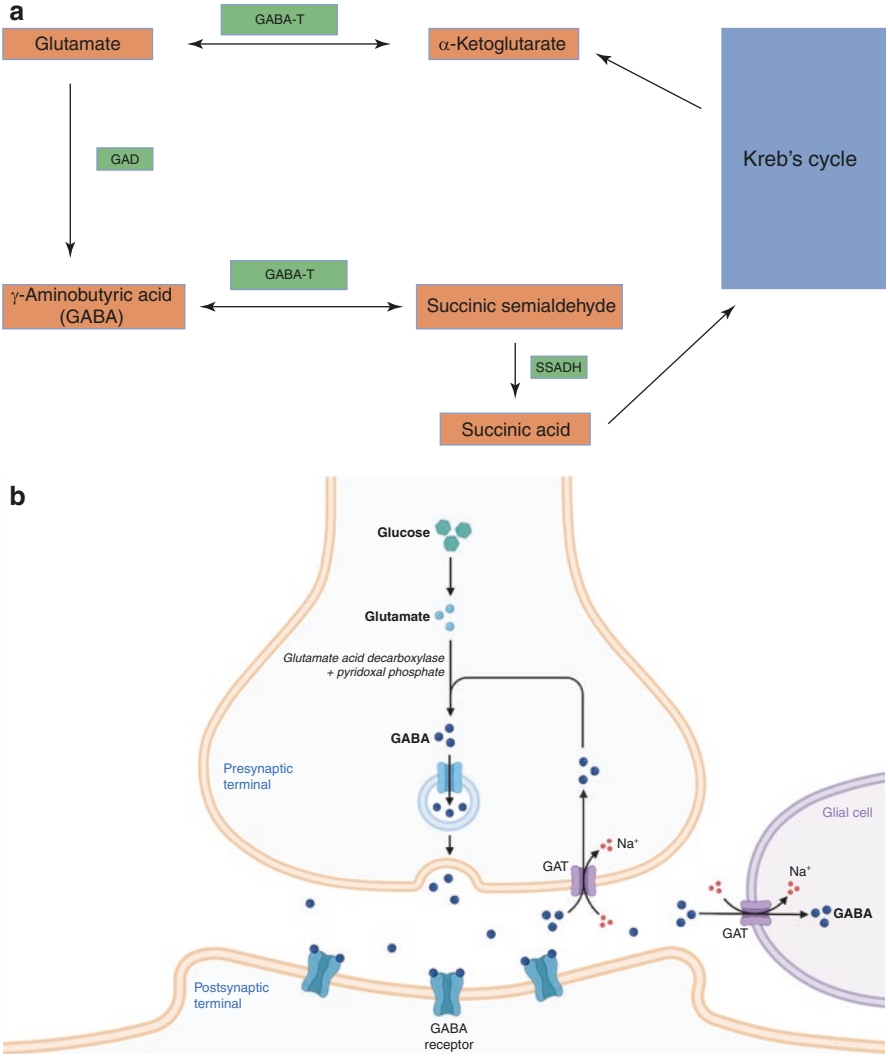

Fig. 4.4 (**a**): Metabolism of GABA [36]. *GAD* glutamic acid decarboxylase, *GABA-T* GABA α-oxoglutarate transaminase, *SSADH* succinic semialdehyde dehydrogenase. (**b**) GABA synthesis and transport in neurons. *GABA* ϒ-aminobutyric acid, *GAT* ϒ-aminobutyric acid transporter, *Na*$^+$ sodium

within the vesicular membrane, which uses H^+ electrochemical gradient to transport GABA into synaptic vesicles; it also uses chloride gradients between the vesicle lumen and presynaptic cytosol [34]. GABA has four transporters with 12 transmembrane-spanning segments, ϒ-Aminobutyric acid transporter (GAT-1), GAT-2, GAT-3, and betaine-ϒ-aminobutyric acid cotransporter (BGT-1) [27].

GABA is released into the synaptic cleft through exocytosis caused by calcium-mediated depolarization [27]. Both transmitters, glutamate, and GABA after being released from the neurons, are mostly returned to the astrocytes [35].

Both GABA and α-Ketoglutarate are transaminated to produce succinic semialdehyde and glutamate by GABA-transaminase (GABA-T) in the mitochondria of neurons and glial cells. In CNS, nearly 90% of the GABA is metabolized through this pathway, which also assists in the energy metabolism in the tricarboxylic acid (TCA) or Krebs cycle [34].

Medications Acting on the GABAergic System

GABA has a crucial role in promoting neuronal development and relaxation, modulation of synaptic transmission, and avoiding depression and sleeplessness. Preventive actions of GABA on neurological diseases are shown in Fig. 4.5. GABA has numerous pharmaceutical attributes on peripheral organs and tissues because of its anti-cancer, anti-hypertension, anti-diabetes, anti-allergy, anti-inflammatory, antioxidant, anti-allergy, anti-microbial, reno-protection, hepato-protection, and intestinal protection [37].

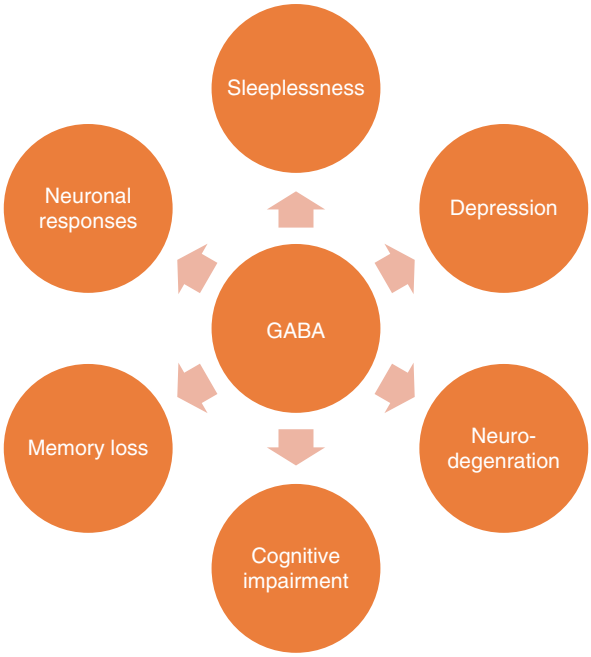

Fig. 4.5 Role of GABA in neurological disorders [37]. *GABA* ϒ-aminobutyric acid

Benzodiazepines

Benzodiazepines are protein-bound and are moderately lipid-soluble [38]. They are commonly used to treat anxiety and sleep disturbances [39] and are also used in inducing sedation and as adjuvants to general anesthetics [38].

The effects of benzodiazepine are mediated by the $GABA_A$ receptor, which is a ligand-gated chloride channel. $GABA_A$ with Alpha-1 (α1) subunit is responsible for sedation, and with α2 subunit are the primary receptors which cause myorelaxation. Benzodiazepines are mainly metabolized in the liver by phase I oxidative reactions, which are primarily excreted from the body through urine. Based on elimination half-life, they are classified into long-acting (>24 h), intermediate-acting (5–24 h), and short-acting (<5 h) [38]. Classification of benzodiazepines, based on pharmacokinetic properties, is given in Table 4.2 [40].

Benzodiazepines are commonly prescribed along with antidepressants in patients with anxiety-related depression or sleep disorders. Compared to placebos, benzodiazepines are better in controlling various symptoms of anxiety and reducing the onset of sleep latency [39]. They are also used in the treatment of alcohol withdrawal syndrome (AWS), which include chlordiazepoxide, diazepam, oxazepam, and lorazepam. Nonbenzodiazepine hypnotics and selective $\alpha -1$ receptor agonists which are commonly used in treating insomnia and alcohol withdrawal delirium (AWD) are zolpidem, eszopiclone, zopiclone, and zaleplon [41].

Benzodiazepines, if used for an extended period, cause issues with withdrawal symptoms and discontinuation and abuse [39]. However, the risk of dependency increases with high-potency benzodiazepines like alprazolam and triazolam. Especially in elderly patients, there is an increased sensitivity to adverse drug effects like daytime sedation, memory problems, impaired motor coordination, high risk of motor vehicle accidents, and falls [7].

Barbiturates

Barbiturates act by enhancing the $GABA_A$ chloride currents by increasing the duration of chloride channel opening [42]. As barbiturates have a small therapeutic window, they can interact with multiple drugs metabolized in the liver [43]. Classification of barbiturates and their clinical indications are shown below in Table 4.3 [44].

Table 4.2 Pharmacokinetic classification of Benzodiazepines [40]

Long-acting	Intermediate-acting	Short-acting
Chlordiazepoxide	Alprazolam	Midazolam
Clorazepate	Bromazepam	Triazolam
Diazepam	Clobazam	
Flurazepam	Clonazepam	
	Nitrazepam	
	Oxazepam	
	Temazepam	

Table 4.3 Classification and clinical indications of barbiturates [44]

Classification	Barbiturates	Clinical indication
Long-acting	Phenobarbital	Sedative
Intermediate-acting	Amobarbital	Hypnotic
Short-acting	Pentobarbital Secobarbital	Hypnotic and anticonvulsant
Ultrashort-acting	Thiopental	Anesthesia inducer

Phenobarbital and butobarbital are commonly used as sedatives in asthmatic patients and functional gastrointestinal disorders; these agents are also used to antagonize the effects of dextroamphetamine, ephedrine, and theophylline. Phenobarbital, primidone, and other barbiturates are used in the treatment of epilepsy and convulsions caused by eclampsia, cerebral hemorrhage, tetanus, status epilepticus, and various poisonings [44].

Although phenobarbital acts as a GABA potentiator, it has a weak hypnotic/anesthetic effect than pentobarbital. Pentobarbital also acts on calcium channels, and with anesthetic levels, it directly opens $GABA_A$ receptor-associated chloride channels [45]. Thiopental and methohexital are the ultrashort-acting barbiturates, which are used as intravenous anesthetic inducers [44].

Barbiturates are also effective in the treatment of AWS. For patients with delirium tremens symptoms who require ICU care, barbiturates act as a good adjutant to benzodiazepine-resistant AWS. The main limitation of the use of barbiturates is respiratory depression [46].

Sodium Oxybate

Sodium oxybate (SXB) is the sodium salt of gamma-hydroxybutyrate (GHB), which is synthesized in the CNS neurons and is an active metabolite of GABA [47]. GHB binds to both high and low affinity G-protein coupled receptors [48]. GHB's effects are mediated via $GABA_B$ effects at thalamocortical, dopaminergic, and noradrenergic neurons [47].

Narcolepsy is a CNS disorder, which is characterized by excessive daytime sleepiness with or without cataplexy, disturbed nocturnal sleep, sleep paralysis, and hypnagogic hallucinations (HH) [49]. Although there is no cure for narcolepsy, SXB is the only medication that is approved by the FDA to treat narcolepsy with cataplexy in both adults and children 7–17 years of age. SXB not only improves sleep continuity and but also decreases the slow-wave sleep duration and delta power. Both postmarketing data and clinical analysis showed a minimal risk of abuse/misuse of SXB. Treatment emergence adverse effects are reported in about 67%, and serious adverse effects such as angina, depression, and suicidal attempt are reported in about 6% of patients [47].

SXB is a safe, effective, and well-tolerated treatment for alcohol withdrawal and in the prevention of relapse in heavily alcohol-dependent patients, with few adverse

side effects and a few abuse cases [50]. Buchele et al. found that SXB showed efficacy and might be a potent drug in treating sleep-wake disturbances in Parkinson's disease (PD). Further clinical trials with a large patient population, follow-ups, and strict monitoring are required to validate these results [51]. Recently, Xywav oral solution has been introduced for the treatment of narcolepsy that contains calcium, magnesium, potassium, and sodium oxybate.

Baclofen

Baclofen is a $GABA_B$ agonist that acts centrally as a skeletal muscle relaxant [52]. Although baclofen was initially introduced to treat epilepsy, it was found to be effective in treating muscle spasticity. Baclofen decreases the release of excitatory neurotransmitters in the presynaptic neurons and stimulates the neuronal inhibitory signals in the postsynaptic neurons, thus relieving the spasticity [53].

It is now widely used in managing spasticity, clonus, flexor spasms, and associated pain, which are commonly seen in neurological conditions such as multiple sclerosis and spinal cord lesions [54]. Some studies showed that oral baclofen has protective effects on the body's musculature deterioration and the metabolic profile seen in spasticity caused by spinal cord injuries [55].

Oral doses that are tolerable sometimes can be insufficient to alleviate muscle hypertonia, in which case, intrathecal baclofen (ITB) is used to increase the drug concentration to higher levels in the lower spinal level. ITB can also help in relieving nighttime spasms and improves the neurogenic bladder by diminishing the bladder tone [55].

Baclofen is also commonly used in the management of alcoholic liver disease, where it decreases alcohol cravings and thus helps in the maintenance of alcohol abstinence and alcohol-related anxiety, hiccups, gastrointestinal disease, and trigeminal neuralgia [56].

Gradual dose reductions of baclofen are necessary to avoid withdrawal symptoms, and abrupt discontinuation might result in seizures and hallucinations [56]. Common side effects include systemic muscle relaxation, fatigue, and sedation. Liver function tests are to be done during baclofen use as there is a potential risk of hepatotoxicity [55].

Other Clinical Aspects

Antibody-Mediated Attack on GABA

Antibodies against CNS proteins are commonly seen in various neurologic disorders (like encephalopathies). Immunoglobulin G (IgG) antibodies are produced against the extracellular components of the proteins, which are presented on the neuronal cells and less commonly against $GABA_B$ receptors [57].

$GABA_A$ receptor α1 and ϒ2 subunits also can become the targets for antibodies in autoimmune neurologic diseases. However, to understand the entire spectrum of clinical presentations, management, and antibody specificity (especially IgM), further studies are needed [57].

The pathophysiology of epilepsy involves an imbalance between GABAergic and glutamatergic processes. Antibodies against GABA have a pro-epileptic effect on both acute and generalized epileptiform activity and also regulate chronic epileptiform activity. Chronic epileptization of the brain coincides with the onset of anti-GABA autoantibodies [58].

Limbic encephalitis (LE) can present as either paraneoplastic or nonparaneoplastic syndromes based on the kind of antibodies; $GABA_B$ receptor antibodies are commonly seen [59]. Patients with $GABA_B$ receptor antibody-associated LE have MRI lesions in the hippocampus or temporal lobe [60]. Antibody testing for $GABA_B$ receptors should be done in all LE patients with or without small cell lung cancer (SCLC), and in some conditions of cerebellar dysfunction and opsoclonus, where no other antibodies are identified. In elderly patients, SCLC should be presumed, who also have onconeuronal antibodies. Höftberger et al. conducted a study and have reported the following findings [59]:

- Most patients with $GABA_B$ receptor antibodies develop LE.
- Two of the patients had a different clinical presentation, opsoclonus and ataxia.
- $GABA_B$ receptor antibodies are found in patients with neurologic dysfunction, with or without SCLC, and are not found in cancer patients without neurologic symptoms.
- Analysis of $GABA_B$ receptor antibodies and other associated autoimmunities revealed that $GABA_B$ receptor epitopes are conformational and the kind of antibodies differ based on the presence or absence of cancer, which might signify the outcome.

GABA Antagonism and Improvement in Alertness: Role of Flumazenil in Hypersomnia

Rye et al. conducted a study on hypersomnolence patients and found a substance in CSF, which enhanced the function of benzodiazepine-insensitive $GABA_A$ receptors while not displacing the benzodiazepines competitively. Furthermore, it was concluded that this naturally occurring substance in CSF is increasing the inhibitory GABA signaling, and thus new pathophysiology of excessive daytime sleepiness (EDS) was explained [61]. This hypothesis of abnormal $GABA_A$ receptor activation causing sleepiness requires further research to definitively establish the role of GABA in idiopathic hypersomnolence [62].

$GABA_A$ receptor antagonists like flumazenil and clarithromycin have been tested in the treatment of idiopathic hypersomnia (IH) [63]. Intravenous flumazenil is used in the reversal of benzodiazepine overdose; it is currently being investigated for neurologic diseases like Parkinson's disease and psychiatric diseases like schizophrenia and hepatic encephalopathy. As the half-life of flumazenil is short, the sublingual or transdermal approach would be compatible for long-term use. Patients treated with sublingual and transdermal flumazenil showed sustained clinical

response in 39% of patients with treatment-refractory hypersomnolence. Although flumazenil is commonly used in the acute management of benzodiazepine overdose, further studies are needed to understand the proper dosing, safety, and effectiveness [64].

GABA in Restless Leg Syndrome (RLS)

Restless leg syndrome (RLS) is a sensory-motor disorder with an urgency to move the legs, along with uncomfortable or unpleasant sensations, which may occur or worsen during rest, either in the evening or at night and disappears with the movement of legs. The major cause of RLS is primary or idiopathic, and secondary causes include end-stage renal disease, iron deficiency, PD, multiple sclerosis, pregnancy, and medications (SSRI, lithium, neuroleptics) [65].

GABA system plays a vital role in RLS, and thalamic GABA levels correlate with RLS symptoms' severity [66]. Thalamic GABA promotes both sensory-motor functions and other cognitive functions in conjunction with working memory and executive functions. In addition, high levels of thalamic GABA improve cognitive control performance [67]. Pregabalin and gabapentin, which are structurally similar to GABA, are commonly used in the treatment of RLS. Their mechanism of action, however, is via antagonism of the calcium channel on the vasculature [65].

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 5
Glutamate

Sireesha Murala, Aditya Boddu, and Pradeep C. Bollu

History

Glutamate is one of the most abundant amino acids in the body and the principal excitatory neurotransmitter in the brain [1]. Glutamate's excitatory action in the brain and spinal cord has been known since 1950s [2, 3]. But it was only in 1970s, the role of glutamate uptake in the control of itsexcitatory action was recognized in the nervous system [4–7]. It took years to recognize glutamate as a neurotransmitter due to its ubiquitous nature in the brain and involvement in multiple metabolic pathways. Glutamate was accepted by the neurochemical community as the major excitatory transmitter in the central nervous system after the review by Fonnum et al. in 1984 [8, 9].

It was speculated that glutamate acts postsynaptically on three families of ionotropic receptors were named N-methyl-D-aspartic acid (NMDA), a-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid (AMPA), and Kainate. These receptors

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would incorporate ion channels that are permeable to cations, with relative permeability to Na^+ and Ca^{2+}, based on the subunit composition of the receptor [10, 11].

Metabotropic receptors are G protein-linked and operate via releasing either second messengers in the cytoplasm or by influencing ion channels through the release of G protein subunits within the membrane [12, 13]. The recent decades have seen a greater understanding of receptor action, role of glutamate in learning, memory, stroke, epilepsy, neurodegenerative and psychiatric disease, and novel therapeutic agents targeting the glutamate receptors that may have implications for clinical practice in the near future.

Neurochemical Profile

Glutamate, being the principal excitatory neurotransmitter, plays a key role in critical functions such as learning and memory. Projected glutaminergic pathways are shown below in Fig. 5.1. However, elevations in extracellular glutamate contributes to neuronal cell death and excitotoxic damage through overactivation of glutamate receptors. Glutamate-mediated neurotoxicity is the primary pathological process in several acute and chronic neurological diseases [1, 14].

Glutamate and Neural Plasticity

The high permeability of NMDA receptors to Ca^{2+} has many implications for cellular function. One of the major consequences of NMDA receptor activation is the generation of long-lasting changes in synaptic function termed synaptic plasticity.

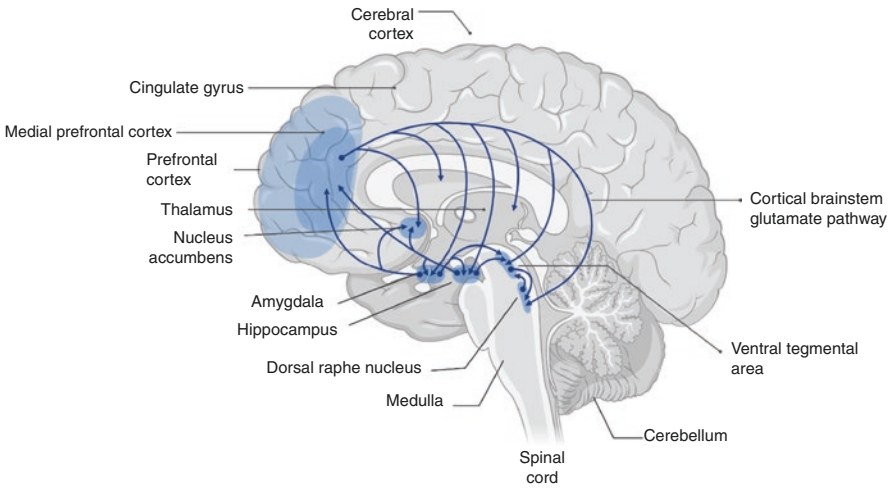


Fig. 5.1 Glutaminergic pathways in the brain

Long-term potentiation (LTP) and long-term depression (LTD) are activity-dependent alterations in synaptic efficacy that can last for weeks or months. Changes in receptor number or changes in receptor function contribute to these changes in synaptic strength. The bidirectional control of synaptic strength by LTP and LTD is believed to be important for many forms of experience-dependent plasticity in the brain [14, 15].

LTP and LTD of excitatory synaptic responses in CA1 pyramidal neurons in the hippocampus have been extensively characterized because of their key role in learning and memory. The triggering of this form of LTP requires activation of NMDA receptors by synaptically released glutamate when the postsynaptic membrane is already strongly depolarized. The depolarization relieves the voltage-dependent block of the receptor channel by Mg^{2+} and allows Ca^{2+} to enter the postsynaptic dendrite spine when the receptor is activated by glutamate. This rise in postsynaptic Ca^{2+} concentration, the critical trigger for LTP, activates complex intracellular signaling cascades that include several protein kinases. The primary mechanism underlying the increase in synaptic strength during LTP is a change in AMPA receptor trafficking where there is an increased number of AMPA receptors in the postsynaptic plasma membrane. Within a few hours, the maintenance of LTP requires protein synthesis and is accompanied by observable enlargements of dendritic spines. Such structural changes may be essential to cement the information storage process initiated at synapses on LTP induction [14, 15].

At many excitatory synapses through the brain, weaker activation of NMDA receptors elicits the opposite phenomenon, NMDA receptor-dependent LTD, which is thought to result from a smaller rise in postsynaptic Ca^{2+} than is required for LTP. The depression of synaptic strength is due to the removal of synaptic AMPA receptors via endocytosis. Interestingly, activation of postsynaptic mGluRs can also lead to a form of LTD that was first described in the cerebellum and also occurs in the hippocampus and neocortex [14, 15].

Glutamate in Neurotoxicity

Excitatory amino acids such as glutamate produce their neurotoxic effects by two proposed mechanisms. Continuous exposure of neurons to toxic concentrations of glutamate, opening the membrane cation channels leading to an excessive influx of Na^+ and a secondary passive influx of Cl^- and water eventually resulting in acute neuronal swelling. The second is a slower process of neuronal degeneration, dependent on extracellular Ca^{2+} where brief exposure of neurons to glutamate or related agonists induces an influx of Ca^{2+} that accumulates in the cell and eventually causes cell death by activation of catabolic enzymes. Under pathologic states, this mechanism is more substantial as neuronal damage occurs in response to a low concentration and a shorter duration of glutamate exposure than that is required to produce an acute neuronal swelling by the Na^+/Cl^- dependent mechanism [16, 17].

Receptors

Glutamate acts through two classes of receptors, which are ion channel–linked inotropic receptors that are gated by agonist binding (iGluR) and metabotropic receptors (mGluR), which contain seven transmembrane domains (TMs) that are coupled with G-proteins inducing intracellular messenger cascades. Classification of receptors was classified based on the affinity to specific agonists and antagonists. Glial cells, particularly astrocytes, in addition to neurons, respond to neurotransmitters. Glutamate, along with its extensive receptor family, is perhaps the most complex and versatile signaling system in the CNS [1, 18].

Ionotropic Glutamate Receptors

Three classes of ionotropic glutamate receptors were named initially based on the ability of these drugs to serve as selective agonists: N-methyl D-aspartate (NMDA), alpha-amino 3-hydroxy 5-methyl 4-isoxazole propionate (AMPA), and kainite [1]. The Glutamate receptor protein subunit composition and their properties are shown below in Table 5.1 [19].

Synaptically released glutamate interacts with postsynaptic receptors on nerve terminals or dendrites of adjacent cells. The binding to NMDA and AMPA receptors opens postsynaptic cation channels to initiate a two-component

Table 5.1 Glutamate receptor protein subunit composition and properties [19]

Receptor	Protein subunit	Receptor properties
Ionotropic receptors		
NMDAR	NR1, NR2A[a], NR2B[a], NR2C, NR2D, NR3A, and NR3B	Heterotetrameric; calcium permeability high; long channel open time
AMPAR	$GluR_1$[a], $GluR_2$ edited, $GluR_2$, $GluR_3$[a], and $GluR_4$[a]	Heterotetrameric; calcium permeability low if edited $GluR_2$, otherwise moderate; short channel open time
Kainate receptor	$GluR_5$[a], $GluR_6$, $GluR_7$, KA1, and KA2	Homotetrameric or heterotetrameric; calcium permeability low; short channel open time
Metabotropic receptors		
Group 1	$mGluR_1$[a] and $mGluR_5$	Homodimeric; signals via phospholipase C; located postsynaptically
Group 2	$mGluR_2$ and $mGluR_3$	Homodimeric; signals via adenylyl cyclase; located mostly presynaptically; agonists and antagonists mostly distinct from group 3
Group 3	$mGluR_4$, $mGluR_6$, $mGluR_7$,and $mGluR_8$	Homodimeric; signals via adenylyl cyclase; located mostly presynaptically; agonists and antagonists mostly distinct from group 2

NMDA N-methyl D-aspartate, *AMPA* alpha-amino 3-hydroxy 5-methyl 4-isoxazole propionate
[a] Glutamate receptor protein subunits for which human autoantibodies have been reported

excitatory postsynaptic current (EPSC) at central synapses. NMDA and AMPA receptors colocalize at most functional excitatory synapses. However, the ratio of NMDA to AMPA receptors at individual receptors varies. On the contrary, small number of kainite receptors are found in several CNS regions. Activation of the ionotropic receptors increases the transmembrane Ca^{2+} and Na^{+} fluxes, while the metabotropic receptors act through the generation of inositol triphosphate and inhibition of adenylate cyclase. AMPA receptors are critical to fast excitatory neurotransmission, while NMDA receptors transmit the slow postsynaptic excitatory postsynaptic potentials (EPSP) essential for global information processing [14].

NMDA Receptors

The NMDA receptor has been widely studied, and they have been demonstrated only in neurons but not glial cells. NMDA receptors have various properties that set them apart from other ligand-gated receptors. These pharmacological binding sites of the NMDA receptor are shown below in Fig. 5.2. These properties are [20] as follows.

1. Transmitter binding site that binds glutamate.
2. A regulatory or coactivator site that binds glycine.
3. Site within the channel that binds phencyclidine and related compounds.
4. Voltage-dependent Mg^{2+} binding site.
5. Inhibitory divalent cation site that binds Zn^{2+}.

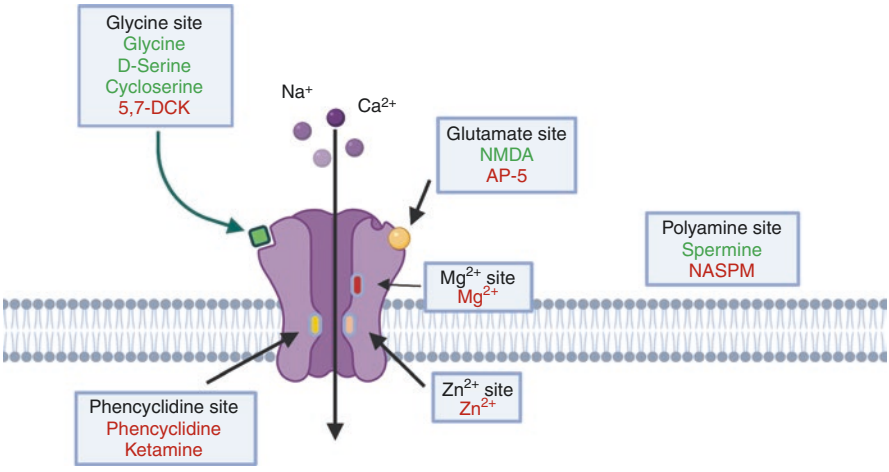


Fig. 5.2 Pharmacological binding sites of the NMDA receptor. Drugs promoting receptor functions appear in green, and those inhibiting receptor functions appear in red. *NMDA* N-methyl D-aspartate

Activation of NMDA receptors produces a nonspecific increase in the permeability to the monovalent cations Na+ and K+; however, these receptors are also highly permeable to Ca^{2+}. They also play a crucial role through which synaptic activity can increase the level of intracellular Ca^{2+} at individual synapses. This receptor is unique as its activation requires the binding of two different agonists simultaneously, glutamate and glycine. Glycine binding seems to be required for receptor activation, and hence glutamate and glycine are referred to as coagonists of the NMDA receptor [21].

Non-NMDA Receptors

AMPA and kainate receptors produce the voltage-independent portion of the synaptic response in several neuronal pathways. They mainly activate the opening of low conductance ion channels that are permeable to Na^+ and K^+ and in general, have low permeability to Ca^{2+}. However, some non-NMDA glutamate-gated channels have substantial permeability to Ca^{2+}, as demonstrated in vitro in hippocampal and retinal bipolar neurons [22].

AMPA receptor activation intermediates a synaptic current with a rapid onset and decay. In contrast, the current conducted by an NMDA receptor has a slower onset and a decay that lasts for several hundred milliseconds. NMDA receptor's decay time is about 100 times longer than the mean open time of its channel. The prolonged activation is caused by the high affinity of glutamate and subsequent slow dissociation from these receptors. On the contrary, glutamate has a low affinity for AMPA receptors and thus rapidly dissociates [22].

AMPA receptors react to single vesicles of glutamate, whereas NMDA receptors require substantial activation from multiple synapses due to their unique voltage dependence. This allows NMDA receptors to act as coincidence detectors that can sense the activity of many independent synaptic inputs converging on the same cell [23].

Metabotropic Glutamate Receptors

The metabotropic glutamate receptors are pharmacologically and functionally different from the ionotropic receptors. The mGluR is coupled to G-protein and evokes a variety of functions mediating intracellular signal transduction. Eight mGluRs, termed mGlu1 to mGlu8, have been cloned so far. The mGluRs are considerably larger than other G-protein coupled receptors, and comparisons of their amino acid sequences with other receptors reveal little homology. They are therefore considered to constitute a separate receptor family. Like other G-protein coupled receptors, mGluRs have seven membrane-spanning domains. Also, like the ionotropic

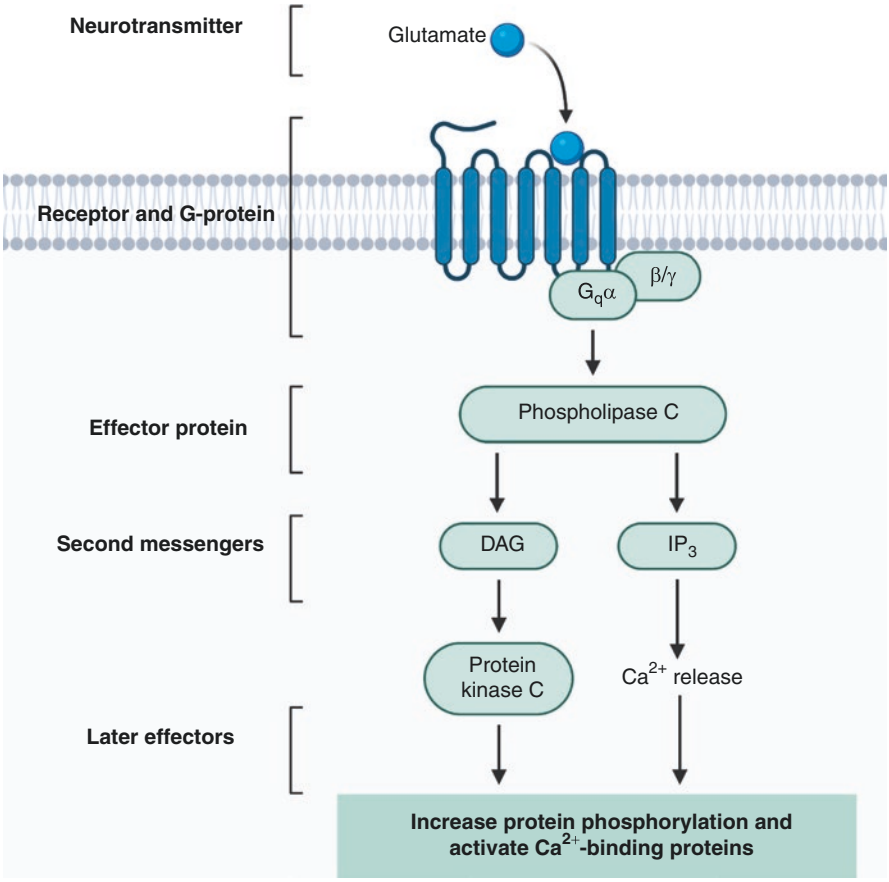


Fig. 5.3 GPCR effector pathway of glutamate

receptors, they possess an unusually large N-terminal extracellular domain that precedes the membrane-spanning segments [24, 25]. The GPCR effector pathway of glutamate is shown below in Fig. 5.3.

There are three functional groups of mGluRs based on amino acid sequence homology, agonist pharmacology, and the signal transduction pathways to which they are coupled. Group 1 includes mGlu1 and mGlu5, found on postsynaptic neurons adjacent to excitatory synapses. Group 2 includes mGlu2 and mGlu3, while Group 3 includes mGlu4, mGlu6, mGlu7, and mGlu8. The mGluRs of groups 2 and 3 are often found on presynaptic terminals where they modulate transmitter release. Glutamate activates all of the mGluRs in addition to highly selective agonists for each of the three groups that have been identified [24, 25].

The mGluRs located on the postsynaptic membrane modulate several ligand and voltage-gated ion channels expressed on central neurons. The activation of each of the three groups of mGluRs has been found to inhibit L-type voltage-gated Ca^{2+}

channels, and groups 1 and 2 also inhibit the N-type Ca^{2+} channels. Additionally, mGluR activation can close voltage-gated K^+ channels resulting in a slow depolarization and neuronal excitation [24, 25].

Several types of mGluRs are also located on the presynaptic terminals of central neurons, and their activation blocks both excitatory glutamatergic and inhibitory GABAergic synaptic transmission in most CNS regions. One mechanism by which activation of mGluRs decreases neurotransmitter release might be through the inhibition of voltage-gated Ca^{2+} channels on the presynaptic nerve terminal, functioning as inhibitory autoreceptors at many glutamatergic nerve terminals [24, 25].

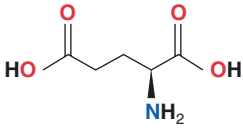
Metabolism

Glutamate plays a vital role not only in protein structure but also in nutrition, metabolism, and signaling. Even though glutamate is the major excitatory neurotransmitter, its byproduct γ amino butyric acid (GABA) is the primary inhibitory neurotransmitter in the CNS. Glutamate is a true functional amino acid [26]. Glutamate cannot cross the blood-brain barrier as it is a charged amino acid and hence synthesized in the brain from glucose and other precursors. Glucose acts as a major source of carbon, and branched-chain amino acids provide nitrogen by transporting them rapidly into the CNS [27].

The average concentration of glutamate in the brain is around 100 nmol/mg protein or 10 Mm. While the concentration of glutamate in CSF is around 1–10 μM and in the extracellular space of the brain where it is only around 0.5–2 Mm [28]. The chemical structure of glutamate is shown below in Fig. 5.4.

Glutamate is in a metabolic pool with α ketoglutarate and aspartate. It is released from the synaptic vesicles in presynaptic terminals via Ca^{2+}-dependent mechanism, which involves both N and P/Q type voltage dependent Ca^{2+} channels that are closely linked to vesicular docking sites [29]. EPSP is produced through the release of glutamate from a single vesicle, and glutamate might also be released through the reverse operation of the glutamate transporters. This process occurs when Na^+ and K^+ gradient across the membrane is decreased during cerebral ischemia. Glutamate's release from the synapse is controlled via a variety of presynaptic receptors, which include not only the metabotropic glutamate receptors but also cholinergic, adenosine, kappa opioid, $GABA_B$, cholecystokinin, and neuropeptide Y receptors [1, 30, 31].

Fig. 5.4 Chemical structure of glutamate



Glutamate Transporters

Glutamate accumulation into synaptic vesicles has a key role in glutamate transmission, which is achieved through vesicular glutamate transporter (VGLUT) coupled to v-type proton ATPase. It is driven by the proton gradient and is selective for L-glutamate [1].

Five glutamate transporters are identified, among which two are expressed predominantly in glia [glial glutamate and aspartate transporter (GLAST) and glial glutamate transporter (GLT)] and three in neurons. These neuronal transporters are called excitatory amino acid transporters (EAAT1–5). These are Na^+ dependent transporters, and the transmembrane gradient of Na^+ and K^+ produces the driving force for glutamate transport [1, 32]. EAAT4 and EAAT5 have the most significant chloride conductance and may also act as inhibitory glutamate receptors than as transporters [33].

The Glutamate–Glutamine Cycle

The key feature of brain glutamate metabolism is the anatomic compartmentation that maintains the two main prerequisites of glutamatergic neurotransmission, which is to maintain a relatively low level of glutamate externally and replenish the neuronal glutamate pool internally [27].

The glutamate–glutamine cycle is crucial in understanding the metabolism of brain glutamate, shown below in Fig. 5.5. It begins with the action potential-driven

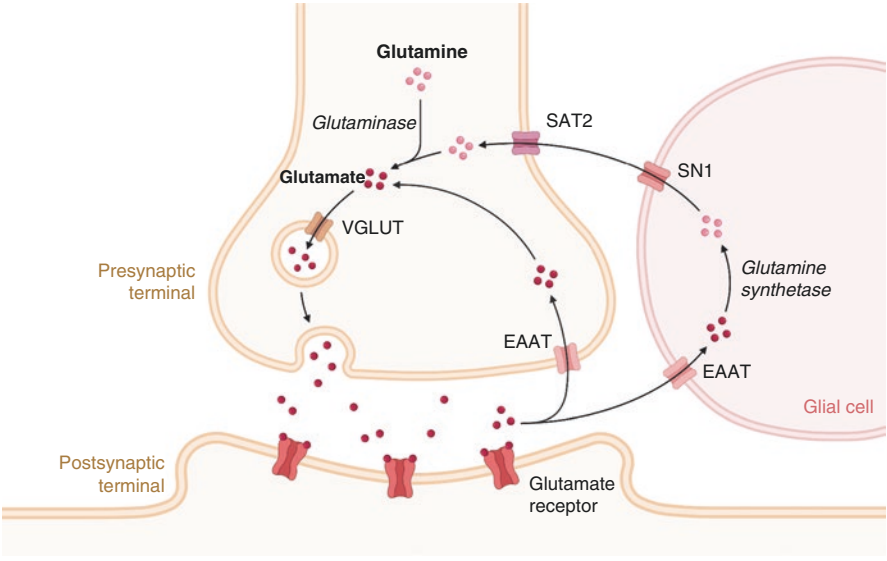


Fig. 5.5 Glutamate–glutamine cycle. *EAAT* excitatory amino acid transporters

release of glutamate from the presynaptic vesicles. The concentration of glutamate in the synapse before release is 2–5 μmol/L, which can rise to as high as 5–100 μmol/L after depolarization [27, 34, 35].

Postsynaptic neurons remove a minute amount of glutamate from the synapse. There is active reuptake of glutamate into presynaptic neurons. However this process plays a negligible role other than astrocytic transport. The membrane potential of astrocytes is low relative to that of neurons which favors efficient glutamate uptake via transporters. Astrocytes convert glutamate to glutamine through the glutamine synthetase pathway. The glia are virtually the only site for glutamine synthetase activity in the brain. The ammonia consumed to generate glutamine is obtained either from brain or blood metabolism [36, 37].

Neuronal glutamine uptake occurs through both Na^+ dependent and independent mechanisms. Primarily, the metabolic fate of glutamine taken into neurons is hydrolysis to glutamate and ammonia through a phosphate-dependent glutaminase, a mitochondrial enzyme. However, not all glutamate derived from glutamine is transported to renew the transmitter pool; a portion of it might be oxidized in the nerve endings primarily through transamination to 2-oxo-glutarate by aspartate aminotransferase. Glutamine hence is not simply a precursor to glutamate but also to glucose, which helps in meeting the neuronal energy requirements [38, 39].

The significance of Glutamate–Glutamine cycle [27]:

- Rapid removal of glutamate from the synapse to maintain a low signal to noise ratio when glutamate is released into synapse.
- Conversion of glutamate to glutamine (nonneuroactive compound), in astrocytes serves as a carrier of glutamate back to neurons.
- Mechanism for the regeneration of glutamate in the neuronal compartment.
- Metabolic substrate, glutamine, acts as fuel for neurons.
- Mechanism for the buffering of ammonia (neurotoxic), through glial glutamine synthetase pathway.

GABA

The enzyme glutamic acid decarboxylase removes the α-carboxyl group of glutamate to produce a γ-carboxyl amino acid (GABA), which is the major inhibitory neurotransmitter in the brain, which requires the cofactor pyridoxal phosphate (Vitamin B6).

Medications

The extensive molecular machinery regulating glutamate signaling provides multiple targets for glutamatergic drug action and drug development. Both preclinical and clinical evidence support the pursuit of small molecule modulators of the

glutamate system as novel therapeutic agents in diseases, particularly epilepsy, neurodegeneration, and mood disorders. In the following section, we briefly review a few medications currently used in clinical practice [40].

Ketamine

Ketamine is a noncompetitive NMDA receptor antagonist currently used as an anesthetic agent, and is classified as a dissociative anesthetic. Several open-label studies and case reports have added to the evidence base for ketamine as a novel glutamatergic antidepressant, particularly for patients with treatment-resistant major depressive disorder. These findings have been extended to bipolar depression, where ketamine added to a mood stabilizer exerted a rapid antidepressant effect in these patients who were in a refractory depressive episode.

Treatment with NMDA receptor antagonists such as ketamine combined with GABA agonists and other agents can diminish experimental status epilepticus. In contrast, ketamine can suppress seizure activity and be neuroprotective after prolonged status epilepticus [40–42].

Memantine

Memantine is an amantadine derivative and voltage-dependent noncompetitive low-affinity NMDA receptor antagonist that is approved for the treatment of moderate to severe Alzheimer's disease [43].

Riluzole

Riluzole is a glutamate modulator with neuroprotective and plasticity enhancing properties. It is approved for the treatment of amyotrophic lateral sclerosis (ALS). It reduces extrasynaptic glutamate by inhibiting glutamate release through presynaptic inhibition of voltage-gated sodium channels, enhancing astroglial uptake of glutamate and increasing AMPA receptor trafficking [44, 45].

Topiramate

Topiramate suppresses excitatory neurotransmission by attenuating non-NMDA glutamate receptor neurotransmission by inhibiting kainate evoked currents. It has also been shown to reduce high basal concentrations of extracellular glutamate in hippocampi of spontaneously epileptic rats [46, 47].

Felbamate

The broad spectrum antiepileptic drug (AED) felbamate has a unique dual mechanism of action as a positive modulator of GABA-A receptors and as an antagonist of NMDA receptors [48].

Pregabalin

Pregabalin selectively binds to the accessory subunit α2δ-1 of voltage-gated calcium channels to block P/Q-type calcium currents, thereby reducing the calcium-dependent release of glutamate [49].

Lamotrigine

In addition to the effects on sodium channels, lamotrigine can modulate P/Q-type, N-type, and R-type calcium channels, all of which are expressed on presynaptic nerve terminals, thereby indirectly modulating glutamate release.

Other sodium channel blockers, including phenytoin and carbamazepine, also reduce evoked glutamate release by their ability to block sodium-evoked calcium influx and excitation-induced glutamate release. Thus, currently approved AEDs show varying degrees of effects on glutamatergic neurotransmission that can be either direct or indirect [50, 51].

Perampanel

Modulation of AMPA receptors may be a promising clinical target as modulation of AMPA but not NMDA receptor signaling exerts fewer effects on neuroplasticity and thus has greater potential to modulate hyperexcitability without the potential for psychosis. Efforts to develop AMPA receptor-selective agents resulted in the clinical approval of perampanel as the first-in-class glutamate system selective drug for epilepsy. It is a noncompetitive AMPA receptor antagonist that decreases neuronal excitability and synchronization characteristic of epileptiform activity [48].

Clinical Aspects

Role of Glutamate in Epilepsy

In epilepsy, chronic dyssynchronous network activity induces extraneous neuronal firing and pathological alterations in signaling that may arise from multiple pathways. One factor that is heavily implicated in much of the aberrant signaling and

resulting pathology of epilepsy is glutamate. Synaptic remodeling contributes to neuronal destabilization because of the increased or decreased ease with which a postsynaptic neuron can be depolarized by presynaptic input. For this reason, aberrant neuronal activity seen within an epileptic network likely leads to long-term rewiring within neuronal networks, an effect that contributes to network hyperexcitability associated with epilepsy. Long-term remodeling of synaptic connectivity and dendritic morphology mediated by NMDA receptors may contribute to the onset of spontaneous recurrent seizures [52–54].

Evidence for the role of glutamate in seizures and epileptogenesis comes from animal models and humans during status epilepticus (SE). SE induced by high doses of nerve agents is associated with excessive acetylcholine accumulation and secondary recruitment of excitatory glutamatergic signaling. SE-induced glutamate release results in overstimulation of glutamate receptors sustained long term seizure activity and development of seizure-induced brain damage. With prolonged SE, GABA receptors are internalized, and NMDA receptors migrate to neuronal synapses, all effects that lead to reduced inhibition and hyperexcitability. The changes in receptor localization highlight why drugs that target GABAergic neurotransmission likely fail to suppress seizures in sustained SE, whereas treatment with NMDA receptor antagonists in combination with GABA agonists and other agents can often successfully attenuate experimental SE [52, 55–57].

Role of Glutamate in Autoimmune Encephalitis

Over the last few decades, several autoimmune encephalitides have been identified with the discovery of antibodies directed against ionotropic glutamate receptors. Anti NMDA receptor antibodies are present in patients with autoimmune anti-NMDA receptor encephalitis. These antibodies are very pathogenic as they can cause a pronounced decrease of surface NMDA receptors expressed in hippocampal neurons and decrease the cluster density and synaptic localization of the NMDA receptors. The antibodies induce these effects by crosslinking and internalization of the NMDA receptors. Such changes can impair glutamate signaling via the NMDA receptors and lead to various neuronal, cognitive and behavioral abnormalities. These patients respond to several modes of immunotherapy [24].

Role of Glutamate in Stroke

NMDA and AMPA receptor antagonists have shown to be neuroprotective in animal models of stroke. In permanent or reversible occlusion of the middle cerebral arteries, these antagonists consistently reduce the volume of cortex that is infarcted 24 h or 1 to several weeks later. They, however do not protect the striatum. The protection is greatest if the antagonist is closest to the time of onset of cerebral ischemia. These preclinical data led to major clinical trials of NMDA receptor antagonists in stroke and head injury, however, these trials have not shown therapeutic clinical benefit [1, 58].

Role of Glutamate as a Dietary Toxin

In humans, domoic acid is the only known dietary toxin, producing pathology through action on a glutamate receptor. Domoic acid is synthesized by marine diatoms and enters the food chain when it is concentrated by mussels feeding on the algae. In an outbreak of such poisoning in eastern Canada in 1987, individuals developed an acute confusional state as the most common presenting feature, with some also having focal seizures. Persistent anterograde amnesia was observed in some cases. Neuropathological studies in those who succumbed after days revealed extensive bilateral limbic system pathology with neuronal loss in cellular zones of hippocampus (CA1, CA3, dentate gyrus), amygdala, claustrum, thalamus, and insular and subfrontal cortex. The pathology is likely a consequence of limbic seizure activity rather than the effect of a direct excitotoxic action of domoic acid [59, 60].

Role of Glutamate in Neurodegeneration

It has been proposed that neurodegeneration in a variety of late-onset neurological diseases is at least partially dependent on endogenous glutamate activating NMDA or AMPA receptors.

In Huntington disease (HD), consistent evidence indicates that NMDA receptors play a key role in HD-related neuronal cell death. One of the first HD mouse models was developed by injecting the NMDA receptor agonist quinolinic acid into the striatum, which triggered HD-like lesions and a phenotype that displayed some aspects similar to what is observed in HD patients. It was demonstrated that mutated huntingtin protein promotes sensitization of the NMDA receptor, which resulted in an increase in extracellular Ca^{2+} entrance into neurons contributing to excitotoxicity [1, 61].

In Parkinson disease (PD), classical therapy consists in administration of 3,4 dihydroxyphenylalanine (L-DOPA), which seeks to provide motor improvements by reestablishing dopamine levels in the striatum. Although L-DOPA administration remains the gold standard therapy for PD, its chronic use leads to L-DOPA-induced dyskinesia (LID) which can be quite disabling to the patient. There is evidence to suggest that LID is caused by dysfunctional neuronal plasticity in the striatum due to an imbalance between glutamate and dopamine signaling. Preclinical studies have shown that ionotropic glutamate receptor antagonists have good anti-parkinsonian responses, however, adverse side effects in humans make its clinical application difficult. An alternative for reducing excessive excitatory activity in the basal ganglia may be provided by targeting mGluRs. mGluRs are widely expressed in the basal ganglia, and their slight inhibition or modulation is believed to be able to regulate neuronal excitability, offering targets for therapeutics in the future [62, 63].

In motor neuron diseases such as ALS, there is evidence to support the involvement of AMPA receptors on spinal motor neurons. There is a reduction in the expression of GLT-1, a glial glutamate transporter in the brain and spinal cord regions showing loss of motor neurons. AMPA receptor antagonists protect against the toxic effects of mutations in Cu/Zn superoxide dismutase in cultured mouse neurons [1, 64].

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Chapter 6
Glycine

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Introduction

Glycine is a nonessential amino acid and is also known as 2-Aminoacetic acid. It is a major amino acid that plays a significant role in the various metabolic functions, antioxidative reactions, and neurological functions. It is chemically neutral and metabolically inert. It is also an essential substrate in the synthesis of a multitude of biomolecules and substances.

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History

The rich history of glycine dates back to as early as 1820, when it was discovered by the French chemist Henri Braconnot while studying acid hydrolysis of sugar-yielding substances. He published a paper describing the results of treating wood sawdust, hay, cotton, and hemp-lime in acid solution. Later on, he tried the same experiment using animal products like muscle fiber, wool, and gelatin. He treated gelatin with sulfuric acid, neutralized it with calcium carbonate, and filtered it. The solution was then concentrated through the process of evaporation over some time. He then separated white sugar-like crystals from the solution and named this sweet substance "sucre de gélatine," meaning sugar of gelatin [1]. He failed to study the compound further and identify its composition.

In 1838, another French chemist Jean-Baptiste Boussingault analyzed glycine composition and deduced a chemical formula, indicating the presence of nitrogen in glycine [2]. The same year, Dutch chemist Gerardus Johannes Mulder isolated leucine and glycine from meat and gelatin through alkaline hydrolysis with potassium hydroxide [3]. He provided the empirical formula of glycine as $C_8H_{14}N_4O_5$ with a molecular weight double the known molecular weight of glycine [4]. He subsequently revised his findings to give the correct chemical formula.

In 1845, French chemist Victor Dessaignes isolated glycine through the hydrolysis of hippuric acid. Knowing the chemical formulae of hippuric acid and benzoic, which is a bi-product of the hydrolysis of the former, he was able to infer the chemical formula of glycine. This, however, lacked substantial proof. He pointed that the name "sugar of gelatin" was unsuitable as there are other sweet tasting substances that were not fermentable [5]. In 1846, American scientist Eben Norton Horsford extensively studied glycine and its derivatives. His findings showed that glycine reacts with both acids and bases. Along with the findings of Dessaignes, Horsford coined the term "glycocoll" to differentiate it from other free sugars [3]. The same year, Horsford, Mugler, and another French chemist Auguste Laurent independently determined the correct chemical composition of glycine—$C_2H_5NO_2$ [6]. In 1848, Swedish chemist Berzelius suggested the more simpler term "glycine" as he claimed that the word was a better fit in comparison to glycocoll, meaning "sweet lime" [7].

The structure of most amino acids was not discovered until late nineteenth century. In 1858, August André Thomas Cahours established the structural formula of glycine chemically by treating chloroacetic acid with ammonia or ammonium carbonate. He debunked the theory of glycine belonging to ammonia type of organic compounds and showed that it was, in fact, a derivative of acetic acid [8].

$$CH_2Cl - COOH + NH_3 = HCl + CH_2 - NH_2 - COOH$$

The theory was later on proved by two British chemists, Sir William Henry Perkin and Baldwin Francis Duppa. They were able to synthesize glycine using bromoacetic acid and ammonia [9]. With some modifications, their method was then used in future studies for the preparation of glycine.

Receptors

Glycine is a major inhibitory neurotransmitter in the brainstem and spinal cord. It also acts as a coagonist at the N-methyl-D-aspartic acid (NMDA) glutamate receptors. Glycine exerts its action in the central nervous system through various receptors, which include the glycine receptor (GlyR), NMDA receptor, and glycine transporters type 1 and 2 (GlyT1 and GlyT2, respectively) receptors.

The Glycine Receptor

In 1982, Pfeiffer et al. used affinity chromatography with strychnine to isolate the glycine receptor from rat spinal cord on strychnine–agarose columns. He identified three polypeptides with a relative molecular mass of 48, 58, and 93 kDa [10]. Graham et al. identified the 48 kDa polypeptide as the α-subunit and the 58 kDa polypeptide as the β-subunit. The two subunits were considered essential for the formation of the GlyR [11, 12]. The 93 kDa polypeptide was regarded as another peripheral membrane protein called gephyrin, located at the postsynaptic GlyR complex [13]. The GlyR is a pentameric anion channel present in the postsynaptic neuronal membrane of the spinal cord [14]. They can also be found on proximal tubule cells of the kidney, endothelial cells, lung, hepatocytes, gastric, and intestinal cells [15]. These receptors have also been identified in specific nuclei of the brainstem like the cuneate, gracile, reticular, trigeminal, cochlear, and hypoglossal motor nuclei [16]. Other areas include the excitatory and inhibitory neurons of the hippocampus [17], retina [18], thalamus [19], and striatum and cerebellar cortex [20].

They are a member of the Cys-loop ligand-gated ion receptors, which also include 5-hydroxytriptamine ($5\text{-}HT_3$), nicotinic acetylcholine, and the γ-aminobutyric acid A ($GABA_A$). Functional GlyR pentamers are composed of the four α- (α_1-α_4) and β-subunits. These receptors can be assembled as homopentamers, including only the α-subunits, or can be heteropentamers where complexes are formed through an association with the β-subunit [21]. The α- and β-subunits are encoded by two genes GLRA and GLRB [22]. The α_2 GlyRs are expressed in abundance on embryonic neurons, and a sharp decline in their numbers is seen by adulthood. The majority of the glycinergic neurotransmission in adults occurs through heteromeric $\alpha_1\beta$ GlyRs. In nociceptive sensory neurons of the dorsal horn of the spinal cord, α_3 GlyRs are mediators of inhibitory glycinergic neurotransmission [23]. The β-subunit cannot form functional receptors without an association with the α-subunits. It interacts with the cytoplasmic protein gephyrin to associate with intracellular microtubules, which is essential for receptor clustering at the postsynaptic neurons [24].

The Cys-loop receptors comprise homomeric or heteromeric pentameric oligomers that are symmetrically arranged around an ion-conducting core. Each GlyR subunit comprises an extracellular amino acid domain which contains the Cys-loop

and the ligand-binding sites. This is connected to a group of four α-helical transmembrane domains (M_1–M_4) with a large intracellular domain between M_3 and M_4, a short extracellular C-terminal tail, and a centrally located ion channel within the M_2 domain [25, 26]. The intracellular domain aids interactions with cytoplasmic proteins, cytoskeleton, and neuroreceptors that are involved in the turnover, clustering, traffic, and modulation of GlyRs [27]. Ligand-binding sites mainly comprise β-sheets connected through flexible loops and at the subunit interface, constitute the agonist binding site [28]. Three flexible loops form the principle binding site while three β-sheets from the adjacent subunit forms the complementary binding site, and three molecules of glycine are sufficient to maximally activate GlyRs [29, 30]. The content of α-subunits determines the characteristics of GlyRs. The β-subunit of the receptor functions by anchoring the receptor to the cytoskeleton, reducing ionic currents, stabilize lower conductance rates, and influence the receptor sensitivity to glycine as well as drugs like tropisetron (5-HT_3 antagonist), pregnenolone sulfate (neurosteroid), and picrotoxin analogs [31].

The N-Methyl-D-Aspartic Acid Glutamate Receptor

Glycine potentiates the action of glutamate at the NMDA receptor. The receptor has three major subunits, which include GluN1/NR1 (grin1), GluN2/NR2 (grin2A-2D), and GluN3/NR3 (grin3A,3B) [32]. It is a tetrameric complex that contains two glycine-binding NR1 subunits and two glutamate-binding NR2 subunits that are encoded by GRIN1 and GRIN2A-D genes, respectively [33]. The NR1 subunits are seen at all stages of development in all the regions of the brain, and NMDA receptors function by potentiating the excitatory synaptic currents in the brain and spinal cord [34].

The Glycine Transporters Type 1 and 2 Receptors

GlyT1 and GlyT2 are receptors with a high affinity for glycine and transport the amino acid through an active electrogenic mechanism coupled with the electrochemical gradient of sodium and chloride ions. Substrate binding causes a conformational change in the receptors, exposing the glycine-binding site to the cytosol, with subsequent release of sodium, chloride, and glycine [35]. The major difference in kinetics of the receptors are that while GlyT1 is driven by glycine binding to conformations that face outwards, GlyT2 is controlled by the binding of sodium to the transporter binding site [36]. Solute carrier 6 (SLC6) genes are a family of sodium and chloride dependent neurotransmitter transporters, and the members of this group, SLC6A9 and SLC6A5, encodes for GlyT1 and GlyT2 receptors, respectively [37]. GlyT1 is distributed in abundance in the glial cells of the olfactory bulb, retina, cortex, diencephalon, thalamus, hypothalamus, and hippocampus, and

neocortex [38, 39]. On the other hand, the expression of GlyT2 is higher in the spinal cord (dorsal and ventral horns), brainstem (cranial nerve nuclei), auditory system, and cerebellum. Both these receptors have been found in neurons and astrocytes of the cortex and hippocampus [40].

Neurochemical Profile

Glycine plays a pivotal role in various metabolic processes of the body. It is utilized in many metabolic pathways to generate purines (nucleic acids), creatinine, heme proteins, collagen, and also other amino acids like serine and glutathione. It is essential for the conjugation of bile acids which is required for digestion and absorption of fats and fat-soluble vitamins [41]. Modulatory effects of glycine like proliferation, differentiation, migration, and cytokine production have been described in immune cells like leukocytes and macrophages, endothelial cells, and macroglial cells. The cytoprotective effects where glycine protects from ischemic cell death have been described in renal cells, hepatocytes, and endothelial cells [42].

Neurotransmitter

Glycine is a major inhibitory neurotransmitter in the spinal cord. The activation of GlyR depends on the extracellular chloride ion concentration. When glycine binds to the receptor, it is activated, resulting in passive chloride influx through the core channel. This results in hyperpolarization of the cell membrane, halting the depolarization and neuronal firing caused by excitatory neurotransmitters [43]. In the brainstem, glycine is considered to be involved in the auditory, cardiovascular, and respiratory functions. The inhibitory effect is essential for voluntary motor control, processing sensory inputs, and generating reflex responses [44]. Glycine also plays a pivotal role in the inhibitory circuits of the spinal reflexes. The glycinergic Ia interneurons inhibit stretch reflex, coordinating the agonist muscles while relaxing the antagonist muscles. Renshaw interneurons utilize glycine to control motor neuron excitability through negative feedback causing recurrent inhibition [45]. Prostaglandin-E2-dependent inhibition of α_3 subunits of GlyRs in the spinal cord have been indicated as the underlying mechanism of central inflammatory pain sensitization [46].

During the developmental period, glycine can act as an excitatory neurotransmitter in the central nervous system. The activation of GlyRs in these locations can increase chloride ion efflux, resulting in cell membrane depolarization and release of neurotransmitter [47]. The excitatory effects of glycine have been implicated in major processes like proliferation and differentiation of neurons, as well as stability of the neuronal network stability [48]. It also plays a crucial role in the development and specialization of postsynaptic glycinergic membrane [49]. The α_2 GlyRs are the

most common type of receptors seen in the central nervous system during the embryonic and immediate post-natal period. This is gradually replaced by α_1 subunit in the second week and then by heteromeric GlyRs in adults, which could probably explain the switch from excitatory depolarization to inhibitory hyperpolarization profile of glycine [50]. Studies have shown this excitatory effect in mature adult neurons as well [51]. It is hypothesized that the effect is seen in neurons with a higher intracellular chloride concentration. In this case, the chloride efflux drives the membrane potentially rapidly towards its equilibrium potential, and relative to the resting membrane potential, the cell can be depolarized or hyperpolarized [51].

Another probable function of glycine is its modulatory role in the striatum, and studies have shown the occurrence of GlyRs in GABAergic interneurons, and active cholinergic interneurons called giant aspiny neurons (GANs) in rat striatum [52]. However, the role has not been extensively explored and is still not well-understood.

Potentiating Action at the NMDA Receptors

Glycine acts on the NR1 subunit of the NMDA receptor, and studies have shown that the excitatory function of glutamate is potentiated at the NMDA receptors in the brain in the presence of glycine [53]. Studies have also shown the affinity of glutamate receptors to glycine varies with the different receptor isoforms [54]. Some studies also show that glutamate receptors can be activated by glycine, even in the absence of glutamate or NMDA [55]. Electrophysiological experiments have shown that NMDA receptor activation increases with inhibition of GlyT1 receptors due to an increase in the glycine concentration at the synaptic cleft [56].

The Role of Glycine Transporters

The glycine transporters function in the central nervous system by transported L-glycine to the synaptic terminals. GlyT1 mainly decreases the extracellular levels of glycine and GlyT2 enhances the presynaptic glycinergic transmission by providing cytosolic glycine to aid vesicular refill [57]. At the cerebellar nerve endings, an increase in the levels of glycine, results in activation of the GlyT2 receptors in the synaptic vesicles, reverses the action of GABA transporter-1 (GAT1), thereby inducing the release of GABA [58].

Metabolism

Glycine is the smallest known amino acid, with a molecular weight of 75.067 g/mol [59]. Traditionally, it is a nutritionally nonessential amino acid in mammals as it is synthesized endogenously. However, some studies have shown that at certain

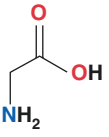
periods, the in vivo synthesis may be insufficient to meet the metabolic demands of an organism, indicating that glycine may be conditionally essential [60, 61]. A chronic glycine insufficiency can adversely affect growth, immune response, health, and nutrient metabolism [62, 63]. According to the European Prospective Investigation into Cancer and Nutrition (EPIC) study, the average daily glycine intake can vary from 2.28 to 3.12 g/day in young adult males in accordance to the dietary protein sources that they consume [64]. The chemical structure of glycine is shown below in Fig. 6.1.

Synthesis

Glycine can be synthesized from serine, threonine, choline, sarcosine (N-methyl glycine), glyoxylate, and as a by-product during the synthesis of L-carnitine. Approximately 2.5 g of glycine/day is produced from serine, probably making it the major contributor to in vivo glycine synthesis [61]. The primary source of serine is through dietary intake, but is also endogenously produced from glucose, especially in the kidneys. Serine is then converted to glycine by serine hydroxymethyl transferase (SHMT) 1 in the cytoplasm and SHMT 2 in the mitochondrial matrix [65]. SHMT expression varies in different tissues and species and has been shown to be the major enzyme for producing glycine to meet the needs of the growing fetus [63]. The cofactors required by SHMT are pyridoxal phosphate and tetrahydrofolate. Mitochondrial enzyme, SHMT2 is ubiquitous and responsible for glycine synthesis in most cell types [59]. Majority of the synthesis occurs in the hepatocytes through SHMT2. The reaction involves transfer of C1 unit from C3 of serine to yield 5,10-methylene tetrahydrofolate and glycine [66]. The produced methyl group is essential in multiple methylation reactions which later on results in regeneration of tetrahydrofolate, provide a constant supply for glycine synthesis [67]. Threonine is not a source of glycine in humans [68] but is an important source in other species like chickens, pigs, and cats [69]. Major sources of 1 carbon units emphasizing glycine synthesis and metabolism are shown in Fig. 6.2 [68].

Choline-dependent glycine synthesis is a minor contributor due to low dietary choline intake. However, the oxidative degradation of choline to glycine provides methyl groups which subsequently is required for cellular methylation processes [41]. The methyl transfer during choline–glycine biosynthesis is from the intermediate metabolites betaine (trimethylglycine), dimethylglycine, and sarcosine (*N*-methylglycine) by cytosolic enzyme betaine-homocysteine S-methyltransferase (BHMT), and the mitochondrial enzymes dimethylglycine dehydrogenase (DMGDH) and sarcosine dehydrogenase (SDH), respectively [59].

Fig. 6.1 Chemical structure of glycine



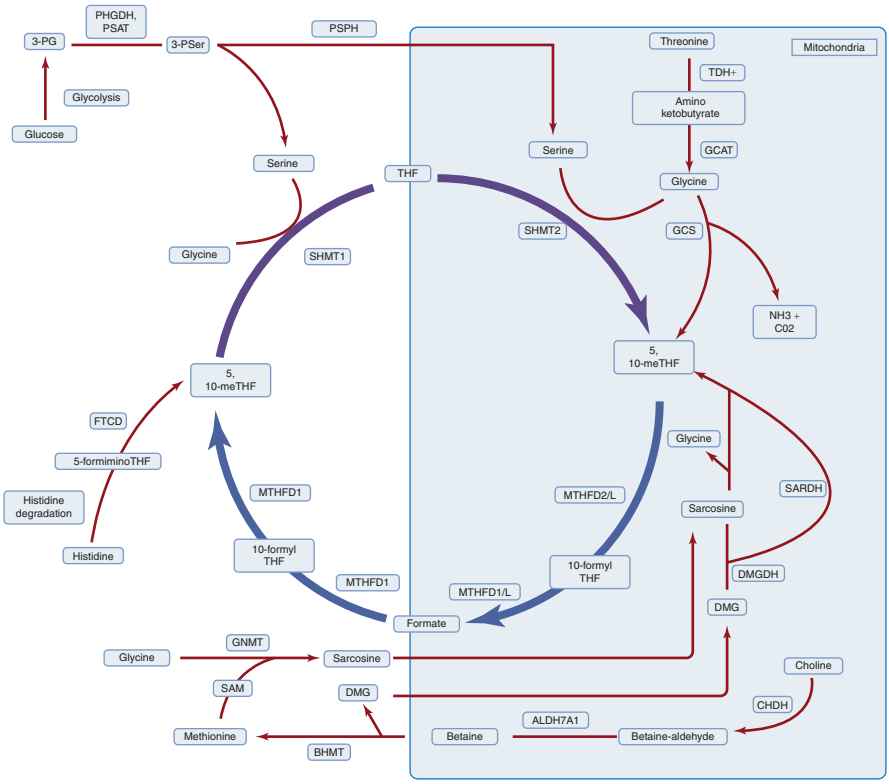


Fig. 6.2 Major sources of 1-carbon units emphasizing glycine synthesis and metabolism [68]. *3PG* 3-phoshoglycerate, *3PSer* 3-phosphoserine, *DMG* dimethylglycine, *TDH* L-threonine dehydrogenase, *GCAT* glycine C-acetyltransferase, *GCS* glycine cleavage system, *SARDH* sarcosine dehydrogenase, *DMGDH* dimethylglycine dehydrogenase, *CHDH* choline dehydrogenase, *ALDH7A1* aldehyde dehydrogenase 7 family member A1, *BHMT* betaine-homocysteine S-methyltransferase, *GNMT* glycine N-methyltransferase, *FTCD* formimidoyltransferase cyclodeaminase, *PHGDH* phosphoglycerate dehydrogenase, *PSAT* phosphoserine aminotransferase, *PSPH* phosphoserine phosphatase

Glyoxylate is another source for the synthesis of glycine through the process of transamination, utilizing alanine as the amino group donor. The reaction is catalyzed by alanine-glyoxylate aminotransferase to produce pyruvate and glycine [70]. The enzyme plays a primary role in the rate-limiting step of oxalate synthesis and is typically found in the peroxisomes [59]. Glyoxylate can be produced as a byproduct of the pentose phosphate pathway in the mitochondria or the breakdown of hydroxyproline and serine [6].

Uptake

The uptake of glycine is carried out by classes of glycine transporters which include SLC36 group in the intestine, SLC6 family in the kidney, intestine, and neural tissue [71]. The SLC38 gene family of glycine transporters is ubiquitously

expressed in the body, especially in rapidly dividing cells as well as in cells actively engaged in amino acid metabolism like neurons, hepatocytes, and cells of the kidney [72].

As mentioned earlier, GlyT1 and GlyT2 are important modulators of neurotransmission. They provide high-affinity transport of glycine and its derivatives and is inhibited by sarcosine [73]. A selective inhibition of GlyT1 increases extracellular glycine levels as well as potentiates NMDA receptor function [74]. GlyT2 has a lower affinity for glycine and functions mainly by modulating the nerve terminal glycine supply required for inhibitory neurotransmission [75].

Degradation

The catabolism of glycine occurs through three pathways [76].

- Glycine cleavage system (GCS)—deamination and decarboxylation of glycine.
- SHMT enzyme—converts glycine to serine.
- D-amino acid oxidase—converts glycine to glyoxylate, though the role of this pathway is insignificant.

When ^{15}N-glycine is orally administered in young adults, the transfer of the nitrogen group was tracked to serine (54%), urea (20%), glutamine or glutamate (15%), alanine (7%), and other amino acids (3–8%—leucine, isoleucine, valine, ornithine, proline, and methionine) [77].

The GCS catalyzes the reversible conversion of glycine into serine, for which it requires tetrahydrofolate or 5,10-methylene tetrahydrofolate as a cofactor. It is a complex enzyme system that is composed of four mitochondrial protein components, three enzymes, and a carrier protein. These include [78] the following.

- P-protein (pyridoxal-containing protein) or glycine dehydrogenase (decarboxylation).
- T-protein or aminomethyl transferase.
- L-protein or dihydrolipoamide dehydrogenase.
- H-protein (lipoic acid-containing carrier protein).

The first step is decarboxylation reaction catalyzed by P-protein by utilizing H-protein as a cosubstrate, producing carbon dioxide and attachment of the decarboxylated glycine to the carrier protein [79]. The decarboxylated aminomethyl moiety attached to the lipoic acid of H-protein is degraded by T protein to produce 5,10-methylene tetrahydrofolate, ammonia, and H-protein with reduced lipoate [80]. In the last step, the reduced form of H-protein is reoxidized by L-protein by using NAD^+ (nicotinamide adenine dinucleotide) as a cosubstrate [78].

Cytosolic SHMT rapidly converts dietary glycine into serine and nearly 50% of 5,10-methylene tetrahydrofolate generated by the GCS is utilized in this reaction [81]. This shows that the GCS and SHMT systems are interconnected in the catabolism of glycine. Approximately 41% of extracellular glycine is directed towards serine biosynthesis in humans [81].

Pharmacology

Drugs Acting on Glycine Receptors

GlyR is activated by glycine, taurine, and β-alanine. These receptors are a target for drugs as well as for some endogenous messenger molecules. Some have direct interactions with the glycine binding site, while some bind to allosteric sites of the receptor complex.

Strychnine

Strychnine is a competitive antagonist of the GlyR as it interacts with the glycine binding site, preventing the inhibitory effects of glycine at the postsynaptic neuron [82]. This selective blocker is a plant alkaloid and a neurotoxin that has been used in early studies to understand the physiological functions and disease pathology of glycine. Strychnine poisoning can cause severe spastic muscle contractions and, less commonly, altered sensory perception like increased sensitivity to auditory or tactile stimuli, as the action potentials are triggered by excitatory neurotransmitters at a lower level. Due to its high selectivity, it is used in physiological experiments to differentiate GlyRs from GABA receptor activity [83].

Ivermectin

Ivermectin is a naturally occurring macrocyclic lactones that belong to the family of avermectins. It is an allosteric modulator or direct activator of glutamate-gated chloride channels which are expressed in the muscles and neurons of nematodes and arthropods [84]. The drug can kill these organisms by paralysis, by enhancing inhibitory neurotransmission; hence is used as an antiparasitic agent in humans [85]. Electrophysiological studies have shown that at submicromolar concentrations (0.39 μM), ivermectin interacts with α_1 and $\alpha_1\beta$ GlyRs [86]. Further studies have shown that substitution of Ala288 (in M3) and Pro230 (in M1) disrupts the sensitivity of GlyRs to ivermectin. While A288G mutation increased the ivermectin sensitivity, A288F substitution abolished the agonistic actions [87]. Ivermectin is not specific for GlyRs as it also exerts allosteric action on other ligand-gated ion channels like α_7 nicotinic acetylcholine and $GABA_A$ receptors [83].

Ethanol

Biochemical and electrophysiological studies have shown that ethanol allosterically potentiates GlyR currents following moderate alcohol intake. The modulating effects occur throughout the central nervous system, including the hippocampus,

hypoglossal nucleus, ventral tegmental area, and the spinal cord [82]. An increase in the postsynaptic glycinergic currents has been observed, indicating that the modulatory effects exerted by alcohol on GlyRs could explain the alterations in motor control and respiratory rhythms associated with ethanol [88]. At concentrations below 100 mM, homomeric α_1 GlyRs (mature neurons) are more sensitive to ethanol than α_2 receptors (neonatal neurons) [89]. Studies have shown that the effects of alcohol on GlyRs can be attenuated by a specific G-protein βγ sequester peptide. It is also shown that the selective modulation of Gβγ, could better explain the differential ethanol sensitivity of α_1 and α_2 receptors [90].

Cannabinoids

The possible role of endocannabinoids and other related molecules as GlyR modulators was first suggested in the year 2005. The direct modulatory effect of the endocannabinoids, N-arachidonoyl ethanolamide and 2-arachidonyl glycerol was reported in the hippocampal neurons. A reduction in the amplitude of glycinergic membrane currents and an alteration in the rise time, desensitization, and deactivation kinetics were reported, depending on the concentration of the endocannabinoids [91]. Later on, endogenous molecules that are structurally related to endocannabinoids which modulated GlyRs like N-arachidonoyl glycine and N-arachidonoyl serine were discovered. Other structurally unrelated molecules like Δ^9-tetrahydrocannabinol (the psychoactive component in marijuana) and synthetic cannabinoid receptor ligands also potentiate or inhibit GlyRs [82]. The allosteric effects of the endocannabinoids were similar in both α_1 and α_2 receptors indicating that the presence of α-subunits was sufficient to produce modulatory effects [92]. However, studies have shown that these molecules lack specificity for GlyRs as they can interfere with the function of other ion channels, including the cannabinoid receptors [93].

General Anesthetics

Volatile anesthetics like isoflurane potentiates homomeric α_1- and α_2-GlyRs [94]. Studies in rat neurons have shown that they potentiate glycinergic currents in the medullary neurons, prolongs the decay kinetics, and increases the postsynaptic membrane currents in trigeminal nucleus and motor neurons of the spinal cord [95–97]. The GlyR interactions of the anesthetic drugs could be one of the mediators that responsible for inducing immobility and depressing the processing of sensory information at the level of dorsal horn in the spinal cord [98, 99]. Though propofol has indicated a potentiating effect at the GlyR, the extent of this modulation is much less in comparison to volatile anesthetics. All the homomeric and heteromeric forms of GlyRs has shown to be equally sensitive to the effects of propofol [100]. However, the binding sites of these drugs on the GlyRs is still not known.

Glutamate

Studies have shown that glutamate, a fast-excitatory neurotransmitter, can allosterically modulate the GlyR. This potentiation by both glutamate and NMDA resulted in an increased single-channel open probability. Glutamate-induced modulation may also provide a rapid feedback mechanism that helps maintain the balance of synaptic excitation and inhibition [101]. The sites on the GlyRs where glutamate interacts are yet to be determined.

Neuroactive Steroids

Endogenous neurosteroids like pregnanolone are cholesterol metabolites produced in the central nervous system that directly interacts with ion channels to induce fast neuronal excitability. Pregnanolone causes significant inhibition of spinal GlyR currents [102]. Synthetic neurosteroids such as minaxolone enhance α_1 receptor and pregnanolone analogues have been shown to potentiate α_3 GlyRs [103, 104]. However, the sites of interaction of these molecules on the GlyRs have not been studied in-depth.

Tropeines

Tropeines like tropisetron are $5HT_3$ receptor antagonists and have shown to allosterically modulate GlyRs with varying subunit composition. While studies have shown GlyR potentiation at nanomolar concentrations of tropeines, inhibition occurred at micromolar concentrations [105]. Further studies determined that the modulatory effects on GlyRs occurred only in the presence of agonist molecules and were dependent on their concentration [106]. It was also observed that tropeines potentiated α_1 and inhibited α_2 GlyRs. The presence of β-subunits enhanced the α_1 potentiation and converted α_2 inhibition to potentiation [107]. They bind to the extracellular domain of the GlyR located close to the agonist binding site [108]. The strong affinity and sensitivity of tropeines for the GlyR, makes it the most promising candidate for the development of drugs that specifically target GlyRs.

Zinc

The cation zinc allosterically modulates GlyR in a dose-dependent manner. The interaction is biphasic, where the glycine currents are predominantly potentiated at lower concentrations (<10 μM) and inhibited at higher concentrations (>10 μM) [109, 110]. Zinc inhibits α_1 GlyR to the greatest degree in comparison to the other receptor isoforms. Zinc increases the affinity of GlyRs to glycine to exert its potentiating action and reduces efficacy to implement the inhibitory effects. Amino acids on the α_1 GlyR that are involved in potentiation by zinc include D80, E192, E194 and those involved with inhibition are H107, H109, T112, and T133 [111, 112].

Gelsemine

Gelsemine is a naturally occurring plant alkaloid that has been shown to elicit antinociceptive effects in animal studies [113]. Studies have shown a biphasic action of the substance on α_1 GlyR, potentiating at lower concentration and inhibiting in higher doses, while all other receptor isoforms are inhibited [114].

Other Allosteric Modulators

The other potent modulators of different GlyR subunits include cyanotriphenylborate, dihydropyridines, ginkgolides, ginkgolic acid, bilobalide, lindane, fipronil, and picrotoxin [83].

Drugs Acting on Glycine Reuptake

Bitopertin

Bitopertin is a selective GlyT1 inhibitor that has been studied since 2014 to determine its efficacy and safety in schizophrenia patients with predominantly negative symptoms who were stable on antipsychotics [115]. The rationale for investigating the drug was its mechanism of action. It inhibits glycine reuptake, thereby increasing its levels at the synaptic cleft. This indirectly may potentiate NMDA receptors, the dysfunction of which is implicated in the pathogenesis of schizophrenia [116]. Though several clinical trials failed to provide satisfying results, no potentially severe side effects were noted [117, 118].

Sarcosine

Sarcosine, also called N-methylglycine is a naturally occurring byproduct in the metabolism of choline to glycine. It is a selective, irreversible inhibitor of GlyT1, causing an increase in the glycine levels and subsequently potentiating NMDA receptors [119]. This modulating effect of sarcosine has made it a drug of interest for the treatment of schizophrenia and depressive disorders [120, 121].

Other Selective Inhibitors

There are multiple selective inhibitors of glycine transporters that inhibit at different half-maximal inhibitory concentrations (IC_{50}), which are mentioned in Table 6.1 [122]. GlyT1 inhibitors increases the activation of NMDA receptors due to increased glycine availability, however, are less likely with GlyT2 inhibitors. This may result in NMDA-associated negative effects on hyperalgesia due to the potential link of NMDA receptors to pain perception and generation of hyperalgesia [123]. Org

25,543 is one of the earliest GlyT2 inhibitors and has been shown to prolong post-synaptic glycinergic currents on the acute application in spinal cord tissue, and the effects diminish drastically with prolonged exposure [124].

Clinical Aspects

The glycinergic system plays crucial role in many vital functions of the body. Hence, there are multiple pathologies that can be associated with the same.

Hyperekplexia

Hyperekplexia or human startle disease is an inherited synaptopathy, which occurs due to mutations affecting the GLRA, GLRB, and SL6A5 genes, which encodes for the α subunits, β subunit of GlyRs and GlyT2 respectively [125]. The GLRA mutation is the major gene of effect; however, the defect has also been identified in the GPHN gene, which encodes the carrier protein gephyrin [126]. The mutations can

Table 6.1 Selective inhibitors of Glycine transporters—GlyT1 and GlyT2 [122]

GlyT1	IC_{50}	GlyT2	IC_{50}
(R)-NFPS (ALX 5407)	0.8–3 nM	O-[2-benzyloxyphenyl-3-flurophenyl] methyl-L-serine (ALX 1393)	–
SSR103800 (structure unavailable)	2 nM	ALX 1405 (Structure unavailable)	–
N-methy-SSR504734	2.5 nM	4-benzyloxy-3,5-dimethoxy-*N*-[1-(dimethylaminocyclopentyl) methyl] benzamide (org 25,543)	20 nM
N-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy) propyl] sarcosine (NFPS)	3 nM	Amoxapine (tricyclic antidepressant)	92 μM
{[2-(4-Benzo [1,3] dioxol-5-yl-2-tert-butylphenoxy) ethyl]-methylamino}-acetic acid (LY2365109)	16 nM	N-arachidonyl-glycine	–
2-Chloro-[*N*-(*S*)-phenyl[(2*S*)-piperidin-2-yl] methyl]-3-trifluoromethyl benzamide (SSR504734)	18–314 nM	–	–
(*N*-[3-phenyl-3-(4′-(4-toluoyl) phenoxy) propyl] sarcosine (NPTS)	37 nM	–	–
R-(−)-*N*-[3-[(4-triflouromethyl) phenoxy]-3-phenyl-propylglycine (org 24,598)	–	–	–
Arachidonic acid	2 μM	–	–
Protons	100 nM	–	–
Zinc	10 μM	–	–

be either recessive or dominant, and a majority of them are loss of function [83]. The disease is characterized by [127]:

- Episodes of generalized stiffness after birth which subsides gradually during the first few years of life.
- Increased likelihood of apnea attacks, delayed speech, and/or intellectual disability.
- Exaggerated startle reflex to sudden, unexpected stimuli that persist throughout life particularly to auditory or tactile stimuli.
- Following these startle responses, patients experience a transient generalized stiffness which can result in serious falls.

The startle reflex is characterized by forceful eye closure, raising flexed arms over the head, followed by flexion of neck, trunk, elbows, hips, and knees while retaining consciousness [128]. Definitive diagnosis can be made by genetic testing, and all forms have been successfully treated with clonazepam, a benzodiazepine that acts by enhancing GABA-mediated neurotransmission [129].

Glycine Encephalopathy

Glycine encephalopathy, also called as nonketotic hyperglycinemia is a severe autosomal recessive disease and the second most common inborn error of metabolism, following phenylketonuria. It occurs due to a defect in the GCS, thereby disrupting glycine catabolism which subsequently results in elevated plasma glycine levels, causing neurological disease [130]. Typically, patients present within the first few days after birth with progressive lethargy, hypotonia, myoclonic jerks, hiccups, and apnea, which, if left untreated, eventually progresses to coma and death [131]. In atypical cases, patients may have nonspecific cognitive or behavioral symptoms, making the diagnosis a challenging task [132]. About 80% of the patients with nonketotic hyperglycinemia have been shown to have deficient glycine decarboxylase or P-protein activity, which is encoded by the GLDC gene [133]. Biochemical tests show elevated levels of glycine in plasma, urine, cerebrospinal fluid, and tissues, especially the brain [41]. Clinical or biochemical studies are not sufficient for diagnosis, and in most cases, a liver biopsy is required for confirmation by enzyme analysis or genetic studies. Due to the complex genetics of the disease, deoxyribonucleic acid (DNA) analysis is a tedious and time-consuming process. More straightforward assays can help diagnosis easier, avoid procedure complications, especially in sick children, and increase the reliability of antepartum detection of the disease [134].

Progressive Encephalomyelitis with Rigidity and Myoclonus (PERM)

PERM is an inflammatory disorder characterized by muscle stiffness, spasms, myoclonic jerks, exaggerated startle reflexes, autonomic dysfunction, cognitive impairment, and brainstem signs like oculomotor dysfunction and altered respiratory

rhythms [135]. It is a more severe variant of the stiff-person syndrome and has a strong association with the occurrence of autoantibodies against α_1GlyR [136]. Though the primary pathology is localized to the central nervous system, no characteristic imaging findings are available, and about 60% of patients show signs of inflammation on cerebrospinal fluid analysis [137]. In general, immunotherapy has been shown to produce significant clinical improvements in affected patients [138].

Temporal Lobe Epilepsy

Temporal lobe epilepsy is the most common form of adult epilepsy and its complex pathology also involves hippocampal formation. GlyT1 regulates the reuptake of glycine in the hippocampus, and mutations resulting in genetic deletion of the transporter can disrupt the electrical balance of the neurons [139]. Animal studies have analyzed GlyT1 dysregulation in chronic temporal lobe epilepsy, which showed an initial downregulation of the transporters followed by pathological overexpression in chronic epilepsy. The suppression of GlyT1 transporters through pharmacological inhibition or genetic ablation in the hippocampus helps in a drastic reduction of both acute and chronic seizure activity [140]. When the extracellular glycine concentration is low, the presynaptic GlyRs are activated, promoting proconvulsant mechanisms [141]. On the other hand, a higher glycine concentration results in a tonic suppression of neuronal excitation due to its interaction with postsynaptic GlyRs [142]. Studies have shown that blocking glycine uptake or transporters resulted in a decrease in the post-synaptic excitatory potentials [143]. Since glycine reuptake is regulated by GlyT1 transporters in the hippocampus, it has become a potential target for therapy. The increased GlyT1 in chronic epilepsy may also affect methylation process of the DNA. The methylation of glycine results in the formation sarcosine, which is an endogenous GlyT1 inhibitor [144]. Therapeutic glycine can increase sarcosine production which can help decrease the occurrences of pathological DNA hypermethylation, control the deleterious effects of GlyT1 overexpression, and halt epilepsy progression [145]. However, the effects of medications are yet to be determined through future research.

Psychiatric Disorders

Glycine is known to potentiate NMDA receptors, and GlyT1 controls the extracellular glycine levels near these receptors. Earlier in the chapter, the modulating function of glycine and the action of GlyT1 inhibitors like bitopertin and sarcosine is discussed. NMDA receptor antagonists elicit schizophrenia-like symptoms; they also dysregulate dopaminergic transmission [146]. Hence, GlyT1 inhibitors were expected to provide additional benefit in patients with schizophrenia; however, no conclusive studies are available to date. These drugs could also be of potential use in other cognitive disorders like obsessive-compulsive disorder and depression

[147]. The α_3 GlyRs mediate inhibitory glycinergic neurotransmission of pain signals to the brain from the spinal cord. The use of glycine transporter inhibitors can increase the availability of glycine, enhancing the neuronal inhibition of GlyRs, thereby suppressing pain signals. Due to the sparse distribution of α_3 GlyRs in the dorsal horn, there could potentially lesser side effects from its potentiation, making a suitable target for treatment of chronic inflammatory pain [148].

The effect of ethanol on GlyRs has been discussed earlier in the chapter. Studies have shown that extra-synaptic GlyRs in the nucleus accumbens play a role in the different phases of alcohol addiction-like initiation, maintenance, and relapse [149]. Some studies have also proposed similar mechanisms of GlyRs in the addictive pathways of nicotine and marijuana [150]. The development of medications that can inhibit the modulating effects of alcohol can be used to treat acute intoxication or addiction therapy.

Conclusion

Glycine is the simplest amino acid and functions in the central nervous system as an inhibitory neurotransmitter and a coagonist at the NMDA receptors. Though the molecule has been extensively studied, the clinical and pharmacological aspects of the molecule is still an area of ongoing research. Future studies are required to assess the function of glycine and its action through various receptors and its role in pathological conditions. The therapeutic role of the glycinergic system also requires large population studies and clinical trials for developing effective medications for future use.

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Chapter 7
Histamine

Sireesha Murala, Mahesh M. Thakkar, and Pradeep C. Bollu

History of Histamine

Histamine is one of the neurotransmitters that was studied extensively in the central nervous system (CNS) and peripheral nervous system (PNS). Histamine is involved in numerous physiological and pathological processes, like learning, homeostasis, sleep–wake cycle, and synaptic plasticity [1, 2]. Windus and Vogt are credited for chemically synthesizing histamine, whereas Ackermann isolated histamine as a metabolite product of bacterial histidine fermentation [3, 4]. Subsequently, several researchers, including Dale, Laidlaw, Popielski, Lewis and Grant, contributed toward elucidating the chemistry and the structure of histamine [5–7]. However, it was Best and later Feldberg who first discovered and documented the physiological properties of histamine [8, 9]. It was in 1952 when Riley and West discovered that mast cells are the main source of histamine [10]. In 1937, Bovet identified the first potent histamine H1 antagonists, for which he was awarded the Nobel Prize in Physiology and Medicine in 1957. The subsequent landmark discovery is attributed to Black, who discovered the H2 receptor and was awarded the Nobel Prize in

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Table 7.1 Historical highlights of last century in regard to histamine, histamine receptors and antihistamines [11]

1907—Histamine synthesis by decarboxylation of histidine described
1910–1911—Physiologic and pathologic effects of histamine described
1937—H1-antihistamines synthesized
1942—H1-antihistamine introduced for clinical use
1943—Adverse CNS effects of first generation H1-antihistamines reported
1955—Antiallergic effects of H1-antihistamines described
1981—Second-generation nonsedating H1-antihistamines introduced for clinical use
1986—Cardiactoxicity of H1-antihistamines announcement
1991—H2-receptor cloning was reported
1993—H1-receptor cloning reported
1999—H3-receptor cloning reported
2000—H4-receptor cloning reported

Physiology and Medicine. The introduction of H2 receptor antagonists revolutionized the treatment of peptic ulcer disease. In 1981, second generation of nonsedating H1 antihistamines were introduced for clinical use. Histamine H4 receptor was discovered followed by cloning of H2, H1 and H3 receptors. Please see Table 7.1 for detailed historical highlights [11, 12].

Neurochemical Profile

Tuberomammillary Nucleus and Histamine Pathways

The tuberomammillary nucleus (TMN) and adjacent areas within the posterior hypothalamus are the primary sources of histamine in the CNS and provide histaminergic innervation to the entire brain [13]. The TMN consists of several dense clusters of large, characteristic neurons, as well as scattered neurons with the same morphology and staining properties in surrounding, more heterogeneous regions. The TMN is localized rostral to the mammillary bodies and caudal to optic chiasm forming the floor of the third ventricle in the posterior hypothalamus [14]. The distribution of histamine in the brain is shown below in Fig. 7.1.

In all mammalian species examined, including humans, the histaminergic neurons are mainly localized within the TMN and/or surrounding regions. In addition to histamine and its synthesizing machinery, some TMN neurons also contain glutamate decarboxylase (GABA synthesizing enzyme), adenosine deaminase (an enzyme involved in the degradation of adenosine; only in rats but not in mice), galanin, substance P, and pro-enkephalin–derived peptides [15–17]. The TMN neurons target almost all major regions of the central nervous system, especially the wake-promoting basal forebrain and the orexinergic lateral hypothalamus, and

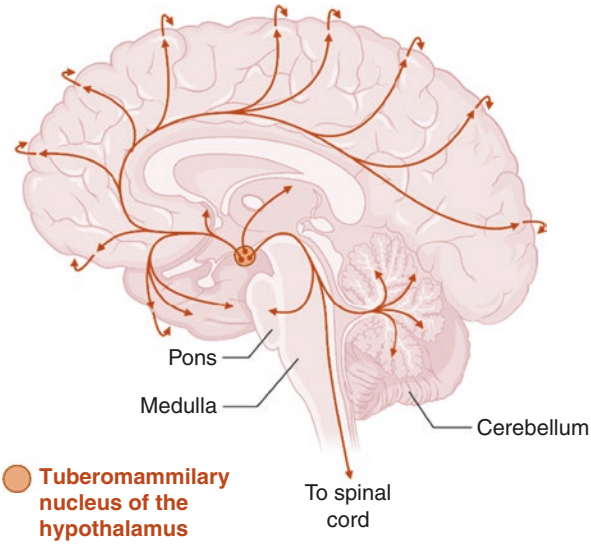


Fig. 7.1 Distribution of histamine in the brain

Table 7.2 CNS functions of histamine

Attention
Learning
Memory
Wake–sleep cycle
Appetite regulation
Excitation

receive strong galanin and gamma amino butyric acid (GABA) inputs from the sleep-promoting regions [14].

TMN neurons have a regular slow firing pattern at 1–4 Hz in separated neurons, even in the absence of synaptic activation. Histamine synthesis, release, and neuronal firing are controlled through feedback inhibition through H3 presynaptic and somatodendritic autoreceptors [13, 18]. TMN neurons have a state-dependent activity by increasing their firing during wakefulness and decreasing the firing before sleep, and resuming right before awakening from sleep [14, 19]. Functions of histamine in the CNS are shown below in Table 7.2 [20].

TMN neurons provide axons to all the CNS areas through two ascending pathways and one descending pathway [13]. One of the ascending pathways travels through the ventral surface of the median eminence to the hypothalamus, the diagonal band, the septum, the olfactory bulb, hippocampus, and cortex. The other ascending pathway leaves the TMN dorsally and runs along the third ventricle to the thalamus, basal ganglia, hippocampus, amygdala, and cortex. The descending pathway travels along with the medial longitudinal fasciculus to the brain stem and spinal cord [16, 21, 22]. The anatomical sites of the CNS affected by the histamine are shown below in Table 7.3 [20].

Table 7.3 Anatomical sites of the CNS affected by histamine [20]

Site	Effect
Dorsal nucleus	Excites serotonergic neurons (↓ serotonin leads to depression)
Basolateral amygdala	Affects emotional memory
Paraventricular nucleus	Can affect appetite via norepinephrine modulation and controls oxytocin secretion
Magnocellular basal nucleus	Affects cognition via modulation of acetylcholine secretion
Orexin	Affects alertness
Locus caeruleus	Can affect alertness via modulation of norepinephrine secretion

Table 7.4 Effects of histamine in the hypothalamus [13]

Effect	Mechanism (Receptor)
Hypothermia	Activation of warm-sensitive GABAergic neurons of the medial preoptic area (H1)
Decreased food intake	Activation of glucose-responsive neurons of the ventromedial nucleus (H1)
Stress response	Activation of CRH neurons in the paraventricular nucleus (H1)
Vasopressin release	Promotion of interneuronal coupling and burst discharge of neurons in the supraoptic nucleus (H1)
Prolactin release	Inhibition of tuberoinfundibular dopaminergic neurons (H2)

CRH corticotropin-releasing hormone

Hypothalamus is the most densely innervated area by histaminergic fibers in the CNS. Effects of histamine in the hypothalamus are shown below in Table 7.4 [13]. The dense network of histaminergic fibers is also evident in periventricular, medial preoptic, suprachiasmatic, supraoptic and supramammillary nuclei. The fibers are less dense in the dorsomedial, ventromedial, paraventricular, arcuate nuclei, ventral tegmentum, and dopaminergic nuclei. Moderate innervation is seen in olfactory nuclei, nucleus accumbens, posterolateral thalamic nuclei, anterior parts of the dorsal striatum, periventricular, paraventricular nucleus, medial habenula, medial geniculate nucleus, and in some subregions of the amygdala. Lower density is seen in the lateral geniculate nucleus, lateral habenula, and in further thalamic nuclei [16].

Through an anterior and a posterior pathway, histaminergic fibers reach the hippocampus to achieve a moderate density in the basal parts of subiculum, cornu ammonis, and dentate gyrus. Moderate histaminergic innervations are observed in the mesencephalic reticular areas of the reticular activating system and the aminergic nuclei, which are moderately innervated. In contrast, few neurons in pontine central gray receive histaminergic projection [16].

Histamine in the Periphery

Histamine is present in the cells of neuroepithelial and hematopoietic origin to serve the following purposes [16].

- Vasodilatation (vascular).
- Immunomodulation.
- Gastric acid secretion.
- Epithelial and endothelial barrier control.
- Smooth muscle contraction (bronchial).

Histamine targets parasympathetic nerve endings in the periphery and is commonly released from glomus cells of the immune system, enterochromaffine cells, mast cells, gastrointestinal system, and chemosensory system [16, 23].

Histamine in Gastrointestinal System

Histamine mobilization from the enterochromaffin-like cells of the stomach is controlled by vagal nerve, via its action on gastrin. Histamine, via its action on H2 receptor, activates the proton pump in parietal cells to regulate gastric acid secretion [16, 24–26]. Although H2 receptor antagonists are commonly used in the treatment of peptic ulcer disease, all the histamine receptors (H1–H4) have excitatory actions on the enteric neurons of the gastrointestinal nervous system [16, 27]. Histamine released from the mast cells plays a vital role to elicit an immune response against gut microbiota, gastrointestinal infection, inflammation, and tumorigenesis [16].

Histamine in Immune System

Histamine plays a key role in allergy and inflammation, both innate and acquired immunity and in immunomodulation and autoimmunity [28–30]. Immune signals regulate histamine signaling and function resulting in the modulation of interferon and cytokine functions [16]. Mast cells have a key role in immunological and allergic responses in the CNS and periphery, thus acting as gatekeepers between the nervous and immune systems [16, 31]. Under pathological conditions, mast cells rapidly enter the brain. Histamine released from mast cells into the median eminence might affect the hypothalamic neurons and thus might also be involved in endocrine and homeostatic regulation [16].

Histamine regulates the neuronal activity in both adrenal gland and sympathetic ganglia. Cutaneous itch is mediated by the C-fibers, which are insensitive to mechanical stimulation, but responds to pruritogens like histamine (seen in urticaria) [16].

Histamine Receptors

Histamine exerts its actions through four subtypes of guanine (G) nucleotide protein coupled receptors (GPCR), which are H1, H2, H3, and H4 [1, 13, 16]. All histamine receptors have seven large transmembrane elements with prototypic domains that

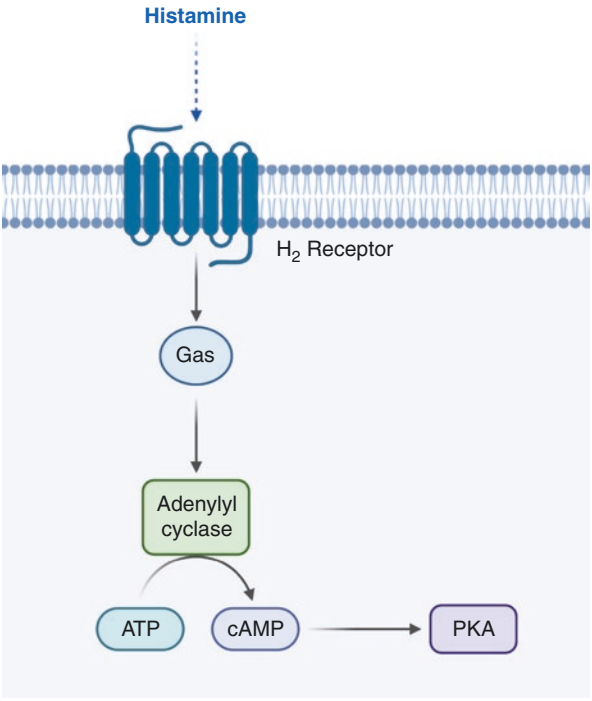


Fig. 7.2 Histamine receptor activation pathway

determines agonist binding, specificity and activation [16]. Several histamine receptors are expressed at both presynaptic and postsynaptic sites. Histamine receptors on presynaptic sites act as either auto or heteroreceptors and provide feedback regulation of neurotransmitter release into the synaptic cleft, shown below in Fig. 7.2 [1]. Histamine receptor subtypes, and their expression and physiological relevance, are shown below in Table 7.5 [2, 11].

Histamine receptors can have excitatory or inhibitory effects depending on the neurotransmitter released and subsequent downstream pathways involved in synaptic plasticity, neuroprotection, or cell death [1, 13]. H1 and H2 receptors are predominantly expressed postsynaptically, while H3 receptors are expressed presynaptically. H4 mainly occurs in peripheral tissue. H1 and H2 receptors have lower affinity for histamine than H3 and H4 receptors [1, 16].

H1 Receptor

H1 receptors are excitatory receptors, coupled with G_q-type proteins, and activate phospholipase C, resulting in an increase of intracellular calcium and reduction in potassium conductance [1, 13]. Histamine acts via H1 receptors in physiological activities like food intake, sleep–wake regulation, bronchoconstriction, and vasodilation [2].

Table 7.5 Histamine receptor subtypes and their expressions [2, 11]

Receptor subtype	GPCR signaling	Expression	Physiologic relevance
H1-receptor	G_q/G_{11} family to phospholipase C stimulation	CNS neurons, CVS, eosinophils, monocytes, neutrophils, macrophages, DCs, T and B cells, smooth muscle cells (vascular, respiratory, and GI), epithelial cells, endothelial cells	Bronchoconstriction, vasodilation, food intake, sleep–wake regulation
H2-receptor	G_s family to adenylate cyclase stimulation and ↑cyclic AMP	Gastric parietal cells, CVS, CNS, smooth muscle, eosinophils, neutrophils, macrophages, monocytes, DCs, T and B cells, epithelial cells, endothelial cells	Gastric acid secretion
H3-receptor	$G_{i/o}$ family to adenylate cyclase inhibition and ↓ cyclic AMP	CNS and peripheral neurons, lungs, CVS, eosinophils, monocytes, endothelial cells	Neurotransmitter release (sleep–wake regulation, attention/cognition, food intake)
H4-receptor	$G_{i/o}$ family to adenylate cyclase inhibition and ↓ cyclic AMP	Eosinophils, monocytes, neutrophils, basophils, mast cells, T cells, DCs, Langerhans cells, bone marrow, fibroblasts, endocrine cells and CNS	Immune responses (chemotaxis, interleukin (IL) and interferon (IFN) modulation)

AMP adenosine monophosphate, *CVS* cardiovascular system, *CNS* central nervous system, *DC* dendritic cells, *GPCR* G-protein-coupled receptor, *GI* gastrointestinal

H2 Receptor

H2 receptors are postsynaptic, G_s protein-coupled receptors. Activation of these receptors result in the stimulation of adenylyl cyclase and triggers cyclic adenosyl monophosphate (cAMP) -dependent activation of protein kinase A, which inhibits calcium activated potassium channels [1, 13]. Histamine acts via H2 receptors in the secretion of gastric acid in the stomach [2].

H3 Receptor

H3 receptors act as presynaptic or somatodendritic inhibitory receptors, which are coupled with G_i-type proteins, to inhibit adenylyl cyclase and subsequently decreasing cAMP levels [1, 13]. Somatodendritic H3 autoreceptors inhibit the firing of TMN neurons, while presynaptic H3 autoreceptors inhibit histamine release from TMN axon terminals. Presynaptic H3 heteroreceptors inhibit the release of other neurotransmitters like serotonin, acetylcholine, norepinephrine, and γ-aminobutyric acid (GABA), thus helping mediate the synaptic cross talk between histaminergic

and other neurotransmitter systems [13, 16]. Histamine acts via H3 receptors in physiological activities like food intake and sleep–wake regulation and in maintaining attention/cognition [2].

H4 Receptor

H4 receptors are coupled with G_i-type proteins, to inhibit adenylyl cyclase and subsequently decreasing cAMP levels. The downstream pathways and pharmacology are similar to H3 receptors. H4 receptors are mainly expressed in the periphery like blood, spleen, lung, liver, and gut [1, 16]. Histamine acts via H4 receptors to induce chemotaxis through different cell types, especially interleukin (IL) and interferon (IFN) modulation [2].

Ionotropic Receptors

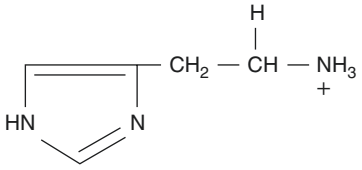
Ionotropic receptors activity is also modulated by histamine [13]. Histamine activates chloride conductances in hypothalamus and thalamus. Histamine promotes NMDA receptors to enhance excitatory transmission via their polyamine modulatory site [16].

Histamine Metabolism

Histamine is synthesized from the precursor histidine by the action of the histidine-decarboxylase, which uses pyridoxal 5′-phosphate as a cofactor [32]. The chemical structure of the histamine is shown below in Fig. 7.3 [18].

Histidine is taken up by the brain through L-amino acid transporters and the bioavailability of histidine determines the rate of histamine synthesis, shown in Fig. 7.4 [13]. Histamine is stored in synaptic vesicles via the vesicular monoamine transporter (VMAT-2), which is released by exocytosis. Histamine is inactivated in the extracellular space by neuronal histamine N-methyltransferase (HNMT) with production of tele-methylhistamine, which goes through oxidative deamination by monoamine oxidase B to tele-methyl-imidazole acetic acid [33].

Fig. 7.3 Chemical structure of histamine



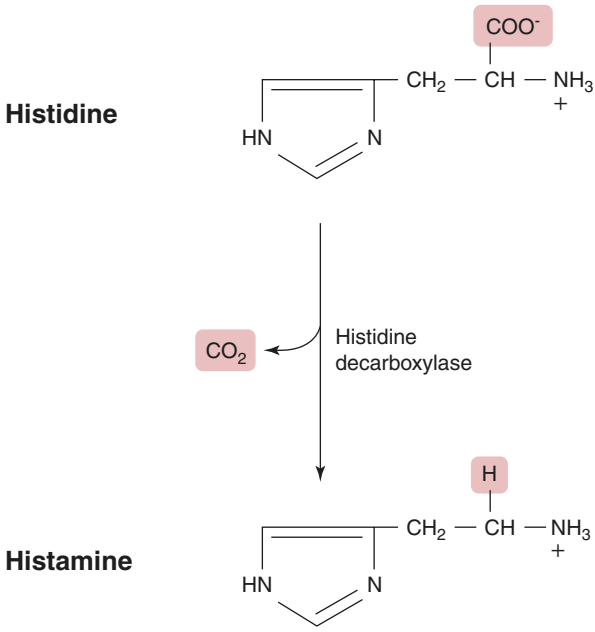


Fig. 7.4 Synthesis of histamine

Histamine synthesis and release are regulated by feedback inhibition via H3 presynaptic and somatodendritic autoreceptors [13]. Brain histamine levels are contributed through the mast cells within the meninges, circumventricular organs and along the blood vessels. Histamine released from the mast cells and blood borne histamine is degraded by the cerebral microvessels expressing HNMT [16, 34]. Among peripheral tissues, (connective tissues, gut) histamine is mainly degraded through diamine oxidase (DAO) enzyme, which transforms histamine to imidazoleacetic acid. Although DAO activity in CNS is low under normal circumstances, it plays a major role when HNMT is inhibited [16].

Medications Acting via Histaminergic System

Medications acting via histaminergic system are discussed below. Four different subtypes of histamine receptors and their main agonists and antagonists are shown below in Table 7.6 [35].

H1 Receptor Antagonists

Histaminergic neurons in the TMN of posterior hypothalamus project profusely throughout the brain, including the vestibular nuclei (VN) complexes. Central histaminergic system is involved in controlling the vestibular functions and

Table 7.6 Four subtypes of histamine receptors and their main agonists and antagonists [35]

Receptors	Agonists	Antagonists
H1	2-Methylhistamine 2-Thiazolyl-ethylamine	Diphenhydramine Promethazine Meclizine
H2	Imipromidine Dimaprit	Cimetidine Ranitidine Zolantidine
H3	α-Methylhistamine	Betahistine Thioperamide
H4	4-Methylhistamine	Thioperamide JNJ 7777120

vestibule-hypothalamic loop plays a major role in the process [35]. H1 receptor antagonists are widely used for the prevention and treatment of central symptoms of vertigo. Commonly used medications from this class include diphenhydramine, meclizine, promethazine, dimenhydrinate and astemizole. They inhibit the signaling pathway transduction through histaminergic neurotransmission from vestibular nuclei to the medullary vomiting center [36].

Betahistine works as a potent histamine H3 antagonist and also as partial histamine H1 agonist [35]. Betahistine affects the histamine release by inhibiting the negative feedback loop and increases the internal ear blood circulation, thus the vasodilatory action improves vestibular function [36].

Chlorpheniramine, acts both centrally and peripherally as H1 receptor inverse agonist and fexofenadine is a peripherally acting H1 inverse agonist, would decrease the mechanical hypersensitivity, in the process of developing neuropathic pain. Thus both blood brain barrier penetrating and poorly penetrating H1 antihistamines are found to block histamine-induced mechanical hypersensitivity, in neuropathic pain patients [1].

H2 Receptor Antagonists

H2 receptor antagonists like cimetidine, ranitidine, zolantidine, and famotidine are widely used in the treatment plan for patients with gastroesophageal reflux disease (GERD), gastric or duodenal ulcers and dyspepsia. Few studies have reported a positive therapeutic effect of oral famotidine in schizophrenia patients [2]. Meskanen et al. conducted a double-blinded, placebo-controlled study with famotidine in treatment-resistant schizophrenia and found a statistically significant improvement on both positive and negative symptom scales [37].

Ranitidine, a centrally permeable H2 receptor antagonist was found to improve the mechanical hypersensitivity associated with neuropathic pain and hence generates the analgesic effect in neuropathic pain patients [1].

H3 Receptor Antagonists

Many clinical trials on H3 receptor antagonists have progressed to phase II efficacy trials for conditions like allergic rhinitis, cognitive disorders in Alzheimer's disease, schizophrenia, attention deficit hyperactivity disorder (ADHD), and sleep disorders. Commonly used antagonists were imidazole based, which include thioperamide, clobenpropit, and ciproxifan. Both thioperamide and clobenpropit have a strong affinity for H4 receptors, while thioperamide is active at $5HT_3$ receptors [2].

Pitolisant, acts on histamine H3 receptors as antagonist/inverse agonist and is the first H3 receptor-targeting compound in clinical use. Pitolisant (1-piperidine, hydrochloride derivative), received an orphan designation from the committee for Orphan Medicinal Products of the EMA in 2007. Pitolisant was approved by the EMA in 2016 as a first-in-class H3 inverse agonist for the treatment of narcolepsy with or without cataplexy in adults [38]. Narcolepsy causes excessive daytime sleepiness and is seen in some neurological disorders like Parkinson's disease. Pitolisant, has an ideal pharmacokinetic profile, given as a once-a-day dose helps in maintaining attention throughout the day and helps patients to sleep at night time. A retrospective study, conducted by Leu-Semenescu et al., [39], reported the benefits of pitolisant in individuals with idiopathic hypersomnia and narcolepsy refractory to stimulants like modafinil [2].

Blocking of H3 receptors causes analgesia, by modulating the levels of histamine in the CNS, as the histamine synthesis is activated by the depolarization in nerve endings, which is regulated by H3 autoreceptors. H3 receptor inverse agonists, analgesic effects in neuropathic pain are partly mediated by α2 adrenoceptor desensitization in the locus coeruleus and spinal cord. Further studies of selective H3 receptor antagonists would provide evidence for the improvement of mechanical and cold sensitivity associated neuropathic pain. These agents not only modulate the levels of histamine but also modulate the release of different neurotransmitters like acetylcholine, noradrenaline, dopamine, and substance P [1].

H4 Receptor Antagonists

Histamine H4 receptors are the new therapeutic target for conditions like asthma, host defense, allergy, neuropathic pain, autoimmune diseases and in cancer. 4-methylhistamine is the first selective H4 receptor agonist. H3 receptor antagonist thioperamide also acts as an antagonist at H4 receptor. H3 agonists imetit and immepip are also H4 receptor agonists [38].

The first highly selective H4 receptor antagonist investigated in acute and chronic inflammation is JNJ 7777120 [1]. JNJ 7777120 acts as an antagonist at G protein-dependent signaling and acts as an agonist in a non–G protein-dependent manner to

recruit β-arrestin to the receptor. Although JNJ 7777120 acts as a partial antagonist at H4 receptor, because of its short half-life and toxicity have prevented the clinical development [38]. H1 antihistamine diphenhydramine along with JNJ7777120 produced a total ablation of the histamine-induced pruritic response [2]. JNJ7777120 reported a reduced mechanical hypersensitivity after systemic administration in neuropathic pain. Selective H4 receptor antagonist, TR-7, evoked a strong analgesic effect after a single dose systemically and was found to be as potent as morphine, which is the choice of drug in pain management [1].

Seliforant, an investigational H4 receptor antagonist, is the first to enter clinical trials in 2010 for seasonal allergic rhinitis. It is currently being investigated in Phase II clinical trials, for acute unilateral vestibulopathy treatment [38].

Adriforant, an investigational H4 receptor antagonist, was originally developed by Pfizer UK. Adriforant is presently in Phase II clinical trials for moderate to severe atopic dermatitis treatment. Interpretation with caution is necessary in experimental findings on the role of H4 receptors [38].

Other Clinical Aspects

Histamine in Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disorder characterized by extracellular amyloid plaque deposits comprising of amyloid-beta peptide and intracellular neurofibrillary tangles composing of hyperphosphorylated tau protein. Cognitive decline in Alzheimer's disease is mainly due to a deficit in cholinergic neurotransmission because of continuous cholinergic neuronal degeneration. There is evidence that neuronal histamine contributes to the progression and maintenance of cognitive deficits in Alzheimer's disease. In patients with pathological changes of histaminergic system are highly predictive of causing cognitive deficits [40].

Grove et al., conducted a randomized, double-blind, placebo-controlled study in patients with mild to moderate Alzheimer's disease were treated with H3 receptor antagonist GSK239512 and found that it had improved episodic memory performance but had no effect on executive functions and working memory. They concluded that H3 antagonists have moderate and selective effects on cognition in patients with mild to moderate Alzheimer's disease [41]. GSK239512 was found to improve cognitive function in a small sample of Alzheimer's patients with mild to moderate disease, by using an increasing dose titration. Recent reviews suggested that histaminergic medications that interact with many targets can be useful in Alzheimer's disease treatment. More clinical trials are necessary to target the neuronal histaminergic system in the treatment of cognitive symptoms [40].

Histamine in Cognitive Disorders

Impaired cognitive functions are seen in neuropsychiatric diseases, ADHD and schizophrenia. Blockade of H3 receptors indirectly increases histamine-mediated attention in cognitive disorders like Alzheimer's disease and ADHD. In schizophrenia patients, along with histaminergic and dopaminergic hyperactivity, postsynaptic H3 receptors also show the activation of striatal dopamine D2 receptors in causing the disease symptoms, supporting that H3 receptor antagonists as antipsychotics along with traditionally used neuroleptics in the treatment. H3 receptor antagonists also induced cognitive enhancing effects at lower doses than usually needed to produce a strong wake enhancement [42, 43].

Neuronal histaminergic system is influenced in both neuropsychiatric and neurodegenerative disorders include Alzheimer's disease, addiction, Gilles de la Tourette syndrome, Parkinson's, and Huntington's disease [40].

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 8
Melatonin

Sireesha Murala, Elanagan Nagarajan, and Pradeep C. Bollu

History of Melatonin

In 1958, Aaron Lerner discovered melatonin or *N*-acetyl-5-methoxytryptamine from bovine pineal extracts [1]. In the 1960s, both Axelrod and Fiske et al. demonstrated that melatonin is synthesized in pinealocytes and is regulated through ambient light in humans, transmitted via a neural pathway from the retina to end in the sympathetic neurons of the superior cervical ganglion [2–4]. In 1965, Hoffman and Reiter proved that the gonadal changes in rodents vanished on exposure to darkness after pinealectomy [5].

In 1965, Axelrod and Wurtman coined the term "neuroendocrine transducer" and also introduced the concept of "melatonin hypothesis," reporting that in reaction to environment light fluctuations, the pineal gland isolates the melatonin hormone and also alters the reproductive functions in humans [6]. Axelford, for his contributions in understanding the monoamines of the CNS and presynaptic norepinephrine reuptake system, was awarded the Nobel Prize for Medicine and Physiology in 1970 [4].

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In 1975, Lynch HJ established that melatonin secretion from pineal glands follows the circadian rhythm [7]. In 1993, Poeggeler B demonstrated the antioxidant effect of melatonin [8]. In 1995, Arendt demonstrated that melatonin displays a rhythmic pattern of synthesis and secretion so that its plasma concentrations are low (10–20 pg/mL) during the day and at night, reaching a remarkable high (80–120 pg/mL), with a fine rise through 24 and 03 h [9].

Neurochemical Profile

Melatonin synthesis and secretion are suppressed by light and intensified by darkness. The circadian profile of the plasma melatonin concentrations is shown below in Fig. 8.1 [10]. The light or luminous data is transmitted through the retina to the pineal gland via the suprachiasmatic nucleus (SCN) of the hypothalamus (see Fig. 8.2), which is the "master circadian clock" [10, 11]. SCN, at night, sends a signal to the pineal gland to discharge norepinephrine (NE) from the post-ganglionic sympathetic neurons toward the pinealocytes to begin and maintain (achieved by β_1-adrenergic receptors) the increased melatonin synthesis [11, 12]. The synthesis of melatonin through the neural pathway is shown below in Fig. 8.3 [13].

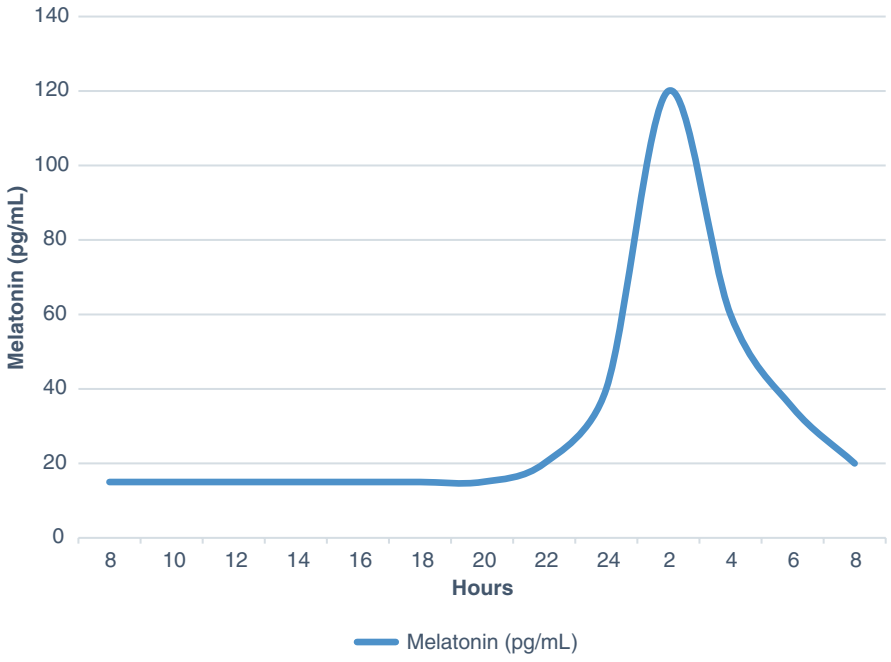

Fig. 8.1 Circadian profile of plasma melatonin concentrations [10]. Note: This is a depiction of melatonin concentrations in the evening hours based on published data and not plotted based on original research by us

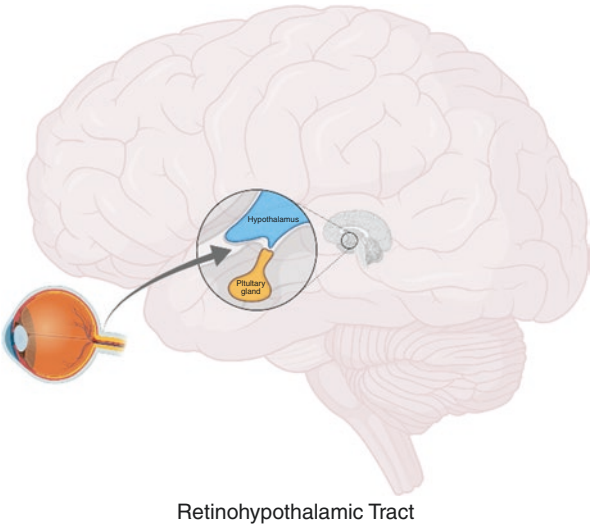

Fig. 8.2 Retinohypothalamic tract carries the visual information from the retina to the hypothalamus. Activation of the suprachiasmatic nuclei of the hypothalamus exerts an inhibitory influence on melatonin release

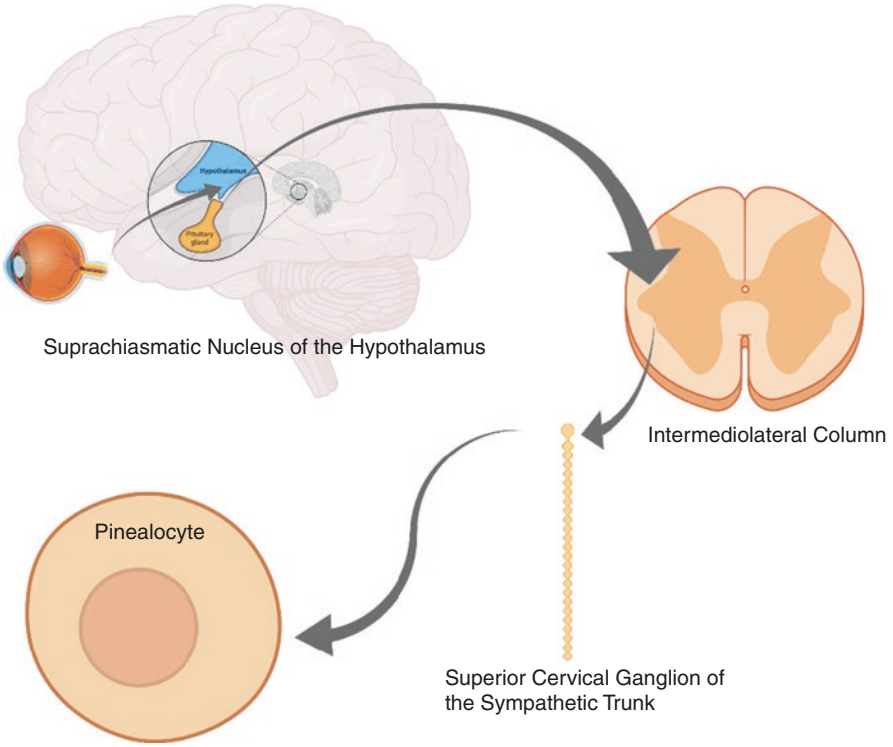

Fig. 8.3 Neural pathway of melatonin synthesis [13]

Table 8.1 Unique features of pineal gland's endocrine function [11]

• Although the synthesis and release of melatonin are controlled by the neural input, other hormones do not influence it
• Pineal gland doesn't store the remaining quantity of melatonin while most of the endocrine organs store huge amounts of the hormones they manufacture
• Both receptor-mediated and receptor-independent actions are attributed to melatonin

Table 8.2 Melatonin and its effects

Nonpineal melatonin local effects are seen on [18] the following:
• Immune system, inflammation
• Metabolism
• Gut function
• Mitochondrial function, membrane fluidity, apoptosis
• Direct antioxidant properties

Neurons between the SCN to the pineal gland are exceptionally intricate, which also comprise neuronal projections from the SCN to the paraventricular nucleus (PVN). PVN axons travel via the brain stem to the intermediolateral cell column (ILCC) in the upper thoracic spinal cord to synapse on the preganglionic sympathetic neurons. These ILCC axons ascend and pass along the sympathetic trunk to relay in the superior cervical ganglia. Nervi conarii, a discrete bundle of nerves is formed in the tentorium cerebelli by the post-ganglionic sympathetic neuronal axons on the pineal gland; these neuronal fibers thus enter the pineal gland to terminate on the pinealocytes [11, 14]. Table 8.1 highlights some unique features of pineal gland's endocrine function.

Infants secrete low levels of melatonin by 3 months of age, which gradually increases as the child develops to form a circadian rhythm. Peak concentrations of melatonin during nights are highest between ages of 4 and 7, which later decline gradually [10, 15].

Suppression of Melatonin synthesis from the pineal gland is achieved through the retinal ganglion cells, which detect and transduce the particular shorter wavelengths (420 nm) of light [16]. Melanopsin responds to blue wavelengths (460–480 nm), which controls the SCN and inhibits the melatonin synthesis [17]. Melatonin effects in various systems and pathologies are shown below in Table 8.2 [13].

Melatonin and Sleep

Melatonin regulates the sleep–wake cycle and a variety of neuroendocrine rhythms (Tables 8.2 and 8.3). This close relationship between the melatonin secretion and circadian rhythm is seen even in blind people without any light perception [10, 19].

Melatonin acts on the SCN to diminish the wake-promoting signals of the circadian clock, thus inducing sleep. Default mode network (DMN) is a mesh of brain areas that are active at rest and comprise of posterior cingulate cortex, medial

Table 8.3 Melatonin effects on various body systems and their physiological effects [13]

Body system	Physiological effect
Sleep modulator	Treatment of circadian rhythm sleep disorders (jet lag and phase shift)
Psychiatric	Antidepressant, antineophobic (fear of anything new), anxiolytic, drug addiction treatment
Central nervous system	Neuroprotective, antiseizure effect in children, pain modulation, anti-inflammatory, brain edema treatment, regulating memory formation
Endocrine system	Ovarian physiology, seasonal reproductive function, regulating reproductive hormone release, type 2 DM, osteoblast differentiation
Autoimmune diseases	Type 1 DM, multiple sclerosis, SLE in females, IBD, autoimmune hepatitis, rheumatoid arthritis
Cardiovascular system	Cardiac syndrome X, antihypertensive
Locomotor system	Locomotor activity–regulating, antinociceptive
Oncology	Antitumor
Other	Antioxidant, hepatoma, retinal, sepsis, pineal calcification

DM diabetes mellitus, *IBD* inflammatory bowel disease, *SLE* systemic lupus erythematosus

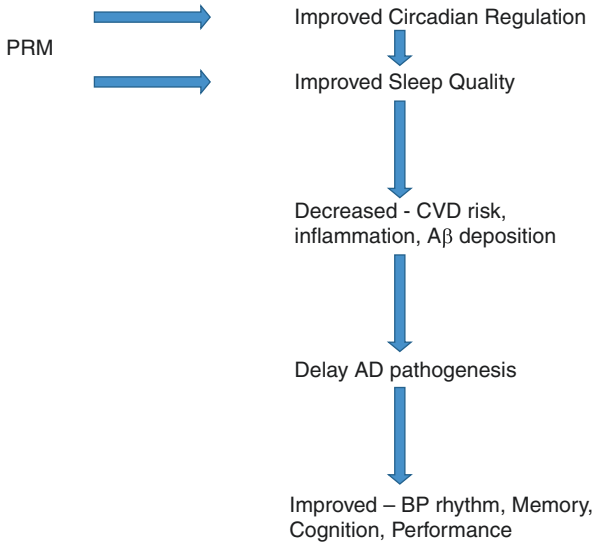

Fig. 8.4 Melatonin mechanism of effects on circadian clocks, sleep, and neurodegeneration in Alzheimer's disease [19] *Aβ, deposition* amyloid-β deposition, *AD* Alzheimer's disease, *BP* blood pressure, *CVD* cardiovascular disease, *PRM* prolonged-release melatonin

prefrontal cortex, inferior parietal lobe, lateral temporal cortex, precuneus, and the hippocampal formation and are pertained to mind wandering and interoceptive awareness. Melatonin acts on the DMN areas of the brain to increase fatigue and induce sleep-like changes with precuneus activation [19, 20].

Atypical circadian rhythms and disrupted sleep–wake cycles are often associated with an increased risk of metabolic, cardiovascular, and cognitive disorders. The sleep–wake cycle regulates the pathogenic amyloid-β peptide levels in the brain, inflammation, and blood pressure rhythms. Melatonin may slow down the AD-related pathology through its desirable effects on the sleep–wake cycle and circadian clock functions. The relationship between melatonin, circadian clocks, sleep, and neurodegeneration is shown in Fig. 8.4 [19, 21].

Melatonin Receptors

Melatonin exhibits its effects via four different mechanisms which are as follows [13]:

- Binding to melatonin receptors on the plasma membrane.
- Binding to intracellular proteins (calmodulin).
- Binding to orphan nuclear receptors.
- Antioxidant effect.

Melatonin exerts its actions through activation of two guanine (G) nucleotide protein-coupled receptors (GPCR), which are melatonin receptor type 1 (MT1) and melatonin receptor type 2 (MT2); GPCR receptors have seven large transmembrane domains [11]. Melatonin receptors and their characteristics are shown below in Table 8.4 [10, 13, 22].

Melatonin receptors are found in various organs of the body, which include the retina, brain, aorta, ventricular wall, cardiovascular system, cerebral and coronary arteries, duodenal enterocytes, colon, cecum, appendix, jejunum, liver and gall bladder, exocrine pancreas, parotid gland, skin, kidney, platelets, immune system, brown and white adipose tissue, prostate and breast epithelial cells, myometrium, ovary/granulosa cells, and placenta [13, 23, 24].

In Alzheimer's disease and the normal aging process, MT1 receptor expression in SCN and cerebral cortex reduces.MT1 receptors also decrease the neuronal discharge rate in SCN which diminishes the secretion of prolactin from pars tuberalis and induces vasoconstriction. MT2 receptors are involved in the antidepressant and anxiolytic effects of melatonin. MT2 receptors are also reduced in Alzheimer's disease. MT2 receptors contribute to the pharmacology of depression, anxiety, sleep disorders, and pain [13, 25].

Table 8.4 Melatonin receptors and their characteristics [10, 13, 22]

Characteristics	MT1 receptor	MT2 receptor
Chromosomal site	4q35.1, comprises 351 amino acids	11q21–q22, comprises 363 amino acids
GPCR signaling	Couples with pertussis toxin-sensitive G_i and insensitive $G_{q/11}$ G proteins; inhibits protein kinase A signaling, forskolin-stimulated cAMP, and CREB phosphorylation	Activates protein kinase C (PKC) in the SCN to reduce Ca+2-dependent dopamine release in the retina; inhibits forskolin-stimulated cAMP production and cGMP formation
Central expression	SCN of the hypothalamus, *pars tuberalis* of the anterior pituitary, thalamus, hippocampus, nucleus accumbens, cortex, substantia nigra, cerebellum, retina, and cornea	Retina, hippocampus, paraventricular nucleus, cortex, and cerebellum

cAMP cyclic adenosine monophosphate, *cGMP* cyclic guanosine monophosphate, *CREB cAMP* response element-binding protein, *GPCR* G protein-coupled receptor, *MT_1 MT_2* melatonin receptors, *SCN* suprachiasmatic nucleus

MT3 or quinone reductase 2 (QR2) enzyme (also called ML2 or NQO2) is a detoxification enzyme and belongs to the reductase group. MT3 receptor is found in the heart, lung, kidney, liver, intestine, brown fat tissues, and muscle. It is involved in preventing oxidative stress through the inhibition of electron transfer reactions of quinones. It also has a role in the regulation of intraocular pressure [26].

Retinoid-related orphan nuclear hormone receptor (RZR/RORα) binds to the transcription factors in the nucleus, which are associated with the superfamily of retinoic acid receptors [23]. These receptors play a key role in cellular growth, bone differentiation, and immunomodulation at the periphery [27].

Melatonin-related Orphan receptor, GPR50: H9, ML1X, is an X-linked inherited receptor that binds to G-protein. It significantly binds Melatonin to MT1 and is expressed both in the brain and periphery. Though GPR50 does not bind to melatonin, it does inhibit the Melatonin signal when it dimerizes with the MT1 receptor. A deletion mutation in GPR50 may be associated with major depression and bipolar disorder. Other functions of GPR50 include neurite outgrow inhibitor (NOGO-A), and TIP60 (glucocorticoid receptor signal activator and histone acetyltransferase [13, 28]. Table 8.3 outlines various physiological effects of melatonin in our body.

Melatonin Metabolism

Melatonin or *N*-acetyl-5-methoxytryptamine (the chemical structure is shown below in Fig. 8.5) is mostly synthesized in the pineal gland (pinealocytes) from the amino acid tryptophan through 5-hydroxytryptophan and serotonin (5-hydroxytryptamine). Serotonin is acetylated by arylalkylamine-N-acetyltransferase (AA-NAT) to N-acetylserotonin. AA-NAT is also known as "Timezyme" and is the rate-limiting enzyme in the synthesis of melatonin. N-acetylserotonin is then methylated through acetylserotonin-O-methyltransferase (ASMT or previously known as hydroxyindole-O-methyltransferase or HIOMT) to melatonin [10, 29]. The synthesis of melatonin is shown below in Figs. 8.6 and 8.7 [10, 13].

Both noradrenergic and neuropeptidergic neuronal projections to the pineal gland control the AA-NAT and ASMT enzyme activities and hence regulating the synthesis and secretion of melatonin into the systemic circulation. By activating adenyl cyclase, norepinephrine increases the production of Melatonin biosynthetic

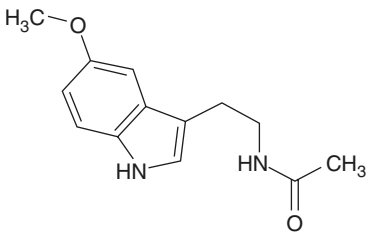

Fig. 8.5 Chemical structure of melatonin [10]

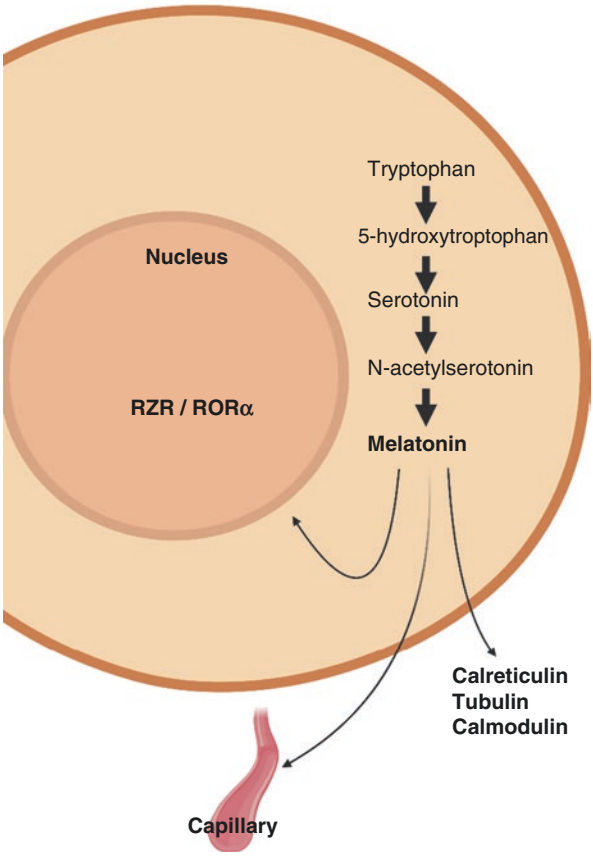

Fig. 8.6 Melatonin biosynthetic pathway [13]. *SCN* suprachiasmatic nucleus, *PVN* paraventricular nucleus of the hypothalamus, *SCG* superior cervical ganglion, *RZR/RORα* retinoid-related orphan nuclear hormone receptor

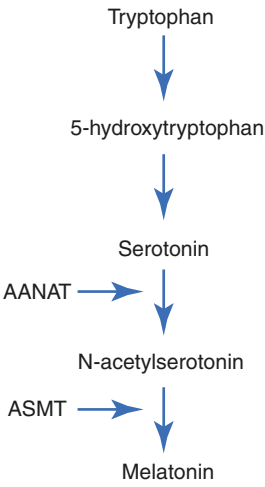

Fig. 8.7 Synthesis of Melatonin [10, 13]. *AANAT* arylalkylamine-N-acetyltransferase, *ASMT* acetylserotonin-O-methyltransferase

enzymes, particularly AA-NAT [10, 30]. The rhythmic synthesis of melatonin is mediated through the clock genes in the SCN, which acts as the clock or the central rhythm-generating system [18].

Melatonin is metabolized to 6-sulfatoxymelatonin (nearly 70%), principally in the liver via 6-hydroxylation and later through sulfate conjugation. Various minor metabolites include glucuronide conjugate, N1-acetyl-n2-formyl-5--methoxykynuramine, and N1-acetyl-5-methoxykynuramine, which are excreted through urine [10, 18].

Intravenous or exogenous melatonin has a short metabolic half-life (20–60 min) due to the hepatic first-pass effect and a biphasic elimination pattern. Prolonged or slow and sustained release preparations increase the time of melatonin circulation peak. The transmucosal route of administration increases the bioavailability by bypassing the hepatic first-pass effect [18, 31, 32].

Medications Acting Via Melatonin System

Activation of melatonin receptors reduces the neuronal firing via MT1 receptors in the SCN and limbic system. This promotes sleep and plays a crucial role in the coordination of both phase and amplitude of circadian rhythms in the body [22, 33]. Melatonin agonists act through MT1, MT2 receptors and are shown below in Table 8.5 [22].

Melatonin in Sleep

Insomnia is the difficulty in initiating/maintaining sleep or perception of nonrestorative sleep along with daytime impairment or distress. Its prevalence is estimated to be around 10% in the world population [34].

Table 8.5 Melatonin agonists, affinity to receptors and their uses [22]

Melatonin receptor agonists	Receptor affinity	Primary use
Melatonin (Circadin®)	MT_1, MT_2	Insomnia in elderly, circadian rhythm disorders
Ramelteon (Rozerem®)	MT_1, MT_2	Primary chronic insomnia
Tasimelteon (Hetlioz®)	MT_1, MT_2	Non-24 h sleep–wake disorder in totally blind patients
Agomelatine (Valdoxan®)	MT_1, MT_2, $5\text{-}HT_{2C}$	Major depressive disorder

MT_1 MT_2 melatonin receptors, *$5\text{-}HT_{2C}$* serotonin

Melatonin and other synthetic analogs (ramelteon, tasimelteon, agomelatine) are used in numerous circadian disorders like shift work, jet lag, seasonal affective disorder, delayed-sleep, and advance-sleep phase syndrome, non-24 h sleep–wake disorder, and major depression. These agonists are lacking the typical side effects of frequently used sleep medications like impairment of memory, motor function, and learning [22, 35].

Circadin (prolonged-release melatonin) imitates the physiological profile of melatonin. It enhances the sleep quality in children and blind patients with non-24 h sleep–wake disorders, latency, and daytime functioning of elderly insomniac patients. Circadian doesn't cause reduced vigilance, memory impairment, or any withdrawal symptoms [22, 36].

Ramelteon, even though nonselective, has a tenfold higher affinity for MT1 than the MT2 receptors and is used in primary chronic insomnia. It also has a 17-fold higher affinity than melatonin. It significantly reduces the latency to persistent sleep and promotes the total sleep time, with no residual effects on the next day. Its usage during the bedtime phase advances and entrains the circadian rhythm after sporadic phase advance of dark onset [22, 37].

Tasimelteon has a high affinity for MT1 and MT2 receptors and is used for non-24 h sleep–wake disorder in totally blind patients, shift work, jet lag, and other circadian rhythm sleep disorders. It shifts the endogenous melatonin rhythm, reduces sleep latency, and enhances sleep efficiency and maintenance [22, 38].

Melatonin in Depressive Disorders

Agomelatine improves the major depression symptoms through activation of MT1 and/or MT2 receptors and blockage of serotonin receptors ($5\text{-}HT_{2C}$). It eases anxiety, depressed mood, neurochemical imbalance, neuronal atrophy, enhances disturbed sleep patterns and circadian rhythm entrainment [22, 39].

Melatonin in Other Disorders

Improved long-term potentiation and performance in learning and memory is achieved by either deletion or blockage of melatonin receptors. Thus melatonin receptor antagonists (Luzindole and K-185) enhance memory and learning and might be useful in Alzheimer's disease in reducing the symptoms of dementia [22, 40].

Melatonin prevents mitochondrial cell death and ischemia–reperfusion injury through its (antioxidant) action on MT1 and MT2 receptors. Melatonin agonists might be useful in diseases like Huntington's disease, improving neurogenesis and in preventing stroke-related ischemia/reperfusion [22, 41].

Melatonin at its peak during the dark phase, there is a reduced response to drugs of abuse. Deletion of MT1 and MT2 receptors of melatonin abolished the methamphetamine-induced locomotor sensitization and reward. Therefore, melatonin receptor antagonists could play a role in improving the symptoms of drug abuse [22, 42].

Melatonin receptors at the cellular level impact cancer pathology. MT1 receptor intermediates the oncostatic effect of melatonin prostate and breast cancer; thus, MT1 receptor agonists may be productive in the treatment of breast and prostate cancer either alone or as adjunct therapy [22, 43].

Other Clinical Aspects

Melatonin in Critical Care

Sleep disturbances are commonly seen in critically ill patients. Melatonin levels in plasma are reduced, and there will be loss of circadian rhythm in intensive care unit (ICU) patients who receive mechanical ventilation, which causes irregular sleep–wake patterns [44].

Sleep interventions in ICU patients intend to coordinate nocturnal sleep and to promote both slow-wake sleep (SWS) and rapid eye movement (REM) sleep. Melatonin use has been shown to significantly improve the nocturnal sleep efficiency in ICU patients with severely compromised nocturnal sleep quality. Melatonin is sleep-promoting (hypnotic) without affecting the normal sleep architecture significantly [44, 45].

Melatonin therapy for closed head injury not also reduced the contusion volume but also extensively improved the recovery rate [46, 47]. In critically ill elderly patients, melatonin levels are low postoperatively, and enteral melatonin is efficient in averting delirium. In pediatric patients, preoperative melatonin also decreased the delirium in a dose-dependent manner when correlated with midazolam [47–49].

In seizure patients, melatonin accelerates the GABA transmission to cause anti-excitatory signals and achieving anticonvulsant activity. Melatonin therapy reduces nocturnal seizures in children and alleviates the seizures in children with epilepsy; thus, melatonin may be used as an analgesic or sedative adjunct in children with lower seizure threshold [47, 50].

Treatment with melatonin in congenital heart disease patients, it showed to lower the postoperative low cardiac output syndrome. In septic patients, usage of melatonin improved sleep–wake cycles, reduced nitrosative stress and inflammatory reactions. Melatonin decreases nocturnal blood pressure, especially in patients at risk or with hypertension [47].

Melatonin in Headaches

Melatonin's benefit in headache might be independent of sleep or hypothalamus due to its antinociceptive and anti-inflammatory properties. Melatonin has a similar indole structure with indomethacin and hence efficient in indomethacin-responsive disorders [51, 52].

Melatonin levels are lower in migraine patients on migraine days compared with nonheadache days, and in chronic migraine patient's lower levels are observed than in patients with episodic migraine. The minimum effective dose of melatonin is 3 mg in migraine for adults; a marginal increase to 4 mg might be helpful in few cases [51, 53, 54]. Melatonin might be a good alternative for migraine prophylaxis, as its efficacy is increasing in migraine prevention [55].

Tension-type headache (TTH) showed a significant decrease in headache frequency and Headache Impact Test Score in an adult cohort of patients on Melatonin 4 mg dose. In the pediatric age group, half of the group had reported more than 50% reduction in headache attack frequency [53, 56].

In episodic cluster headache patients, nocturnal melatonin levels are lower during the cluster period and also in cluster remission. Melatonin therapy helps in replenishing the endogenous levels during the cluster period and helps to phase shift sleep. However, few case reports have reported that lower doses of melatonin might help in relieving cluster headaches [51].

In hemicrania continua (HC) is a rare headache disorder, and therapeutic efficacy is found between 3–30 mg of melatonin. In patients with HC along with migraines, melatonin is gaining evidence as one of the treatments of choice [51, 57, 58], although clinical studies did not show significant benefit consistently.

Hypnic headache commonly affects the elderly population due to dysfunction of the SCN of the hypothalamus. Few case reports showed improvement in symptoms from 6–12 mg of melatonin [51, 59].

In primary stabbing headaches, melatonin relieved pain from the range of 3–12 mg of melatonin [60]. Paroxysmal hemicrania, an indomethacin-responsive disorder, melatonin might also be useful in treating the symptoms, but more trials are required [51].

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 9
Norepinephrine

Sireesha Murala and Pradeep C. Bollu

Introduction

History of Norepinephrine

Norepinephrine (NE) is a neurotransmitter of the brain that has a vital role in the modulation of cognition, arousal, attention, and stress responses. In the periphery, NE functions as a hormone as a part of the "fight or flight" response in the sympathetic nervous system [1]. NE was discovered in the peripheral nervous system (PNS), along with epinephrine. German physiologist, Otto Loewi, demonstrated that a substance released into the blood subsequent to sympathetic ganglion stimulation increased the heart rate, which was later identified as epinephrine. NE was considered an inactive precursor of epinephrine; however, Ulf von Euler, a Swedish physiologist, demonstrated that NE was also involved in the sympathetic response in 1940 [2, 3].

Marthe Vogt, in 1954, expanded the role of NE as a neurotransmitter in the central nervous system (CNS). In 1962, Falck and Hillarp applied histochemistry to brain structures, and NE was identified in both PNS and CNS. Dahlstrom and Fuxe,

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in 1964, demonstrated that locus coeruleus (LC) is the primary source of NE in the CNS through their experiments [2, 4]. Alpha-2 adrenoceptors were first identified on peripheral nerve endings in 1974 and on cell bodies and dendrites of central noradrenergic neurons in 1975 [5, 6].

Neurochemical Profile

The noradrenergic system in the brain has two primary ascending projections that arise from the brainstem: the dorsal noradrenergic bundle (DNB) and ventral noradrenergic bundle (VNB). DNB originates from LC and is mainly composed of noradrenergic neurons that project to the cerebral cortex, hippocampus, and cerebellum and intersects with the projections from the VNB to innervate the hypothalamus amygdala, and spinal cord. The VNB also innervates the midbrain and medulla along with the amygdala and hypothalamus. VNB plays a crucial role in controlling vegetative functions and endocrine regulation [7–9].

NE is released mainly from the LC in the brain. LC, a small pontine nucleus that is located in the lateral wall of the brainstem by the fourth ventricle. LC has about 20,000 neurons that produce central NE, while the medullary nuclei primarily function peripherally. Most of the noradrenergic neurons of the brain are found in LC. LC projects to most parts of the brain, forming both synaptic and nonsynaptic contacts, with projections to the forebrain, cerebellum, brainstem, and spinal cord. NE pathways have a wide innervation in the brain from olfactory tubercle to spinal cord and are shown below in Fig. 9.1 [10–12].

LC neurons produce NE from two types of NE cells, the large multipolar cells (35 μM) and smaller fusiform cells (20 μM). Their distribution in the LC is distinct—fusiform cells are located in the dorsal LC and multipolar cells in the ventral

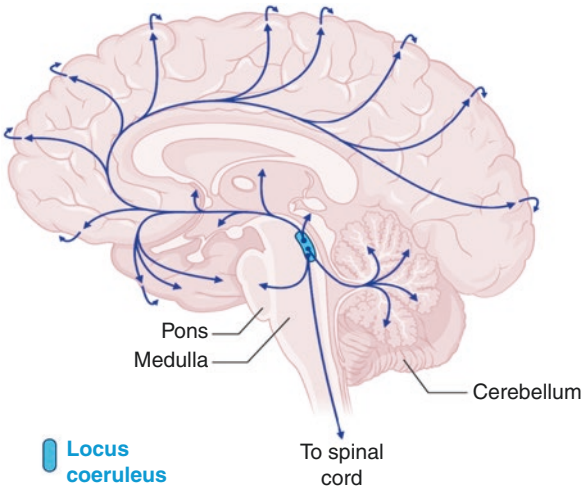


Fig. 9.1 Norepinephrine pathways in the brain

LC [13]. In addition to NE, galanin is also expressed in up to 80% of LC neurons, which is involved in regulating wake/sleep states, feeding, nociception, and parental behavior [14, 15].

The unique characteristic of LC is the diversity of projections and intense collateralization of the axons. LC projects to most parts of the brain and modulates information from various systems like sympathetic, parasympathetic, cortical, and limbic centers [12]. The sympathetic and parasympathetic nervous systems and their neurotransmitters are shown below in Fig. 9.2. The sympathetic nervous system and neuroendocrine chromaffin cells of the adrenal medulla synthesize and release NE and other catecholamines [2, 16].

Factors contributing to noradrenergic transmission are the diversity of noradrenergic receptors, tonic and phasic neurotransmission, and the nonlinear relationship between innervation and performance [17]. The noradrenergic system promotes wakefulness, arousal and facilitates sensory signal detection. Most studies have shown that NE influences cognition and behavior like working memory, attention, behavioral flexibility, and long-term mnemonic processing [18].

During anxiety or stress, the released NE and epinephrine are bound to adrenergic receptors throughout the body that exert effects like dilating pupils and bronchioles, increasing heart rate and constricting blood vessels, increasing renin secretion from the kidneys, and inhibiting peristalsis. NE plays a crucial role in metabolic effects like stimulating glycogenolysis and gluconeogenesis and inducing ketogenesis and lipolysis [19, 20].

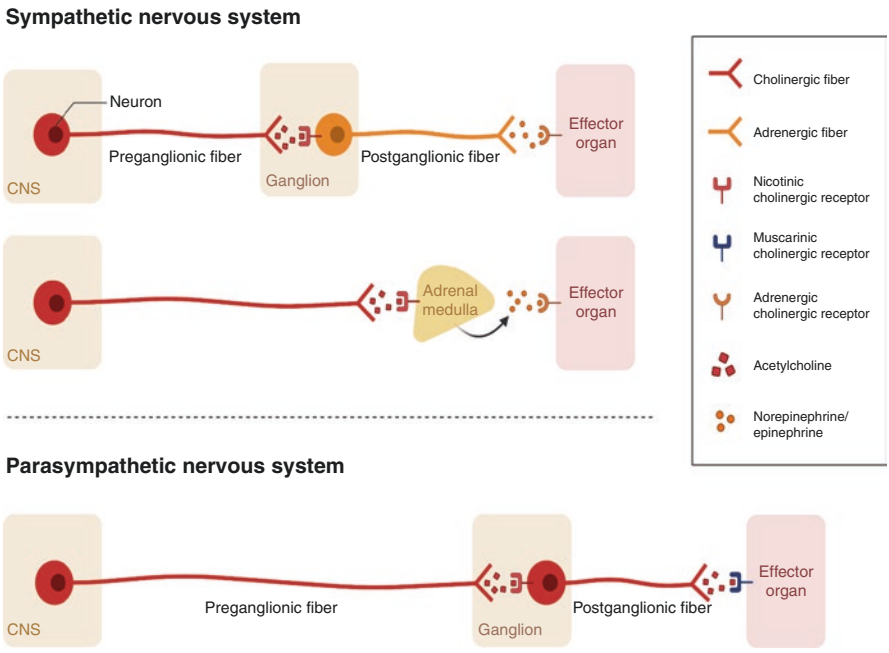


Fig. 9.2 Sympathetic and parasympathetic nervous system neurotransmitters

Norepinephrine Receptors

NE acts through alpha and beta receptors, which are α1, α2, β1, β2, β3 adrenoreceptors. The various types of α1 receptors include α1a, α1b, and α1d receptors, which are found in LC, amygdala, thalamus, cerebral cortex, olfactory bulb, and dentate gyrus. α1 receptors act through the Gq protein signaling pathway, and the receptor pathway is shown below in Fig. 9.3. NE receptor and their mechanism of action are shown below in Table 9.1 [21, 22].

Noradrenaline has an excitatory action through its post-synaptic α1 and β-adrenoreceptors and inhibitory action through presynaptic α2-adrenoreceptors [17]. α2-adrenoreceptor is commonly found in prefrontal cortical areas, and NE has the highest affinity and lower affinity for α1 and β-adrenoreceptors, respectively.

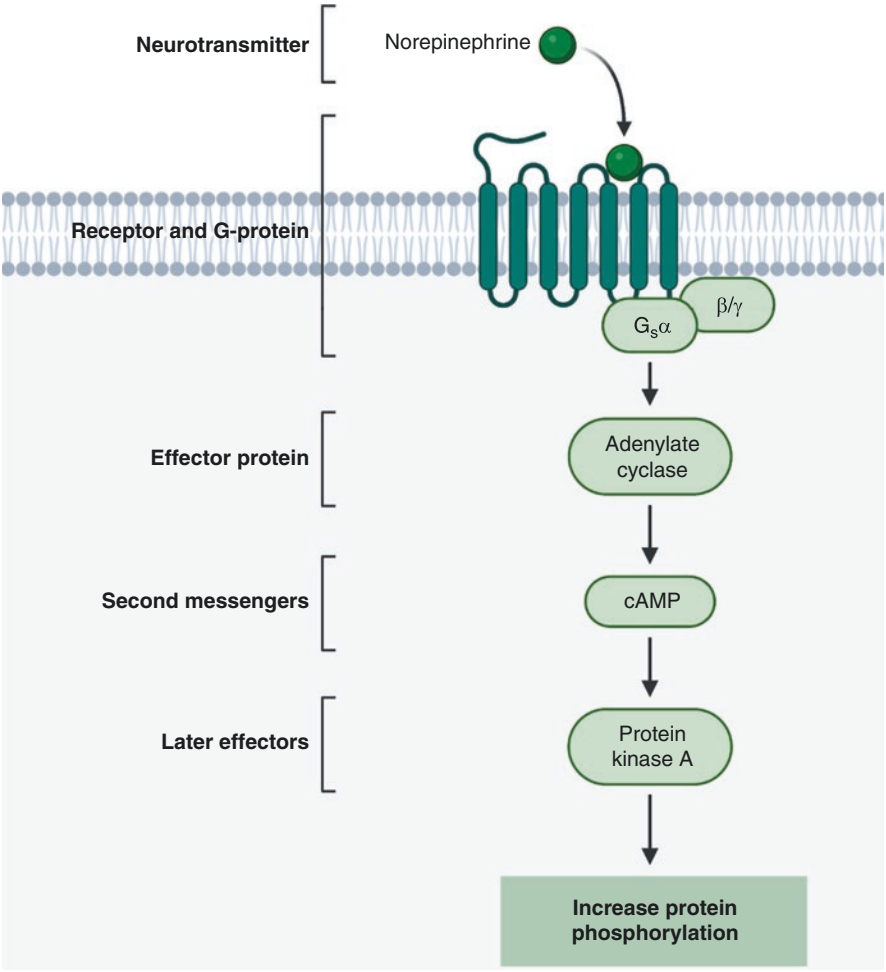


Fig. 9.3 Norepinephrine receptor pathway

Table 9.1 NE receptor, mechanism of action and their effects [21]

Receptors	Subunits	Mechanism	Effects
α-Receptor	α1—α1a, α1b, α1d	Phospholipase C is activated, that leads to the formation of IP3 and DAG—intracellular calcium ↑	Smooth muscle contraction, mydriasis
	α2—α2a, α2b, α2c	Adenylate cyclase is inactivated, which leads to a ↓ intracellular cAMP	Mixed smooth muscle effects
β-Receptor	β1	Adenylate cyclase is activated, and intracellular cAMP ↑	Increased cardiac chronotropic and inotropic effects
	β2	Adenylate cycle becomes activated through the Gs-protein-coupled receptors, ↑ intracellular cAMP. Gi protein-coupled receptors are also activated, and ↓ intracellular cAMP	Bronchodilation
	β3	Adenylate cyclase is activated, and intracellular cAMP ↑	Increased lipolysis

cAMP cyclic adenosine monophosphate, *DAG* diacylglycerol, *IP3* inositol triphosphate

Thus the distribution and relationship of adrenoreceptors are variable, with moderate levels of NE activating α2 receptors, while higher levels activate lower-affinity α1 and β-adrenoreceptors [23–25].

α1 and α2 receptors have been found to influence attention, fear, working memory, and spatial learning. β1 and β2 have been found to work on fear memory, auditory fear, memory retrieval, and spatial reference [18].

The stimulation or inhibition of these functions is dependent on the agonism or antagonism of the adrenergic receptors. The α1 and β-adrenoreceptors promote neurotransmission and plasticity, thus enhancing the stimulatory effects in the CNS. At the same time, α2 receptors have inhibitory effects in the CNS, like decreasing NE release and reducing neuronal excitability [18].

In the periphery, acetylcholine (Ach) stimulates the release of adrenaline and noradrenaline. Ach binds to nicotinic receptors on adrenal chromaffin cells that generate action potentials via voltage-gated sodium and potassium channels. Calcium influx into the cytosol leads to binding of NE vesicles to the cell membrane causing NE release into the blood [26].

NE is removed from the synaptic cleft through presynaptic reuptake. NE transporters (NET) are found on presynaptic terminals that mediate the NE reuptake that can either be stored in the vesicles or undergo degradation [18, 27].

Norepinephrine Metabolism

NE is a monoamine neurotransmitter, and the chemical structure of Norepinephrine is shown below in Fig. 9.4. Tyrosine is an aromatic amino acid that acts as a precursor for the synthesis of dihydroxyphenylalanine (DOPA) by the action of tyrosine hydroxylase. Tyrosine hydroxylase is the rate-limiting enzyme in NE and dopamine

Fig. 9.4 Chemical structure of Norepinephrine

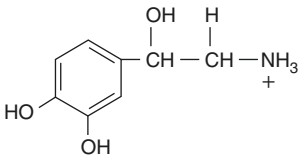
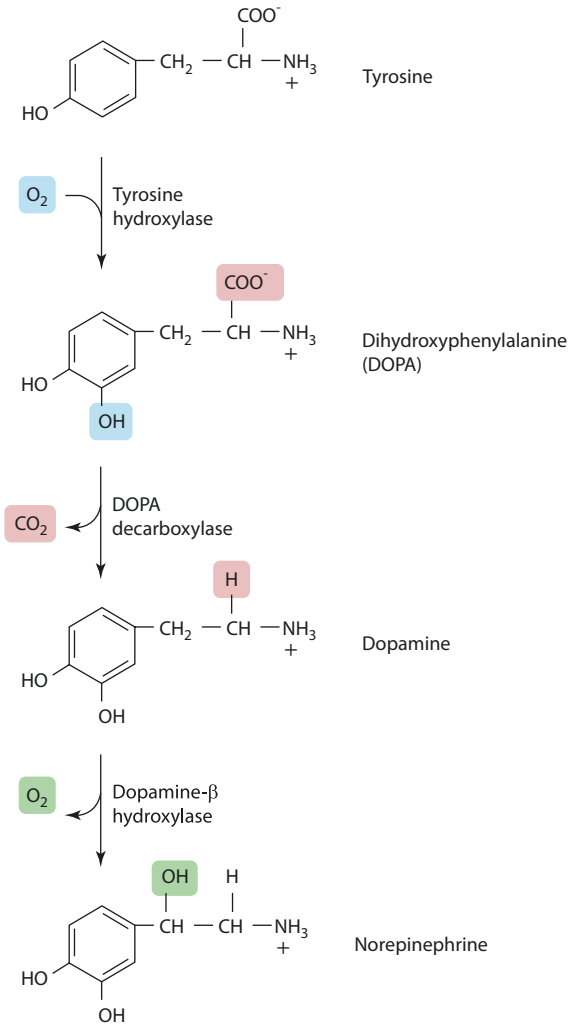


Fig. 9.5 Synthesis of Norepinephrine



synthesis. Dopamine is synthesized by the action of DOPA decarboxylase; dopamine is transported into vesicles through vesicular monoamine transporter (VMAT). Dopamine β hydroxylase (DBH) converts dopamine to NE. NE is converted to epinephrine via phenylethanolamine-*N*-methyltransferase, both in the CNS and adrenal medulla [2, 28]. The synthesis of NE is shown below in Fig. 9.5.

NE is degraded intracellularly or in the synaptic cleft by the enzymes monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT). MAO oxidizes norepinephrine, and COMT metabolizes deaminated norepinephrine by O-methylation. MAO and COMT are found in adrenal chromaffin cells, while sympathetic nerves have only MAO. MAO has two isoforms—MAO-A and MAO-B. MAO-A is mainly found in noradrenergic neurons, while MAO-B is found in serotonergic cells. COMT is found in all organs, and degradation of NE to vanillylmandelic acid (VMA), is completed in the liver [2, 7, 16, 29].

Medications Acting on the Norepinephrine System

Adrenergic drugs can bind to one or more of the receptors to produce physiological actions. Based on the specific receptor affinity, they are classified into selective or direct-acting drugs and nonselective or indirectly acting drugs [21]. Medications acting on the NE system are shown below in Table 9.2 [30]. Direct-acting drugs are bronchodilators, and vasopressors, while indirect-acting drugs are amphetamines, desipramine, atomoxetine, and phenelzine [31, 32].

Table 9.2 Medications acting on NE system [30]

Receptor	Drugs	Action	Indication
Agonists			
α1	Phenylephrine	Vasoconstriction	Hypotension and nasal congestion
α2	Clonidine Brimonidine	↓ Blood and intraocular pressure	Hypertension and glaucoma
β1	Dobutamine	↑ Cardiac output	Cardiogenic shock
β2	Albuterol, terbutaline, ritodrine	Relaxes smooth muscle in lung and uterus	Asthma and premature labor
Indirect acting	Amphetamine	CNS stimulant	Narcolepsy, hyperactivity
Indirect acting	Desipramine, atomoxetine	Blocks NE reuptake	Depression, ADHD
Indirect acting	Phenelzine	Inhibits NE metabolism by MAO	Depression
Antagonists			
α1	Prazosin, terazosin	Vasodilation	Hypertension, benign prostatic hypertrophy
α2	Mirtazapine	Antidepressant	Depression
B1, β2	Propranolol, timolol	↓ cardiac output and intraocular pressure	Hypertension, glaucoma, angina, migraine, tremors, and anxiety
B1	Atenolol, metoprolol	↓ Cardiac output	Hypertension

Selective Drugs

Alpha-1 Receptor Drugs

Phenylephrine, an alpha-1 receptor agonist, is commonly used as a decongestant and vasopressor in cases of hypotension due to septic shock. Another drug, oxymetazoline, is used as a decongestant and to treat rosacea. Common side effects include reflex bradycardia [33, 34].

Alpha-1 receptor antagonists like prazosin and terazosin are used in the treatment of hypertension and benign hypertrophy of the prostate because of their vasodilatory effect [30].

Alpha-2 Receptor Drugs

Clonidine, an alpha-2 receptor agonist, is used to treat hypertension and attention deficit hyperactivity disorder (ADHD). Nonapproved indications include posttraumatic stress disorder (PTSD), sleep disorders, anxiety, hot flashes associated with menopause, restless leg syndrome, and other illnesses. Methyldopa is a centrally acting sympatholytic and is used in the treatment of hypertension and gestational hypertension. It decreases the adrenergic outflow by alpha-2 agonistic action from CNS, thus reducing the total peripheral resistance and systemic blood pressure. As alpha-2 agonistic activity does not affect the cardiac output or renal blood flow, it is preferred in hypertensive patients with renal insufficiency. Dexmedetomidine is used in the intensive care unit for sedation, which does not induce respiratory depression. Common adverse effects of alpha-2 agonists include dry mouth, sedation, hypotension, respiratory depression, and somnolence [35, 36].

Alpha-2 receptor antagonists like mirtazapine are used as an antidepressant. It is a noradrenergic and specific serotonergic antidepressant, which acts by antagonizing both alpha-2 autoreceptors and heteroreceptors and also blocks the 5-HT2 and 5-HT3 receptors [30].

Beta-1 Receptor Drugs

Beta-1 receptors are commonly found in cardiac myocytes, cardiac nodal tissues, cardiac conduction pathways, and kidneys. Dobutamine is a beta-1 receptor agonist that is indicated in the treatment of cardiogenic shock and heart failure. Common side effects include hypertension, tachycardia, palpitations, anxiety, and tachyarrythmias [37, 38].

Beta-1 blockers: Cardio-selective blockers include atenolol, betaxolol, bisoprolol, esmolol, metoprolol, and nebivolol. They are clinically used for hypertension, heart failure, chronic stable angina, post-myocardial infarction, and decreased left ventricular function after myocardial infarction. They are also used

in the treatment of arrhythmias, glaucoma, migraine prophylaxis, anxiety, and essential tremor [21, 39]. Common adverse effects include hypotension, bradycardia, heart failure, decreased exercise capacity, and atrioventricular nodal block. Noncardiac side effects include headache, fatigue, dizziness, nausea, vomiting, abdominal discomfort, dry mouth and eyes, sexual dysfunction, confusion, and memory loss.

Nonselective beta-1 blockers include propranolol, timolol, sotalol, and nadolol. Beta-1 blockade decreases heart rate, myocardial contractility by slowing AV conduction and suppresses automaticity. Beta-2 blockade reduces peripheral vascular resistance and can cause bronchospasm and hypoglycemia. Propranolol is used in hypertension, myocardial infarction, angina, idiopathic hypertrophic subaortic sclerosis, migraine, and vascular headaches. It also used for anxiety and in improving tremors by blocking the peripheral beta-2 adrenergic receptors. Common side effects include hypotension, bradycardia, dizziness, depression, memory loss, impotence, and rebound hypertension with sudden withdrawal. It is contraindicated in asthma, bradycardia, and heart failure [21, 40].

Beta-2 Receptor Drugs

Beta 2 receptors are commonly found in airway smooth muscles and cardiac muscles, uterine muscles, alveolar type II cells, mast cells, mucous glands, epithelial cells, vascular endothelium, eosinophils, lymphocytes, and skeletal muscles [41]. Short-acting Beta-2 agonists (SABAs) are albuterol, levalbuterol, metaproterenol, and terbutaline. Beta-2 agonists that act as bronchodilators are indicated for the treatment of obstructive lung diseases, such as COPD, asthma, or emphysema [41, 42].

Other uses of SABAs like albuterol are in the treatment of hyperkalemia, and terbutaline is used to postpone preterm labor and in the management of vascular extravasation. Long-acting B2 agonists (LABAs) are used in the maintenance treatment of patients with COPD, chronic bronchitis, and emphysema. Common LABAs include salmeterol, formoterol, and arformoterol. Olodaterol is an ultralong-acting beta-2 agonist used in the management of COPD and asthma [43, 44]. Adverse effects include anxiety, tachycardia, tremors, palpitations, nausea, vomiting, constipation, dizziness, and fatigue [37].

Beta-3 Receptor Drugs

Beta-3 receptors are located in the gallbladder, urinary bladder, and brown adipose tissue. They enhance lipolysis in adipose tissue and promote thermogenesis in skeletal muscle. They cause relaxation of the urinary bladder, thus preventing urination. Mirabegron is used in the treatment of overactive bladder (urinary incontinence, urinary frequency) [21].

Indirect Acting Agonists

Atomoxetine is a selective noradrenaline reuptake inhibitor, which is used as a first-line alternative when unresponsive to stimulants or in tic disorders that are aggravated by stimulants. As atomoxetine does not affect the dopamine level associated with motor activity, hence they do not worsen Tourette's or tics. As it has a sedative effect, the dose is split for the evening to improve sleep symptoms, and it has a 24-h duration of action that can take up to 4–6 weeks to see the clinical benefit [45, 46].

Nonselective Drugs

Norepinephrine is used for the treatment of shock and hypotension. Epinephrine (adrenaline) is used in the treatment of cardiac arrest, anaphylaxis, and croup, while dopamine is used in the treatment of hypotension, bradycardia, and cardiac arrest. Isoprenaline is used in treating bradycardia and heart block [40].

Carvedilol and labetalol are nonselective and have both beta-receptor and alpha-blocking activity. Beta-1 blockade decreases heart rate, myocardial contractility by slowing AV conduction and suppresses automaticity. Beta-2 blockade reduces peripheral vascular resistance and can cause bronchospasm and hypoglycemia. Alpha-1 receptor blockade causes arterial smooth muscle relaxation and vasodilatation. Carvedilol is used in hypertension and heart failure and reduces cardiovascular mortality after myocardial infarction. It is also used in the treatment of migraine and vascular headaches [21].

Other Clinical Aspects

LC neuronal loss is the most commonly seen early feature in many neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and progressive supranuclear palsy.

Alzheimer's Disease

Alzheimer's disease is the most common neurodegenerative dementia that is characterized by progressive loss of memory and cognition. Although the disease pathology is considered multifactorial, the main factor might be the interaction between noradrenaline and acetylcholine. Proposed mechanisms of disease development and progression are tau protein hyperphosphorylation assembles into neurofibrillary tangles, which accumulate in LC, causing neurodegeneration and cell death. The rostral cortically projecting neurons are widely affected, leading to impaired cognition and dementia. Alzheimer's disease is seen as a cholinergic dysfunction due to nucleus basalis of Meynert (nbM) degeneration. However, there is a significant loss

of LC neurons and is directly correlated with the severity of the disease. Other factors include decreased NE levels that affect the anti-inflammatory and neuroprotective effects within the CNS. Impaired transmission of NE within the hippocampus leads to hyperphosphorylated tau protein and noradrenergic axonal degeneration [47, 48].

In Alzheimer's disease pathogenesis, noradrenaline plays a crucial role as a neuroinflammatory moderator through microglial activation. Furthermore, it negatively affects the transcription of the inflammatory gene in microglia and astrocytes, which express adrenergic receptors [49].

Patients with Down's syndrome and Alzheimer's disease and patients with Down's syndrome who later on develop Alzheimer's disease have decreased levels of noradrenaline metabolites, which correlate with behavioral and psychological signs and symptoms of dementia [50, 51].

Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disease and a movement disorder that is characterized by bradykinesia, rigidity, tremor, postural instability, and cognitive decline in late stages. Loss of the dopaminergic nigrostriatal neurons is the main neuropathological abnormality that underlies the motor symptoms of the disease. In addition to the dopaminergic system, the noradrenergic system is also involved in the impairment of cognitive and emotions seen in Parkinson's disease. The pathogenesis involves noradrenergic neuronal degeneration in LC and alpha-2 autoreceptor losing its protective effects on both noradrenergic and dopaminergic systems. Within LC, alpha-synuclein accumulates, leading to low NE levels and nigrostriatal pathway degeneration due to loss of NE neuroprotective effects [47].

Lewy body pathology is the unique feature of Parkinson's disease, which occurs commonly in substantia nigra pars compacta and LC, that is associated with cell loss. Furthermore, Lewy body pathology in substantia nigra is preceded by the pathology in LC, indicating the dysfunction of noradrenaline even before affecting the dopaminergic systems in Parkinson's disease [48, 52, 53].

Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is a neurobehavioral disorder characterized by inattention, hyperactivity, and impulsivity, affecting 8% to 12% of children worldwide [54, 55]. The proposed mechanism involved in ADHD is an imbalance in dopaminergic and noradrenergic systems metabolism and neurotransmission in the prefrontal cortex and other subcortical regions. The deficiency in phosphoinositide 3-kinase (PI3K-gamma) enzyme causes an imbalance in norepinephrine/dopamine ratio and synaptic plasticity dysregulation. Impaired NET function and prefrontal dysfunctional noradrenergic transmission is involved in inattention and hyperactivity [56].

For parents of preschool children, a parent training program (PTP) is recommended. For moderately severe symptoms of ADHD in school-age children, consider PTP for parents and cognitive behavioral therapy (CBT) or social skills training, while CBT should be considered for adolescents [46]. Stimulants are the first-line agents in their treatment because of their rapid onset of action, safety, and efficacy in both children and adolescents [56]. Methylphenidate is the first-line therapy with a response rate of 60–80%. Atomoxetine is used if the child is intolerant to methylphenidate or has tics and anxiety.

Schizophrenia

Schizophrenia is a chronic mental illness characterized by both positive and negative symptoms, which include inattention, delusions, hallucinations, blunted affect, and disorganized thoughts. Alterations in the signaling of the dopaminergic and glutaminergic system appear to be the main cause, and there is evidence of noradrenergic system involvement as increased NE levels in CSF and blood in patients with paranoia and elevated NE markers in the post-mortem of schizophrenic patients [47].

Noradrenergic drugs can either treat or worsen the symptoms of schizophrenia. Blockage of alpha-1 adrenoceptors decreases the positive symptoms, while alpha-2 adrenoceptors blockage has been found to decrease the negative and cognitive symptoms [57]. Clonidine, methyldopa, and propranolol reduce the positive symptoms in schizophrenia by reducing the NE transmission in the brain. Desipramine and methylphenidate are found to worsen positive symptoms by increasing NE levels [47].

Depression

Depression is a common disorder that affects thoughts, mood, cognition, and behavior. The most common clinical symptoms include depleted energy, sadness, guilt, hopelessness, impaired concentration, and suicidal ideality [58, 59]. NE levels are elevated among other biomolecules in the periphery. Tyrosine hydroxylase expression was found to be increased in the LC, and levels of urinary NE were also increased [60].

Acknowledgments All the pictures in this chapter were prepared using BioRender premium software. The tables were redrawn using information from the reference article mentioned alongside them.

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Chapter 10
Orexins

Safoor Fathima, Sireesha Murala, and Pradeep C. Bollu

History of Orexins

Orexin, a peptide neurotransmitter also known as hypocretin, is expressed exclusively by lateral hypothalamic area (LHA) neurons. Orexin is involved in various complex processes like feeding, sleep and wakefulness, energy homeostasis, autonomic regulation, and reward processing [1].

In 1998, two different groups independently discovered this neurotransmitter. De Lecea and colleagues, in their attempt to identify specific genes that could possibly play a role in wide-ranging functions of the hypothalamus, discovered an mRNA that encodes the precursor for two secreted peptides (Hcrt 1 and Hcrt 2). These mRNAs were expressed only in cells of perifornical region of the lateral hypothalamus, and these cells projected widely throughout the brain. One of these peptides was found to be excitatory to cultured hypothalamic neurons, and this suggested the neurotransmitter nature of these peptides. They named it as "hypocretin" due to its localization in the *hypo*thalamus and structural similarity to *incretin* family of peptides [2].

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In the same year, T Sakurai and colleagues, in an attempt to identify the natural ligands for orphaned G-protein coupled receptors, discovered two peptides that activated two related orphaned GPCRs (OX1R and OX2R). These neuropeptides, on intracerebroventricular (icv) administration, increased food intake in rats, and in fasting state, showed an increased expression. They named these peptides "orexins" after the Greek word *Orexis* meaning appetite [3].

Later in 1998, Peyron et al. demonstrated the projection of axons of hypocretin neurons throughout the brain and suggested the role of hypocretin in varied physiological functions in addition to food intake [4]. Nambu et al. in 1999 supported this finding and proposed that orexin system provides a link between the hypothalamus and other regions of the brain and hence its role in cognitive, motivational, and emotional aspects of feeding behavior [5]. The presence of orexinergic neurons in areas outside the lateral hypothalamus that are involved in arousal hinted that hypocretins play a role in sleep–wake regulation [4].

In 1999, a mutation in the orexin receptor gene was identified to be associated with canine narcolepy [6]. Involvement of Orexin in sleep–wakefulness cycles was first reported in prepro-orexin knockout mice [7]. In the 2000s, deficiency of orexin neurons in narcolepsy patients highlighted its importance in the regulation of sleep–wakefulness cycle in humans [8, 9]. Nishono in 2000 showed deficient orexin neurotransmission in some people with narcolepsy [10].

In 2001, the first selective OX1 receptor antagonist, SB-334867-A, was identified and characterized. It was shown that SB-334867 binds to recombinant human OX1 receptors and inhibit the OX1-mediated calcium response. SB-334867-A also inhibited the OX2-mediated calcium response, but only at higher concentrations [11]. Over the past decade, many clinical studies fuelled a strong interest in developing an orexin antagonist as a novel approach for treating insomnia and promoting sleep.

Mignot and his colleagues, in 2002, reported that 90% of the narcolepsy with cataplexy patients had low levels of orexin in the cerebrospinal fluid [12]. Subsequently, orexins were also described as regulators of feeding behavior. In 2003, Yamanaka et al., found that orexin neuronal activity is inhibited by glucose and leptin and stimulated by ghrelin and that orexin expression is correlated negatively with changes in food intake, blood glucose, and leptin, thus demonstrating that orexin neurons were associated with energy balance and mediate adaptive augmentation of arousal in response to fasting [13].

In 2005, Harris et al. showed that activation of orexin neurons in the lateral hypothalamus is strongly linked to preferences for cues associated with drug and food reward. He demonstrated that chemical activation of orexinergic neurons reinstates an extinguished drug-seeking behavior [14].

In 2014, the first dual orexin receptor antagonist, Suvorexant, gained FDA approval to treat primary insomnia [15]. In 2017, it was demonstrated that orexin 2 receptor agonist YNT-185 ameliorates narcolepsy symptoms in mice, which inspired hope for effective orexin therapy in treating narcolepsy [16].

Neurochemical Profile of Orexins

Orexin neuropeptides present in the neurons of the hypothalamus that project throughout the central nervous system and are involved in sleep/wakefulness, feeding and energy homeostasis, autonomic regulation, and reward system and have many functions at the peripheral organs.

1. *Sleep–Wakefulness and Narcolepsy*: Orexins are crucial in coordinating sleep and wakefulness states [17]. Sleep and wakefulness are influenced by neurotransmitters including GABA, acetylcholine, adenosine, dopamine, histamine, glutamate, noradrenaline, glycine, serotonin, galanin, and prostaglandins [18]. In rodents, central administration of OA reduced REM, nREM sleep, increased the duration of wakefulness [19], and direct photostimulation of orexin neurons increased the transition to wakefulness from slow-wave sleep or REM sleep [20]. In humans, the orexin system regulates behavioral state by modulating the arousal threshold and maintains adequate wakefulness to cope with the changes in the internal and external environment [21]. A definite causal relationship has been established between orexin neurons and transitions of sleep/wakefulness states [20]. Orexins are more active during active wakefulness as compared to quiet wakefulness [22]. Though OX2R are more significant, than OX1R in behavioral and electroencephalographic findings in mice, both OX1R and OX2R are critical in the regulation of this process [17]. A complex interaction between OX1R and OX2R was revealed in the regulation of sleep and wakefulness when OX1R blockade attenuated the effects of the OX2R antagonist [23]. Figure 10.1 shows the extent of orexinergic projections in the brain and impact of other structures on orexin neurons.

   Orexin deficiency causes narcolepsy, which is characterized by chronic daytime sleepiness, cataplexy, hypnogogic hallucinations, and sleep paralysis [7]. Narcolepsy patients had abnormally low concentration of OA in CSF and reduced orexin neurons in the lateral hypothalamus (LH) [9, 10]. Narcoleptic effects of orexin are thought to be mediated mainly by OX2R or a combination of OX1R and OX2R but not OX1R alone [25]. Figure 10.2 shows the relationship between orexins and narcolepsy.
2. *Feeding, energy homeostasis and obesity*: Orexins increase food intake and energy expenditure [27]. Yamanaka demonstrated that orexin system integrates metabolic state into locomotor activity [13], and Thrope showed that orexin-induced feeding is affected by caloric challenge [28]. Orexin neurons are activated during negative energy balance state such as decreased glucose, decreased leptin, and increased ghrelin level [13]. Orexin administration increases energy expenditure by increasing wakefulness, locomotor activity, and sympathetic tone [29]. It inhibits glucoreceptors in ventral medial hypothalamus and enhances feeding behavior [30]. Orexin -mediated hyperphagia is mediated through area postrema and nucleus of tractus solitarius [31]. Orexinergic sys-

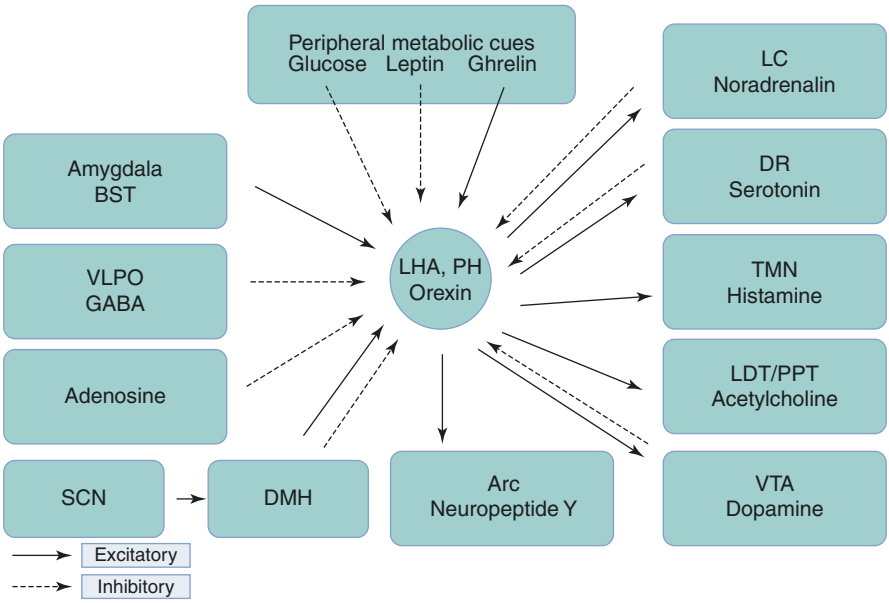

Fig. 10.1 Inputs/outputs of orexin neurons and their role in sleep, reward system, and energy homeostasis [24] *DR* dorsal raphe, *TMN* tuberomammillary nucleus, *VLPO* ventrolateral preoptic area, *VTA* ventral tegmental area, *PPT* pedunculopontine nucleus, *LHA* lateral hypothalamic area, *LC* locus ceruleus, *LDT* laterodorsal tegmental nucleus, *DMH* dorsomedial hypothalamic nucleus, *BST* bed nucleus of the stria terminalis, *SCN* suprachiasmatic nucleus

tem regulates muscle glucose metabolism by enhancing sympathetic tone and activating β_2 adrenergic signaling [32]. Orexin enhances OX2R mediated leptin sensitivity and improves insulin sensitivity [33]. During fasting, orexin neurons evoke adaptive behavioral arousal [13]. This arousal increases sympathetic and hypothalamic-pituitary-adrenal tone that further helps to execute adaptive behavior. These findings suggest the role of the orexinergic system in maintaining a proper balance between feeding, energy intake, or storage and expenditure.

Orexins are protective against obesity. Orexinergic neurotransmission promotes obesity resistance through enhanced spontaneous physical activity and energy expenditure regulation. Dysfunction of these neurons in animals leads to obesity despite low-calorie intake [34]. OX1R selective receptor antagonist SB334867 was found to ameliorate obesity in leptin-deficient mice [35]. Narcoleptic patients were found to have increased prevalence of obesity [36].

3. *Reward system and Addiction*: Orexins play an important role in reward mechanisms system and drug addiction. Activation of orexin neurons in lateral hypothalamus is strongly associated with preferences for cues associated with drug and food reward [14]. Projection of dopaminergic neurons from ventral tegmental area (VTA) to nucleus accumbens (NAc) is identified as a reward pathway.

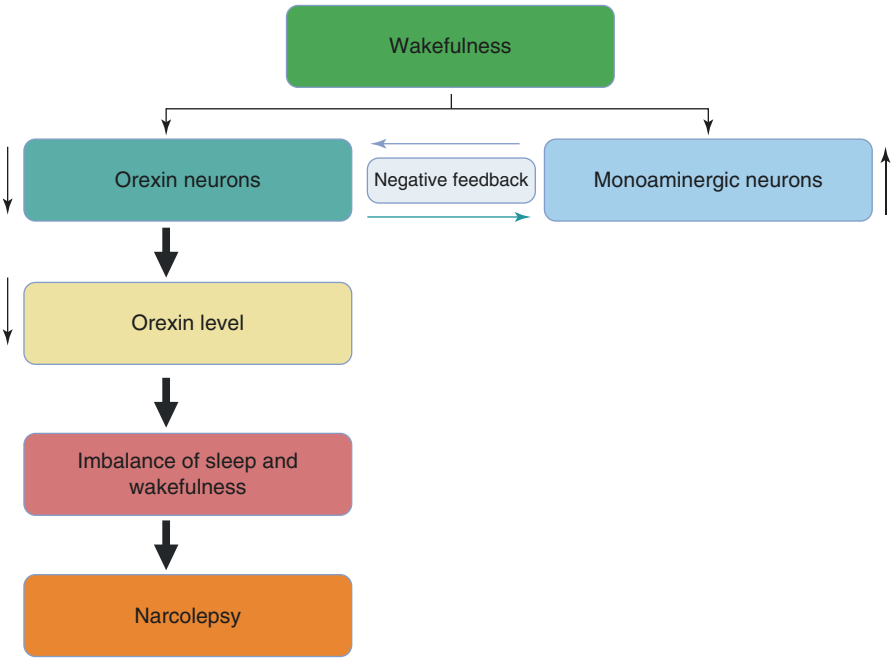

Fig. 10.2 Relationship between orexin and narcolepsy [26]

Orexin neurons have reciprocal connections with both these areas of the brain. These neurons function as an input to VTA and NAc for behavioral effects that are associated with reward-paired stimuli [37].

A direct role of orexins in the reinstatement of opioid, cocaine, alcohol, and nicotine seeking behavior is established [14, 38, 39]. However, different subregions of the brain are involved corresponding to different addictive substances. LH is linked to alcohol and morphine addiction [38], VTA is linked to cocaine addiction [40], and insula and LH are linked to nicotine addiction [41]. The role of both OX1R and OX2R signaling is reported in drug-seeking behavior [42, 43]. Blockade of OX1R eradicated the reinstatement responses to conditioned cues without suppressing food intake and wakefulness [37, 38, 40]. JNJ-10397049, an OX2R antagonist, reverses alcohol withdrawal and blocks the rewarding effects of alcohol in rodents [43]. Treatment of narcoleptic patients with amphetamine-like drugs did not lead to addiction to these drugs [44, 45].

4. *Role in cardiovascular and respiratory system*: The cardiovascular responses induced by orexins involve rostral ventromedial medulla, nucleus ambiguous, area postrema, and nucleus tractus solitarius (NTS) [46]. Intracerebroventricular administration of orexin increases heart rate (HR), mean arterial pressure (MAP), renal sympathetic nerve activity, and plasma catecholamine levels. However, these effects are not seen with intravenous administration [47]. This suggests

that the cardiac effects of orexins are mediated through a central mechanism. It was observed that orexin in NTS act in a dose-dependent manner. Higher doses of orexin increase MAP and HR, and lower doses reduces these variables. Their action is also site dependent in this region as MAP and HR declined with microinjections of orexins into caudal lateral and medial subnuclei of NTS and when injected into commissural nucleus of NTS, both MAP and HR increased [48, 49]. OA signaling into nucleus ambiguous produced bradycardia by increasing vagal excitation [50], and in the subfornical organ, it produces a similar effect through reduction of sympathetic tone [51]. In the nucleus ambiguous, it also enhances the inhibitory input and attenuates excitatory synapses to vagal neurons [52].

Role of orexinergic system in the hypercapnic chemoreflex response, phrenic and ventilatory long-term facilitation, the elevation of respiratory frequency, tidal volume, and minute respiration is demonstrated [53–55]. It facilitates upper airway patency and increases the respiratory frequency [56]. It also augments the phrenic nerve discharge and the electromyographic activity of the diaphragm when applied to the pre-Botzinger region [57]. The activation of orexin neurons is enhanced significantly by ambient levels of H^+ and CO_2 [58]. Orexin neurons are involved in sleep apnea as decreased levels of OA are found in patients with obstructive sleep apnea syndrome [59].

1. *Gastrointestinal tract*: Local direct role of orexins in gastrointestinal function has been demonstrated by the presence of orexin peptide and orexin receptors in the GI tract [61–64]. Orexin receptor expression in the gut is regulated by the metabolic state [65]. OX1Rs are found in the submucosal and myenteric plexus of neurons and OX2Rs in the enteroendocrine cells [64]. OA immunoreactivity was detected in thoracic sympathetic trunk ganglion cells, myenteric plexus, and GI endocrine cells [63]. Orexin and orexin receptors modulate gastrointestinal motility through central and peripheral mechanisms. Its central action increases gastric motility, and its peripheral action increases intestinal motility in isolated segments of the small bowel and colon and modulates small bowel motility [66]. OA stimulates gastric acid secretion in the stomach through the vagal pathway. It also stimulates the duodenal bicarbonate secretion [60].
2. *Pancreas*: Endocrine pancreas express both OA and OB, and their presence suggest that these peptides are involved in glucose control and control of pancreatic hormone secretion. It was reported that OA colocalizes mainly with insulin and to a lesser extend with somatostatin and glucagon cells [63]. But in 2005, Ehrstrom found that OA immunoreactivity is present in β and pancreatic polypeptide-containing cells. It was absent in α or δ cells in human islets [61]. The functional role of orexins in glucose metabolism is unclear due to discrepancies in the reported results of various studies. In rats, low glucose levels stimulated OA release, and high glucose levels inhibited its release. Ouedraoga yielded that OA increases glucagon secretion and decreases glucose-stimulated insulin release [67], but Ehrstrom reported contrasting results. He found that IV

infusions of OA in fasted rats decreased plasma glucagon without affecting insulin levels. Novak found that both OA and OB stimulate insulin secretion at different glucose concentrations [68]. OA elevated plasma insulin and glucose on subcutaneous injection, and OB increased only plasma insulin without any effect on plasma glucose [69]. A significant increase in postprandial plasma insulin was observed without any changes in plasma glucose and glucagon in fasted humans following OA infusion before a meal [61]. Postmeal, OX1 antagonist increased plasma glucose levels, but no such effect was seen with OA [70].

Exocrine pancreas: Studies in rats revealed that icv injection of OA stimulates pancreatic fluid and protein output, but this effect is not seen with iv injection [71]. OA also affects the exocrine pancreas by stimulating amylase release from pancreatic tumor cells [72].

3. *Kidney*: The increased levels of plasma OA in hemodialytic patients suggest renal clearance of orexin from plasma [73]. Prepro-orexin, OX1R, and OX2R mRNA have been found in human kidneys [63, 74]. But the role of orexin in renal function is yet unclear. Studies have demonstrated involvement of OA in pelvic-urethral reflex [75] and micturition reflex [76].
4. *Reproductive system*: In humans, OX1R and OX2R mRNA was detected in testis, epididymis, penis, and seminal vesicles, whereas PPO mRNA was detected only in epididymis and penis [77]. Expression of orexin receptors in testis suggests the role of orexin peptides in the male reproductive system.

   Studies in rats showed Expression of both OX1R and OX2R in ovaries. During certain times of proestrus, the expression of both receptors increased significantly not only in ovaries but also in the hypothalamus and pituitary, suggesting that ovarian receptor expression is regulated specifically and timely in a specific hormonal environment during the female cycle further indicating a possible role in female reproduction [60, 78].
5. *Adipose tissue*: Functional orexin receptors are present in both subcutaneous and omental adipose tissue. The role of orexins in adipose tissue metabolism and adipogenesis was demonstrated by its effect on inhibition of lipolysis. OA reduced glycerol release, and both OA and OB reduced the hormone-sensitive lipase in omental tissue [79]. OA have proliferative effect while OB have inhibitory effect on preadipocyte proliferation [80].
6. *Plasma orexin*: Orexin levels in plasma are affected by changes in energy homeostasis [60]. Energy status and body composition affect the plasma concentration of OA. Komaki reported a significant increase in plasma OA levels in normal weighted subjects after fasting for 10 days [81]. Low levels of plasma OA in the obese and a negative correlation between BMI and OA plasma levels is also demonstrated [82, 83], but a positive correlation was seen among morbidly obese individuals undergoing bariatric surgery [84]. Narcolepsy patients had significantly low levels of basal plasma OA [85]. Low levels are also seen in untreated and treated patients with the obstructive apnea–hypopnea syndrome [59].

7. *Neuroendocrine*: Orexins play an important role in the regulation of endocrine function [86]. This includes the following.
   (a) *Growth Hormone (GH)*: OA enhances growth hormone-releasing hormone-stimulated GH secretion, and OB directly increases GH secretion. But none had any effect on GH secretion from the pituitary [87–89]. ICV administration of OA inhibits spontaneous GH secretion and reduces plasma GH levels [88]. OA stimulates somatostatin release [90] and dampens the GH responses to ghrelin markedly [88]. The normal rate of pulsatile and basal GH secretion is seen in narcoleptic patients with orexin deficiency [91].
   (b) *Prolactin*: Orexin reduces basal plasma levels of prolactin [92]. It also decreases domperidone-induced levels of plasma prolactin [90]. OA increases secretion of prolactin on long days (month of May) and reduces it during the short days (month of December), suggesting day-length dependent regulation of prolactin secretion by OA [93]. There is a lack of effect of orexins on prolactin secretion [90, 94, 95].
   (c) *Thyroid hormone*: OA inhibits the release of hypothalamic thyrotropin-releasing hormone, thereby decreasing the plasma TSH level on icv administration. But it did not show any effect on TSH release or plasma thyroid hormone levels after peripheral infusion [95, 96]. OB increases plasma TSH level after icv administration [92]. In narcoleptic patients, plasma TSH concentration is low but plasma thyroid level is normal [97].
   (d) *Adrenal gland*: Orexin receptors induce activation of hypothalamic-pituitary-adrenal axis. ICV infusion of OA increases plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone. It involves both OX1R and OX2R receptors [86, 95, 98]. OA directly stimulates corticosterone release through OX1Rs coupled to the adenylate cyclase or protein kinase-A dependent signaling cascade [99] and suppresses ACTH secretion that is stimulated by corticotrophin-releasing factor [95]. Its effect on catecholamine release from the adrenal medulla or phaeochromocytoma cells is not consistent [100–102].
   (e) *Gonads*: Luteinizing hormone (LH) release was decreased in orexin deficient narcolepsy patients [103]. Studies in ovariectomized female rats showed an increase in LH secretion in the presence of estradiol and progesterone following icv administration of orexins [104] and suppression of LH release in the absence of estradiol and progesterone [105]. Orexins also increases LH release when microinjected into the rostral preoptic area but suppressed LH release when microinjected into the medial preoptic area or arcuate/median eminence suggesting region-dependent effects of OA on LH release [106]. Figure 10.3 shows the various functions of orexin neurons.

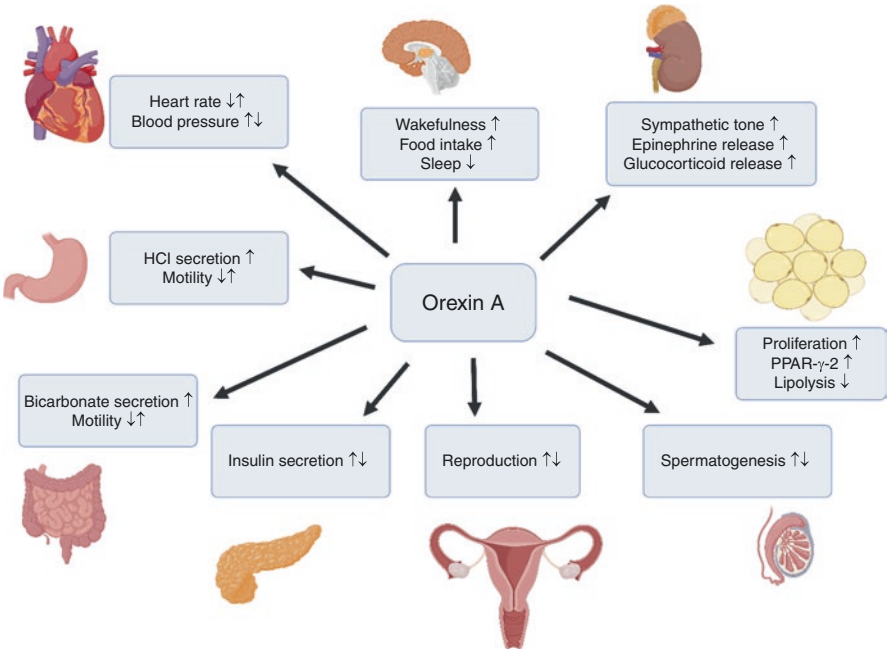

Fig. 10.3 Various functions of Orexin A [60]

Orexin Receptors

The actions of orexins are mediated by two G-protein-coupled receptors termed orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R). These receptors have seven transmembrane domains and some similarity to other neuropeptide receptors. The mRNA of human OX1R is 1564 bp in length that translates into a protein of 425 amino acid, and mRNA of OX2R is 1843 bp in length translating into 444 amino acid protein. Orexin-1 receptor binds with high affinity to orexin A but it has low affinity for orexin B. Orexin 2 receptor shares 64% homology with orexin 1 receptor, but binds to both orexin A and orexin B with high affinity [3]. These receptors have some structural similarities to other neuro peptide receptors but none of them have affinity to other orexinogenic factors like secretin, neuropeptide Y, or similar peptides [107].

Distribution of Orexin Receptors in the Brain

Lateral hypothalamus contains neurons that innervate entire neuraxis including monosynaptic projections to several areas of limbic system, brain stem, and cerebral cortex (see Fig. 10.4), suggesting that this area integrates information about nutritional homeostasis and transmits that to various regions in the brain [109, 110]. Orexin-containing neurons are found in these projections [5]. OX1R and OX2R exhibit marked differential distribution. OX1R is predominantly found in ventromedial hypothalamic nucleus, while OX2R is abundantly found in paraventricular nucleus of hypothalamus. Tenia tecta, dorsal raphe, locus coeruleus, and hippocampal formation have high levels of OX1R mRNA expression. Cerebral cortex, nucleus accumbens, paraventricular thalamic nuclei, tuberomammillary nucleus, subthalamic nucleus, and anterior pretectal nucleus express mainly OX2R [4].

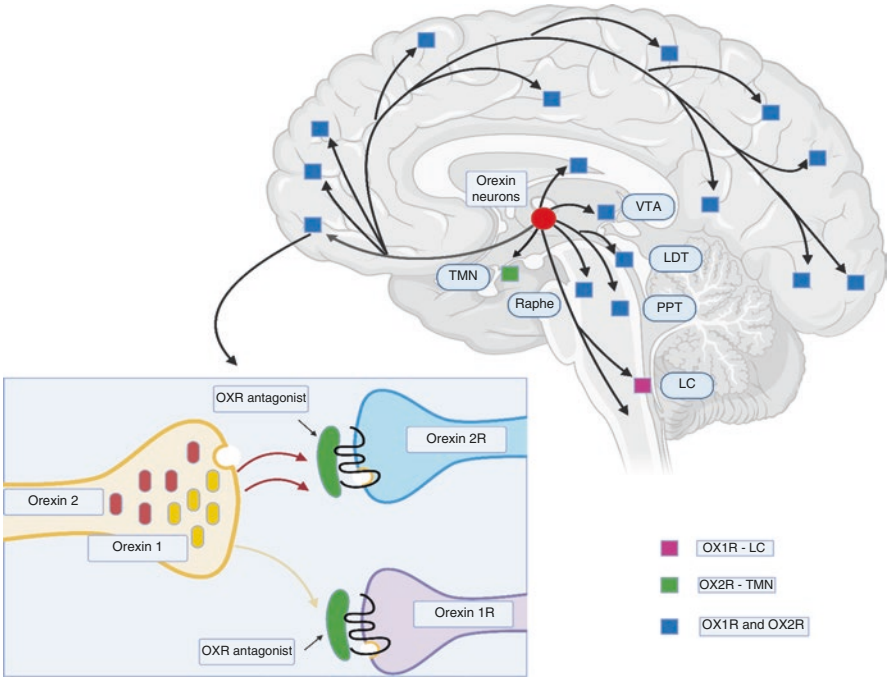

Fig. 10.4 Projection of Orexin neurons and the distribution of Orexin Receptors [108] Red circle = orexin neurons in the lateral and posterior hypothalamus. Squares = orexin projections and receptor localizations. Blue squares = sites where OX-1R and OX2R are collocated.Purple and green squares = nuclei where OX-1R and OX-2R predominate. *TMN* tuberomamillary nucleus (histaminergic), *LDT/PPT* laterodorsal/pedunclopontine tegmental nucei (cholinergic), Raphe nucleus (serotonergic), *LC* locus ceruleus (norepinehprinergic), and *VTA* is ventral tegmental area (dopamine). The call-out shows the preferential binding of orexin 1 (OA) to OX-1R and OX-2R and orexin 2 (OB) to OX-2R, respectively

Orexin Receptor Signaling Mechanisms

The coupling mechanism of orexin receptors differs by cell type. OX1R couple to Gq (G Protein) and OX2R couple to either Gq or Gi/G0 [111, 112]. Multiple mechanisms are involved in the orexin-induced postsynaptic excitation, to exert various actions of orexin. These mechanisms are listed below and are shown in Fig. 10.5.

(a) Reduction in potassium conductance [114].
(b) Activation of electrogenic Na^+/Ca^{2+} exchanger and a Ca^{2+} current [115].
(c) Inhibition of GIRK (G-protein-coupled inward rectifier) channel [116].
(d) Induction of TTX (tetrodotoxin) insensitive Na^+ invert current [117].
(e) Rapid and sustained rise in the intracellular calcium via transient receptor potential channel 3(TRPC3), voltage-gated calcium channels, or intracellular calcium stores [118, 119].

In addition to these post-synaptic effects, orexins induce the release of neurotransmitters GABA or glutamate presynaptically and generate more complicated effects on downstream neurons [119, 120]. Orexins excites the neurons in the ventral tegmental area by increasing the number of N-methyl-D-aspartate (NMDA) receptors in the cell membrane and produce a long-lasting increase in neuronal excitability [40, 121].

The binding of Orexin A and Orexin B with OX1R and OX2R activates Gq/PLC/PKC/ERK, Gs/AC/cAMP/PKA/ERK (and/or CREB, p38 MAPK) and Gi cascades.

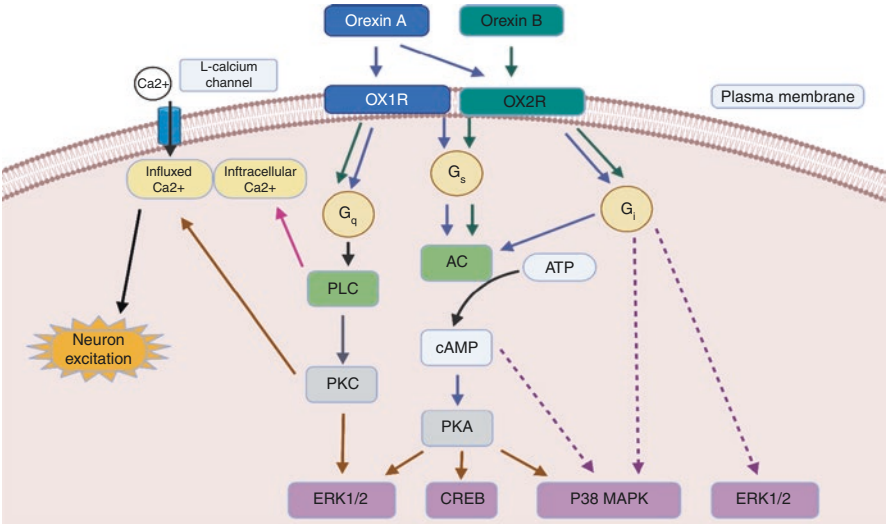

Fig. 10.5 Schematic summary of signaling pathways mediated by the orexin–receptor system [113]

Orexin Metabolism

Orexin peptides are of two types—orexin-A is a 33 amino acid peptide with two intrachain disulfide bonds, and orexin-B is a 28 amino acid linear peptide (see Fig. 10.6). Both these forms are derived from the cleavage of prepro-orexin [2, 3]. They were identified as endogenous ligands of two orphan G-protein coupled receptors. These peptides are produced by neurons in the hypothalamus with extensive projections to different regions in the brain [9, 122]. Orexin system, through these projections, coordinates the activation of many other neural systems involved in various processes [4]. Most of the projections are to the neurons of locus coeruleus, tuberomammillary nucleus, raphe nuclei, ventral tegmental area, and nuclei that regulate arousal and motivation.

The orexin neurons respond to a variety of neural signals [123]. They receive inputs from the amygdala and insular cortex to mediate stress response and autonomic tone. They also receive inputs from nucleus accumbens to regulate reward and motivation. They are influenced by the dorsomedial nucleus of the hypothalamus through the information related to wakefulness and circadian rhythms [124].

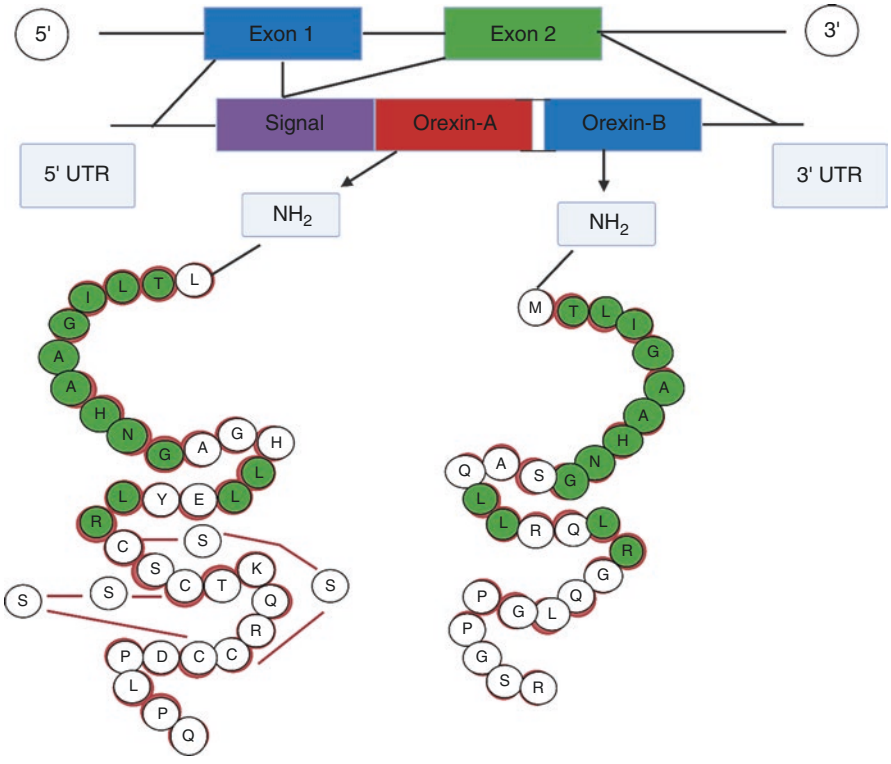

Fig. 10.6 Molecular structures of the orexin precursor and orexin [26]

Medications Acting Via Orexin System

The therapeutic perspective of medications that work via the orexinergic system is currently being studied actively. Greater number of orexin antagonists have been and are being developed as compared to orexin agonists. These drugs act via orexin receptors (OX1R and OX2R). Some of these antagonists and agonists are discussed below and shown in Table 10.1.

Orexin Antagonist

After the discovery of orexins, several groups developed orexin antagonists among which SB-334867 is the first and widely studied drug. It is a selective orexin 1 receptor antagonist [11]. Tables 10.2 and 10.3 outline the various orexin receptor antagonists.

Table 10.1 Orexin receptor antagonists and their selectivity

Adipose tissue	Selectivity	Possible application
1. SB-334867	OX1R	Withdrawal, substance abuse, obesity, panic disorder
2. SB-408124		
3. SB-674042		
4. ACT-335827		
5. TCS-OX2–29	OX2R	Sleep promotion
6. JNJ-10397049		
7. EMPA		
8. Antagonist 26		

Table 10.2 Dual orexin receptor antagonist and their applications

Dual Orexin receptor antagonist	Possible application
1. Almorexant	Treatment of insomnia
2. SB-649868	
3. Suvorexant	
4. MK-6096	–
5. DORA 30	Sleep promotion

Table 10.3 Orexin receptors and antagonists [125]

Drug group	Compounds	Target nuclei
Orexin-1 receptor antagonist	SB-334867 SB-408124 SB-674042 ACT-335827	Dorsal raphe Locus coeruleus Laterodorsal tegmental nucleus Pedunculopontine tegmental nucleus
Orexin-2 receptor antagonist	TCS-OX2–29 JNJ-10397049 EMPA	Dorsal raphe Tuberomammillary nucleus

(continued)

Table 10.3 (continued)

Drug group	Compounds	Target nuclei
Duel orexin receptor antagonist	Almorexant SB-649868 Suvorexant MK-6096	Dorsal raphe Locus coeruleus Laterodorsal tegmental nucleus Tuberomammillary nucleus Pedunculopontine tegmental nucleus

Orexin 1 Receptor-Selective Antagonist

The first discovered orexin 1 receptor antagonist SB-334867 inhibits the orexin A and orexin B calcium response [126]. Its affinity for OX1R is approximately 50 times higher than that of OX2R, but very high doses may block the binding of the agonist to both the receptors [127]. It is devoid of any agonist like activity [11]. The effect of this selective orexin receptor antagonist (SORA) on sleep promotion was poor, but showed some promise in the treatment of obesity, panic disorder, substance abuse, and withdrawal [128–132]. SB-334867 also affects norepinephrine and monoamine transporters and binds vaguely to serotonin and adenosine receptors [133]. Later compounds like SB-408124, SB-674042, and AK-335827 also exhibited selective OX1R antagonism, but none of these were found to help promote sleep [23, 134, 135]. When administered alone, they lack the sleep-promoting effect but can prevent the sleep inhibiting effects of orexin [130]. Interestingly, when coadministered along with OX2R antagonist, the sleep induction effect was milder compared to administration of OX2R antagonist alone, indicating that they reduce the sleep-promoting effect of other antagonists [23].

Orexin 2 Receptor-Selective Antagonist

This is a less common class where few molecules have been studied. These are EMPA, TCS OX2 29, and JNJ-10397049 [125].

During initial hours of intraperitoneal administration of EMPA, it selectively increased NREM sleep without increasing REM sleep. It did not show any reduction in latencies of either stage of sleep [134]. Intracerebroventricular infusion of TCS OX2 29 in rats increased their sleep time secondary to an increase in REM sleep [136]. JNJ-10397049 produced an increase in total sleep time with an increase in REM and nREM sleep and induced a decrease in REM sleep latency without significant changes in its duration [23, 137].

Dual Orexin Receptor Antagonist (DORA)

DORAs, drugs with dual orexin receptor antagonistic action were developed for the treatment of insomnia. They inhibit both receptor subtypes OX1R and OX2R. Some of them are as follows.

Almorexant (ACT-078573), the most widely studied DORA, was the first to enter phase 3 clinical trials. It is a competitive antagonist of OX1R and a noncompetitive antagonist of OX2R [138]. It is well tolerated in humans. It elicited decrease alertness when administered at a dose of 200 mg or more. It caused increase fatigue, drowsiness, and sleepiness [139]. It boosts sleep by increasing total sleep time and reducing the frequency of awakening and REM sleep latency in insomnia patients without comorbid conditions [140]. Its side effects include headache, blurred vision, and dizziness. The molecular structure of Almorexant is shown in Fig. 10.7.

Suvorexant (MK-4305) is a small molecule diazepane-based orexin receptor antagonist which is used successfully in the treatment of insomnia [15]. It has the same potency as OX1R and OX2R, and is effective in sleep stimulation [141, 142]. It increases the total sleep time which is attributed to increase in REM sleep and reduce sleep latency without reducing the frequency of awakenings. Dose-dependent side effects include headaches, dizziness, somnolence, and abnormal dreams [125]. Its effects in insomnia patients are dose-proportional and significant, thus establishing the role of DORAs in the treatment of insomnia [143]. The molecular structure of suvorexant is shown in Fig. 10.8.

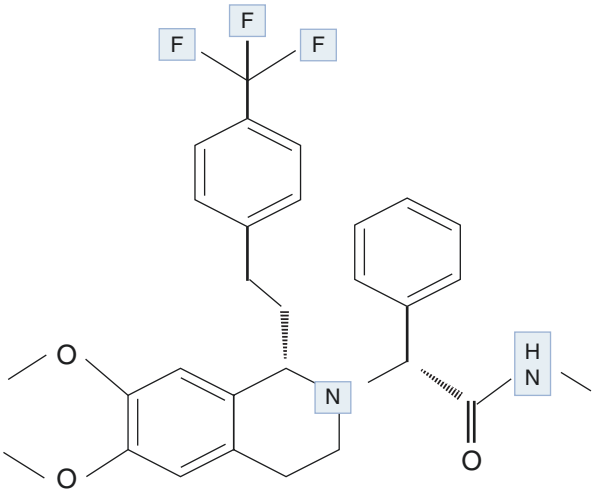

Fig. 10.7 Structure of Almorexant

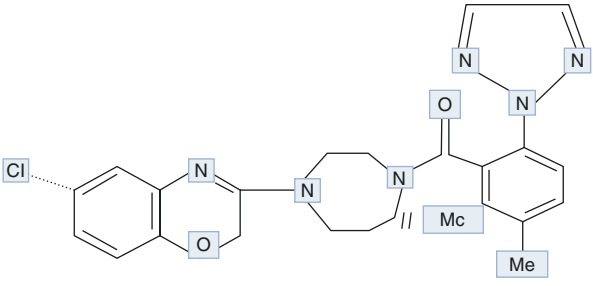

Fig. 10.8 Structure of Suvorexant [15]

Another orally active potent DORA is SB-649868, which is effective in promoting sleep. Compared to Almorexant, the efficacy of SB-649868 in vivo is tremendous. It significantly improves sleep quality but reported side effects are headache, dry mouth, and nasopharyngitis [144].

Orexin Agonist

[Ala^{11}, D-Leu^{15}] orexin-B is a potent and selective orexin-2 receptor agonist. Asahi and colleagues in 2003 identified that this agonist showed 400-fold selectivity for OX2R and enhanced its potency. It paved the way for further exploration of OX2R physiological roles and pharmacology [145]. Studies by Deadwyler in 2007 highlighted the possibility of using orexin-A agonist in the treatment of cognitive impairment-related conditions and arousal disorders [146]. OBt-9, a small molecule compound, positively potentiates the activity of orexin-A on OX1R by upregulating orexin signaling [147].

Most of the compounds in this class are known to be weak [148, 149]. Compound 26 showed 70-fold selectivity, and compound 31 (YNT-185) showed 100-fold selectivity for OX2R in animal cells [16, 150]. In disorders with reduced orexinergic activity, orexin receptor agonist might be useful, but unfortunately none of the compounds of this class are available commercially.

Other Clinical Aspects

1. *Depression*: Projection of orexinergic neurons to the dopaminergic ventral tegmental nucleus and substantia nigra suggests the role of the orexin system in the pathophysiology of depression. Orexins excite serotonin neurons directly and, at high concentrations, inhibit serotonin by exciting GABAergic interneurons speculating a certain relationship between them in the process of depression [120, 151]. The level of CSF OA was higher in patients with depression, and this level declined after the administration of antidepressants, confirming the correlation between orexin-A and depression [152]. In rodents with depressive symptoms, a reduced number and reduced size of orexin neurons were observed [153], and OA mRNA was detected in the peripheral blood cells of patients with depression [154]. Depending on the receptor subtype activated (OX1R or OX2R), orexin can act either like an antidepressant or pro-depressant [155]. In the hippocampus there is a negative correlation between orexin expression and depressive behavior whereas in the amygdala, there is a positive correlation between orexin expression and depressive behavior, suggesting that orexin receptor pathways play different roles in different regions of the brain [156]. The antagonistic roles of dynorphin and orexin regulate the brain reward system and also modulate the pathophysiology of depression [157]. The effect of orexin neurons on serotonergic and dynorphin neurons is showin in Fig. 10.9.

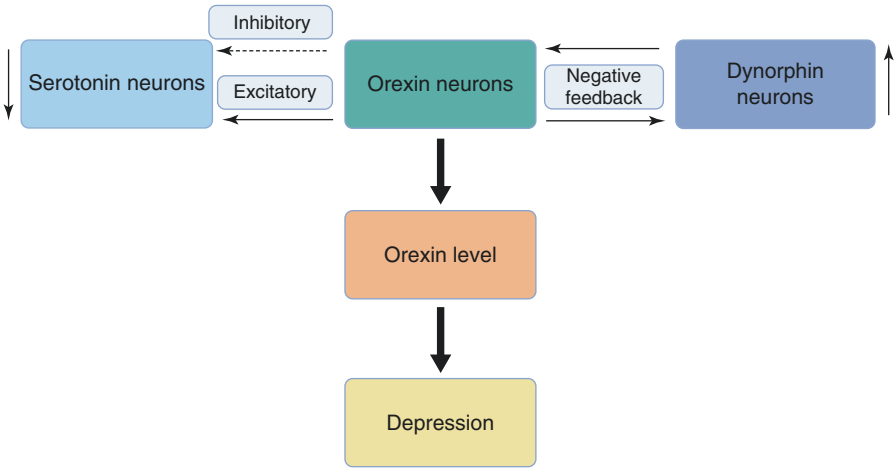

Fig. 10.9 Role of Orexin in depression [26]

2. *Alzheimer's Disease (AD)*: The role of orexinergic neuron system is reported in the pathogenesis of Alzheimer's disease. Alzheimer's disease is a progressive neurodegenerative disease characterized by progressive cognitive dysfunction and behavioral impairment. Orexins modulate amyloid-β pathology in the brain and overexpression of orexin causes Aβ accumulation in the brain [158].

   Alzheimer's Disease patients suffer from sleep disorders due to some effect on the secretion of orexins which further enhance amyloid-β levels contributing to Alzheimer's Disease [159]. Correlation between amyloid β42 level and CSF OA upregulation is also established [160]. Some studies have shown a low concentration of OA in CSF of patients with AD [161], while others have demonstrated increased CSF orexin levels in AD patients [162]. Though it is unclear, there seems to exist a relationship between the orexin system and Alzheimer's disease. Further studies can pave way for orexin receptor antagonists as a potential treatment option against AD.
3. *Cancer*: Orexins are involved in the proliferation and apoptosis of cancer cells. In human colon cancer cell lines, orexin induces apoptosis which reduced cell growth (see Fig. 10.10). This was mediated by OX1R. It was also observed in neuroblastoma cells [164]. But OA was also found to stimulate growth of tumor cells in the adrenal gland [165]. The role of OA and its receptors in angiogenesis under pathophysiological conditions is also established [166]. Hence, it is strange that in some cell lines, orexin signaling induces apoptosis while in other cell lines it increases proliferative activity. Clinical studies reveal low levels of CSF OA in patients with hypothalamic tumors [167], and low levels of serum OA with elevated expression of OX2R in patients with benign prostatic hyperplasia [168]. Epigenic silencing of OX2R was found to be associated with endometrial cancer [169]. Studies suggesting the expression of OX2R in a certain type of cancer cells but not in normal cells offer OX2R as a potential target for cancer therapy in OX2R-positive cancer cells [170].

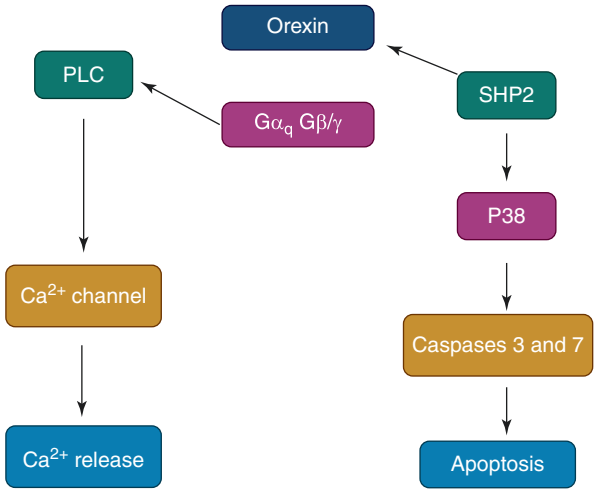

Fig. 10.10 Proapoptotic signaling pathway induced by orexins [163]

4. *Stroke*: Orexinergic neuronal system is protective against ischemia–reperfusion injury of the brain. It regulates antiapoptotic and inflammatory responses following cerebral ischemia. Orexin-A and OX1R play an important role in ischemia pathology [171]. The levels of OX1R mRNA and OX1R protein were elevated in rat ischemic cortex post middle cerebral artery occlusion [172]. OA levels in CSF decreases persistently in cerebral infarction [173]. The dynamics of orexin and OX1R in the neuronal damage caused due to transient ischemia was further demonstrated by Dohi, where the declining level of CSF OA correlated with increased Expression of OX1R on second and fourth day post ischemia [174]. The ischemic side contained a greater number of orexin-A containing neurons than nonischemic side [175]. The neuroprotective action of OA is mediated not only through reduction in the number of apoptotic cells and activation of HIF-1α [176] but also by promoting the expression of brain-derived neurotrophic factor [177]. Treatment with OA in animals altered the mRNA expression of TNF-α and IL-6, indicating an involvement of chronic inflammatory response [178]. Figure 10.11 shows the role of orexins in neuroinflammation and depression.
5. *Pain*: Many studies have described the analgesic properties of the orexin system. Orexins can block thermal, chemical, mechanical, and nociceptin-induced behavioral responses through the involvement of the adenosine pathway, which is independent of the opioid system [180]. Clinical observations have shown an association between changes in orexin receptors and headache [181]. Outside the CNS, OA sensitizes nociceptive responses modulating the peripheral sensory transmission by activation of intracellular calcium signaling through the protein kinase C pathway [182]. Orexins coordinate with nociceptin/orphanin FQ system and regulate stress-induced analgesia [183]. Chronic pain is common in narcolepsy patients with cataplexy [184]. Figure 10.12 outlines the role of orexins in antinociceptive pathways in the brain.

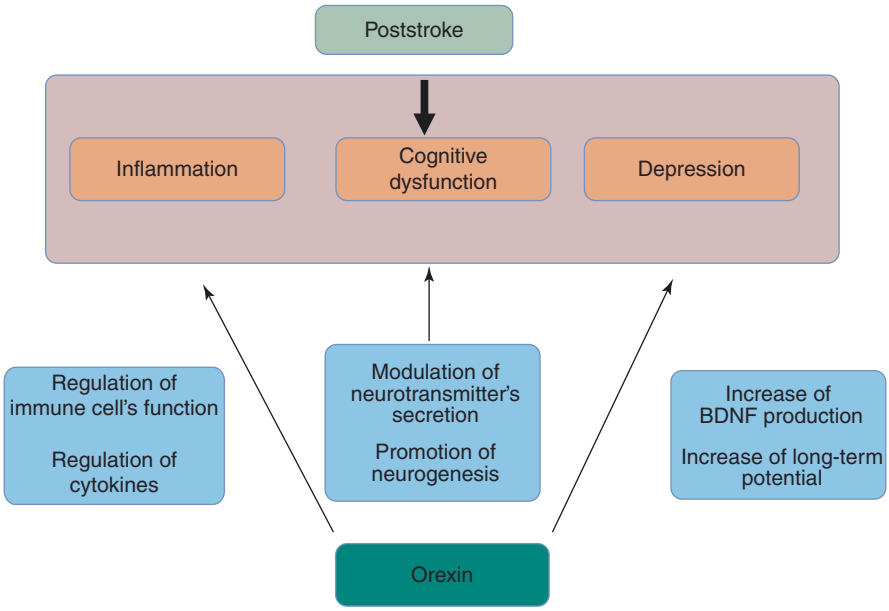

Fig. 10.11 Role of Orexin poststroke [179]

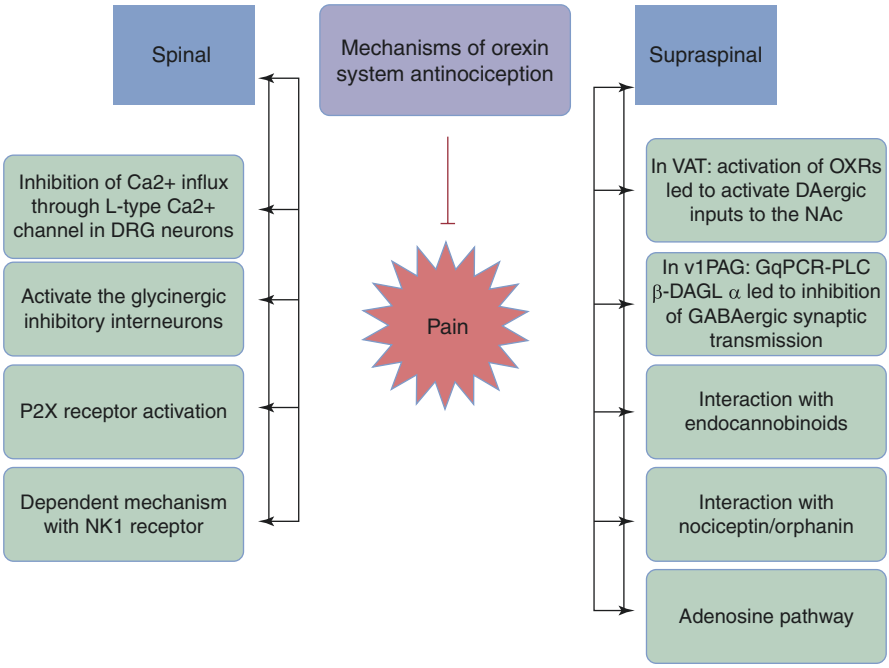

Fig. 10.12 Mechanism of orexin antinociception action [185]

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 11
Acetylcholine

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History

Acetylcholine (Ach) is a well-known neurochemical, and the tale of its discovery is a rather remarkable one. In 1867, Adolf Von Baeyer synthesized acetylcholine by acetylating choline at very low temperatures [1]. As his experiment could not be repeated, the exploration of the structure of Ach and its biological action came to a halt. In 1899, Mott and Halliburton detected choline in cerebrospinal fluid of patients with diseases causing severe atrophy of the brain, epilepsy, and other nervous system diseases. They injected the fluid into animals and noted a fall in their blood pressure with little to no effect on respiration [2]. In 1906, Hunt and Taveau studied substances occurring in the adrenal glands that can lower blood pressure and found choline to be the most prominent among these substances [3].

In 1906, Henry Hallett Dale and his colleagues extracted ergotoxine from ergot, a fungus that grows on Rye. Later on, he demonstrated the occurrence of amines (tyramine, histamine) and Ach in these ergot extracts [4]. In 1914, Arthur J Ewins, a colleague of Dale, extracted a derivative from an ergot fungus that was chemically and physiologically identical to Ach. This was the first time Ach was discovered in a natural organism [5]. The same year, Dale published a paper based on the observations made while studying the effects of choline esters and ethers. This article served

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as the basis for future studies to determine the role of acetylcholine in chemical transmission. He determined that the effects of acetylcholine, in particular, could be broadly classified into two types—"muscarine-like" and "nicotine-like" effects [6]. Though the effect of Ach on the parasympathetic system was established, its occurrence as a normal body constituent was not demonstrated until 1929 by Dale [7].

In 1926, Otto Loewi conducted a series of experiments on frog heart preparations to demonstrate the chemical transmission of vagal impulses. He observed the occurrence of an active substance which he named "Vagusstoff" since it was released from the stimulated vagus nerve of the heart. On further studying, he found that the substance had a close resemblance to Ach [8]. However, since Ach was known only as a synthetic derivative, many scientists did not concede to its role as a naturally occurring chemical. In 1929, Dale and Dudley chemically identified Ach after they successfully extracted it from the spleens of oxen and horses [9]. The results drawn from Loewi's experiments as well his own research, helped Dale reach the conclusion that Ach played a pivotal role in neurotransmission at the autonomic ganglion cells and the neuromuscular junction. In 1933, Dale wrote an article titled "Nomenclature of fibers in the autonomic system and their effects," in which he suggested the terms "cholinergic" and "adrenergic." Neuronal fibers including pre- and postganglionic autonomic fibers that functioned through the release of Ach-like substance were described as "cholinergic." Those that operated through release of adrenaline-like substance were termed 'adrenergic' [10]. In 1936, Dale and Loewi were awarded the Nobel Prize in Physiology or Medicine for their discoveries related to the chemical transmission of nerve impulses [11].

Neurochemical Profile

Ach is an excitatory neurotransmitter that mediates synaptic transmission in the central, peripheral and autonomic nervous systems. The organization of the sympathetic and parasympathetic nervous systems is shown below in Fig. 11.1.

Central and Peripheral Nervous System Distribution of Ach

The function of Ach in the brain is rather intriguing and includes alteration of the synaptic plasticity [12], presynaptic release of neurotransmitters [13], and neuronal excitability. These diverse effects in the brain are due to the location of cholinergic neurons, different receptor subtypes, and the target neurons. Until the 1980s, the topography of cholinergic neuronal distribution was studied based on the histochemical staining of acetylcholine esterase (AchE). With the development of antibodies against acetylcholine transferase (AchT), it became evident that neurons secrete and utilize Ach as a neurotransmitter [14]. Other methods employed to map the cholinergic system include immunohistochemical staining and in situ hybridization for detecting vesicular acetylcholine transporter and AchT.

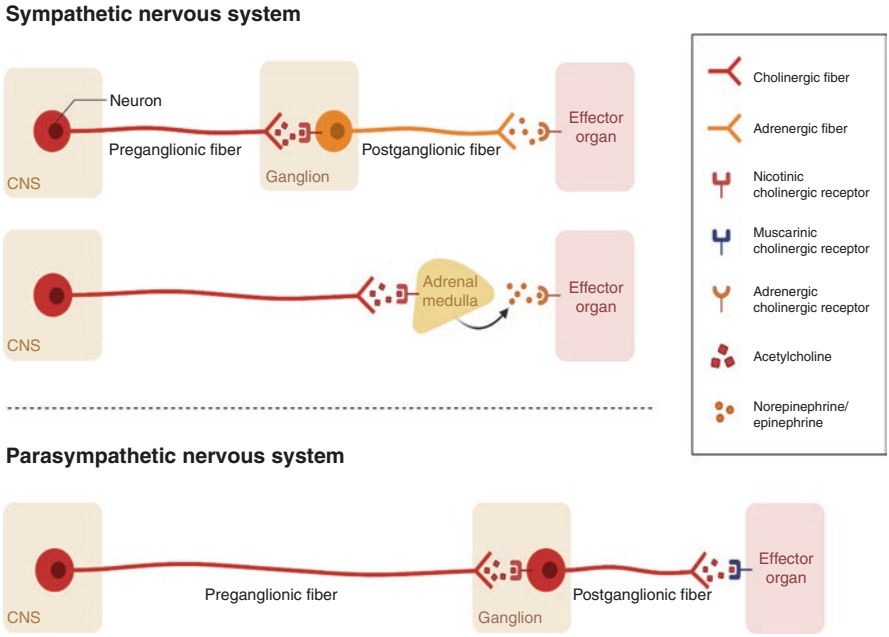

Fig. 11.1 Organization of the sympathetic and parasympathetic nervous systems

In the central nervous system, cholinergic neurons exist as interneurons, projections, pathways, and part of major cell groups [15]. They are classified into the following types:

1. Cholinergic projection neurons of mesopontine region

   Although cholinergic neurons constitute less than 1% of the total neuron population, the extent of its influence is vast. The estimated number of cholinergic neurons in the mesopontine region is approximately 20,000 [16], and at the nucleus basalis is around 2,00,000 [17]. Mesopontine cholinergic neurons are located in the pedunculopontine nucleus and lateral dorsal tegmental nucleus. They are intermingled with GABAergic and glutaminergic neurons and give rise to descending and ascending projections. The descending projection is known to reduce muscle tone during REM sleep [18]. The ascending projections to the subthalamic nucleus are involved in regulating basal ganglia functions [19].
2. Cholinergic projection of basal forebrain

   Cholinergic neurons in the basal forebrain form a complex integrative projection into the neocortex, which has a significant role in arousal [20], learning, and memory [21]. These widespread projections into the neocortex are categorized into different pathways-.

   (a) Septohippocampal pathway, formed by the projections of vertical diagonal band and medial septal nucleus into the hippocampus [22].

   (b) The second group innervates amygdala, olfactory bulb, cingulate, entorhinal and perirhinal, retrosplenial, insular cortices, and parts of the frontal cortex [23].

(c) Cholinergic neurons in the substantia innominata, magnocellular preoptic nucleus, and nucleus basalis (of Meynert) project to all parts of the brain, including visual (primary and secondary), auditory, somatosensory, and higher association cortex [24].

3. Medial habenula group

   The one group of cholinergic neurons that is exceptional to the usual ventral location is the medial habenula, located dorsally [25]. The projections terminate largely in the interpeduncular nucleus and play a significant role in nicotine dependence [26].
4. As interneurons in islands of cajella complex, and striatum

   Cholinergic neurons exist in both the dorsal (neostriatum) and ventral striatum as interneurons. The neostriatum corresponds to the caudate nucleus–putamen complex [27, 28]. The ventral striatum consists of the nucleus accumbens and the islands of Calleja, which consists of smaller neurons than the striatum [27, 29]. Though the striatum forms a part of the extrapyramidal system, they are involved with cognitive functions.
5. Cholinergic motor neurons in the hindbrain and spinal cord [30]

   Cholinergic neurons exist majorly in the ventral horn of the spinal cord. The motor neurons, regardless of the type of muscle innervated, are invariably cholinergic. In the periphery, cholinergic neurons are organized as clusters or bundled fibers and are distributed as follows:
   (a) Pre- and postganglionic parasympathetic innervation.
   (b) Preganglionic sympathetic ganglia.
   (c) Postganglionic sympathetic innervation (piloerector muscle & sweat glands).
   (d) Somatic (voluntary) motor neurons.

Acetylcholine in Sleep–Wake Cycle/Brenner's Experiments

Sleep is a fundamental physiologic state characterized by physiological, behavioral, and electrophysiological parameters. It occupies one-third of human lives and serves multiple functions [31]. In 1930, Von Economo first examined the neural regulation of sleep/wake cycle [32]. Changes in the brain activity alter cognition and motor control during transitions between sleep-wake cycles. Jouvet reported the role of acetylcholine and other monoamines in the arousal period. For decades, various experimental techniques like lesional and electrical stimulations, electrophysiological recordings, and drugs were utilized to determine the functions of the sleep-wake circuit, which are regulated by multiple heterogeneous neuronal populations [33]. The technology of identifying genetically defined neuronal groups enabled scientists to specifically stimulate specific cell types and understand their function with high precision [34].

In the paper titled "The genetics of Caenorhabditis elegans," the Nobel laureate Sydney Brenner quoted, "How genes might specify the complex structures found in higher organisms is a major unsolved problem of biology." His pioneering experiments on a nematode, Caenorhabditis elegans (C. elegans) led to the discovery of a complete set of synaptic connections in the nervous system of an animal for the first time [35]. His primary goal was to identify an animal whose nervous system could be pinned down to the synaptic level, and C. elegans had the smallest nervous system, containing 302 neurons. He reconstructed the pictures of the entire system, which constituted about 5000 chemical synapses and 2000 neuromuscular junctions, using an electron microscope. Since C. elegans was readily amenable for genetic analysis, many mutants were mapped and isolated, which facilitated the study of the functional changes in the mutant nervous systems [36]. Various wiring diagrams reported through his work revealed how the nervous system could control behavior. His work on genome mapping laid the foundation for a more precise novel brain stimulation technique, devoid of side effects occurring from the traditional methods. Neuronal genomic mapping helps obtain information to study the role of specific neuronal populations and is being widely used by sleep researchers to study genes involved in sleep–wake cycle regulations [37].

The complex interactions between various regions of the brain like the hypothalamus, brainstem, midbrain, cortex, thalamus, and basal forebrain play a crucial role in sleep–wake states. Wakefulness is a spectrum of behavioral states associated with voluntary muscle activity in response to external/internal stimuli. During this state, low amplitude, fast frequencies are detected on electroencephalograms (EEG), and muscle activity is detected on electromyogram (EMG) [38].

The sleep cycle constitutes three stages of NREM (non–rapid eye movement) and REM (rapid eye movement) sleep as seen on EEG. As the stages increase, the stimulus required for arousal also increases [39].

1. N1—Early stage of quiescent sleep associated with spindle waves that occur at a frequency of 7–14 Hz.
2. N2—A slightly deeper sleep where the frequencies become slower.
3. N3—A deeper sleep state dominated by delta (0–4 Hz) and theta (4–7 Hz) waves.
4. REM sleep which is similar to a state of wakefulness but with markedly inhibited motor output and lasts around 90 min.

The basal forebrain consists of neurons that are 55% glutaminergic, 35% of GABAergic, and 5% cholinergic. The stimulation of the area results in a heterogeneous sleep–wake transition [40] and the neuronal firing is maximum during wakefulness and REM sleep. There have been reports that lesions affecting this area can result in a coma-like state. The projections from this region to the cortex excite the cortical pyramidal neurons. In addition to arousal, it is involved in other cortical functions like memory, attention, and processing sensory information [39]. These projections also constitute GABAergic neurons in addition to cholinergic neurons [41]. They also project into the amygdala and medial septum, which drive the hippocampal theta activity [39]. The optogenetic stimulation of cholinergic neurons in

the basal forebrain aids the transition from NREM sleep to wakefulness [42], and this is genetically controlled by the channel rhodopsin 2. They induce sustained cortical activation when stimulated during wakefulness state or REM sleep and, when activated during NREM sleep, prolongs the period of REM sleep [40]. Optogenetic experiments on cholinergic stimulation were complicated by the possible interactions within the basal forebrain neurons. The GABAergic neurons that accompanied the cortical projections of the basal forebrain were also stimulated during these studies, making the interpretation of the results difficult. Zant et al. were able to overcome these obstacles using an opto-dialysis probe. In his experiment, the cholinergic neurons were optically stimulated resulting in a wakefulness state, and using the technique of simultaneous, reverse microdialysis with a cholinergic antagonist, he noted abolishment of the wakefulness state [42].

Ach in REM Sleep Generation

Current understanding of REM sleep is based on the historical works of Eugene Aserinsky, Nathaniel Kleitman, Michel Jouvet, and William Dement. In 1953, Kleitman and Aserinsky reported that human infants showed periods of active REM sleep alternated by periods of quiescent sleep [43]. Later on, Kleitman and Dement reported the correlation of particular brain wave patterns and dreams during REM sleep [44]. Jouvet and his colleagues used the term "paradoxical sleep" for the first time. He explained that dreams and oneiric behaviors are secondary to cyclical activation of brainstem reticular neurons during REM sleep [45].

Polysomnographic studies have shown fast spike potentials within the pontine tegmentum, dorsal lateral geniculate bodies, and occipital cortex, called "ponto-geniculo-occipital" (PGO) spikes; associated with REM sleep. The phasic activity that occurs within the visual system determines the content of dreams [46]. The spikes in the dorsal lateral geniculate bodies, occipital cortex, and phasic thalamocortical system activation can be eliminated by lesions of the lateral and dorsolateral midbrain [47]. Further studies have shown that REM sleep is characterized by low amplitude, fast frequency cortical activity on EEG. A hippocampal EEG demonstrates high amplitude theta waves. The arousal threshold is elevated during REM sleep. It is also associated with intermittent muscle twitches, markedly inhibited skeletal muscle activity, fluctuations in body temperature, and activation of both the autonomic and respiratory systems [44].

Animal studies involving cats have demonstrated the role of Ach in REM sleep generation. Scientists could induce a natural REM sleep state in cats by injecting cholinergic drugs into the mesopontine tegmentum [48]. The destruction of cholinergic neurons in the dorsolateral pontine tegmentum (DLT) by injecting kainic acid showed the abolishment of REM sleep [49]. Neurons in the mesopontine tegmentum are active during REM sleep and during the state of wakefulness. The DLT and pedunculopontine tegmentum (PPT) contains glutaminergic, GABAergic, and cholinergic neurons. The cholinergic neurons project into the thalamus and are

implicated in REM sleep genesis [50, 51]. These neurons also project into the pontine reticular formation, where they act through muscarinic receptors [52]. Multiple studies have shown the role of Ach produced in the pontine area in promoting REM sleep [51]. The cholinergic neurons of DLT and PPT play a pivotal role in REM sleep genesis through their interaction with the glutaminergic neurons in the subdorsolateral nucleus of the pons. This relationship is essential to generate atonic muscles during REM sleep and has no role in determining its duration [53]. Studies have shown that activating DLT and PPT during NREM sleep increased the frequency of REM sleep episodes without affecting the duration. Distinct mechanisms control REM sleep genesis and maintenance, and both DLT and PPT are the modulators of REM sleep initiation [50].

Acetylcholine in Memory Systems

In 1950, Platt and Wickens reported the effects of diisopropylfluorophosphate, an irreversible AchE inhibitor, through their experiments on rats [54]. In 1959, McGaugh and other scientists studied the relationship between AchE in the brain and the performance of animals in a 'maze'. These studies demonstrated that Ach levels in the brain varied with the degree of training [55]. Drugs like atropine and scopolamine impaired task performance in rats. Effects of the central cholinergic system on learning were established when methyl-atropine or methyl-scopolamine, a peripherally acting compound, was injected into rats and showed no influence on the behavior of learning [56].

Cellular mechanisms that enhance memory encoding secondary to Ach stimulation have been shown in various computational studies. The process of encoding is mediated by a combination of two processes:

1. The excitatory afferent input is secondary to nicotinic enhancement [57] and suppression of excitatory feedback executed through presynaptic inhibition of the muscarinic receptors (predominantly M1) [58].
2. Long-term potentiation to strengthen synapses following an activity.

Ach favors the process of long-term potentiation in the entorhinal cortex, hippocampus, and piriform cortex. The process involves muscarinic receptor-activated through phospholipase C signal cascade and stimulation of nicotinic receptors. Animal studies using rats demonstrated an increased calcium activity on stimulation of α7 subunits of nicotinic receptors in CA_1 and CA_3 regions of the hippocampus of normal rats in comparison to receptor knockout mice that showed no response [59].

Ach enhances excitatory synaptic transmission in the CA_3 region through inputs from the dentate gyrus and entorhinal cortex [57, 60]. The cholinergic projections from the medial septum to the entorhinal cortex increase hippocampal Ach, generate 'theta' oscillations, and facilitate encoding by activating the pathway from dentate gyrus to CA_3 region [61]. The dynamic change of encoding and retrieval with each cycle of "theta" rhythm is responsible for the enhancement of memory. The

modulatory effect of hippocampal interneuron depolarization can also enhance this "theta" rhythm [62].

According to the Hasselmo, memory consolidation depends on the level of Ach present during the sleep–wake cycle. The CA_1 region of the hippocampus receives input from the entorhinal cortex which is encoded by enhancing the input from the CA_3 region to increase the working memory [63]. The maintenance of newly acquired information is seen as a persistent spike of cortical neuron activity, specifically the entorhinal cortex. This mechanism applies to both short-term as well as long-term memories. Studies have shown an increase in the frequency and duration of these spikes in the presence of carbachol, an Ach agonist [64]. These occur due to a regenerative cycle of cation current in the entorhinal cortex, resulting in an increase in the calcium influx and subsequently persistent spikes [65].

An intact central cholinergic neurotransmission is essential for cognitive and behavioral functions [66]. A defect in the system can affect impair information processing in the hippocampus resulting in memory problems. Ach plays a crucial role in the pathogenesis of Alzheimer's disease, and anticholinesterases are implicated in the treatment of the same [67, 68]. This is discussed in detail elsewhere in the chapter.

Acetylcholine Receptors

Ach exerts various modulatory functions in the CNS and PNS (peripheral nervous system) through its receptors. Based on the agonist selectivity, there are two main receptors—muscarinic and nicotinic. The majority of the receptors are associated with the postsynaptic membrane of the neuromuscular junction and presynaptic and extra-junctional areas of the denervated muscle fibers and embryonic tissue [69]. They are large integral membrane glycoprotein molecules that are assembled in the Golgi apparatus about 15 min after its biosynthesis. Over the next 3 h, the receptors get embedded into the plasma membrane with the help of microtubular elements [70]. This is in an active process, utilizing ATP, and is inhibited by low temperatures. In homeotherms, the average lifespan of extrajunctional Ach receptors is around 8–30 h, and the process of degradation is energy-dependent as well [69].

Muscarinic Receptors

Muscarinic receptors are broadly divided into five subtypes—M1-M5, based on their selective agonist activity (Table 11.1). They mediate excitatory and/or inhibitory effects on the CNS and PNS through G-protein (guanine nucleotide-binding protein). The G-protein coupled receptors have a characteristic seven-transmembrane amino-acid sequence [71]. Depending on the cell type, stimulation of the muscarinic receptors can result in depolarization or hyperpolarization by promoting the

Table 11.1 Types and characteristics of muscarinic receptors

Subtype	Nature of receptor and transducer mechanisms	Location	Pertussis toxin (PTX) susceptibility	Function
M1	$G_{\alpha q}$ • IP3/DAG—Increase in cytosolic Ca^{2+} • Increase PLA2-phospholipase A2	• Autonomic ganglia • CNS—Cerebral cortex, hippocampus, thalamus, caudate-putamen, amygdala • Exocrine glands	None	• Involved in learning and memory • Secretion of the glands • Depolarization (slow EPSP)
M2	$G_{\alpha i}$—Inhibits adenylyl cyclase • Decreased Ca+ conductance • Increased K+ conductance • Decreased cAMP	• Heart • CNS—Basal forebrain, caudate-putamen, hippocampus, hypothalamus, amygdala, and pontine nuclei	Susceptible	• Reduced chronotropy • Reduces contractility of the heart • Decreased AV nodal conduction • Reduces acetylcholine release (homotropic inhibition) • Analgesia
M3	$G_{\alpha q}$ • IP3/DAG—Increase cytosolic Ca^{2+} • Increase PLA2-phospholipase A2	• Lungs—Bronchial smooth muscle • Eyes—Iris and ciliary muscle • Cerebral vasculature • CTZ—Chemoreceptor trigger zone (area postrema) • Exocrine glands • Visceral smooth muscle • CNS—Olfactory tubercle, cerebral cortex, hippocampus, medial thalamus, caudate-putamen, and amygdala	None	• Increase lung secretions and bronchoconstriction • Role in accommodation and miosis • Increased gut secretions and gut motility • Emesis • Cerebral vasodilatation
M4	$G_{\alpha i}$—Inhibits adenylyl cyclase • Decreased Ca+ conductance • Increased K+ conductance	• CNS—Cerebral cortex, hippocampus, thalamus and caudate-putamen	Susceptible	• Facilitation/inhibition of transmitter release in the brain
M5	$G_{\alpha q}$	• CNS—Hippocampus, substantia nigra, amygdala, thalamus, hypothalamus, lateral habenula medial mammillary nucleus	None	• Facilitate dopamine release • Mediates reward behavior

opening or closing of calcium, potassium, or chloride channels [72, 73]. M1 and M3 receptors are postsynaptic, and M2 (auto)receptors are presynaptic, regulating the acetylcholine release [74]. They also modulate synaptic transmission by regulating calcium channel activity [75, 76]. Stimulation of muscarinic receptors produces excitability, especially in the cortex, which involves K^+ suppression in the postsynaptic sites [72]. Presynaptic receptor activation results in the inhibition of the GABAergic transmission [77]. NMDA (N-methyl-D-aspartate) currents are increased by the stimulation of Gαq coupled receptors [78]. The combination of promoting glutaminergic transmission coupled with inhibition of GABAergic transmission results in cortical excitation. However, it has been demonstrated that in the hippocampus, these receptors modulate both excitatory and inhibitory transmissions, which plays a key role in the process of learning and memory [79].

Nicotinic Receptors

Nicotinic receptors are ion gated receptor channels similar to GABA and glycine receptors. It is a pentameric structure made of five subunits, namely, alpha, beta, epsilon, gamma, and delta, that are homologous with a symmetrical, pseudo-fivefold arrangement [80, 81]. It is found in skeletal muscles, autonomic ganglia (pre- and postganglionic parasympathetic), adrenal medulla (preganglionic sympathetic), spinal cord, and certain areas of the brain [82].

Nicotinic receptors are broadly divided into two subtypes—N_N and N_M, based on their selective agonist and antagonist activity (Table 11.2). The properties and functions of the receptors are influenced by subunit compositions. Muscular nicotinic receptors are mostly postsynaptic, and their activation results in synaptic transmission [83]. Neuronal nicotinic receptors are widely distributed in autonomic and central synapses and modulate neurotransmitter release. Due to the heterogeneous expression of these receptors, they have diverse functionality. Receptors in the CNS are mainly presynaptic, regulating neurotransmitter release and exhibit a

Table 11.2 Types and characteristics of Nicotinic receptors

	N_N	N_M
Location	Neuromuscular junction	• Autonomic ganglia • Adrenal medulla • CNS
Structure	Ion channel—Pentamer of all subunits	Ion channel—Pentamer of only alpha or alpha and beta subunits
Transducer mechanism	Open channel for cation (K+, Ca+, and Na+) conductance.	Open channel for cation (K+, Ca+, and Na+) conductance
Function	Skeletal muscle contraction by depolarization of the motor endplate	• Postganglionic impulse • Catecholamine release • Excitation/inhibition-site specific in the CNS

modulatory influence [84]. Stimulation of these receptors increases presynaptic Ca^{2+} levels and eventually results in the release of serotonin, dopamine, GABA, and norepinephrine [57]. The α7 subunit expressed in the CNS receptors increases its permeability to calcium, and higher receptor concentrations are seen in the olfactory areas, amygdala, hypothalamus, hippocampus, and cerebral cortex [85]. The α7 subunit has the lowest sensitivity to nicotine, whereas the α4 subunits are most sensitive [86]. Cognitive performance is enhanced by nicotinic agonists and impaired by the antagonists. The interaction between the cholinergic and the glutaminergic systems is neuroprotective and enhances the cognitive process [87].

Acetylcholine Metabolism

The chemical formula for acetylcholine is $C_7H_{16}NO_2$. Proteins implicated in the synthesis, storage, transportation, and degradation of Ach regulate the cholinergic neurotransmission.

Synthesis

Ach is synthesized in the cytoplasm of the nerve terminals, and the enzyme AchT catalyzes the reversible transfer of acetyl groups from acetyl-coenzyme A (acetyl-CoA) to choline forming acetylcholine [88–90]. Mitochondria is the source of acetyl CoA, and choline is obtained from three different sources [90].

1. The breakdown of choline-containing phospholipids.
2. Free plasma choline.
3. Reuptake of choline after hydrolysis of acetylcholine.

Choline is an essential nutrient as it is required to make essential membrane phospholipids, and the majority comes from the diet [91]. It is exclusively found in cholinergic neurons (except human placenta), localized within the cytoplasm and a fraction of the enzyme is presumably found attached to membranes of the vesicles [92]. It is a 69 kDa enzyme, and two other longer forms have been identified, secondary to alternate splicing of mRNA [93]. The 82 kDa enzyme form is localized in the nuclei and is the first form of nuclear enzyme reported to synthesize a neurotransmitter [94]. The expression of the longer form in the neuronal nuclei has been demonstrated with the help of immunohistochemistry [95]. The synthesis of acetylcholine is regulated by various factors which include demand, neuronal depolarization, and the influx of calcium and sodium [96].

$$\text{AcetylCoA} + \text{Choline} \xrightarrow[\text{AchT}]{} \text{Acetylcholine}$$

Storage

The synthesized acetylcholine gets pumped into synaptic vesicles, which are the structural elements for storage. ATP, calcium ions, and protons play an active role in this stage. The uptake of acetylcholine is regulated by vesicular ATPase, which possibly provides the proton-motive force. In exchange for two vesicular protons, one molecule of acetylcholine gets transported [97]. The vesicular transporter gene is crucial for the normal function of the transporter and if mutated can be lethal [98]. Gene mapping has enabled us to establish the relationship between the vesicular transporter and choline acetyltransferase genes; where the former was nested in the first long intron of the latter gene [99]. The location of the transporter is responsible for the type of vesicles in which neurotransmitters are stored. Protein kinase C regulates vesicular localization through phosphorylation of the transporter [100]. The genetic role of the transporter was established by studying a mouse model with homozygous knockdown of the transporter gene, which showed a 65% decrease in the transporter protein levels. A minimal reduction in the transporter levels was also found to be associated with mild cognitive impairment [101]. Vesamicol is a non-competitive inhibitor of choline uptake and inhibits vesicular transport [102].

Hydrolysis

The enzyme, AchE secreted by cholinergic neurons is responsible for the inactivation of acetylcholine [103]. It is encoded by a single gene located on chromosome 7 and is secreted into the synapse. The posttranscriptional and translational modifications are responsible for the diverse nature of this enzyme [104]. The unique aspect of this enzyme is that it gets secreted into the synaptic cleft, where it associates with the plasma membrane [105]. The gene can generate multiple protein products through alternative mRNA splicing, which explains the structurally diverse patterns of plasma membrane association. The transcript is mainly seen in the brain, muscles, and developing blood cells [104, 106].

Many compounds inhibit AchE activity and are discussed elsewhere in this chapter -

1. Competitive AchE inhibitors are used in the treatment of Alzheimer's disease.
2. Organophosphates commonly used as insecticides are irreversible inhibitors of AchE.

Choline Reuptake

An active transport system is responsible for choline uptake after the hydrolysis of Ach [107]. This is the rate-limiting step of Ach synthesis [108].

Two types of choline transporters have been identified.

1. Low-affinity, sodium-independent transporter.
2. High-affinity, sodium-dependent transporter, sensitive to hemicholinium (CHT).

In 2000, Okuda et al. identified the CHT gene, and the corresponding proteins are predominantly localized to the synaptic vesicles and early endosomes [109]. The CHT transporter is recycled back to the plasma membrane after internalization by a depolarization modulation, which is calcium-dependent [110]. It is also demonstrated that increased neuronal firing promotes choline uptake and Ach synthesis. In an experiment with rats that were exposed to performing attention-based tests, results exhibited an increase in the levels of CHT at the synaptic membrane and increased choline uptake in the prefrontal cortex [111].

Medications Acting on Acetylcholine System

Cholinomimetics/Parasympathomimetics [112]

Drugs that act directly on the Ach receptors and mimic its action are termed cholinergic agonists (Table 11.3). Choline esters exhibit their actions through both muscarinic and nicotinic receptors. Acetylcholine and methacholine are hydrolyzed by acetylcholinesterase and butyrylcholinesterase, respectively, and they have limited clinical use. Cholinomimetic alkaloids are naturally occurring extracts. Pilocarpine is obtained from the leaves of Pilocarpus microphyllus, muscarine is from Amanita muscaria (poisonous mushroom), and arecoline is from Areca catechu (the betel nut). These drugs exhibit muscarinic effects predominantly although nicotinic effects have been reported as well.

Drugs that act indirectly increase the availability of Ach by inhibiting the degradation of AchE are termed anticholinesterases (Table 11.4). Acetylcholinesterase enzyme gets inactivated reversibly and irreversibly by acetylation/carbamylation and phosphorylation of the esteric site, respectively. The cholinergic actions on various systems result in a side effect profile that includes increased secretions,

Table 11.3 Cholinergic agonists, mechanism of action, uses, and side effects

Drug classification	Drug name	Mechanism of action	Side effects	Uses
Cholinergic Direct-acting (choline esters)	• Acetylcholine • Methacholine • Carbachol • Bethanechol	Agonist activity on both muscarinic and nicotinic receptors	Colic, involuntary urination/defecation, flushing, belching, sweating, fall in BP, bronchospasm	Rarely used clinically Bethanechol—Postoperative ileus (not used anymore)
Cholinergic Direct acting (alkaloids)	• Muscarine • Pilocarpine • Arecoline	Pilocarpine-predominantly muscarinic	Sweating, salivation and increase in secretions	Pilocarpine—Drug in open-angle glaucoma

Table 11.4 Anticholinesterases and their clinical uses

Drug classification	Drug name	Uses
Reversible anticholinesterases (indirect-acting)	Carbamates • Physostigmine • Neostigmine • Pyridostigmine • Edrophonium • Rivastigmine • Donepezil • Galantamine Acridine • Tacrine	• Myasthenia gravis, autonomic dysfunction—Pyridostigmine • Dementia—Rivastigmine, donepezil, galantamine • Physostigmine—Miotic eyedrops, belladonna poisoning • Neurotoxic snake poisoning—Neostigmine
Irreversible anticholinesterases (indirect-acting)	Organophosphates • Echothiopate • Parathion • Malathion • Diazinon • Tabun • Sarin • Soman Carbamates • Carbaryl • Propoxur	• Insecticides and nerve gas • Clinical implication in treating insecticide poisoning

bronchospasm, diaphoresis, increased gut motility, bradycardia, and AV conduction blocks. The clinical use of anticholinesterases in the management of dementia is discussed in detail elsewhere in this chapter.

Synthesis and Release Inhibitors [112]

The uptake of choline is the rate-limiting step in the synthesis of Ach. Drugs like hemicholinium and vesamicol block the uptake and inhibit the synthesis. Although they are not used clinically, they have been implicated in many experimental studies. Botulinum toxin blocks the release of Ach from the vesicles that store them through axonal protein interactions. Toxin from the black widow spider also interferes with the cholinergic transmission by inducing a massive release of acetylcholine and depleting it. Botulinum is an exotoxin produced by Clostridium botulinum, and both A and B-type toxins are highly potent with a wide variety of clinical uses. It is used for treating spastic neurological conditions like blepharospasm, migraine, nystagmus, torticollis, strabismus, hemifacial spasm, stroke spasticity. Side effects include ptosis, diplopia, dry mouth, dysphagia, muscular weakness, and even respiratory paralysis in higher doses.

Anticholinergics/Parasympatholytics

Anticholinergics are drugs that block the actions of Ach (Table 11.5). Atropine is the prototype drug that predominantly acts through muscarinic receptor blockade. The most common side effects of these drug compounds are blurry vision, confusion, urinary retention, dry mouth, dry skin, hyperthermia, excitement, psychotic behavior, ataxia, delirium, and visual hallucinations. They are clinically used for their anti-muscarinic actions, and the central action of certain compounds enables their use in the treatment of Parkinson's disease. They are sometimes used in cases with drug-induced parkinsonism and are used in conjunction with dopaminergic drugs in patients with Parkinson's disease Trihexyphenidyl is the most commonly used medication to treat Parkinson's disease, and it improves tremor more than rigidity or bradykinesia [113].

Anticholinergic drugs that act predominantly on the nicotinic receptors are classified based on their site of action. Ganglionic agents (Table 11.6) act on neuronal

Table 11.5 Anticholinergic agents and their clinical uses

Drug classification—Anticholinergics	Drug name	Uses
Natural alkaloids	Atropine Scopolamine	• Organophosphate poisoning • Motion sickness
Semisynthetic compounds	Homatropine Atropine methonitrate Hyoscine butyl bromide Ipratropium bromide Tiotropium bromide	• Bronchial asthma • Mydriatic eye solutions
Synthetic compounds	Cyclopentolate, tropicamide, oxyphenonium, clidinium, pipenzolate methyl bromide, isopropamide, glycopyrrolate, dicyclomine, valethamate, pirenzepine. Oxybutynin, flavoxate trihexyphenidyl	• Bronchial asthma • Mydriatic eye solutions • Antispasmodic (colic, dysmenorrhea) • Parkinson's disease • Drug-induced parkinsonism • Preanesthetic antisecretory

Table 11.6 Types of (cholinergic) drug acting on autonomic ganglia

Ganglionic stimulants	Ganglionic blockers
Nicotine Varenicline Dimethyl phenyl piperazinium (DMPP) Tetramethyl ammonium (TMA)	Mecamylamine Pempidine Trimethaphan Hexamethonium Pentolinium

nicotinic receptors, and neuromuscular agents act on muscular nicotinic receptors [112]. Nicotine and varenicline have significant use in smoking cessation and are discussed elsewhere in this chapter.

Neuromuscular blockers are primarily used as skeletal muscle relaxants in conjunction with anesthetic medications during surgeries. They are classified into [114] two types.

- Depolarizing blockers—succinylcholine, decamethonium.
- Nondepolarizing (competitive) blockers—d-Tubocurarine, pancuronium, vecuronium, doxacurium, atracurium, cisatracurium, rocuronium, mivacurium.

Depolarizing blockers depolarize the muscle endplates, producing an initial twitch. Prolonged depolarization and inactivation of sodium channels eventually ensue flaccid paralysis. They are not hydrolyzed by AChE and do not dissociate from the receptor resulting in desensitization of the receptor to acetylcholine. Nondepolarizing blockers have a high affinity for neuromuscular receptors without any intrinsic activity. In 1856, Claude Bernard described the site of action of curare, the prototype drug. These drugs bind to the two alpha subunits of the receptor, the same site as Ach, and prevent the generation of endplate potential. However, their action can be antagonized by increasing the levels of acetylcholine both in vivo and in vitro. The side effect profile of neuromuscular blockers includes respiratory paralysis, flushing, cardiac arrhythmias, asthma precipitation, malignant hyperthermia (succinylcholine) [114].

Other Clinical Aspects

Antibodies to Ach Receptors in Myasthenia Gravis

Myasthenia Gravis is an autoimmune disease where antibodies are produced against the acetylcholine receptors resulting in defective neuromuscular transmission [115]. About 80–85% of patients with generalized myasthenia have positive antibodies and around half of the affected patients have ocular myasthenia [116]. In 1960, Simpson hypothesized that the disease resulted from antibodies blocking the endplate receptor. This was made while he was studying the presence of autoimmune diseases in patients and/or their families. He found that babies born to mothers diagnosed with myasthenia gravis developed transient myasthenic signs after birth [117]. In 1973, Fambrough et al. used ^{125}Iα-Butx (Bungarotoxin), to demonstrate a decrease in the number of Ach receptors at the neuromuscular junction in a patient with myasthenia, compared to healthy controls [118]. In 1976, Lindstrom et al. was the first to report the presence of antibodies in the serum of myasthenia patients. The classical picture of the disease constitutes fluctuating weakness of the muscles, often presenting as dysphagia, dysphonia, diplopia, weakness of extremities, and respiratory muscles [119].

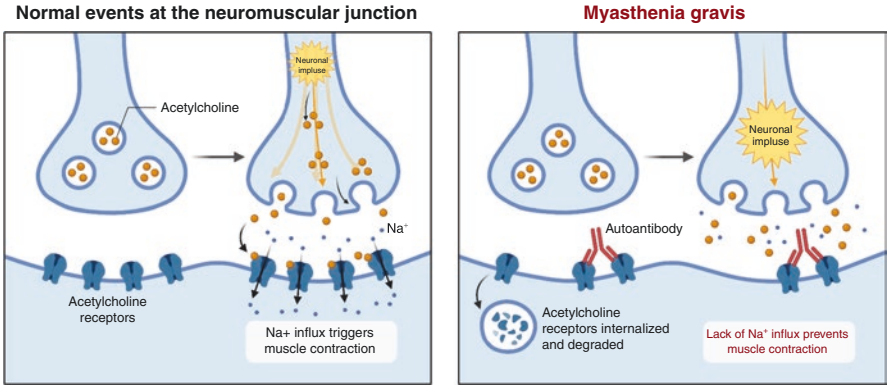

Fig. 11.2 Myasthenia gravis—autoantibodies against receptors cause disease by blocking the receptor function

MuSK (muscle-specific kinase) antibodies are detected in around 6% of patients, a condition now called seronegative myasthenia due to the absence of Ach receptor antibodies [120]. Ach receptors are composed of five homologous subunits with $\alpha 2\beta\gamma\delta$ form found in fetal or denervated muscles and $\alpha 2\beta\delta\varepsilon$ seen in adults. Each subunit is structured with four transmembrane domains, an extracellular domain, and a partly structured intracellular domain. The autoantibodies are targeted against the extracellular domain of the Ach receptor subunit, resulting in receptor degradation. Though the disease severity has no correlation with the antibody titer levels, the latter can be used to monitor response to therapy [120]. Ach receptor antibodies are heterogenous and predominantly belong to the IgG1 subclass [121]. There are similarities in the immunogenicity between the various subunits of the Ach receptor, and antibodies against the α-subunits are the most pathogenic [120, 122].

These autoantibodies disrupt the postsynaptic membrane leading to loss of voltage-gated sodium channels. This further increases the depolarization threshold, generating an action potential in the muscle fiber [123]. The synthesis of Ach receptors at the mRNA (messenger ribonucleic acid) level and its associated proteins increases as a compensatory mechanism in patients with myasthenia [124]. RIPA (Radioimmunoprecipitation assay) is the gold standard for antibody detection and is highly sensitive and specific for generalized myasthenia [125]. Figure 11.2 shows the differences between a neuromuscular junction in normal people and patients with myasthenia gravis.

Dementia and Treatment with Cholinesterase Inhibitors

Dementia is a disorder associated with significant cognitive decline, interfering with an individual's domestic, occupational, and social functioning. It affects about 5% of the elderly population (>65 years) and is more predominant among females. The

major risk factors for dementia include advanced age, systemic vascular disease, and genetics. The lower rates of Alzheimer's disease in a highly educated population can be attributed to compensatory strategies that these individuals develop, preventing early detection [126]. Dementia is classified into two forms based on the etiology [126]:

1. Neurodegenerative causes—This is the most common etiology among the elderly population and comprises of Alzheimer's disease, Lewy body dementia, frontotemporal degeneration, vascular dementia, and Parkinson's disease.
2. Nonneurodegenerative causes—hypothyroidism, chronic alcohol abuse, vitamin deficiencies, infections, trauma, normal pressure hydrocephalus, and psychiatric illness.

Alzheimer's disease is the most common neurodegenerative cause of dementia that presents with memory decline and other cognitive deficits. Neurofibrillary tangles and amyloid plaques are the hallmarks of the disease [126, 127]. Loss of cholinergic neurons in the basal forebrain, especially from the nucleus basalis of Meynert, is also a characteristic finding [128]. The deficits in cholinergic transmission cause defective processing of cortical and hippocampal information resulting in impaired cognition-attention, decision-making capacity, and behavior [129, 130].

In 1993, tacrine was the first drug that was approved for treating dementia; and is no longer used because of the associated hepatotoxicity [131]. Currently, cholinesterase inhibitors are the mainstay of treatment preventing the breakdown of Ach and increasing its concentration in the synaptic cleft. The three approved reversible cholinesterase inhibitors are donepezil, galantamine, and rivastigmine. These drugs enhance synaptic transmission while not altering the disease progression.

Donepezil is a centrally acting cholinesterase inhibitor that is highly efficacious and reportedly has lesser adverse effects [132]. It exhibits significant cognitive and functional benefits in patients with moderate to severe Alzheimer's disease [133]. Galantamine is a reversible cholinesterase inhibitor and an allosteric modulator of alpha3beta4, alpha4beta2, and alpha6beta4 nicotinic receptors [134, 135]. The use of galantamine for 2 years showed mortality benefit and reduced cognitive decline in mild to moderately severe Alzheimer disease [136]. Rivastigmine is well-tolerated and is approved as an oral form (2000) and a transdermal patch (2007) [131].

Pyridostigmine in Autonomic Dysfunction

The autonomic system plays a crucial role in the maintenance of normal blood pressure (BP) through various neurohumoral mechanisms and reflexes. Orthostatic hypotension is defined as drop in the BP of at least 20 mmHg systolic or 10 mmHg diastolic within 3 min of standing [137]. Autonomic dysfunction is characterized by sympathetic and parasympathetic failure, and the most of the times is idiopathic. A clinical symptom that is commonly encountered occurs due to BP fluctuations resulting from sympathetic system dysfunction [138, 139]. The effector limb of

baroreceptor reflex involved in BP regulation utilizes Ach at the level of the autonomic ganglion. Since minimal BP changes occur when supine, there is minimal sympathetic activation. Typically, orthostatic stress increases ganglion transmission, which is impaired in patients with autonomic dysfunction. They commonly present with symptoms of orthostatic hypotension along with supine hypertension.

Pyridostigmine is an AchE inhibitor that augments the efferent activity and sensitivity of the baroreceptors, thereby improving the BP [137, 140]. Midodrine was the first drug to demonstrate symptomatic relief for orthostatic hypotension, but was discontinued due to the adverse effect of dose-dependent supine hypertension. These side effects are also seen with drugs like fludrocortisone acetate, used for neurogenic orthostatic hypotension. Pyridostigmine provides symptomatic relief by increasing upright BP without causing supine hypertension [137, 138, 140]. It is postulated that the benefit of pyridostigmine is produced by harnessing the residual sympathetic tone in patients. This was supported by a study where the combination of atomoxetine and pyridostigmine elicited a synergistic effect on the BP in patients with neurogenic hypotension [139].

Paroxysmal Nocturnal Dystonia/ADFE

In 1981, Lugaresi et al. first described paroxysmal nocturnal dystonia (PND) as "hypnogenic paroxysmal dystonia" in five patients with recurrent brief nocturnal episodes of dystonic limb movements during slow-wave sleep. They had normal EEG recordings and responded well to carbamazepine therapy [141].

The typical motor phenomenon includes dystonic and dyskinetic movements of the head, trunk, and limbs. The episode lasts for a few seconds to minutes and is accompanied by vocalizations. A postictal state is absent, and the patient typically returns to sleep. Though the semiology is stereotyped for a given patient, it can differ from one patient to another. The events tend to occur during the NREM sleep and can be recorded using polysomnography [142].

Currently, PND is regarded as NREM sleep-related frontal lobe epilepsy or sleep-related hyper motor epilepsy. Autosomal dominant chromosomal mutations of genes encoding nicotinic Ach receptors have been identified. The genes CHRNA2, CHRNA4, CHRNB2, CRH, DEPDC5, and KCNT1 that encode for nicotinic receptor subunits disheveled Egl-10, corticotropin-releasing hormone, and a sodium-gated potassium channel have been identified [143]. Since the identification of the CHRNA4 gene that codes for the α4 nicotinic receptor subunit, PND is recognized as a channelopathy (nicotinic receptors are ion channels), causing epilepsy. The most commonly encountered mutations are those of α4 and β2 subunits. The heteromeric nicotinic receptors stimulate glutaminergic transmission and regulate GABAergic interneurons resulting in more complex mechanisms of disease pathophysiology [144, 145]. Multiple studies identify gain of function mutations as the major type, resulting in increased receptor sensitivity to acetylcholine. However, loss-of-function mutations have also been rarely identified.

Video polysomnography has high sensitivity and specificity for PND diagnosis, and ictal SPECT studies are useful in doubtful cases. Antiepileptic therapy is the standard, and most patients respond well to carbamazepine [143].

Nicotine and Varenicline [112]

Nicotine is an alkaloid obtained from tobacco (*Nicotiana tabacum*) and is an agonist at both types of nicotinic receptors. In small doses, it is a ganglion stimulant and in large amounts, exerts a blockade effect by causing persistent depolarization. It is a highly abused drug causing both physical and psychological dependence. Nicotine stimulates its receptor that is located centrally, inducing dopamine release, producing the feelings of satisfaction, and a reinforcement effect. The effect is exerted through α4β2 nicotinic receptors in nucleus accumbens and other mesolimbic areas. Withdrawal symptoms include sleep disturbances, stress, anxiety, irritability, and difficulty concentrating. The only indication for clinical use is as a short-term replacement in the form of gums and patches in people quitting smoking.

Varenicline is a partial α4β2 nicotine receptor agonist used to aid with smoking cessation. It helps reduce cravings and keep withdrawal symptoms to a minimum. The abstinence rates of varenicline are comparable to other drugs like bupropion (atypical antidepressant) and nicotine patches used for smoking cessation. The commonly reported side effects are irrational behavior, mood changes, agitation, sleep disturbances, and changes in appetite. Some patients also report the promotion of suicidal thoughts.

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Chapter 12
Endorphins

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Introduction

Endorphins are naturally occurring polypeptides that are predominantly found in the central nervous system in humans. They have been shown to modulate the perception of pain and are known to mimic the activity of substances like morphine. There are three types of endorphins: alpha-endorphins, beta-endorphins, and gamma-endorphins. These endorphins interact with μ-receptors to exert analgesic effects [1].

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Endorphins are involved in the regulation of various functions such as the following [2–5]:
- Respiration.
- Cardiovascular regulation.
- Gastrointestinal activity.
- Temperature regulation.
- Learning.
- Memory.

Endogenous opioid peptides were first discovered in 1973 by two independent investigators—John Hughes and Kosterlitz, who isolated a set of compounds—"enkephalins" from a pig's brain and identified them as metenkephalin and leu encephalin [6–8]. Samintov et al. isolated morphine-like peptides from the calf brain [9]. Simon, who had in the past discovered opioid receptors, termed these peptides as endorphins, derived from the Greek word *éndon*, meaning from within and Morpheus after a Greek God of dreams by the same name [10]. The word endorphin thus meant "morphine from within." Soon, research was targeted to find more details about these peptides. In 1976, Li and Chung isolated α-, β-, and γ-endorphin from the pituitary gland of a camel and found that β-endorphin produced the strongest analgesic effect amongst the three [11]. A similar comparative study in 1977 by Feldberg and Smyth concluded that β-endorphin was a stronger opiate than morphine and exhibited chemical resemblance to the same as it was removed from opioid receptors by naloxone, a morphine antagonist [12].

Endogenous opioid peptides can be broadly divided into endorphins, enkephalins, and dynorphins. These sub-groups have similar biochemical properties to opiates such as morphine and heroin but differ in their receptor selectivity and distribution in the body. These endogenous peptides act on various receptors such as mu, kappa, and delta receptors.

Metabolism: Synthesis and Degradation in the Body

Endorphins consist of three types of polypeptides: alpha-endorphins, beta-endorphins, and gamma-endorphins [1]. Beta-endorphins, the most important of all endorphins, are synthesized from its precursor protein—proopiomelanocortin (POMC), a 241 amino acid chain in the anterior pituitary where it is stored after production [13]. Early studies have also shown that the immune system is capable of the production of beta-endorphins since they possess mRNA transcripts for POMC [14]. Studies have also shown key cells involved in inflammation, such as the T cells, B cells, macrophages, and monocytes, to have endorphins during the inflammatory process [15, 16].

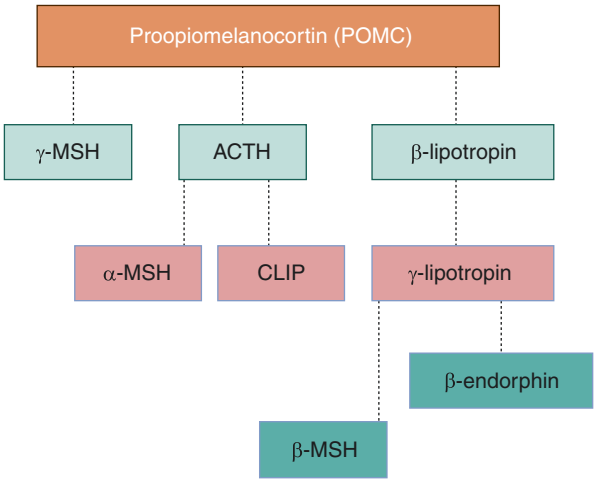

Fig. 12.1 Synthesis of β-endorphins from its precursor Proopiomelanocortin (POMC) [17]. *ACTH* adrenocorticotrophic hormone, *CLIP* corticotrophin-like intermediate peptide, *POMC* proopiomelanocortin, *MSH* melanocyte-stimulating hormone

The POMC production in the anterior pituitary starts as a response to a signal from the hypothalamus, which responds to stressors such as pain. POMC further cleaves due to action by the enzyme convertase into beta-lipoprotein, which further breaks down into smaller proteins such as alpha-melanocyte-stimulating hormone, adrenocorticotropin, beta-endorphin, and others. The synthesis of β-endorphins from its precursor Proopiomelanocortin (POMC) is shown below in Fig. 12.1 [17].

Mechanism of Action

Beta-endorphins primarily act on the central nervous system (CNS) and the peripheral nervous system (PNS) of humans to produce its analgesic effects. In the CNS, they bind to mu-opioid receptors to exert their primary action at presynaptic nerve terminals. In the CNS, the mu receptors are found primarily in the amygdala, mesencephalic reticular formation, rostral ventral medulla, and periaqueductal gray matter, which are key areas involved in descending pain control circuits. This is possible by the inhibition of gamma acetyl butyric acid (GABA), an inhibitory neurotransmitter that leads to increased production of dopamine [18]. The production of dopamine leads to a sense of pleasure, thereby promoting analgesia.

In the PNS, beta-endorphins produce their analgesic effect by binding to mu receptors at both the pre- and postsynaptic nerve terminal. The mu receptors are found principally in the central terminals of primary afferent neurons, dorsal root ganglia, and primary afferent neurons [4]. Of these two locations, they bind principally to the presynaptic receptors, leading to a cascade of events resulting in the inhibition of the release of tachykinins, including substance P, which is a crucial protein involved in the transmission of pain [15, 18, 19].

Physiological Effects

Endorphins exert their major effects by binding to μ-opioid receptors (MOP), which also serve as a target for opioids. Besides their principal distribution in the limbic system, brainstem, dorsal horn cells of the spinal cord, and other areas of the CNS and PNS, they are also found in other organ systems, including cardiovascular, gastrointestinal, and immune systems. Activation of these receptors alters pain perception and transmission [20, 21].

Clinical Aspects

Pain

Endorphins are involved in the modulation of pain perception in both the peripheral and central nervous systems by working on the same site as that of opioid medications such as fentanyl, morphine, and Vicodin [22, 23]. Utilization of these medications has been shown to decrease the levels of beta-endorphins postsurgery. This was further confirmed by a study involving analyzing the levels of endorphins. The study showed that patients who were given lidocaine alone had increased levels of beta-endorphins during and after surgery as compared to patients who received fentanyl along with lidocaine. The study also reflected better analgesia in patients where fentanyl was administered [24, 25].

Animal studies have confirmed that chronic administration of opiate medications in animals has been shown to downregulate the POMC gene leading to decreased production of endorphin [26–28]. Early studies have also reported postsurgical patients to experience hyperalgesia and allodynia on cessation of morphine use [29]. Another vital set of chemicals referred to the anti-opioid peptides such as cholecystokinin (CCK), neuropeptide FF (NPFF), and orphanin FQ/nociception also act on the mu receptors by binding to them and decreasing the affinity for endorphins and similar opioids [30]. While all of these processes occur, the patient's body needs increased levels of opioids to induce the same level of analgesia—a phenomenon referred to as tolerance [31]. These mechanisms lead the patient to eventually be addicted to opiates, thereby needing to employ drug-seeking behavior to maintain dopamine homeostasis, which is active in the reward center of the brain formed by the ventral tegmental area, nucleus accumbens system, prefrontal cortex, and extended amygdala [26]. Furthermore, studies have also confirmed that analgesia induced from the ancient Chinese art of acupuncture is naloxone reversible, thereby implicating the role of endogenous opioids in its etiology [32].

Exercise

Exercise has numerous beneficial effects on physical and emotional well-being. Regular exercise has been shown to improve mood and decrease the pain threshold. "Runner's high," a well-known phenomenon amongst athletes, has been primarily attributed to the effects of endorphins and endocannabinoids after exercise [33]. Studies have also shown that regular exercise in depressed individuals showed an improvement in depression levels, although the endorphin levels in this study remained inconclusive [34]. Another study concluded that when addicted runners withdrew from running for 2 weeks, there was a high correlation to endorphin levels on resumption of exercise [35].

Mood and Psychiatric Disorders

Apart from the abovementioned studies where the effect of exercise on endorphin release and subsequently on the mood of a person is discussed, there have been other studies that have hinted at a potential relationship between the two. Early studies showed that ectopic secretion of beta-endorphin was associated with ectopic production of ACTH and suggested its role in nonmetastatic complications of cancer such as depression, anorexia, and psychosis [36].

Although not explored further, studies from the 1970s have demonstrated changes in opiate concentrations in the cerebrospinal fluid (CSF) of schizophrenia patients and improvement in their mental state after an infusion of naloxone or naltrexone [37].

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 13
Neuropeptides and CGRP

Sireesha Murala, Elanagan Nagarajan, and Pradeep C. Bollu

History of Neuropeptides

Neuropeptides are small proteinaceous substances produced and released by neurons via a regulated secretory route and acting on neural substrates [1, 2]. In 1905, Ernest Sterling coined the term "hormones" for substances that convey chemical messages generated for physiological needs and transported via the bloodstream to the target organ. These hormonal substances include insulin, secretin, oxytocin, vasopressin, and substance P [1, 3].

Since the 1950s, hormones' chemical homology was analyzed, and most of them were found to be of peptides (a short chain of amino acids) [1]. In the 1970s, David de weid introduced the term "neuropeptides" to assign neuroactive peptide hormones and fragments [4, 5]. In 1975, John Hughes and Hans Kosterlitz discovered

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enkephalins based on their biological activity [6]. In 1977, Guillemin and Schally were awarded the Nobel Prize for analyzing the chemical homology of releasing factors from the hypothalamus in the 1960s [7].

The history of neuropeptides demonstrates the following scientific highlights (1):
- Peptide hormones as regulators in the endocrine system.
- Neurosecretion of peptidergic substances.
- Responsiveness of nerve cells to peptides.

Neurochemical Profile

Neurons release neuropeptides to send signals to adjust neurons and target organs [1]. Neuropeptides are grouped into families based on their structural homologies. A few of the neuropeptide families are shown below in Table 13.1 [2]. Effects of neuropeptides on neurogenesis and neuritogenesis are shown below in Table 13.2 [8].

Table 13.1 List of neuropeptide families [2]

CGRP: CGRP (α, β), calcitonin, amylin, adrenomedullin
Glucagon/secretin: PACAP, VIP, glucagon, secretin, GHRH, GIP
Opioids: Enkephalins, dynorphin, endorphins, nociceptin
Somatostatin/cortistatin
Natriuretic factors: ANF, BNF, CNP
GRP, neuromedins
Endothelins
CCK/gastrin
Insulins: Insulin, IGFs, relaxins
Motilin/ghrelin
Galanins
Gonadotropin-releasing hormone
Neuropeptide B/W/S
Neurexophilins
Cerebellins
Granins: Chromogranins, secretogranins
Vasopressin/oxytocin
F- and Y-amides: NPY, PPY, NPFF
Tachykinins: Sub P, neurokinin A, neuropeptide K, neuropeptide gamma
Tensins: Angiotensin, neurotensin, bradykinin
CRH-related: CRH, urocortins, urotensins
Adipose neuropeptides: Leptin, adiponectin, resistins
Family-less: Orexins, MCH, TRH, PTHrP, CART, AGRP, prolactin, diazepam-binding inhibitor peptide, kisspeptins, and so on

Table 13.2 Effects of neuropeptides on neurogenesis and neuritogenesis [8]

Neuropeptides	Size of the amino acids	Neurogenesis effects	Neuritogenesis effects
Orexins	33	Primary hippocampal cells, ↑ gyrus dentatus	↑ Primary cortical cells
Melanin-concentrating hormone	19	Unknown	↑ SH-SY5Y cells
Melanocyte-stimulating hormone (α, β subtypes)	13 and 18	↑ Gyrus dentatus	↑ Dorsal root ganglia neuron culture
Substance P	11	↑ Spinal neural stem cells	Unknown
Enkephalins	5	↑ SH-SY5Y cells, neuro-2A cells	↑ Neuro-2A cells
Neuropeptide Y	36	↑ Gyrus dentatus	↑ Dorsal root ganglia neuron culture
Thyrotropin releasing hormone	3	Unknown	↑ Ventral spinal cord
Corticotrophin-releasing hormone	41	↑ Neural stem/progenitor cells	Unknown
Oxytocin	9	↑ Hippocampus	↑ SH-SY5Y cells
Vasopressin	9	↑ Gyrus dentatus	Unknown

The characteristics of neuropeptides include the following [1].

- Gene expression and biosynthesis by neurons.
- Storage and regulated release on demand.
- Ability to mediate or modulate neural functioning directly via neural receptors.

Gene expression and protein synthesis of the neuropeptides takes place in the cytoplasm. They are transported via axon hillock to the nerve terminals. Glial cells and astrocytes have a regulated secretory pathway to release either intact or propeptides. Peptides produced by the nervous system other than neurons are growth factors and cytokines [1, 9, 10].

Controlled release through neurons is achieved by regulating chemical communication and secretion of peptides from the large dense-core vesicles. Secretion routes are stimulated via a single peptide sequence (20–25 amino acids). Short motifs of amino acids achieve proteolytic processing into active peptides. C-terminal amidation is accomplished through modification of the peptides by peptidylaminotransferase. Posttranslational modifications of the peptides include N- and O-glycosylation, sulfation, phosphorylation, and acetylation [1, 11, 12].

Neuropeptides have biological activities at the cellular, biochemical, genetic, behavioral, and organism levels (metabolism, food intake, analgesia, reward, social behaviors, reproduction, memory, and learning). Neuropeptides' cellular actions are often slow compared to the fast neurotransmitters, as they are stored in the

dense-core vesicles and are not anchored to the cellular release area like the neurotransmitters. Slow responses are also due to the G-protein coupled receptors (GPCR), which trigger multiple intracellular molecular enzymatic reactions to elicit a response, which takes longer than neurotransmitters [1, 2, 13].

Calcitonin Gene-Related Peptide (CGRP)

CGRP Structure, Receptors, and Metabolism

Calcitonin Gene-Related Peptide (CGRP) is a multifunctional neuropeptide, a 37-amino acid peptide with an N-terminal disulfide bond and amidated C terminus(see Fig. 13.1). It is formed from gene encoding calcitonin and CGRP based on alternative splicing [14, 15]. CGRP has two isoforms: α-CGRP (CGRP1), encoded by CALCA (Calcitonin Related Polypeptide Alpha) gene and β-CGRP (CGRP2), encoded by CALCB (Calcitonin Related Polypeptide Beta) gene [15–17].

CGRP has an amphiphilic α-helical structure similar to other family members like calcitonin, α- and β-CGRP, amylin, adrenomedullin, and adrenomedullin 2 (intermedin) [15]. CGRP is a potent vasodilator and discovered in the trigeminal neuronal cells, which innervate the cell bodies and are considered as a marker for trigeminal activity. CGRP is found abundantly both in the central and peripheral nervous systems [18]. CGRP is seen in Aδ and C fiber sensory nerves, smooth muscles of vasculature and heart, immune cells, adipocytes, and keratinocytes [19, 20].

CGRP is stored at the sensory nerve endings in the dense vesicles, which is released along with substance P. Nerve depolarization through

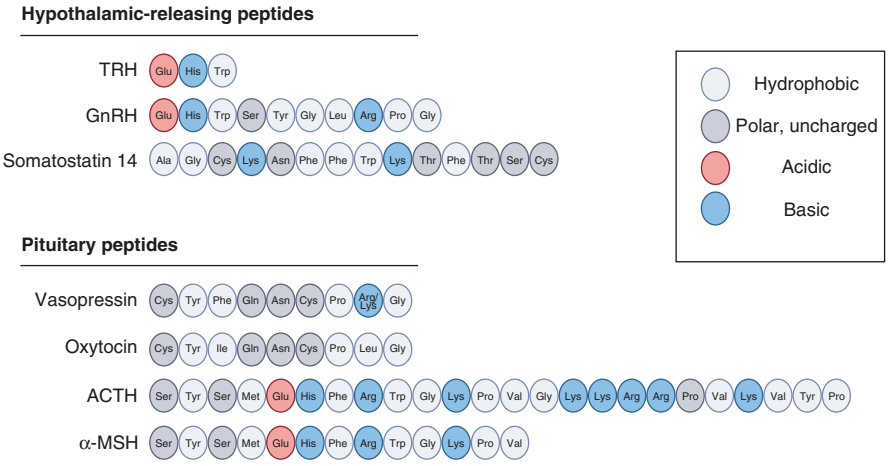

Fig. 13.1 Neuropeptide amino acid sequences—hypothalamic and pituitary peptides

calcium-dependent pathways leads to the release of CGRP through exocytosis; angiotensin and sympathetic noradrenergic transmitter also cause the CGRP release via sensory fibers activation by transient receptor potential (TRP) or electrical stimulation [19, 20].

CGRP has a G-protein coupled receptor, with three subunits which are as follows [21].

- Calcitonin-like receptor (CLR).
- Receptor activity–modifying protein 1 (RAMP1).
- Receptor component protein (RCP).

CLR, with its seven-transmembrane protein, needs RAMP1 for its trafficking to the plasma membrane and also in binding to CGRP; RCP accelerates the G-αs coupling. The rate-limiting subunit of the receptor is RAMP1 [21, 22]. CGRP is metabolized through proteases and other mechanisms; it has a short half-life with a biphasic clearance rate of 6.9 min half-life and 26.4 min slower decay [19, 20].

CGRP has an integral part in migraine, pain transmission, cardiovascular regulation, arteriolar dilation, and gastrointestinal physiology [23]. CGRP vasodilator effect is due to nitric-oxide in some tissues and also decreases the endothelin vasoconstrictor effect through G-protein βϒ subunit activation [19, 24]. The role of CGRP and the trigeminal system in migraine pathophysiology is shown below in Fig. 13.2.

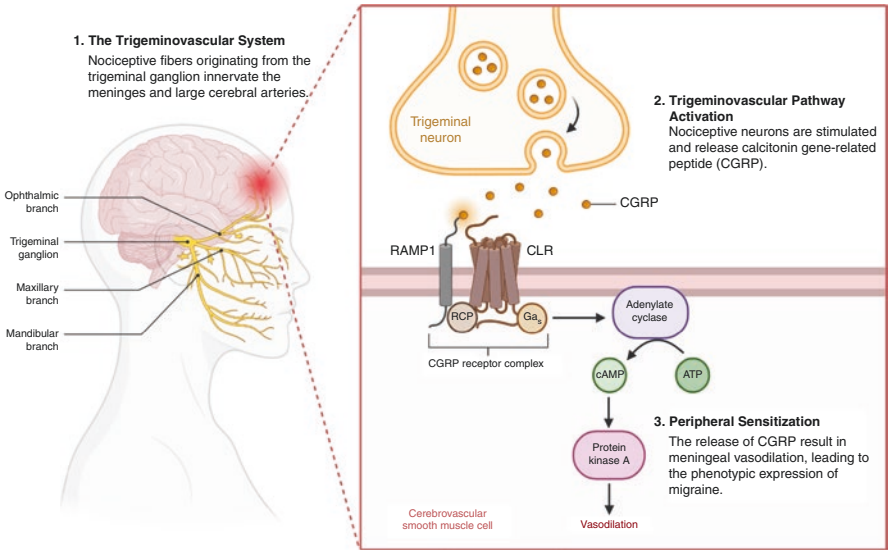

Fig. 13.2 Role of CGRP and the trigeminal system in migraine pathophysiology

Table 13.3 High CGRP-induced hypersensitivity to stimuli [15]

Stimuli	Response
Light	Photophobia
Sound	Phonophobia
Smell	Nausea
Somatosensory	Pain

CGRP calcitonin gene-related peptide

Table 13.4 Current CGRP monoclonal antibodies and their targets [19]

Monoclonal antibody	Target
Erenumab (AMG-334)	CGRP receptor
Eptinezumab (ALD403)	CGRP peptide
Fremanezumab (TEV-48125)	CGRP peptide
Galcanezumab (LY2951742)	CGRP peptide

CGRP calcitonin gene-related peptide

CGRP plays an essential role in migraine; intravenous CGRP produces delayed moderate-severe headaches in migraine patients [25]. Several clinical trials have demonstrated that triptans block the CGRP release, and the plasma levels of CGRP are significantly raised in migraine patients with and without aura [18, 26]. High levels of CGRP cause hypersensitivity to the somatosensory stimuli (light, sound, smell), which are shown below in Table 13.3 [15].

Medications Acting Via the CGRP Pathway

CGRP receptor antagonists and monoclonal antibodies might play an important role in migraine treatment. Olcegepant, a CGRP receptor antagonist, when given at 2.5 mg intravenous dose, it decreased the headache by 66% compared to 27% in the placebo group. It also reduced phonophobia, photophobia, nausea, and a 2-h pain-free rate compared to the placebo group [15, 27].

Other receptor antagonists are MK-3207 (200 mg, oral, 69% reduction of pain compared to 36% with placebo), BI 44370 TA (400 mg, oral, similar efficacy to eletriptan, better tolerability), BMS-927711 (75–300 mg, oral) and MK-1602. These antagonists have a better safety profile, particularly with the absence of coronary vasospasm, which makes them a better treatment option for acute migraine. Although no receptor antagonists have higher efficacy than triptans, CGRP has therapeutic potential for migraine [15].

Currently available anti-migraine monoclonal antibodies (mAbs) are biological drugs against CGRP peptide or the receptor. Migraine prevention can be achieved through mAbs because of their long half-life by avoiding the host immune responses [15, 28]. A few of the current mAbs and their targets are shown below in Table 13.4 [19]. These mAbs are efficient in preventing frequent episodes of migraine (4–14 attacks per month) and chronic migraine (>15 attacks per month) [15].

Other Clinical Aspects

Other therapeutic actions of CGRP are seen in hypertension, as it reduces blood pressure (BP) in pathological states but not physiologically by regulating the RAAS system and inhibiting sympathetic activity. In heart failure, CGRP releases in a compensatory manner, thus causing vasodilatation, which reduces the afterload to enhance the stroke volume; positive inotropic effects also increase the stroke volume and ejection fraction. In ischemia, CGRP activates the transient receptor potential vanilloid 1 (TRPV1) channels, thus protecting them from reperfusion injury while mediating preconditioning [19]. CGRP reduces the pain and itching seen in arthritis and skin diseases [15].

Acknowledgments All the pictures in this chapter were prepared using Biorender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 14
Galanin, Substance P, and Melanin-Concentrating Hormone

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Galanin

Galanin (GAL) is a 29-amino acid neuropeptide (30 in humans), which performs various physiological functions in both CNS and PNS [1]. In the 1980s, Victor Mutt et al. discovered GAL at the Karolinska Institute in Stockholm; GAL was first identified in the porcine intestinal extracts [2, 3].

GAL effects are mediated via GAL receptors (GALRs), which are Galanin receptor 1 (GalR1), Galanin receptor 2 (GalR2), and Galanin receptor 3 (GalR3). These receptors belong to G-protein coupled receptor (GPCR) superfamily, with seven transmembrane proteins spanning across the plasma membrane [1, 4].

While the three receptors are similar by belonging to the GPCR superfamily, they are distinguished by their binding properties and their effects on intracellular signaling [1, 5]. GalR1 receptor was initially cloned from human bowes melanoma cells, and high levels were expressed in the cerebral cortex, olfactory nucleus, and

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bulb of the olfactory system, thalamus, dorsal dentate gyrus, and ventral subiculum of the hippocampus [1]. GalR1 mRNA is most significantly seen GAL receptor transcript in the brain (in cortical areas also) [6]. Receptor activation causes an inhibition in the adenyl cyclase (AC) production through Gi/0 pathway, which leads to a reduction in the production of cyclic adenosine monophosphate (cAMP), a second messenger [7].

GalR2 was first cloned in rat hypothalamus cells, later on from spleen cells of mice [8, 9]. GalR2 receptors are primarily expressed in the dorsal root ganglia (DRG), hypothalamus, hippocampal formation, and cerebellar cortex [10]. Receptor activation via an agonist leads to an effector activation, phospholipase C (PLC), through which phosphatidylinositol, 4, 5-bisphosphate is transformed to diacylglycerol (DAG) and inositol triphosphate (IP3). Activation of stimulatory G-protein leads to an increase in cAMP levels, which induces AC activation accompanied by an increase in cAMP levels [1, 11].

GalR3 receptor was initially isolated from the rat hypothalamus. Human GalR3 is composed of 368 amino acids, which has 90% sequence similarity to rat GalR3. These receptors are diffusely distributed in medulla oblongata, median, ventromedian, ventrolateral, and medial preoptic nuclei [1, 12–14].

Various functions affected by galanin are [1] as follows:

- Endocrine secretions.
- Metabolism.
- Nociception.
- Intestinal motility.
- Gut secretion.
- Contractility.
- Feeding behavior.
- Cognition.
- Addiction.
- Hormone secretion.
- Memory.
- Nerve regeneration.
- Neuroendocrine release.

GAL produced both mixed depressive and antidepressant effects based on receptor dimers (iso and hetero) and the location of subtypes of receptors in various brain areas. GAL binding to GalR1 and GalR3 elicits antidepressant effects, and GalR2 produces depressive effects [1]. Hypothetical mechanisms by which GAL is involved in human depression are shown below in the Fig. 14.1 [6].

Neuropeptides regulate the neurotransmitters released from the raphe nucleus and locus coeruleus (LC) via the GalRs signal transduction in depressive states [1, 15]. Thus receptor agonists acting on the LC neurons have an important role in treating depressive disorders. GAL is highly expressed in the LC neurons and is found

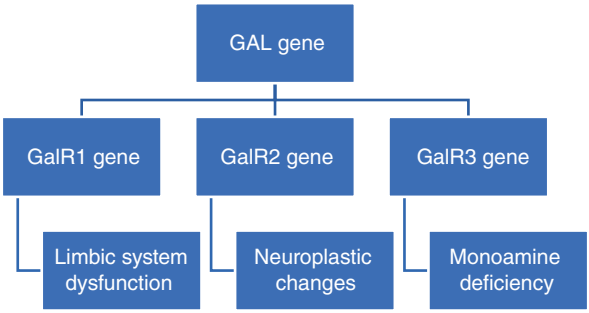

Fig. 14.1 Galanin hypothetical mechanisms involved in human depression [6]. Environmental stressors like recent negative life events and adverse childhood act on the GAL genome stimulation. The ultimate phenotypic expression includes anxiety and depression

in the noradrenergic projections in the cortex and hippocampus. GAL receptor antagonists have an inhibitory action on LC neurons and dorsal raphe to exert antidepressant activity [1].

GAL 1–15 ligands have shown encouraging results in mood disorders. As ligands lack receptor selectivity, they have demonstrated limited effects on different receptors in depression. GAL promotes the selective serotonin reuptake inhibitors (SSRIs) actions, therefore enhancing the transcription proteins' availability [1].

Substance P

Substance P (SP) is an 11-amino acid neuropeptide (undecapeptide), which is present in both CNS and PNS. SP is produced by both neuronal, nonneuronal cells like microglia and astrocytes, along with immune cells [16, 17]. SP is the most diffusely distributed tachykinin and brain-gut peptide which is involved in various pathophysiological functions [18].

In 1931, SP was isolated from the equine brain and gut; later, in 1970, from bovine hypothalamus tissue, and in 1971, its amino acid sequence was identified [18–20]. In the mid-1970s, SP immunoreactivity in the CNS was demonstrated [16].

SP neuroanatomical analysis showed the highest levels in the medial amygdaloid nucleus and substantia nigra and the superficial dorsal horn of the spinal cord. The existence of SP in the sensory neurons of the brain stem, cranial nerve nuclei, and spinal cord dorsal horn shows the role of SP as a sensory neurotransmitter for pain perception. SP is also found in several other areas of the CNS, which are the cortex, hypothalamus, hippocampus, and basal ganglia [16, 21]. SP regulates the function of the immune cells by acting either through autocrine or paracrine fashion. SP is expressed in the soma of the neurons and is transported via large dense-core vesicles, which are released by exocytosis at axon terminals [17].

SP exerts its actions through a high-affinity neurokinin 1 receptor (NK1R), which is a G-protein coupled receptor [16]. NK1R has two isoforms, namely full-length NK1R and truncated NK1R (NK1R-T) [17]. The binding of SP to NK1R stimulates the C-terminus phosphorylation through G-protein receptor kinases and protein kinase C, which activates the mitogen-activated protein kinase (MAPK) [16, 22].

SP is degraded through neprilysin, a metalloproteinase, and hence has a short half-life within the tissues [17]. SP half-life depends on the extracellular neuropeptide degradation kinetics and through desensitization dynamics and cellular internalization which is accompanied by the receptor recycling [23].

SP and various other tachykinin family members are found along with glutamate in primary afferent fibers [24]. SP released in the intercellular space diffuses from the origin site and reaches the targets via high-affinity receptors to regulate the synaptic transmission in the various neuronal population through volume transmission [25]. Activation of microglial cells has a crucial role in nociceptive sensitization development [23].

SP exerts its anti-inflammatory effects through its high-affinity NK1R, which leads to cell-type dependent reactions like vascular smooth muscle dilation and endothelial cells retraction [16]. SP plays a key role in the pathophysiological functions of the respiratory system, digestive system, urogenital system, immune system, nervous system, skin, and tumors [18].

SP controls the intestinal excitatory rhythm by activating nonselective calcium channels of the Cajal interstitial cells [26]. SP binding NK1R increases the release of the neurotransmitter degrading enzymes, vasoactive intestinal peptide (VIP), nitric oxide, and M2 muscarinic receptors in the respiratory tract membranes; hence has a significant role in allergic reactions [18, 27]. SP also controls the inhaled antigenic responses by promoting the aggregation of the dendritic cells [28].

SP enhances the intercellular adhesion molecule-1-mediated inflammation and reactive oxygen species (ROS) production, causing bladder hypersensitivity [18]. SP promotes the nerve growth factors and pro-inflammatory factors in keratinocytes to aid in mucosal and cutaneous nerve regeneration, thus enhancing wound healing [29, 30]. SP also promotes the growth of various cancers, and hence NK1R antagonists constrain the cancer cell growth [31].

SP released along with glutamate plays a key role in pain conduction; therefore, NK1R antagonists are used to inhibiting pain. SP promotes bacterial phagocytosis by enhancing the release of matrix metalloproteinases, chemokines, cytokines and ROS from neutrophils [18].

Infectious and other CNS disorders are most frequently associated with severe inflammatory reactions because of the immune responses by the neuronal cells and leukocytes; SP binding to NK1R augments the release of inflammatory mediators and also decreases the anti-inflammatory cytokine release from astrocytes and microglia [16].

Treatment with NK1R antagonists have been shown to reduce the neuroinflammation, demyelination, and reactive gliosis in animal models [32]. Latest oral, NK1R antagonists can easily cross the blood–brain barrier to exert central effects;

aprepitant, an NK1R antagonist, is currently approved as an antiemetic in chemotherapeutic patients. More research is needed to add NK1R antagonists as an adjuvant agent for infectious and sterile CNS inflammatory conditions [16].

Melanin-Concentrating Hormone

Melanin-concentrating hormone (MCH) is a 19-amino acid cyclic peptide (see Fig. 14.2), which is encoded from 165-amino acid preprohormone [33]. MCH is the first peptide specified to be involved in skin pigmentation control; it also has a significant role in motivated behaviors like drinking, feeding, mating, and maternal behavior [34].

Although MCH existence was stipulated in the 1930s, it was first isolated from the chum salmon hypophysis, where it was professed to be involved in skin pigmentation via inciting melanin concentration in melanophores which causes pallor [34, 35].

MCH is synthesized in the soma of neurons, mainly in dorsomedial hypothalamus, the lateral section of posterior hypothalamus, and zona incerta. Few MCHergic neurons are also found in the pontine reticular formation and olfactory tubercle; MCH is also found pancreas and gastrointestinal tract [36, 37].

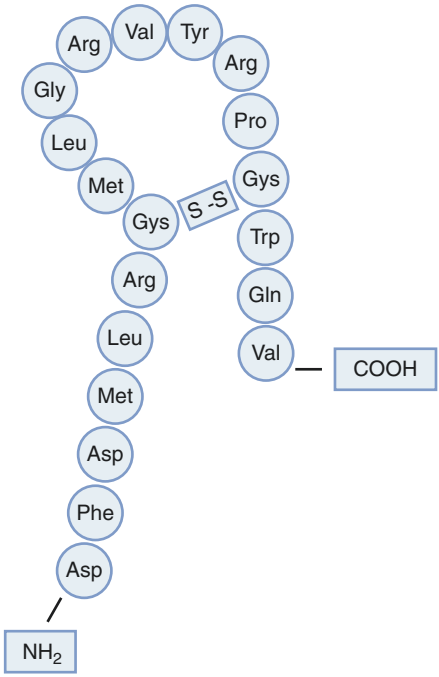

Fig. 14.2 The amino acid structure of melanin-concentrating hormone [38]

MCH functions are mediated via two G-protein coupled receptors, namely MCHR-1 and MCHR-2. MCHR-1 activates the Gi and Gq proteins and inhibits calcium currents; MCH exerts an inhibitory role both at pre- and postsynaptic levels to reduce the release of GABA and glutamate [36, 37].

Infusion of MCHR-1 agonist induced obesity in mice, followed by hyperphagia, a reduction in body temperature, and an increase of lipogenic activity in the liver and white adipose tissue [38]. While animals with increased expression of MCH are found to be obese, animals that lack MCH are found to be lean and hypophagic. All this data suggest that MCH is involved in body energy conservation by increasing food intake and facilitating anabolism [37].

One of the presumed functions of sleep is to conserve energy. And as MCHergic neurons are important in controlling energy homeostasis, they also have some presumed role in sleep regulation. MCH promotes sleep, mainly rapid eye movement (REM) sleep, by regulating both activating and somnogenic systems. The relationship between MCH and sleep through MCH microinjections in animal models is shown below in Table 14.1 [37, 39].

It is also shown that infusion of MCHR-1 antagonist in mice decreased the REM and NREM sleep and promoted wakefulness [37]. MCHergic neurons have a low frequency of discharge during wakefulness, and the firing rate increases during the NREM sleep and achieves the highest level of activation during REM sleep [40].

MCH's peptidergic system is highly affected by leptin, which is produced by the adipocytes [41]. MCH neurons regulate the feeding behavior through a complex mechanism that involves, metabolic rate, the energy balance of the organism, rewarding circuits, arousal, locomotor activity, olfactory cues, spatial memory, and other complex relations with peptidergic systems [34].

Regarding foraging and predation, MCH is associated with three main aspects [42]:

- Sensory integration.
- Decision-making.
- Memory.

MCH impacts the sexual physiology, and steroid hormones (mainly via gonadotrophin release), are also found to impact the MCH peptidergic system [34, 43].

Table 14.1 Melanin-concentrating hormone and sleep [37]

MCH microinjection site	Main effect
Intracerebroventricular (rat)	Increases REM sleep; moderate increase in NREM sleep
Dorsal raphe (rat)	Increases REM sleep; moderate increase in NREM sleep
Locus coeruleus (rat)	Increases REM sleep
Nucleus pontis oralis (cat)	Increases REM sleep
Basal forebrain (rat)	Decreases wakefulness; increases REM sleep in the first 2-h of the recordings
Ventrolateral preoptic nucleus (rat)	Increases NREM sleep

NREM nonrapid eye movement, *REM* rapid eye movement

Both sleep and arousal play an important interacting role with other motivating behaviors, particularly via hypothalamic circuits. MCH also has effects on both maternal behavior and the lactation period. MCH encourages the maternal behavior expression via the medial preoptic area, thus promoting maternal behavior in early stages and at late stages selectively inhibits the appetitive elements [34].

Acknowledgments All the pictures in this chapter were prepared using BioRender premium software. The tables were redrawn using information from the reference article mentoned alongside them.

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Chapter 15
Neuroreceptors

Susan C. McKarns

Corpora non agunt nisi fixate
Compounds do not act unless bound

—Paul Ehrlich, 1908 Nobel Prize in Medicine

Abbreviations

12(R)-HPETE	12R-hydroperoxyeicosatetraenoic acid
4-TM	Four transmembrane-spanning α-helices
5-HT	5-Hydroxytryptamine
7-TM	Seven transmembrane-spanning α-helices
A1	Adenosine A1 receptor
A2A	Adenosine 2A receptor
A2B	Adenosine 2B receptor
A3	Adenosine 3 receptor
ACh	Acetylcholine
AChBP	Acetylcholine-binding protein
ACKR	Atypical chemokine receptor
ACTH	Adrenocorticotropic hormone
ADAR	RNA-specific adenosine deaminase
ADGR	Adhesion G protein-coupled receptor
ADP	Adenosine diphosphate
AF-1	Activation function-1
AM	Adrenomedullin

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AMP	Adenosine monophosphate
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMY	Amylin
AN	Arcuate
ANF	Atrial natriuretic factor
ANGPT	Angiopoietin
ANP	Atrial natriuretic peptide
AR	Androgen receptor
ASIC	Acid-sensing ion channel
AT2	Angiotensin II receptor type 2
AXL	AXL receptor tyrosine kinase
BAI	Brain-specific angiogenesis inhibitor
BB	Bombesin BB receptor
BDNF	Brain-derived neurotrophic factor
BLT	Leukotriene B4 receptor
C3a	Complement peptide receptor C3a
CAH	Carbonic anhydrase
CAK	CDC-activating kinase
CAKAK	CAK-activating kinase
CaMK	Calcium/calmodulin-dependent protein kinase
cAMP	Cyclic adenosine monophosphate
CAR	Constitutive androstane receptor
CAS	Ca^{2+}-Sensing
CASK	Calcium/calmodulin-dependent serin protein kinase
Ca_V	Voltage-gated calcium channel
CB	Cannabinoid receptor
CCK	Cholecystokinin
CCL	C-C chemokine ligand
CCR	C-C chemokine receptor
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
CELSR	Cadherin EGF LAG seven-pass G-type receptor
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene-related peptide
CLR	C-type lectin receptor
CNS	Central nervous system
CO	Colliculi
COL3A1	Collagen type III alpha 1 chain
COUP-TF	Chicken ovalbumin upstream promoter-transcription factor
CP	Caudate Putamen
CRF	Corticotropin-releasing factor receptor type
CSF1R	Colony stimulating factor 1 receptor
CT	Calcitonin
CTP	Cytidine triphosphate
CX_3CL	C-X3-C chemokine ligand

CX_3CR	C-X3-C chemokine receptor
CXCL	Chemokine (C-X-C motif) ligand
CXCR	Chemokine (C-X-C) motif receptor
Cys	Cysteine
D	Dopamine receptor D
DAG	Diacylglycerol
DAX-1	Dosage-sensitive sex reversal adrenal hypoplasia congenital critical region on the X chromosome, gene1
DBD	DNA binding domain
DDR	Discoidin domain receptor
DP	Prostaglandin D2 receptor
DRD	Dopamine receptors
ECD	Extracellular domain
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMR1	EGF-like module-containing mucin-like hormone receptor-like 1 (also known as F4/80)
ENaC	Epithelial sodium channel
EP	Prostaglandin E2 receptor
Eph	Erythropoietin-producing
Ephrin	Eph receptor-interacting ligand
ErbB4	Erb-B2 receptor tyrosine kinase 4
ERK	Extracellular signal-regulated kinase
ERK	Extracellular signal-regulated protein kinase
ERR	Estrogen-related receptor
ESRRG	Estrogen-related Receptor γ
ET	Endothelin receptor type
F2L	Formylpeptide receptor (FPR)-like (FPRL)-2 ligand
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FLT3	FMS-like tyrosine kinase 3
FLT3L	FMS-like tyrosine kinase 3 ligand
FN	Fibronectin
FN-III	Fibronectin type III domain
FP	Prostaglandin F receptor
FPR	Formyl peptide receptor
FSH	Follicle stimulating hormone receptor
FXR	Farnesoid X receptor
FZD	Frizzled
GABA	Gamma-aminobutyric acid
GABAA	Gamma-aminobutyric acid A receptor
GABAB	Gamma-aminobutyric acid B receptor
GABP	GA-binding protein
GAL	Galanin
GAL	Galanin receptor

GC	Guanylate cyclase
GCNF	Germ cell nuclear factor
GHRH	Growth hormone–releasing hormone
GHRHR	Growth hormone–releasing hormone receptor
GIP	Glucose-dependent insulinotropic polypeptide
GIRK	G protein-coupled inwardly-rectifying potassium channels
GLEPP	Glomerular epithelial protein
GLP	Glucagon-like peptide
Glu	Glutamate
GluA	Ca^{2+}-permeable AMPA receptor
GluK	Kainate receptor
GluN	NMDA receptor
GluR	Glutamate receptor
Gly	Glycine
GlyR	Glycine receptor
GlyT1	Glycine transporter 1
GlyT2	Glycine transporter 2
GnRH	Gonadotropin-releasing hormone
GPBA	G protein-coupled bile acid
GPCR	G protein-coupled receptor
GPER	G protein-coupled estrogen receptor
GR	Glucocorticoid receptor
GRK	G protein receptor kinase
GRP	Gastrin-releasing peptide
GTP	Guanosine triphosphate
Gα	Gα Protein subunit
Gαgustducin	Gα Protein, gustducin subunit (taste specificity)
Gαi	Gα Protein, inhibitory subunit; adenylate cyclase inactivated, cAMP down
Gαo	Gα Protein, other subunit
H	Hippocampus
H	Histamine receptor H
HAT	Histone acetyltransferase
HB-EGF	Heparin-binding *EGF-like* growth factor
HCA	Hydroxycarboxylic acid receptor
HCF	Heregulin
hCG	Human chorionic gonadotropin
HDAC	Histone deacetylase
HGF	Hepatocyte growth factor
HIV	Human immunodeficiency virus
HNF4	Hepatic nuclear factor 4 receptor
HR	Hormone receptor
HY	Hypothalamus
IA2	Islet tyrosine phosphatase
IGF	Insulin-like growth factor

iGlu	Ionotropic glutamate
iGluR	Ionotropic glutamate receptor
INaC	Intestine sodium channel receptor
IP	Prostaglandin receptor
IP_3	Inositol 1,4,5- trisphosphate
IP_3R	Inositol 1,4,5-trisphosphate receptor
JNK	c-Jun amino-terminal kinase
KIM	Kinase interaction motif
Kit	Tyrosine protein kinase
LAR	Ligand-binding domain
LBP	Ligand-binding pocket
LH	Luteinizing hormone
LL-37	Antimicrobial peptide belonging to the cathelicidin family
LMR	Lemur kinase receptor subfamily
LPA	Lysophosphatidic acid receptor
LRH-1	Liver receptor homolog-1
LTB	Leukotriene
LTK	Leukocyte tyrosine kinase
LTX	α-Latrotoxin
LXA_4	Lipoxin A_4
LXR	Liver X receptor
M	Muscarinic acetylcholine receptor M
mAChR	Muscarinic acetylcholine receptor
MAM	Meprin-A5-μ domain
MAP2K	Mitogen-activated protein kinase
MAPK	Mitogen-activated protein kinase
MC	Melanocortin
MCH	Melanin-concentrating hormone receptor
MEK	Mitogen-activated protein kinase
MER-TK	Mer tyrosine kinase receptor
Mg	Magnesium
mGlu	Metabotropic glutamate
mGluR	Metabotropic glutamate receptor
MR	Mineralocorticoid receptor
MSH	Melanocyte stimulating hormone
MT_1	Melatonin receptor type 1A
MuSK	Muscle-associated receptor tyrosine kinase
Myt	Membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase
N/OFQ	Nociceptin/orphanin Fq
nAChR	Nicotinic acetylcholine receptors
NCOA1	Nuclear receptor coactivator 1
NCoR	Nuclear receptor corepressor
NF-κB	Nuclear factor-κb
NGF	Nerve growth factor

NK1	Tachykinin receptor 1
NMDA	*N*-methyl-D-aspartate
NMU_1	Neuromedin U receptor
NO	Nitric oxide
NOP	Nociception opioid peptide receptor
NOR	Neuron-derived orphan receptor
NPBW	Neuropeptide B/W receptor
NPFF	Neuropeptide FF receptor
NPS	Neuropeptide S
NPY	Neuropeptide Y
NR0B	Nuclear receptor subfamily 0 group B member
Nrg	Neuregulin
NT	Neurotrophin
NTD	N-terminal domain
NTS	Neurotensin receptor type
NURR1	Nurr-related receptor factor 1
oGPCR	Orphan G protein-coupled receptor
OPN1LW	Opsin 1, long wave sensitive
OPN1MW	Opsin 1, medium wave sensitive
OPN1SW	Opsin 1, short wave sensitive
OX	Orexin receptor type
P2X	Adenosine triphosphate–gated cation channel P2X purinoceptor
P2Y	G Protein-gated P2Y purinoceptor
PAC	Pituitary adenylate cyclase
PAC1	Pituitary adenylate cyclase–activating polypeptide-selective type I receptor
PACAP	Pituitary adenylate cyclase–activating polypeptide
PAF	Platelet-activating factor
PAM	Positive allosteric modulator
PAR	Protease-activated receptor
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PGR	Progesterone receptor
PHM	Peptide histidine methionine
PHV	Peptide histidine valine
PI3Kγ	Phosphoinositide 3-kinase γ
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
PKR_1	Prokineticin receptor 1
PKR_2	Prokineticin receptor 2
PLC-β	Phospholipase C-β
PNR	Photoreceptor cell-specific nuclear receptor
PP1	Protein phosphatase 1
PPAR	Peroxisome proliferator-activated receptor

PR	Progesterone receptor
PrP	Prion protein
PrRP	Prolactin-releasing peptide
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTH	Parathyroid hormone receptor
PTHrP	Parathyroid hormone–related protein
PTK	Protein tyrosine kinase
PTP	Protein tyrosine phosphatase
PXR	Pregnane X receptor
PYY	Peptide YY
QRFP	Neuropeptide 26RFa
RACK	Receptors for activated C kinase
RAMP	Receptor activity-modifying proteins
RAPGEF3	Rap guanine nucleotide exchange factor 3
RAR	Retinoic acid receptor
RARα	Retinoic acid receptor
RDGS	Peptides containing the sequence Arg-Gly-Asp-Ser
RET	Ret proto-oncogene
REV-ERB	Reverse-Erb receptor
REV-ERBα	Reverse-Erb-A
REV-ERBβ	Reverse-Erb-B
rGC	Receptor guanylyl cyclase
Rho	Rho family of GTPases belong to the Ras superfamily of proteins
RhoA	Ras homolog family member, a Rho family GTPase
RIP 140	Receptor-interacting protein 140
ROR	Retinoic acid–related orphan receptor
ROS	Proto-oncogene that encodes an orphan receptor-type tyrosine kinase
RPTP	Receptor-like protein tyrosine phosphatase
RSTK	Receptor serine–threonine kinase
RXFP	Relaxin family peptide
RXR	Retinoid X receptor
RYK	Transmembrane receptor tyrosine kinase
S1P	Sphingosine-1-phosphate
SAP	SLAM-associated protein
SAPK	Stress-activated protein kinase
SCAM	Substituted cysteine accessibility method
SCF	Stem cell factor
SEA	Stearoylethanolamide
SF-1	Steroidogenic factor 1
SFK	Src family of protein tyrosine kinases
SHH	Sonic hedgehog
SHP	Small heterodimer partner
SHP-2	SH2 domain–containing protein tyrosine phosphatase-2
SK3	Glycogen synthase kinase 3

SMO	Smoothened
SMRT	Silencing mediator of retinoic acid thyroid hormone receptors
SNARE	Soluble-N-ethylmaleimide-sensitive-factor accessory-protein receptor
SRC	Steroid receptor coactivator
SRIF	Somatotropin release–inhibiting factor; also known as somatostatin
SST	Somatostatin receptor
STEP	Striatal-enriched phosphatase
STYK1	Serine/threonine/tyrosine kinase 1
SUMO	Small ubiquitin–related modifier
TAM	Typo3, Axl, and Mer family of tyrosine kinase receptors
TAS1R	Taste 1 Receptor
TGF	Transforming growth factor
THRA	Thyroid hormone receptor α
THRB	Thyroid hormone receptor β
Tie	Tyrosine kinase with immunoglobulin like and EGF-like domains
TKAR	Tyrosine kinase–associated receptors
TLX	The human orphan nuclear receptor tailless; also known as NR2E1
TNF	Tumor necrosis factor
TP	Thromboxane receptor
TR	Thyroid hormone receptor
TRH	Thyrotropin-releasing hormone
TrK	Tropomyosin receptor kinase
TYRO3	TAM family receptor tyrosine kinase
Ucn	Urocortin
UDP	Uridine diphosphate
UT	Urotensin
UTP	Uridine-5′-triphosphate
VDR	Vitamin D receptor
VEGFR	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VIPR	Vasoactive intestinal peptide receptor
VPAC	Vasoactive intestinal polypeptide receptor
Wnt	Coined from the drosophila gene *Wingless* and the mouse oncogene *Int-1*.
WNT	Superfamily of Wnt signaling proteins
Y	Neuropeptide Y receptor
ZAC	Zinc-activated channel receptor
Zn	Zinc
α12A	Adrenoreceptor type α2A
α12AB	Adrenoreceptor type α2B
α12AC	Adrenoreceptor type α2C
α1A	Adrenoreceptor type α1A
α1B	Adrenoreceptor type α1B
α1D	Adrenoreceptor type α1D
β	β-adrenergic receptor
βARK	β-adrenergic receptor kinase

Overview

Neuroreceptors—also known as neurotransmitter receptors—are protein complexes that are activated in response to neurotransmitters. Regardless of how a signal initiates, the cellular receptors are proteins that serve dual functions: (1) recognize chemical or environmental stimuli, and (2) transduce these stimuli to cellular responses—which may be either excitatory or inhibitory. Given their widespread involvement in physiological and pathological processes, receptors are a successful target for the drug discovery. Currently 70% of all available drugs target cellular receptors. In the simplest of terms, receptors can be classified into four categories (Fig. 15.1):

- Membrane bound ligand-grated ion channels.
- G protein-coupled receptors.
- Enzyme-linked receptors.
- Intracellular nuclear receptors.

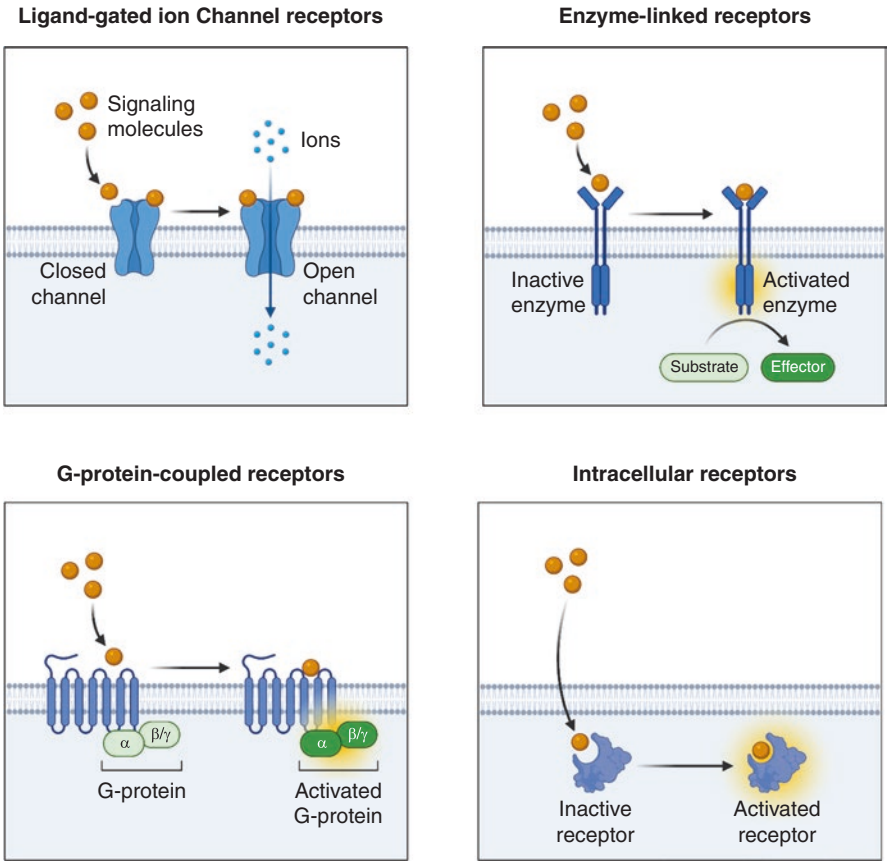

Fig. 15.1 Types of receptors

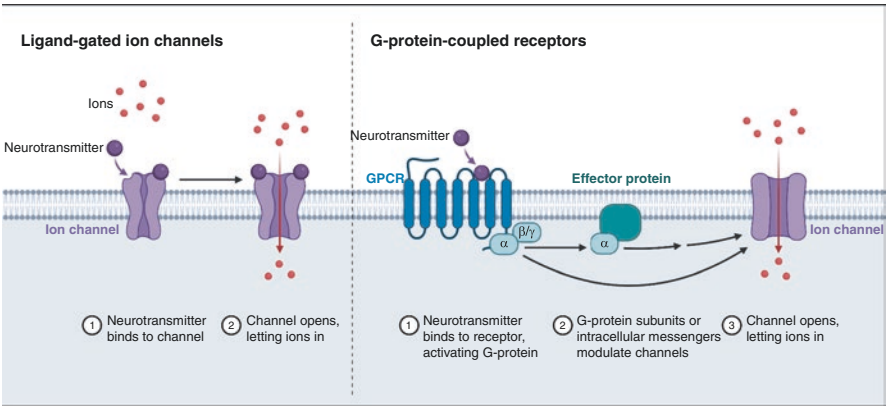

Fig. 15.2 Two types of neurotransmitters

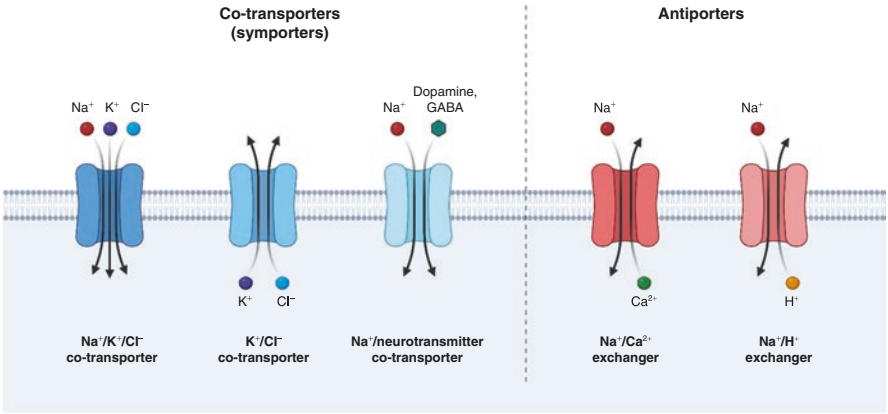

Fig. 15.3 Different types of secondary active transporters

The majority of neurotransmitters mostly interact with postsynaptic receptors, but some receptors are located on presynaptic neurons (Fig. 15.2). The family of ionotropic receptors (e.g., *N*-methyl-D-glutamate, kainate, nicotinic acetylcholine, glycine, and gamma-aminobutyric acid receptors) are ion channels (see Fig. 15.3) that open when bound to the neurotransmitter and induce very rapid responses. In contrast, metabotropic receptors (e.g., serotonin, α- and β-adrenergic, and dopaminergic receptors) interact with G proteins to activate a second messenger(see Fig. 15.4) (e.g., cAMP) that catalyzes a much slower cascade of neurological responses. Albeit, the endogenous ligands for a large number of orphan neuroreceptors remain unidentified, most known neurotransmitters activate specific receptors rather than second messenger signaling cascades. Figure 15.2 shows the main difference between ionotropic and metabotropic receptors.

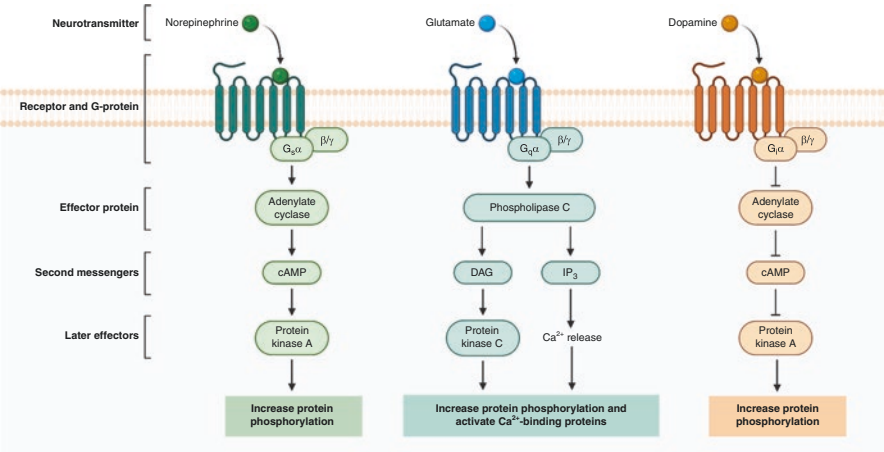

Fig. 15.4 Neurotransmitters and GPCR effector pathways

Neuroreceptors that are continuously stimulated by neurotransmitters or drugs can become desensitized (downregulated). Alternatively, other receptors may by upregulated by their endogenous neurotransmitter or drug blockade. The downregulation or upregulation of receptors strongly influences the development of tolerance and physical dependence. Withdrawal symptoms can be explained, at least in part, by a rebound phenomenon due to altered receptor affinity or density.

G-Protein Coupled-Receptors (GPCRs)

G-protein-coupled receptors (GPCRs; also called metabotropic receptors to imply coupling to metabolism) represent the largest superfamily of signaling receptors in the human proteome. GPCR-driven signaling depends on which G protein it couples and involves most physiological and many pathological processes. Alterations in the expression and activity of GPCRs occur during normal development and aging. The central nervous system (CNS) is particularly affected by these alterations, which result in decreased brain function, neurodegeneration, and increased vulnerability to neuropathology such as Alzheimer's disease, and inflammatory neurological disorders such as multiple sclerosis (MS).

GPCRs mediate a wide array of intracellular signaling pathways and are targeted by 25–50% of currently marketed pharmaceutical drugs [1]. Pharmacological differences among receptor subtypes are exploited therapeutically through the development and use of receptor-selective drugs. For example, β2 adrenergic agonists such as terbutaline are used to control bronchodilation in the treatment of asthma to minimize cardiac side effects in response to stimulation of the β1 adrenergic receptor. Conversely, β1-selective antagonists lessen bronchoconstriction in patients

under treatment for hypertension. Prominent therapeutic neurological GPCRs applications include μ opioid agonists, antihistamines, anticholinergics, antipsychotics, antimigraine drugs, and antihypertensives. Most neurological GPCR-targeting therapeutics are small molecules and target a small subset of GPCRs and compete for binding with endogenous GPCR ligands. The more common GPCR ligands in the nervous system include monoamines (e.g., adrenaline, noradrenaline, serotonin, dopamine, and histamine), other small neurotransmitters (acetylcholine, gamma aminobutyric acid, glutamate, ATP, adenosine, and cannabinoids), peptide neurotransmitters and hormones (opioids, somatostatin, NPY, oxytocin, vasopressin, neurotensins, VIP, galanin, kinins, and releasing hormones), and sensory modalities (e.g., light, odorants and tastants).

GPCR Classification

Structurally, all GPCRs share a similar three-dimensional architecture characterized by an extracellular N-terminus, seven transmembrane-spanning α-helices (7-TM), an intracellular C-terminus, and variable extracellular and intracellular elements. The International Union of Pharmacology (IUPHAR) classification categorizes GPCRs into five main groups based on structural similarity and phylogenetic analyses.

- Class A (Rhodopsin family) is the largest group, followed by
- Class B (Secretin family),
- Class C (Glutamate family),
- Class F (Frizzled/Taste family), and
- Adhesion family.

More than 140 unclassified GPCRs, including orphan GPCRs, for which an endogenous ligand is not yet know, have also been identified. Presently, most GPCR modifying pharmaceutical drugs target Class A and Class B GPCRs [2].

Functionally, integral plasma membrane GPCRs transduce extracellular stimuli into intracellular signals (Fig. 15.1). Endogenous peptides that bind to the extracellular surface of membrane-spanning GPCRs spatiotemporally extend over paracrine and autocrine signaling and include long-acting hormones as well as locally released mediators of cellular functions and neurotransmitters. Low-molecular-weight ligands normally bind within the hydrophobic region of GPCRs. In contrast, protein and peptide agonists bind to N-terminal and extracellular hydrophilic loops joined to the transmembrane domains. The second and third cytoplasmic loops and C-terminal are particularly important for ligand-G protein interactions and receptor stabilization.

Under the current paradigm, ligand binding to GPCRs stabilizes conformations with increased affinity for intracellular heterotrimeric guanine nucleotide-binding proteins (G proteins, comprised of three distinct α, β, γ subunits), GPCR kinases (GRKs), and *β*-arrestins. This receptor activation, induces various events such as second-messenger modulation by several families of G proteins, phosphorylation of

intracellular receptor residues by GRKs, or desensitization and internalization mediated by *β*-arrestins. "Freed" active GPCR can bind and activate another G protein molecule to further propagate signal transduction. Hence, the activation of a single effector molecule induces the movement of numerous ions across plasma membrane or the conversion of several substrate molecules into product, providing further signal amplification. Among GPCR-mediated signaling, amplification is the greatest in rod photoreceptors, as demonstrated by the ability of cells to respond to a single photon. Notedly, most endogenous ligands are agonists, but some are antagonists (e.g., agouti and agouti-related peptide are antagonists of melanocortin receptors).

Depending on the affinity for the different *β*-arrestin isoforms (*β*-arrestin 1 or *β*-arrestin 2) and the stability of the GPCR–*β*-arrestin complex during internalization, GPCRs are referred to as class A (transient interaction) or class B (sustained interaction). In addition, *β*-arrestins can act as important scaffolds and promote G protein–independent signaling such as extracellular signal–regulated kinase (ERK)1/2 phosphorylation.

Gα-GTP Amplification

Heterotrimeric G proteins are named by the type of α subunit they contain. Five Gα subunits have been identified, and they can bind to multiple Gβ and Gγ subunits.

1. $G\alpha_s$ and $G\alpha_{olf}$ (s for stimulatory, olf for olfactory) subunits activates plasma membrane adenylyl cyclase to increase the cytosolic second messenger cyclic AMP (cAMP) and stimulate the phosphorylation of target proteins by cAMP-dependent protein kinase.
2. $G\alpha_i$ and $G\alpha_o$ (i for inhibitory and o for other) subunits inhibit adenylyl cyclase and reduce cellular cAMP levels. $G\alpha_o$ is the most abundant heterotrimeric G protein in CNS.
3. $G\alpha_q$ and $G\alpha_{11}$ subunits activate phospholipase C (PLC)-β to increase the levels of several second messengers including inositol trisphosphate (IP_3) that release Ca^{2+} from intracellular stores and diacylglycerol (DAG) that activates phosphorylation by protein kinase C (PKC). Strong activation of PLC-β by $G\alpha_q$ depletes $PI(4,5)P_2$ lipid from the plasma membrane, thus altering the function of lipid-sensitive ion channels and transporters.
4. $G\alpha_{12}$ and $G\alpha_{13}$ subunits enhance RhoA, Rho kinase and slow dephosphorylation of myosin light chain.
5. $G\alpha_{transducing}$ and $G\alpha_{gustducin}$ subunits activate cyclic GMP (cGMP) phosphodiesterase (transducin), which can deplete cytoplasmic cGMP or cAMP phosphodiesterase (gustducin), which can deplete cAMP.

Of these G protein subunits, G_s, G_i, and G_q-mediated signaling are the most commonly encountered in the nervous system. The long cascades of signaling may take up to tens of seconds to be completed. However, some cases, such as vision using rhodopsin and transducin, take only tens of milliseconds.

Downstream Coupling of Gβγ

The Gβγ subunits are well recognized to (1) activate G-protein coupled inwardly rectifying K^+ (GIRK) channels, (2) inhibit the opening of several voltage-gated Ca^{2+} channels of the Ca_V family, and (3) bind to the SNARE complex of the exocytotic machinery in synapses and reduce exocytosis of neurotransmitters. The latter two of these signaling pathways play a major role in receptor-dependent presynaptic inhibition by reducing Ca^{2+} entry and blocking neurotransmitter exocytosis. Further, Gβγ dimers can directly stimulate PLC-β and phosphoinositide 3-kinase γ (PI3Kγ).

Termination of Signaling

Rapid termination of GPCR signaling is accomplished by a conserved two-step mechanism: phosphorylation of active GPCR by GPCR kinases followed by binding of arrestin proteins. In addition to blocking GPCR signaling via G proteins, GPCR kinases and arrestins contribute to several GPCR-initiated as well as GPCR-independent intracellular signaling pathways. Hence, selective regulation of GPCR kinases and arrestins are promising therapeutic targets. For example, cardiac failure can manifest from a loss of heart tissue responsiveness to pro-contractile stimuli, predominantly adrenalin, a nonselective β1 and β2 adrenergic receptor agonist. Both β1 and β2 receptors are desensitized in response to GPCR kinase 2 (GRK2). Thus, heart failure can be alleviated by overcoming GRK2-mediated β-adrenergic signaling suppression.

Specificity of GPCR Signaling

The specificity of ligand-GPCR signaling tightly regulated by several mechanisms.

1. Individual cell types expresses only a subset of the available receptors.
2. Individual cell types expresses only a specific subset of downstream protein targets that respond to second messengers activated in response to GPCRs.
3. GPCRs are localized to only selective regions of the plasma membrane.
4. Finally, convergence of signaling pathways enables subsets of diverse cells to response to individual nutritional and energy needs.

Class A (Rhodopsin family) GPCRs

The largest of the GCPR families (Class A Rhodopsin family) influences virtually every aspect of human physiology and can be subdivided into eight groups, according to the type of ligand (e.g., light, amine, peptide, lipid, nucleotide/nucleoside, amino acid, proton, or olfactory) and 19 subgroups, based upon phylogenetic cluster and the GPCR region to where the ligand binds [3, 4]. Table 15.1 summarizes the

Table 15.1 Class A (Rhodopsin-like) of G protein-coupled receptors

Family	Receptor(s)	Endogenous ligand(s)
5-Hydroxytryptamine (5-HT) receptors	5-HT_{1A}, 5-HT_{1F}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_4, 5-HT_{5A}, 5-HT_{5B}, and 5-HT_6	5-Hydroxytryptamine (also known as serotonin)
	5-HT_{1B} and 5-HT_{1C}	5-HT-Moduline, 5-Hydroxytryptamine, and Tryptamine
		5-HT-Moduline and 5-Hydroxytryptamine
	5-HT_{1E} and 5-HT_{2A}	5-Hydroxytryptamine and Tryptamine
Acetylcholine receptors (muscarinic)	M_1, M, M_3, M_4, and M_5	Acetylcholine
Adenosine receptors	A_1, A_{2A}, A_{2B}, and A_3	Adenosine
Adrenoceptors	α_{1A-}, α_{1B-}, α_{1D-}, α_{2A-}, α_{2B-}, and α_{2C}	(−)-Adrenaline (−)-Noradrenaline
	β_1	(−)-Adrenaline, Noradrenaline, and (−)-Noradrenaline
	β_2	(−)-Adrenaline, Noradrenaline, (−)-Noradrenaline, and Zn^{2+}
	$\beta_{3\alpha\alpha}$	(±)-Adrenaline, (−)-Adrenaline, and (−)-Noradrenaline
Angiotensin receptors	AT_1 receptor	Angiotensin A, Angiotensin II, Angiotensin III, and Angiotensin IV
	AT_2 receptor	Angiotensin-(1–7), Angiotensin II, and Angiotensin III
Apelin receptors	Apelin receptor	Aapelin-36, Apelin-13, Apelin-17, Elabela/Toddler-32, Elabela/Toddler-21, Elabela/Toddler-11, [Pyr1] apelin-13
Bile acid receptor	GPBA receptor	Chenodeoxycholic acid, Cholic acid, Deoxycholic acid, and Lithocholic acid
Bombesin receptors	BB_1 receptor	Gastrin-releasing peptide, gastrin releasing peptide (14–27), GRP-(18–27), and Neuromedin B
	BB_2 receptor	Gastrin-releasing peptide, gastrin releasing peptide (14–27), GRP-(18–27), Neuromedin B, and Neuromedin C
	BB_3 receptor	
Bradykinin receptors	B_1 and B_2 receptors	Bradykinin, [des-Arg9] Bradykinin, [des-Arg10] Kallidin, [Hyp3] Bradykinin, Kallidin, Lys-[Hyp^3]-Bradykinin, and T-kinin
Cannabinoid receptors	CB_1 and CB_2	Anandamide and 2-Arachidonoylglycerol
Chemerin receptors	Chemerin receptor 1	Chemerin and Resolvin E1
	Chemerin receptor 2	Chemerin

(continued)

Table 15.1 (continued)

Family	Receptor(s)	Endogenous ligand(s)
Chemokine receptors	CCR1	CCL 3–5, 7–8 13–16, and 23
	CCR2	CCL 2, 5, 7–8, 11, 13, 15, 24, 26, and 28
	CCR3	CCL 2, 5, 7–8, 11, 13, 15, 24, 26, and 28 and CXCL 9–11
	CCR4	CCL17 and 22
	CCR5	CCL 2–5, 7–8, 11, 13–14, and 16
	CCR6	Beta-Defensin 4A and CCL 20
	CCR7	CCL19 and CCL21
	CCR8	CCL1
	CCR9	CCL25
	CCR10	CCL27 and CCL28
	CXCR1	CXCL 1, 6, and 8
	CXCR2	CXCL 1–3, and 5–8
	CXCR3	CCL5, 7, 11, 13, 19–20 and CXCL9–11 and CXCL12α
	CXCR4	CXCL 12α, 12β, 12γ, 12δ, 12φ, and 12ε
	CXCR5	CXCL13
	CXCR6	CXCL16
	CX_3CR1	CX3CL1
	CXCR1	CXCL1 and CXCL2
	ACKR1 and ACKR2	All inflammatory CC-chemokines
	ACKR3	CXCL11 and CXCL12α
	ACKR4	CCL19, 21, and 25
	CCRL2	CCL19
Cholecystokinin receptors	CCK1 receptor	CCK-58, 39, 4, 33, and 8 and Gastrin-17
	CCK2 receptor	CCK-4, CCK-33, CCK-8, desulfated cholecystokinin-8, desulfated gastrin-14, desulfated gastrin-17, desulfated gastrin-34, desulfated gastrin-71, Gastrin-34, Gastrin-71, Gastrin-14, Gastrin-17
Complement peptide receptors	C3a receptor	C3a and C5a
	$C5a_1$ receptor	C3a, C5a, C5a des-Arg, and ribosomal protein S19
	$C5a_2$ receptor	C5a and C5a des-Arg
Dopamine receptors	D_1 and D_5 receptors	Dopamine, 5-Hydroxytryptamine, and noradrenaline
	D_2, D_3, and D_4 receptors	Dopamine
Endothelin receptors	ET_A	Endothelin-1 and Endothelin-2
	ET_B	Endothelin-1, Endothelin-2, and Endothelin-3

Table 15.1 (continued)

Family	Receptor(s)	Endogenous ligand(s)
Formyl peptide receptors	FPR1	Annexin I and Cathepsin G
	FPR2	Annexin I, Humanin, LL-37, LXA4, PrP106–126, Resolvin D1, and serum amyloid A
	FPR3	Annexin I-(2–26), Humanin, and F2L
Free fatty acid receptors	FFA1	Docosahexaenoic acid, α-linolenic acid, Myristic acid, oleic acid, and long chain carboxylic acids
	FFA2	Acetic acid, butyric acid, 1-Methylcyclopropanecarboxylic acid, Propanoic acid, and Trans-2-Methylcrotonic acid
	FFA3	Butyric acid, 1-Methylcyclopropanecarboxylic acid, and Propanoic acid
	FFA4	Linoleic acid, α-linolenic acid, Myristic acid, oleic acid, and free fatty acids
	GPR42	Orphan
Galanin receptors	GAL_1, GAL_2, and GAL_3	Galanin and Galanin-like peptide
Ghrelin receptor	Ghrelin receptor	Ghrelin and [des-Gln14] ghrelin
Glycoprotein hormone receptors	FSH receptor	FSH
	LH receptor	LH and hCG
	TSH receptor	TSH
Gonadotrophin-releasing hormone receptors	$GnRH_1$ and $GnRH_2$	GnRH I and GnRH II
GPR18, GPR55 and GPR119	GPR18	N-Arachidonoylglycine
	GPR55	Anandamide, 2-Arachidonoylglycerol, 2-Arachidonoylglycerolphosphoinositol, Lysophosphatidylinositol, and N-Palmitoylethanolamine
	GPR119	N-Oleoylethanolamide, N-Palmitoylethanolamine, and SEA
G protein-coupled estrogen receptor	GPER	17β-estradiol
Histamine receptors	H_1, H_2, and H_3	Histamine
	H_4 receptors	Histamine and CCL16
Hydroxycarboxylic acid receptors	HCA_1 receptor	L-lactic acid
	HCA_2 receptor	Butyric acid and β-D-Hydroxybutyric acid
	HCA_3 receptor	3-Hydroxyoctanoic acid
Kisspeptin receptor	Kisspeptin receptor	Kisspeptin-10, Kisspeptin-13, Kisspeptin-14, and Kisspeptin-54

(continued)

Table 15.1 (continued)

Family	Receptor(s)	Endogenous ligand(s)
Leukotriene receptors	BLT_1 receptor	20-Hydroxy-LTB4, LTB4, and 12R-HETE
	BLT_2 receptor	12-epi LTB4, 12-Hydroxyheptadecatrienoic acid, 20-Hydroxy-LTB4, LTB4, 12R-HETE, 15S-HETE, 12S-HETE, and 12S-HPETE
	BLT_3 and BLT_4 receptors	LTC4, LTD4, and LTE4
	BLT_5 receptor	5-oxo-C20:3, 5-oxo-ETE, 5-oxo-20-HETE, 5-oxo-12-HETE, 5-oxo-15-HETE, 5-oxo-ODE, 5S-HETE, and 5S-HPETE
	BLT_6 receptor	Annexin I, aspirin triggered Lipoxin A4, aspirin triggered Resolvin D1, Humanin, LL-37, LXA4, PrP106–126, Resolvin D1, and serum amyloid A
Lysophospholipid (LPA) receptors	LPA_1 and LPA_6 receptors	LPA
	LPA_2 and LPA_3 receptors	LPA, farnesyl diphosphate, and farnesyl monophosphate
	LPA_4 receptor	LPA, farnesyl diphosphate
	LPA_5 receptor	LPA, farnesyl diphosphate, farnesyl monophosphate, and N-Arachidonoglycine
Lysophospholipid (S1P) receptors	$S1P_1$ receptor	LPA, sphingosine 1-phosphate, and Sphingosylphosphorylcholine
	$S1P_2$, $S1P_3$, $S1P_4$, and $S1P_5$ receptors	Sphingosine 1-phosphate and Sphingosylphosphorylcholine
Melanin concentrating hormone receptors	MCH_1 and MCH_2 receptors	Melanin concentrating hormone
Melanocortin receptors	MC_2 receptor	ACTH
	MC_1, MC_3, MC_4, and MC_5 receptors	ACTH, β-MSH, α-MSH, and γ-MSH
Melatonin receptors	MT_1 and MT_2 receptors	Melatonin
Motilin receptor	Motilin receptor	Motilin
Neuromedin U receptor	NMU1 and NMU2 receptors	Neuromedin S-33, U-25
Neuropeptide FF and AF receptors	NPFF1 and NPFF2 receptors	Neuropeptide AF, neuropeptide FF, neuropeptide SF, RFRP-1, and RFRP-3
Neuropeptide S receptor	NPS receptor	Neuropeptide S
Neuropeptide W and B receptors	NPBW1 receptor	Des-Br-neuropeptide B-23, des-Br-neuropeptide B-29, and neuropeptide B-23, neuropeptide B-29, neuropeptide W-23, and neuropeptide W-30
	NPBW2 receptor	Neuropeptide B-23, neuropeptide B-29, neuropeptide W-23, neuropeptide W-30

Table 15.1 (continued)

Family	Receptor(s)	Endogenous ligand(s)
Neuropeptide Y receptors	Y2 receptor	Neuropeptide Y, neuropeptide Y-(3–36), pancreatic polypeptide, peptide YY, and PYY-(3–36)
	Y_1, Y_4, and Y_5 receptors	Neuropeptide Y, pancreatic polypeptide, and peptide YY
Neurotensin receptors	NTS receptor	Large Neuromedin N, large Neurotensin, Neuromedin N, and Neurotensin
	NTS_2 receptor	Neuromedin N, Neurotensin, and Xenin
Opioid receptors	δ-Opioid receptor	Dynorphin A-(1–13), Dynorphin A, Dynorphin A-(1–8), Dynorphin B, Endomorphin-1, β-endorphin, [Leu]enkephalin, [Met]enkephalin, and α-Neoendorphin
	κ-Opioid receptor	Big Dynorphin, Dynorphin A-(1–13), Dynorphin A, Dynorphin A-(1–8), Dynorphin B, β-Endorphin, [Leu]enkephalin, [Met]enkephalin, α-Neoendorphin, and β-Neoendorphin
	μ-Opioid receptor	Dynorphin A-(1–13), Dynorphin A, Dynorphin A-(1–8), Dynorphin B, Endomorphin-1, Endomorphin-2, β-endorphin, [Leu]enkephalin, and [Met]enkephalin
	Nociceptin opioid peptide receptor (NOP)	Nociceptin/Orphanin FQ (N/OFQ)
Opsin receptors	Rhodopsin, OPN1LW, OPN1MW, OPN1SW, OPN3, OPN4, and OPN5 receptors	Photons
Orexin receptors	OX_1 and OX_2 receptors	Orexin-A and Orexin-B
Oxoglutarate receptor	Oxoglutarate receptor	α-Ketoglutaric acid
P2Y2 receptors	$P2Y_1$ and $P2Y_{13}$ receptors	ADP and ATP
	$P2Y_2$, $P2Y_4$, and $P2Y_{11}$ receptors	ATP and uridine triphosphate
	$P2Y_6$ receptor	Uridine diphosphate and uridine triphosphate
	$P2Y_{12}$ receptor	ADP
	$P2Y_{14}$ receptor	UDP-galactose, UDP-glucose, UDP-glucuronic acid, UDP N-acetyl-glucosamine, and uridine diphosphate
Platelet-activating factor receptor	PAF receptor	Methylcarbamyl PAF and PAF
Prokineticin receptors	PKR_1 and PKR_2 receptors	Prokineticin-1, Prokineticin-2, and Prokineticin-2β

(continued)

Table 15.1 (continued)

Family	Receptor(s)	Endogenous ligand(s)
Prolactin-releasing peptide receptor	PrRP receptor	Neuropeptide Y, PrRP-20, PrRP-31, and PTHrP
Prostanoid receptors	DP_1 receptor	PGD2, PGE1, PGE2, PGF2α, PGI2, and PGJ2
	DP_2 receptor	PGD3, PGD2, PGE2, PGF2α, PGI2, and PGJ2
	EP_1, EP_2, EP_3, EP_4, and IP receptors	PGD2, PGE1, PGE2, PGF2α, and PGI2
	FP receptor	PGD2, PGE2, PGF2α, and PGI2
	TP receptor	PGD2, PGE2, PGF2α, PGI2, and Thromboxane A2
Proteinase-activated receptors	PAR1 and PAR3 receptors	Thrombin
	PAR2 receptor	Serine proteases
	PAR4 receptor	Cathepsin G and Thrombin
QRFP receptor	QRFP receptor	QRFP26 and QRFP43
Relaxin family peptide receptors	RXFP1 receptor	Relaxin-1, Relaxin, and Relaxin-3
	RXFP2 receptor	INSL3, Relaxin-1, Relaxin, and Relaxin-3
	RXFP3 receptor	INSL5, Relaxin-3, and Relaxin, Relaxin-3
	RXFP4 receptor	INSL5, INSL5, and Relaxin-3
Somatostatin receptors	SST_1, SST_2, SST_3, and SST_4 receptors	CST-17, SRIF-14, and SRIF-28
	SST_5 receptor	
Succinate receptor	Succinate receptor	Succinic acid
Tachykinin receptors	NK and NK_2 receptors	Neurokinin A, Neurokinin B, Neuropeptide γ, Neuropeptide K, and Substance P
	NK_3 receptor	Neurokinin A, Neurokinin B, and Substance P
Thyrotropin-releasing hormone receptors	TRH_1 and TRH_2 receptors	Thyrotropin-releasing hormone (TRH)
Trace amine receptor	TA_1 receptor	Dopamine, 3-iodothyronamine, Octopamine, β-phenylethylamine, and Tyramine
Urotensin receptor	UT receptor	Urotensin II and Urotensin II-Related Peptide
Vasopressin and Oxytocin receptors	V_{1A}, V_{1B}, V_2, and OT receptors	Oxytocin and Vasopressin

eight groups and 19 subgroups of the GPCR Rhodopsin family groups. Rhodopsin, the prototype of this GPCR family, is activated in response to photons and functions as the dim-light photoreceptor in the rod cell. All Class A GPCRs share primary structural homology as characterized by a conserved disulfide bridge that connects the second extracellular loop and the third transmembrane segment with a high degree of sequence homology. All Class A GPCRs have a ligand-binding pocket between the α-helices, which may reside either in close proximity to the extracellular surface or buried almost to half the depth of the membrane.

Class B (Secretin Family) GPCRs

The Class B secretin receptor-like family of GPCRs include 20 receptors, which can be grouped into five subfamilies (based upon their physiological role), are activated by 15 endogenous peptide hormones (Table 15.2). Class B GPCRs are particularly important drug targets for multiple sclerosis, schizophrenia, anxiety, depression, migraine, and a host of metabolic disorders, such as type 2 diabetes, osteoporosis, and cardiovascular disease. Based upon the ligand that they bind to, Class B GPCRs can be grouped in the following six families.

Table 15.2 Class B (Secretin receptor-like) G protein-coupled receptors

Family	Receptor	Endogenous ligand(s)
Calcitonin (CT) receptors	CT, Amylin $(AMY)_1$, AMY_2, and AMY_3 receptors	Adrenomedullin (AM), Adrenomedullin 2/intermedin, Amylin, Calcitonin, α-Calcitonin gene-related peptide (CGRP), and β-CGRP
	Calcitonin receptor-like receptor	Adrenomedullin and CGRP
	CGRP, AM_1, and AM_2 receptors	Adrenomedullin, Adrenomedullin 2/intermedin, α-CGRP, and β-CGRP
Corticotropin-releasing factor receptors	CRF_1 and CRF_2 receptors	Corticotrophin-releasing hormone, Urocortin 2, and Urocortin 1
		Corticotrophin-releasing hormone, Urocortin 1, Urocortin 2, and Urocortin 3
Glucagon receptor family	GHRH receptor	Growth hormone-releasing hormone (GHRH)
	Glucose-dependent insulinotropic polypeptide (GIP) receptor	Glucose-dependent insulinotropic polypeptide (GIP)
	Glucose-like peptide (GLP) -1 receptor	Glucagon, glucagon-like peptide 1-(7–37) and glucagon-like peptide 1-(7–36) amide
	GLP-2 receptor	Glucagon-like peptide 2 and glucagon-like peptide 2-(3–33)
	Glucagon receptor	Glucagon
	Secretin receptor	Secretin and Vasoative intestinal peptide (VIP)
Parathyroid hormone (PTH) receptors	PTH1 and PTH2 receptors	PTH, PTHrP-(1–36), PTHrP, and TIP39
VIP and pituitary adenylate cyclase–activating polypeptide (PACAP) receptors	PAC_1 receptor	PACAP-38, PACAP-27, peptide histidine methionine (PHM), peptide histidine valine (PHV), and VIP
	Vasoactive intestinal peptide $(VPAC)_1$ receptor	GHRH, PACAP-38, PACAP-27, PHM, secretin, and VIP
	$VPAC_2$ receptor	GHRH, PACAP-38, PACAP-27, secretin, and VIP

1. The glucagon subfamily includes glucagon, glucagon-like peptide 1 (GLP-1), GLP-2, and glucose-dependent insulinotropic polypeptide which play roles in regulating glucose homeostasis. Secretin, a member of the glucagon subfamily, stimulates the secretion of bicarbonate, pepsin and other hormones from the pancreas and duodenum.
2. Corticotropin-releasing factor (CRF) and urocortin (Ucn) 1 and Ucn2 are ligands for CRF_1 and CRF_2. Whereas CRF_1 receptor antagonists are anxiolytic and antidepressant, CRF_2 and Ucn 1–3 receptor mainly target the cardiovascular and renal systems.
3. The calcitonin (CT) family is comprised of two receptors, namely, the calcitonin receptor and the calcitonin receptor-like receptor (also known as CLR). Both of these receptors interact with receptor activity-modifying proteins (RAMPs), which modulate ligand-binding specificity. CLR modulates vasodilation and is an important target for migraine. In contrast, the CT receptor regulates glucose homeostasis and is a target for osteoporosis and diabetes.
4. The pituitary adenylate cyclase activating polypeptide (PACAP) family is comprised of PAC_1, $VPAC_1$ and $VPAC_2$, and PTH1 and PTH2. These receptors are activated by PACAP, parathyroid hormone (PTH), or vasoactive intestinal peptide (VIP), neuropeptides that are widely distributed throughout the central and peripheral nervous system and function as anti-inflammatory and neuroprotective agents.
5. Finally, growth hormone-releasing hormone (GHRH), the ligand for the GHRH receptor (GHRHR), stimulates the release of growth hormone. Recombinant GHRH is currently used to treat metabolic dysregulation that coincides with human immunodeficiency virus (HIV) infection.

Structurally, Class B GPCRs consist of a large N-terminal extracellular domain (ECD), which contains the high affinity part of the ligand-binding site, and the signature 7-TM α-helices. Alternatively, a lower affinity ligand-binding site lies within the extracellular pockets positioned between the α-helices. G-protein-dependent Class B GPCR signaling primarily activates adenylate cyclase to increase intracellular levels of cAMP.

Class C (Glutamate Receptor-Like) GPCRs

Glutamate serves as the brain's primary excitatory neurotransmitter and a key neuromodulator to control synapse and circuit function over a wide range of spatial and temporal scales. The Class C GPCR family is comprised of approximately 24 receptors which can be subdivided into five subclasses (Table 15.3).

1. Metabotropic glutamate receptors (mGluRs).
2. $GAGA_\beta$ receptors.
3. Single calcium-sensing receptors.
4. Taste receptors.
5. Orphan receptors coupled to the heterotrimeric G Protein, G_o.

Table 15.3 Class C (Metabotropic Glutamate Receptor-like) G protein-coupled receptors

Family	Receptor	Gα protein	Endogenous ligand(s)
Metabotropic glutamate receptors (mGluRs)	$mGluR_1$, $mGluR_2$, $mGluR_3$, $mGluR_4$, $mGluR_5$, $mGluR_6$, $mGluR_7$, and $mGluR_8$	G_o	L-glutamic acid, L-aspartic acid, L-serine-O-phosphate, L-cysteine sulfinic acid, and N-acetylaspartylglutamate (NAAG)
$GABA_B$ receptors	$GABA_{B1}$ and $GABA_{B2}$	G_o	Gamma aminobutyric acid (GABA)
Ca^{2+}-sensing (CAS) receptor	CaS receptor	G_o	Ca^{2+}, L-tryptophan, Mg^{2+}, and spermine
Taste 1 receptors (TAS1Rs)	TAS1R1, TAS1R2, TAS1R3	G_o	Sweet and savory tastes

Structurally, Class C GPCRs are distinguishable by a characteristically large extracellular domain and constitutive dimerization. The known endogenous ligands of this family bind to the N-terminal region, but allosteric ligands have also been shown to interact with TM3, TM5, TM6, and TM7. In addition, Ca^{2+} can bind to the extracellular region to enhance the effects of glutamate in some Class C GPCR receptors. Therefore, numerous Ca^{2+} interacting residues also have potential significance in drug design targeting depression learning, and memory.

Class F (Frizzled/Taste Family) GPCRs

The Frizzled family of GPCRs consists of ten Frizzled (FZD) receptors and a smoothened (SMO) receptor (Table 15.4) which are key for cell proliferation, cell differentiation, and embryonic development. FZD receptors bind to secreted Wingless/Int-1 (WNT) glycoproteins. In contrast, SMO, although structurally similar to FZD receptors, signals in a ligand-independent manner in response to the formation of a heterodimeric SMO/sonic hedgehog (SHH) signaling complex. The neurobiology surrounding Class F GPCRs is not well understood; however, WNT and SHH signaling are independently essential for coordination of cell fate and tissue renewal in the nervous system. Dysregulated WNT and SHH signaling pathways manifests in neurological disorders, including pathology associated with a compromised blood–brain barrier, neuropsychoses, Alzheimer's and other neurodegenerative diseases, as well as neurodevelopmental disorders.

Adhesion Family GPCRs

The adhesion family, the second largest subfamily of GPCRs, consists of 33 members which can be subdivided into nine subfamilies based on the architecture of the N-terminus and phylogenetic relationships (Table 15.5). Although the majority of Adhesion family GPCRs are orphan receptors, more than half are expressed in

Table 15.4 Class F (Frizzled) G protein-coupled receptors

Family	Receptor	Endogenous ligand(s)
Class frizzled (FZD)	Frizzle class receptor class 1 (FZD_1)	Wnt-1, Wnt-2, Wnt-3a, Wnt-5a, and Wnt-7b
	Frizzle class receptor class 2 (FZD_2)	Wnt-5a
	Frizzle class receptor class 3 (FZD_3)	Wnt-3a and Wnt-5a
	Frizzle class receptor class 4 (FZD_4)	Wnt-2, Wnt-3a, Wnt-5a, and Wnt-7b
	Frizzle class receptor class 5 (FZD_5)	Wnt-3a, Wnt-5a, and Wnt-7b
	Frizzle class receptor class 6 (FZD_6)	Wnt-3a, Wnt-4, and Wnt-5a
	Frizzle class receptor class 7 (FZD_7)	Wnt-3, Wnt-3a, Wnt-5a and Wnt-7a
	Frizzle class receptor class 8 (FZD_8)	Wnt-2, Wnt-3a, Wnt-9
	Frizzle class receptor class 9 (FZD_9)	Wnt-2 and Wnt-7a,
	Frizzle class receptor class 10 (FZD_{10})	Wnt-7a and Wnt-7b
	Smoothened (SMO)	Cholesterol, 20(S)-hydroxycholesterol, and 20(S)-yne (a 20(S)-hydroxycholesterol derivative)

the CNS and are key for cortical development, neurite growth, synaptogenesis, dendritic spine formation, and myelination. Mutations in several adhesion GPCRs associate with neurological disorders including neural tube defects, Parkinson's disease, schizophrenia, and addiction. Structurally, adhesion GPCRs contain a long N-termini that binds extracellular matrix molecules, which are involved in cellular adhesion. A better understanding of adhesion GPCR biology in the nervous system is likely to identify novel therapeutic targets for neurological disorders.

Orphan G Protein-Coupled Receptors (oGPCRs)

A large distinct subset of the GPCRs remains "orphans," lacking a defined endogenous ligand. Several of these orphan receptors are expressed in the CNS and associate with neurological disorders (Table 15.6). The discovery of novel neurotransmitters that act as natural ligands of orphan GPCRs is an exciting ongoing field of research as drug targets for neurological disorders including anxiety, addiction, depression, Alzheimer's disease, Parkinson's disease, and schizophrenia.

Table 15.5 Adhesion Class of G Protein-Coupled Receptors

Group	Receptor	G Protein	Endogenous ligand(s)
I	Latrophilin 1	Gq, Go	LTX, teneurin-2, neurexin, and FLRT proteins
	Latrophilin 2	TBD	LTX
	Latrophilin 3	TBD	FLRT proteins
	ETL	TBD	Orphan
II	CD97	G12/13	Chondroitin sulfates, CD55, and CD90
	EMR1	TBD	Orphan
	EMR2	TBD	Chondroitin sulfates
	EMR3	TBD	Orphan
	EMR4	TBD	Orphan
III	GPR123	TBD	Orphan
	GPR124	TBD	Integrins $_{AND}$ glycosaminoglycans
	GPR125	TBD	Orphan
IV	$CELSR_1$	TBD	Orphan
	$CELSR_2$	Gq?	Celsr2-N terminus
	$CELSR_3$	Gq?	Celsr3-N terminus
V	GPR133	GS	Orphan
	GPR144	TBD	Orphan
VI	GPR110	TBD	Orphan
	GPR111	TBD	Orphan
	GPR113	TBD	Orphan
	GPR115	TBD	Orphan
	GPR116	TBD	Orphan
VII	BAI1	TBD	Phosphatidylserine on apoptotic cells
	BAI2	TBD	Orphan
	BAI3	TBD	Complement C1q-like protein
VIII	GPR56	G12/13	Transglutaminase 2, CD9, CD81, GPR56 N terminus, and collagen III
	GPR97	Go	Orphan
	GPR112	TBD	Orphan
	GPR114	Gs	Orphan
	GPR126	Gs	Orphan
	GPR64	TBD	Orphan
None	GPR98	TBD	Orphan
	GPR128	TBD	Orphan

BAI brain-specific angiogenesis inhibitor, *CD* cluster of differentiation, *CELSR* Cadherin EGF LAG seven-pass G-type receptor, *EMR1* EGF-like module-containing mucin-like hormone receptor-like 1 (also known as F4/80), *ETL* EGF, latrophilin and seven transmembrane domain-containing protein, *Gα12/Gα13 subunits enhance RhoA* Rho kinase and slow dephosphorylation of myosin light chain, *Gαo* subunit inhibit adenylyl cyclase and reduce cellular cAMP levels, *Gαq* subunits activate phospholipase Cβ (PLCβ), *Gαs* s for stimulatory, *GPR* G-protein coupled receptor, *LTX* α-latrotoxin

Table 15.6 Major Orphan G protein-coupled receptors associated with neurological disorders

Orphan GPCR	Gα protein	Expression in the brain	CNS disorder
GPR3	$G\alpha_s$	Habenula region, hippocampus, amygdala, cerebral cortex	Alzheimer's disease, anxiety, depression
GPR6	$G\alpha_s$	Striatopallidal neurons	Parkinson's disease, schizophrenia, depression, Alzheimer's disease
GPR17	$G\alpha_i$	Neurons, parenchymal quiescent oligodendrocyte precursor cells	Traumatic injury, Alzheimer's disease
GPR26	$G\alpha_s$	Olfactory area, amygdala, hippocampus, cerebral cortex	Anxiety, depression, substance abuse
GPR37		Corpus callosum, cerebellum, caudate nucleus, putamen, substantia Nigra, hippocampus	Autism, depression, bipolar disorder, Parkinson's disease, anxiety, epilepsy
GPR39	$G\alpha_q/G\alpha_{11}$	Amygdala, hippocampus	Mood disorders, anxiety, depression, epilepsy
GPR40	$G\alpha_q$	Midbrain, hippocampus, hypothalamus, cerebral cortex, olfactory bulb, medulla oblongata, cerebellum, spinal cord	Anxiety
GPR50		Hypothalamus, pituitary, locus Coeruleus	Stress and anxiety disorders
GPR52	$G\alpha_s$	Striatum	Schizophrenia
GPR54	$G\alpha_q/G\alpha_{11}$	Cortical and medial nucleus of amygdala, dentate gyrus of hippocampus	Alzheimer's disease
GPR55	$G\alpha_q$, $G\alpha_{12/13}$	Hippocampus, frontal cortex, cerebellum, striatum, hypothalamus, brain stem	Anxiety, substance abuse
GPR85		Dentate gyrus of hippocampus	Schizophrenia
GPR88	$G\alpha_i$	Nucleus Accumbens, olfactory tubercle, thalamus, cortex, inferior olive	Anxiety, schizophrenia
GPR103	$G\alpha_{i/o}$, $G\alpha_q$	Paraventricular and magnocellular hypothalamic nuclei, bed nucleus of Stria terminalis, lateral septum, medial Supramammillary nucleus, olfactory bulb, brain stem	Anxiety
GPR139	$G\alpha_s$	Lateral striatum, hypothalamus	Parkinson's disease, schizophrenia

Ligand-Gated Ion Channels

Ligand-gated ion channels (also referred to as ionotropic neurotransmitter receptors) are homomultimeric or heteromeric proteins that span the cell membrane and contain an intrinsic ion-conducing pore in addition to a neurotransmitter binding site. Upon activation, ligand-gated ion channel receptors rapidly change the cell membrane potential and cytoplasmic ionic composition to transiently increase membrane permeability in response to external signals (Fig. 15.1). These receptors regulate the fastest (fraction of a millisecond time scale) events in the nervous

system. The number, density, and function of ionotropic neurotransmitter receptors and their activity at synapses define synaptic strength and contribute to learning and memory formation. Dysregulated ligand-gated ion channels associate with diseases including Alzheimer's disease, Huntington's Disease, and Myasthenia Gravis.

Ligand-Gated Ion Channel Classification

Two different types of ligand-gated ion channels (excitatory, cation-selective and inhibitory, anion-selective) control the flow of ions, e.g., Na^+, K^+, Ca^{2+}, and Cl^- to regulate membrane depolarization and hyperpolarization, respectfully. These ionotropic neurotransmitter receptors can be divided into the following families: (1) 5-HT_3 receptors, (2) acid-sensing (proton-gated) ion channel (ASIC) receptors, (3) epithelial sodium channel (ENaC) receptors, (4) $GABA_A$ receptors, (5) glycine receptors, (6) ionotropic glutamate receptors, (7) inositol 1,4,5-trisphosphate receptors (IP_3Rs), (8) nicotinic acetylcholine receptors (nAChR), (9) P2X receptors, and (10) zinc-activated channel (ZAC) receptors (Table 15.7). These families are all pore-forming proteins that transduce rapid neurotransmission at chemical synapses within the CNS and at the neuromuscular junctions. The nAChR cation channel, which mediates neuromuscular transmission and excitatory synaptic neurotransmission, is a prototype ligand-gated ion channel receptor. The nAch and 5-HT_3 receptors are the two major excitatory ligand-gated ion channel receptors. $GABA_A$ and glycine receptors constitute the two major inhibitory receptors.

Ligand-Gated Ion Channel Structure

Most all ligand-gated ion channels are pentameric, comprised of five homologous subunits arranged to form an aqueous pore through the membrane. Exceptions include ionotropic glutamate and P2X receptors, which are tetrameric and trimeric structures, respectively. Characteristically, neurotransmitter-gated ion channel receptors are composed of a long extracellular N-terminal domain (which contains a hydrophobic signal sequence), four membrane-spanning segments (M1–M4), a large intracellular loop that separates M3 and M4 segments (and contains sites for protein phosphorylation), and a relatively short C-terminus. The current consensus is that the C-terminus of acetylcholine, $GABA_A$ and glycine receptor subunits lies on the extracellular side of the lipid membrane, but intracellularly in subunits of the glutamate receptors. The receptor recognition sites for neurotransmitter binding are likely located on the N-terminal extracellular domain of receptor subunits. Ion channel function appears to be regulated by the second transmembrane segment (M2) of each receptor subunit. The flow of cations or anions is determined by the type of electric charges at the M2 segment. Ligand-gated ion channels receptors are formed by multi-subunit associations. The actual subunit composition of neuronal heteromeric or homomeric receptors of most neurotransmitter-gated ion channels expressed by neuron is uncertain.

Table 15.7 Major ligand-gated ion channel receptors associated with the nervous system

Family	Receptor(S)	Endogenous ligand(s)
Gamma aminobutyric Acid ($GABA_A$)	$GABA_A$ receptor α1, α2, α3, α4, α5, and α6 subunits	GABA, 5α-pregnan-3α-ol-20-one, tetrahydrodeoxycorticosterone, and Zn^{2+}
	$GABA_A$ receptor β1, β2, β3, γ1, γ2, γ3, δ, ε, θ, π, ρ1, ρ2, and ρ3 subunits	GABA
Glycine (GlyRs)	Glycine receptor α1 subunit	Glycine, taurine, Zn^{2+}, Cu^{2+}, and H+
	Glycine receptor α2 and α3 subunits	Glycine, taurine, Zn^{2+}, and Cu^{2+}
	Glycine receptor α4 subunit	Glycine, taurine, and Zn^{2+}
	Glycine receptor β subunit	Glycine, taurine, and Zn^{2+}
Nicotinic acetylcholine (nAchRs)	nAchR α1, α2, α3, α4, α5, α6, α7, α9, α10, β1, β2, β3, β4, γ, δ, and ε subunits	Acetylcholine
5-hydroxytryptamine type-3 ($5\text{-}HT_3$)	5-HT3A, 5-HT3B, 5-HT3AB, 5-HT3C, 5-HT3D, and 5-HT3E	5-hydroxytryptamine
	5-HT3A	5-hydroxytryptamine, Ca^{2+} Mg^{2+}, and Zn^{2+}
Zinc-Activated Channel (ZAC)	ZAC	Cu^{2+}, H+, and Zn^{2+}
Glutamate (Glu) receptors	GluA1, GluA2, GluA3, GluA4, GluD1, GluD2, GluK1, GluK2, GluK3, GluK4, and GluK5	L-glutamic acid
	GluN1, GluN2A, GluN2B, GluN2C, and GluN2D	L-glutamic acid, D-aspartic acid, D-serine, glycine, and L-aspartic acid
	GluN3A and GluN3B	L-glutamic acid
Inositol 1,4,5-trisphosphate (IP_3)	IP3R1	ATP, Ca^{2+}, and IP3
	IP3R2 and IP3R3	Ca^{2+} and IP3
P2X	P2X1, P2X2, P2X3, P2X4, P2X5, P2X6, and P2X7	ATP
Acid-sensing ion channels (ASICs)	ASIC1, ASIC2, and ASIC3	H+
Epithelial sodium channels (ENaCs)	ENaCαβγ, ENaCα, ENaCβ, ENaCγ, and ENaCδ	Na^+

Ligand-Gated Ion Channel Signaling

Ligand-gated ion channels open and close in response to binding of chemical neurotransmitters that are released from pre-synaptic neurons. Upon ligand binding, the receptor undergoes a conformational change to activate receptors located at post-synaptic neurons and propagate electrical signals along the membrane of the post-synaptic neuron to mediate a fast-synaptic transmission. A prolonged presence of agonist at pre-synaptic neurons induces a rapid rise in signal transduction, and this is followed by gradual receptor desensitization and the deactivation (loss of current). Excitatory neurotransmission occurs in response to acetylcholine, glutamate, 5-HT_3, and P2X receptors and is mediated through cation-selective ion channels. In contrast, inhibitory neurotransmission is initiated in response to anion-selective glycine or $GABA_A$ receptors.

Ligand-Gated Gamma Aminobutyric Acid $(GABA)_A$ Receptors

GABA is the principal inhibitory neurotransmitter in the CNS. $GABA_A$ receptors are the primary mediators of fast inhibitory neurotransmission in the CNS. GABA inhibitory neurotransmission is essential for normal brain function ranging from neuronal activity and network synchronization to information processing and plasticity. GABAergic therapeutics are commonly used to treat anxiety, alcohol withdrawal, epilepsy, and to induce sedation and anesthesia.

$GABA_A$ receptors exist as pentamers of 4-TM subunits that form an intrinsic anion selective channels and belong to the same Cys-loop family as the nicotinic acetylcholine, 5-HT_3 and strychnine-sensitive glycine receptors (Table 15.7). Nineteen distinct $GABA_A$ receptors have been identified based upon subunit composition, ligand binding, and receptor function. Given the ability of selective $GABA_A$ receptors to mediate phasic (synaptic) or tonic (extrasynaptic) currents that are important for neuronal excitability in response to ambient concentrations of GABA, this receptor family holds great promise for the discovery of new therapies to treat neurodevelopmental disorders, schizophrenia, depression, epilepsy, and stroke. The underlaying mechanisms of action for how $GABA_A$ receptors regulate extrasynaptic inhibition remains unresolved and is a focus of ongoing research.

Ligand-Gated Glycine Receptors (GlyRs)

Glycine is the predominant neurotransmitter of many inhibitory interneurons in the adult CNS and directly activates ionotropic GlyRs, which are the predominant inhibitory receptors at post-synapses in the adult spinal cord, brainstem and retina. GlyRs are abundantly expressed in the spinal cord (ventral and dorsal horns), brainstem (motor, auditory, vestibular, and sensory nuclei), superior colliculus, granular cell layer of the cerebellum, hippocampus, retina, and olfactory bulb. In addition to glycine, zinc, $_D$-alanine, and the sulfuric acid taurine also activate GlyRs. Blockade

of GlyRs in the dorsal horn is a probable major mechanism of inflammatory and neuropathic pain. Genetic abnormalities, autoantibodies, tetanus toxin, and strychnine toxicities that impair GlyR function give rise to neurological disorders and highlight the importance of Gly receptors in promoting motor neuron hyperexcitability. Importantly, glycine also controls post-synaptic glycine availability through direct modulation of two glycine transporters, GlyT1 and GlyT2, located throughout the nervous system.

Inhibitory GlyRs are expressed either as homo-pentamers of α subunits or heteromeric combinations of two α and three β subunits that form the intrinsic anion channel (Table 15.7). Under the current paradigm, heteromeric α1β GlyRs mediate the majority of glycinergic inhibition in the adult CNS. The N-terminus of the α-subunit contains both agonist and antagonist binding sites. The β-subunit binds to the scaffolding protein, gephyrin (which itself is bound to the actin cytoskeleton) and is required for postsynaptic clustering of GlyRs. GlyR subunits also contain four α-helical TM domains and a short extracellular C-terminus. G-protein βγ subunits also influence GlyR signal transduction. In contrast to heteromeric α1β GlyRs that are highly expressed in the adult, homomeric α2 subunits are the abundant GlyR expressed in embryonic neurons. The endogenous ligand, glycine, binds at the strychnine-sensitive glycine-A binding site to open GlyR channels and initiate a transient flux of chloride ions, which silences neurons (in a manner similar to $GABA_A$).

In mature adults (conditions of low intracellular Cl^- concentrations), agonistic activation of GlyR elicits a rapid Cl^- influx, membrane hyperpolarization and postsynaptic inhibition. In contrast, the activation of GlyRs in immature neurons (conditions of high intracellular Cl^- concentrations), propagates Cl^- efflux and neuronal depolarization to facilitate the development of the nervous system. GlyR degradation at synaptic sites controls receptor availability to modulate synaptic plasticity.

Ligand-Gated Nicotinic Acetylcholine Receptors (nAcRs)

Acetylcholine (ACh) is synthesized by and released from cholinergic neurons (and nonneuronal tissues) and exerts its effects throughout the central and peripheral nervous systems. Within the CNS, cholinergic sources of Ach include local interneurons that are present throughout the brain and in projections originating from the brainstem. Endogenous Ach can signal through two distinct types of receptors: ligand-gated nicotinic Ach receptors (nAchRs) and G-protein-coupled muscarinic ACh receptors (mAChRs). As discussed previously, mAChRs play a key role in regulating complex behaviors such as cognition, movement, and reward, making them ideally situated as potential drug targets for the treatment of several brain disorders. In addition to contributing to a wide range of brain activities that include cognitive functions such as learning, memory, arousal, reward, and motor control, nAChRs play a key role in neuronal development and regeneration. Nicotinic AChRs are the target of numerous drugs that target cognitive function and analgesia.

Structurally, all nicotinic AchRs are members of the Cys-loop family of transmitter-gated ion channels, which also include the $GABA_A$, glycine and 5-HT_3 receptors. Pentameric nAchR channels, consisting of a total of 17 subunits (α1–7, a9–10, β1–4, γ, δ, and ϵ) (Table 15.7) are widely expressed in both pre- and post-synapses. Homopentameric (α7 or α9) or heteropentameric (α2-α6 with β2-β4) share a common basic structure, but have selective pharmacological and functional properties. The most widely expressed nAchR subtypes in the brain are heteromeric α4β2 and homomeric α7. In contrast, α3β4 is the most widely expressed nAchR subtype in the peripheral nervous system. Most AchRs found at somatic neuromuscular junctions in adults exist as $(\alpha1)_2\beta1\delta\epsilon$ subunit assemblies; whereas the stoichiometry of $(\alpha1)_2\beta1\gamma\delta$ predominates in embryonic extra-junctions, denervated skeletal muscle, and other pathological states. All α subunits contain two tandem cysteine residues that are located proximal to the two Ach-binding sites positioned at the interface between the extracellular N-terminus and the transmembrane helices.

All human nAChR subtypes share the general functional property of being permeable to small covalent and divalent cations. Under normal physiological conditions, the main conducting cations include Na^+, K^+, and Ca^{2+}. Endogenous Ach (or exogenous nicotine) directly binds to and stabilizes an open conformation of the Ach channel enabling transient permeation of cations for milliseconds, before closing back to either a resting or desensitized state, that is unresponsive to agonist-induced activation.

Ligand-Gated 5-Hydroxytryptamine Type-3 (5-HT_3) Receptors

The 5-hydroxytryptamine type-3 (5-HT_3) receptor is an excitatory member of the Cys-loop family of ionotropic receptors that share a five pseudosymmetrically arranged subunit structure. Other members of this family include nAChR, $GAGA_A$, strychnine-sensitive glycine receptors, and zinc-activated channel receptors. The 5-HT_3 receptors differs from other serotonin receptors (5-HT_1 to 5-HT_7) whose actions are mediated via G-proteins. The five 5-HT_3 receptor subunits as well as the homo-oligomeric assemblies of 5-HT_3A and hetero-oligomeric assemblies of 5-HT_3A and 5-HT_3B subunits are summarized in Table 15.7. Given the wide distribution of 5-HT_3 receptors throughout the central and peripheral nervous systems and the ability of 5-HT_3 antagonists to freely pass the blood–brain barrier, there is much interest in the therapeutic potential of 5-HT_3 receptor antagonists for antipsychotic, antinociceptive and other psychiatric disorders.

Ligand-Gated Zinc-Activated Channel (ZAC) Receptors

Zinc is an essential metal. The zinc-activated channel (ZAC) receptor is a novel type of cationic channel belonging to the pentamer superfamily of Cys-loop receptors gated in a concentration-dependent manner by zinc (Table 15.7). ZAC is the least understood member of this superfamily and displays little amino acid sequence

homology with other members. ZAC mRNA is expression in fetal and adult brain and spinal cord (and pancreas, placenta, prostate, thyroid, trachea and stomach). Aged patients with a metabolic syndrome have elevated serum zinc concentrations, raising the possibility that zinc might be associated with pathophysiologic processes leading to major neurodegenerative diseases including Alzheimer's and amyotrophic lateral sclerosis.

Structurally, ZAC is thought to exist as a homopentamer of four TM subunits that form an intrinsic cation selective channel. Although denoted ZAC, the channel is more potently activated by protons and copper, with greater and lesser efficacy than zinc, respectively. Among its pharmacological features, ZAC is permeable to Na^+, K^+, and Cs^+, but impermeable to Ca^{2+} and Mg^{2+}. ZAC is constitutively active and elicits an outward rectifier current that is inhibited by high concentrations of Ca^{2+} and tubocurarine, a nicotinic receptor antagonist.

Ligand-Gated Glutamate Receptors (GluRs)

Glutamate is the major excitatory neurotransmitter in the CNS, and as such, ionotropic GluRs is essential for excitatory synaptic transmission, which is important for learning and memory. Disrupted glutamatergic synapse function is a common feature of many cognitive and neuropsychiatric disorders which lead to excitation/inhibition imbalances. Despite many outstanding mechanistic questions, iGluRs (and metabotropic glutamate receptors (mGluRs)) have emerged as potential drug targets for a number of neurological disorders, including schizophrenia, depression, and addiction. Glutamate does not distinguish between iGlu and mGlu receptor families that are both present in many cell types and subcellular compartments.

Ionotropic GluRs can be divided into four subfamilies on the basis of their ligand binding properties and sequence similarity: (1) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), (2) kainite, and (3) *N*-methyl-D-aspartate (NMDA) receptors, and (4) delta receptors (Table 15.7). Ionotropic GluRs assemble as tetramers comprised of four intertwined subunits forming a nonselective cation channel. Each subunit has an extracellular N-terminus, an extracellular ligand binding domain, three transmembrane pore-forming domains, and an intracellular C-terminus. Receptor heterogeneity within each class arises from the homo-oligomeric, or hetero-oligomeric, assembly of distinct subunits into cation-selective tetramers. The expression of different iGluR subtypes varies in different CNS regions, but their expression is widespread, and individual cells typically express multiple different iGluR subtypes.

AMPA, NMDA, and kainite glutamate receptors are non-selective cation channels, that mediate Na^+ and K^+, and in some cases small amounts of Ca^{2+} influx to produce excitatory postsynaptic responses. Glutamate delta receptors are similar to the other three classes of iGluRs in that they play a key role in synapse formation, but they are distinct in that they do not exhibit a typical agonist-induced ion channel current. The expression pattern of different iGluR subtypes varies in different brain

regions, but their expression is widespread, and individual cells typically express multiple different iGluR subtypes.

Ligand-Gated Inositol 1,4,5-Trisphosphate (IP_3) Receptors (IP_3Rs)

Inositol 1,4,5-trisphosphate (IP_3) is an important second messenger that is produced from membrane phospholipids. The inositol 1,4,5-trisphosphate receptors (IP_3Rs) are ligand-gated Ca^{2+}-release channels located on intracellular Ca^{2}+ storage sites, such as the endoplasmic reticulum. Upon IP_3 binding to tetrameric IP3Rs on the endoplasmic reticulum, Ca^{2+} is released from the endoplasmic reticulum into the cytosol. Thus, IP_3Rs play a key role in the mobilization of intracellular Ca^{2+} stores and play an important role in intracellular Ca^{2+} signaling in a wide variety of cell types.

Three IP_3R subtypes have been identified: IP_3R1, IP_3R2, and IP_3R3 (Table 15.7). These three IP_3R subtypes are similar in that they each form a Ca^{2+} channel that is coregulated by IP_3 and Ca^{2+}, but they differ in their distinct expression pattern. Of the three types of IP3Rs, the type 1 receptor (IP_3R1) is dominantly expressed in the brain and is important for brain function.

Ligand-Gated P2X Receptors

Exogenous ATP elicits pain and enhances pain sensations associated with inflammation. P2X receptors open a membrane ion channel in response to extracellular ATP, much less so by ADP, and not activated at all by AMP or adenosine or other purines (e.g., GTP) or pyrimidines (e.g., UTP and CTP). P2X receptor subunits assemble to form homotrimeric (P2X1, P2X2, P2X3, P2X4, P2X5, and P2X7) and heterotrimeric structures (Table 15.7).

The ability of ATP to modulate pain-associated neuronal excitability associated with nociception is mediated directly by activation of homomeric and heteromeric P2X3 receptors on peripheral nerves and indirectly by activation of P2X and P2Y receptors on glial cells in the periphery and spinal cord. In addition to its actions as a fast neurotransmitter, ATP can also function as a danger signal in response to tissue trauma. Within the CNS, ATP acting at P2X receptors on both neurons and a variety of glial cells participates in communication with conditions of neuronal hyperexcitability, chronic inflammation, and injury. Structurally, each P2X receptor subunit has two TM domains separated by a large extracellular domain, and intracellular N- and C-termini. The bulk of the P2X receptor lies within the extracellular space and undergoes substantial conformational rearrangement upon channel opening. The channel opens within a few milliseconds of ATP binding, permitting passage of cations Na^+, K^+, and Ca^{2+}, and closes within tens of milliseconds when the application is discontinued (deactivation).

Ligand-Gated Acid-Sensing (Proton-Gated) Ion Channel (ASIC) Receptors

Acid-sensing ion channels are members of a Na^+ channel superfamily that includes the epithelial Na^+ channel and the orphan channel, human intestine Na^+ channel (INaC). ASIC channels are primarily expressed in central and peripheral neurons including nociceptors and participate in neuronal sensitivity to acidosis. Although, not completely clear, there is a high likelihood that ASICs also participate in other CNS functions including learning, fear perception, seizures, pain, and axonal degeneration associated with autoimmune inflammation. Three ASIC receptors (Table 15.7) are comprised of homo- or heterotrimeric subunits to form proton-gated, voltage-insensitive, and Na^+ permeable channels that differ in kinetics, ion selectivity, pH-sensitivity, and blockade sensitivity.

Ligand-Gated Epithelial Sodium Channel (ENaC) Receptors

Epithelial sodium channel (ENaC) receptors are critical for sodium absorption in neuronal (mechanosensory neurons, brain, and dorsal root ganglia) and nonneuronal tissues. These five ligand-gated receptors (Table 15.7) are voltage-independent, amiloride-sensitive, and selectively Na^+ permeable. A pharmacological function for ENaC channel modulation in the nervous system has not been well defined. Structurally, ENaC channels are assembled as heterotrimeric complexes comprised of three homologous subunits (α, β, and δ). Each trimeric protein complex contains two membrane-spanning segments per subunit and form a pore that is lined by a second transmembrane helix of each subunit.

Enzyme-Linked Receptors

Enzyme-linked receptors are a large group of proteins which act both as a receptor (with intrinsic activity) and as an enzyme, such as a kinase or a phosphatase, which rapidly respond to extrinsic ques. The binding of an extracellular ligand causes intracellular enzymatic activity. Enzyme-linked receptors are composed of three key structural elements

- A large extracellular ligand binding domain—This positioning of the ligand binding stie on the extracellular domain binding permits ease of access for receptor activation.
- A single transmembrane domain—which is composed of a series of hydrophobic amino acids that function to tether the receptor to the lipophilic cell membrane.
- A variable-length intracellular enzyme activation domain—that contain intrinsic kinases or phosphates that modify selective serine, threonine, or tyrosine amino residues to either stimulate or inhibit downstream second messenger transcriptional activity.

The enzyme-linked receptors can be subdivided into five groups of receptors.

- **Receptor Tyrosine Kinase (RTK)**—RTKs (e.g., EGFR and VEGFR) contains intrinsic tyrosine kinase activity.
- **Receptor Serine–Threonine Kinase (RSTK)**—RSTKs (e.g., PKA and PKC) contains intrinsic serine–threonine kinase activity.
- **Receptor Guanylyl Cyclases (rGC)**—rGCs (e.g., ANP and NO) contain intrinsic cyclase activity.
- **Tyrosine Kinase–Associated Receptor (TKAR)**—TKARs (e.g., JAKs and STATs) associate with proteins that have tyrosine kinase activity.
- **Receptor Tyrosine Phosphatase (RTP)**—RTPs (e.g., cdc25 and PTEN)—contain intrinsic tyrosine phosphatase activity.

Receptor Tyrosine Kinases (RTKs) and Tyrosine-Kinase Associated Receptors (TKARs)

RTKs are transmembrane proteins that typically transduce signals originating at the cell surface, which then influence numerous cellular processes, in many cases by ultimately impacting on gene expression (Table 15.8).

RTK signals can be divided into two groups: canonical RTK signaling and direct nuclear signaling through the release of an RTK intracellular domain. Canonical RTK signaling involves ligand binding of the RTK at the cell surface, which leads to transphosphorylation of tyrosine residues within the receptor itself. Then, the activated receptor recruits downstream signaling molecules to activate a signaling cascade that often regulates gene transcription. Alternatively, ligand binding to the receptor induce proteolytic cleavage to release an activated intracellular tyrosine kinase–containing domain that attaches to a cytoplasmic substrate an undergoes nuclear translocation to regulate transcription of target genes. An example of this RTK direct nuclear signaling through a released RTK intracellular domain is well documented in neuronal development. In particular, the direct binding of neuregulin to ErbB4 at the CNS synapses causes the proteolytic release of the intracellular domain fragment of the receptor (ICD- ErbB4) to activate a MAPK kinase cascade to regulate gene transcription. Erythropoietin-producing Eph proteins constitute the largest receptor tyrosine kinase family. Eph receptors and ephrin ligands have attracted considerable attention in the CNS. The expression of both Eph receptors and ephrin ligands and are distributed in most regions, including the amygdala and hippocampus, and cell types, change markedly during CNS development, and play key roles in hippocampal plasticity, CNS angiogenesis, and certain types of pain. Eph/ephrin signaling are also upregulated following CNS injury and contribute to subsequent astrocytic gliosis, neural regeneration, vascular remodeling, and neuroinflammation. Many other growth factors, cytokines, and hormones are receptor tyrosine kinases.

In contrast to RTKs, tyrosine kinase–associated receptor members are nonreceptors tyrosine kinases. These proteins lack integral kinase activity and activate

Table 15.8 Major receptor tyrosine kinases (RTKs) associated with the nervous system

Family	Receptor	Endogenous ligand(s)
Epidermal growth factor receptor (EGFR) family	EGFR	EGF, heparin-binding *EGF-like* growth factor (*HB-EGF*), transforming growth factor (TGF)-β, Tumor necrosis factor (TNF)-α, *Amphiregulin*, and Heregulin (HCF)
	Human *EGF2* (HER2)	
	Human *EGF3* (HER3)	
	Human *EGF4* (HER4)	
Tropomyosin receptor kinase (Trk) family	Trk-A	Nerve growth factor (NGF)
	Trk-B	*Brain-derived neurotrophic factor (*BDNF) and Neurotrophin (NT)-4.
	Trk-C	Neurotrophin (NT)-3
Vascular endothelial growth factor receptor (VEGFR) family	VEGFR-1 (Flt-1)	VEGF-A
	VEGFR-2 (Flk-1)	VEGF-A, VEGF-C, and VEGF-D
	VEGFR-3	VEGF-C and VEGF-D
Fibroblast growth factor receptor (FGFR) family	FGFR-1, isoform IIIb	FGF-1, 2, 3, 10, and 22
	FGFR-1, isoform IIIc	FGF-1, 2, 4, 5, 6, 19, 20 and 21
	FGFR-2, isoform IIIb	FGF-1, 3, 4, 6, 7, 10, and 22
	FGFR-2, isoform IIIc	FGF-1, 2, 4, 5, 6, 8, 9, 17, 18, 19, 21, and 23
	FGFR-3, isoform IIIb	FGF-1 and 9
	FGFR-3, isoform IIIc	FGF-1, 2, 4, 8, 9, 17, 18, 19, 21, and 23
	FGFR-4	FGF-1, 2, 4, 6, 8, 9, 16, 17, 18, and 19
Tyrosine kinase with Ig and EGF-like domains (Tie) family	Tie-1	Angiopoietin 1 (Ang1) and angiopoietin (Ang4)
	Tie-2	Angiopoietins 1–4 (Ang1–Ang4)
Insulin-like growth factor receptor (*IGFR*) family	IGF1R	IGF-1 and IGF-2
	IGF2R (catalytic inactive)	IGF-2
Type III receptor tyrosine kinase family	Platelet-derived growth factor (PDGF) receptor α and β	PDGF-A, PDGF-B, PDGF-C, and PDGF-D
	Colony stimulating factor 1 (CSF) receptor	Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and CD34
	Stem cell factor (SCF) receptor (also KIT)	PDGF-A, PDGF-B, CSF, and SCF
	FMS-like tyrosine kinase 3 (FLT3) receptor	FMS-related tyrosine kinase 3 ligand (FLT3L)

separate kinases that are associated with the receptor. For example, the binding of the cytokine, interleukin (IL)-2, to the cell-surface high-affinity IL-2 receptor causes receptor dimerization, which brings the receptor-associated Janus kinases (JAKs) into close proximity to phosphorylate the receptor. STAT proteins then bind to the phosphorylated receptor and initiate JAK-STAT signaling.

Receptor Serine–Threonine Kinase (RSTK)

A serine–threonine protein kinase is a kinase that phosphorylates the OH group of serine or threonine. The major classes of protein serine–threonine kinases in the nervous system are listed in Table 15.9. Signal transduction in the nervous system is heavily dependent on three multifunctional second messenger-dependent serine–threonine protein kinases: protein kinase A (PKA), protein kinase C (PKC), and calcium calmodulin-dependent kinase II (CaMKII). Other second messenger-independent protein serine–threonine kinases are essential and include mitogen-activated protein kinases (MAPKs), cyclin-dependent kinases (cdks), and G protein receptor kinases (GRKs).

Protein Kinase A (cAMP-dependent protein kinase; PKA) is composed of catalytic and regulatory subunits, both of which are widely distributed throughout the brain. The holoenzyme of the kinase, which consists of a tetramer of two catalytic and two regulatory subunits, is inactive. The binding of cAMP to the regulatory units activates the holoenzyme, causing dissociation of the holoenzyme into free regulatory and free active catalytic subunits. PKA activity is highly compartmentalized throughout the cell, associated with the plasma membrane and the cytoplasmic and nuclear fractions. Several anchoring proteins tether both the regulatory and catalytic subunits to specific subcellular sites, for example, postsynaptic dendrites, in order to keep the protein kinase in close proximity to the cascade of signal-transduction proteins it phosphorylates to regulate synaptic transmission.

Calcium/calmodulin-dependent protein kinases (CaMKs) are one of two major classes of calcium-dependent kinases in the nervous system. The brain contains at least six major types, each with very different properties. Protein kinase C (PKC) comprises the other major class of Ca^{2+}-dependent protein kinases and is activated in conjunction with diacylglycerol (DAG) and phosphatidylserine. The brain is known to contain at least seven variant forms of PKC, which exhibit different cellular distributions throughout the brain and different regulatory properties.

PKC is single polypeptide chain and contains a regulatory domain, which, in the resting state, binds to and inhibits the catalytic domain. The binding of Ca^{2+} or DAG to the regulatory domain relieves this inhibition, enabling the enzyme to translocate from the cytosol to associate with the membrane. PKC exhibits a broad substrate specificity and mediates numerous second-messenger functions of Ca^{2+} in neurons. cGMP-dependent protein kinase (PKG) is also second messenger-dependent protein kinase. Similar to PKA, cGMP activates the inactive holoenzyme by binding to the regulatory domain of the molecule; however, PKA, activation of the cGMP-dependent holoenzyme is not accompanied by dissociation of the subunits. PKG also exhibits a more limited cellular distribution and substrate specificity relative to PKA.

Table 15.9 Major protein serine–threonine kinases associated with the nervous system

Class	Kinases	Cell localization	Substrates	Functions	Regulator(s)
Second messenger-dependent protein kinases	cAMP	Membrane, cytoplasm	Ion channels and cyclic nucleotide-binding proteins (Epac1 and RAPGEF2)	Intracellular signal transduction; activates protein kinases	ATP, adenylate cyclase, phosphodiesterases
	cGMP	Membrane, cytoplasm	Protein kinase G (PKG)	Ion channel conductance, glycogenolysis, cell apoptosis, vascular relaxation	Hormones, ANF, nitric oxide, phosphodiesterases
	Ca^{2+}/calmodulin kinase (CaMKII)	Cytoplasm, subcellular compartments	Neurogranin, Cdc25c, MEK/Erk, myosin light chain kinase (MLCK), microtubules, RyR2 channels, PDE, PKC, calcineurin, GABAa, NMDA	Long-term potentiation of synaptic strength, memory; learning, hippocampal and cortical plasticity	Ca/calmodulin complex
	Protein kinase C (PKC)	Cytoplasm, membrane, subcellular compartments	B-catenin, ion channels, myelin peptides, p53, vitamin D receptor, GRK2, and FXR	Ion channel neurotransmitter release, gene transcription	Ca^{2}+, diacylglycerol, phosphatidylserine
	Protein kinase A (PKA)	Membrane, subcellular compartments	Phosphorylase kinase, ion channels, histone H1, CREB, acetyl-CoA carboxylase, pyruvate dehydrogenase	Glycogen, sugar, and lipid metabolism; activate reward system, memory	Glucagon, epinephrine, dopamine, histamine, vasopressin, adrenergic agonists, ATP, cAMP

Mitogen-activated protein kinase (MAPK)	Extracellular signal-regulated protein kinase (ERK)	Cytoplasm, nucleus	Transcription factors, nuclear translocation proteins, chromatin remodeling protein complexes, cytoskeleton	Synaptic plasticity, development, immunity, metabolism, memory formation	Excitatory glutamatergic signaling, specific kinases and phosphatases, growth factors
	c-Jun kinase (JNK)	Cytoplasm, nucleus	Transcription factors, nuclear translocation proteins, chromatin protein complexes, cytoskeleton	Gene expression, immune responses, cell growth, and tissue repair	Excitatory glutamatergic signaling, specific kinases and phosphatases
	Stress-activated protein kinase (SAPK)	Cytoplasm, nucleus	Transcription factors, nuclear translocation proteins, chromatin protein complexes, scaffolding proteins	Gene expression, immune responses, cell growth, and tissue repair	Stress, inflammation, growth factors, GPCR agonists
MAPK kinase (MAP2K)	MEK	Cytoplasm, nucleus	Genes associated with glial cell activation, differentiation, survival, and function	Phosphorylates and activates ERK, p38, and JNK	SHP-2, epigenetics, Ras, RAF kinase
	SAPK kinase (SEK)	Cytoplasm, nucleus	Phosphorylation of serine and threonine residues at positions 257 and 261 by MEKK	Activates SAPK and JNK in response to stress and inflammation	Sphingomyelinase, C2-ceramide GTP-binding proteins Rac1 and Cdc42H, tyrosine kinase Pyk2
	Raf	Membrane, Cytoplasm	MEK 1, MEK 2	Initiates the cascade to activate the MAPK pathway	Extracellular mitogen, Ras, RAF kinase
	MEK kinases	Cytoplasm, subcellular compartments	Cytoplasm, specific subcellular compartments	Phosphorylates the MAPKs: ERK, p38, and JNK	

(continued)

Table 15.9 (continued)

Class	Kinases	Cell localization	Substrates	Functions	Regulator(s)
CDK-regulating kinases	Cyclin-dependent kinase 5 (Cdk5)	Cytoplasm, nucleus	β-Catenin, p,27(kip1), RasGRF2, TrKB, CASK, Synapsin 1, CaV2.1, Prx2, PPARγ, mSds3, GR, NR2A, ephrins, Sox6	Neurite development, cytoskeletal structure, neuronal migration, synaptic transmission	p35, p39, calpain
G protein-coupled receptor kinases (GRKs)	βARK1	Cell membrane, cytoplasm	Src, Gβ/γ subunits, GPCRs, arrestins, PDGF, IGF, CCR5	GPCR densensitization, viral entry, muscle contraction	PKC, PKA, Gβ/γ subunits
	βARK2	Cell membrane, cytoplasm	GPCRs, arrestins, Gβ/γ subunits	GPCR densensitization	Gβ/γ subunits
	GRK6	Cytoplasm	Arrestins; D2 dopamine and leukotriene B4 receptors	Immune responses, platelet activation, neuropathic pain	ATP
Others	90 kDa ribosomal S6 kinase (RSK)	Nucleus, cytoplasm	Ribosomal protein s6, PP1, GSK3, li cam, Myt, CREB	Gene expression, cell survival and growth, cognitive function	MAPK, ATP
	Casein kinase 1 (CK1)	Plasma membrane, cytosol, nucleus	NMDA activity, mGluR1, Wnt/β-catenin	Neurodegeneration, cell cycle, apoptosis, metabolism	ATP, glutamate/mGluRs
	Casein kinase 2 (CK2)	Nucleus, cytoplasm	P53, MAPK, casein, Maf1, NMDA activity	DNA repair, cell cycle, impairs apoptosis	ATP, Wnt/frizzled

AD Alzheimer's disease, *ANF* atrial natriuretic factor, *βARK1* β-adrenergic receptor kinase 1, *βARK2* β-adrenergic receptor kinase 2, *CASK* calcium/calmodulin-dependent serin protein kinase, *$Ca_V2.1$* P/Q-type voltage-gated calcium channel, *JNK* c-Jun amino-terminal kinase, *CREB* cAMP response element-binding protein, *ERK 1/2* extracellular signal-regulated kinases 1 and 2, *EPAC1* exchange factor directly activated by cAMP 1, *GPCRs* G protein-coupled receptors, *GRK6* G protein-coupled receptor kinase 6, *GRK2* G protein-coupled receptor kinase2, *FXR* Farnesoid X receptor, *IGF* insulin-like growth factor, *Li Cam* a neural cell adhesion molecule, *Myt* an inhibitor of cdc-2, *PDGF* platelet-derived growth factor, *PP1* protein phosphatase 1, *PPARγ* peroxisome proliferator-activated receptor gamma, *SAPK* stress-activated protein kinase, *SHP-2* SH2 domain-containing protein tyrosine phosphatase-2, *RACK* receptors for activated C kinase, *RAPGEF3* Rap guanine nucleotide exchange factor 3, *SK3* glycogen synthase kinase 3

Receptor Guanylate Cyclase (rGC)

Guanylate Cyclase (GC) is the intracellular receptor of nitric oxide (NO). The activation of GC results in the conversion of Guanosine Triphosphate (GTP) to the second messenger cyclic Guanosine Monophosphate (cGMP). The diversity of targets allows cGMP to have wide-ranging effects that can differ by cell and tissue type. There are three known targets for cGMP which mediate the transmission of the cGMP pathway signal downstream from guanylate cyclase: cGMP-dependent protein kinase, cGMP-regulated phosphodiesterase, and cGMP-gated ion channel. The nitric oxide-GC-cGMP signaling pathway plays a fundamental role in regulating diverse physiological processes within the CNS, including blood flow, inflammation, neuroprotection, and metabolism, and is believed to be key for neuronal information transmission and synaptic plasticity, which is a foundation for learning and memory.

Receptor Tyrosine Phosphatase (RTP)

In the nervous system, receptor tyrosine phosphatases (PTPs) have not been studied extensively, but their targets and functions are now beginning to emerge (Table 15.10). For example, signals through the epidermal growth factor (EGF) and fibroblast growth factor (FGF) receptors regulate proliferation and survival of postmitotic neurons. Further, neuregulin (Nrg) receptors and oligodendrocyte-intrinsic CD45 are critical for myelination. In accordance RTPs are exciting pharmacological targets for CNS development and disorders including neural tube defects, gliomas and glioblastomas, and multiple sclerosis and other autoimmune demyelinating disorders.

RTPs, also often recognized as proto-oncogenes, are cell-surface proteins with a single TM region and intracellular tyrosine phosphatase activity. Family members include both tyrosine-specific and dual-specific phosphatases. Based on their cellular localization they are also classified as receptor-like or intracellular PTP. The phosphorylation state of tyrosine residues on proteins is fundamental for the control of different functions of the cell and is the result of the balance between protein tyrosine kinase (PTK) and protein tyrosine phosphatase (PTP) activities.

Receptor tyrosine phosphatases are classified into subfamilies according to their extracellular domains and include: immunoglobulin domains, fibronectin (FN)-like type-III repeats, carbonic anhydrase (CAH)-like domains, meprin-A5-μ (MAM) domains, and highly glycosylated domains. Many family members exhibit constitutive activity in heterologous expression, dephosphorylating intracellular targets such as the Src family of protein tyrosine kinases (SFKs) to activate downstream signaling cascades.

Nuclear Receptors (NRs)

Nuclear receptors (NRs), in contrast to ligand-gated ion channels, G-protein-coupled receptors, and enzyme-linked receptors, are soluble proteins that are found either within the cell cytosol or nucleus (Fig. 15.1). Accordingly, the NR superfamily is a group of ligand-activated transcription factors that regulate gene expression to modulate a host of biological processes, including development, growth, cholesterol-related and diet-derived lipid metabolism, inflammation, and circadian rhythm. Nuclear receptors are activated by ligands, that include fat-soluble vitamins, hormones, steroids, fatty acids, phospholipids, bacterial metabolites, and

Table 15.10 Major protein tyrosine phosphatase (PTP) receptors associated with the nervous system

Type	PTP receptor	Binding partner(s)	Extracellular domain	Cytoplasmic domain
I	CD45	Galectins	1 fibronectin type III (FNIII) domain	2 PTP domains
IIA	LAR	Heparan sulfate proteoglycans and Chondroitin sulfate proteoglycans	8–9 FNIII repeats and 3 N-terminal Ig domains	1–2 PTP domains
	PTP-σ			
	PTP-δ			
IIB	PTP-μ	Cadherins and catenins	4 FNIII repeats, 1 Ig domain and 1 meprin/A5/PTP-μ (MAM) domain	2 PTP domains
	PTP-κ			
	PTP-ρ			
	PTP-γ			
III	PTP-β	Phosphatidylinositol moieties	8 FNIII repeats	1 PTP domain
	PTP-η			
	SAP1			
	GLEPP1			
	PTPS31			
IV	PTP-α	Contactins and Pleiotrophin	Short and heavily glycosylated	2 PTP domains
	PTP-ε			
V	PTP-γ	Contactins and Pleiotrophin	1 FNIII domain and 1 carbonic anhydrase (CA) domain	2 PTP domains
	PTP-ζ			
VI	PTPP	N-methyl-D-aspartate (NMDA) and*α*-Amino-3-hydroxy-5--methyl-4-isoxazolepropionic acid (AMPA)	Short and nonglycosylated	1 PTP domain and 1 kinase interaction motif (KIM)
	STEP			
VII	IA2 (catalytic inactive)	Type IV PTP receptors: RPTPα and RPTPε	1 RDGS adhesion recognition motif	1 PTP domain
	IA2β (catalytic inactive)			

CD cluster of differentiation, *LAR* leukocyte common antigen related protein, *PTP* protein tyrosine phosphatase, *RPTP* receptor tyrosine phosphatase, *STEP* striatal-enriched phosphatase

heme. Dysregulated nuclear receptor signaling associates with an array of neurological disorders, including migraine, metabolic syndromes, autism, Parkinson's disease, Alzheimer's disease, Schizophrenia, and depression. Structurally, all nuclear receptors, with the exception of two atypical receptors DAX-1 and SHP, share a common overall architecture.

- An unstructured N-terminal domain (NTD)—The NTD contains the Activation Function 1 (AF-1) region, which interacts with a variety of coregulator proteins, such as chromatin remodeling complexes. The NTD is the target for numerous posttranslational modifications including phosphorylation, sumoylation, and acetylation.
- A zinc finger DNA binding domain (DBD)—The DBD contains two subdomains, each containing one zinc finger. The first subdomain interact with the DNA major groove and the second subdomain participates in DBD dimerization.
- A short, flexible hinge region—The hinge region is positioned between the DBD and the LBD, functions as a site for post-translational modifications, and contains a nuclear localization signal.
- A ligand-binding domain (LBD)—The LBD binds to ligands and interacts with co-regulator proteins through the Activation Function 2 (AF-2) region. The LBD is structurally conserved and commonly contains 11 α-helices and four β-strands that fold into three parallel layers to form an alpha helical sandwich that creates a hydrophobic ligand-binding pocket (LBP).
- A C-terminal domain (CTD)—The CTD participates in the folding of the receptor into a transcriptionally active form, enabling binding to DNA response elements.

Nuclear Receptor Superfamily Classification

Nuclear receptors can be subdivided into seven subgroups (Table 15.11).

- *Subgroup 0*—Includes the atypical NRs, dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, Gene 1 (DAX-1) and small heterodimer partner (SHP). These two NRs uniquely contain only a ligand-binding domain (LBD). The sequence motifs of these LBDs are commonly seen in NR coactivators and readily interact with other NR LBDs to regulate transcription.
- *Subgroup 1*—Includes the thyroid hormone receptor (TR), retinoic acid receptor (RAR), peroxisome proliferator activated receptor (PPAR), reverse-Erb receptor (REV-ERB), retinoic acid related receptor (ROR), farnesoid X receptor (FXR), liver X receptor (LXR), and the vitamin D receptor (VDR) members. In general, these NRs are regulated by a variety of lipophilic signaling molecules including thyroid hormone, commensal bacterial metabolites, fatty acids, bile acids, and steroids.
- *Subgroup 2*—Includes the orphan receptors retinoid X receptor (RXR), chicken ovalbumin upstream promoter transcription factor (COUP-TF), and hepatocyte

Table 15.11 The major nuclear receptors associated with the nervous system

Group	Family	Receptor(s)	Gene	Endogenous ligand(s)	CNS location(s)
0	0B	Dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, Gene1 (DAX-1)	NR0B1	Orphan	HY, AN
		Short heterodimeric partner (SHP)	NROB2	Orphan	OA, BC, H, T, HY, AN, CO, C, BS
1	1A	Thyroid hormone receptor-α (TRα)	THRA	Thyroid hormones	OA, BC, CO, H, T, HY, CO, C, BS
		Thyroid hormone receptor-β (TRβ)	THRB	Thyroid hormones	OA, BC, CP, H, HY, AN, CO, C, BS
	1B	Retinoic acid receptor-α (TARα)	RARA	Retinoic acids	OA, BC, CP, H, HY, CO, C, BS
		Retinoic acid receptor-β (TARβ)	RARB	Retinoic acids	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Retinoic acid receptor-γ (TARγ)	RARG	Retinoic acids	OA, BC, H, HY, CO, C, BS
	1C	Peroxisome proliferator-activated receptor-α (PPARα)	PPARA	Fatty acids	BS, C, BS
		Peroxisome proliferator-activated receptor-β (PPARβ)	PPARD	Fatty acids	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Peroxisome proliferator-activated receptor-γ (PPARγ)	PPARG	Fatty acids	OA, BC, CO, C
	1D	Reverse-Erb-α (REV-ERBα)	NR1D1	Heme	OA, BC, CP, H, T, HY, CO, C, BS
		Reverse-Erb-β (REV-ERBβ)	NR1D2	Heme	BC, CP, H, T, HY, AN, CO, C, BS
	1F	Retinoic acid-related orphan-α (ROBα)	RORA	Sterols	OA, BS, CP, H, Y, HY, AN, CO, C BS
		Retinoic acid-related orphan-β (ROBβ)	RORB	Sterols	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Retinoic acid-related orphan-γ (ROBγ)	RORC	Sterols	BC, H, HY, CO, C, BS
	1H	Farnesoid X receptor (FXRα)	NR1H4	Bile acids	OA, BC, CP, H, T, HY, AN, CO, C, BC
		Farnesoid X receptor-β (FXRβ)	NR1H5P	Orphan	Low expression
		Liver X receptor-α (LXRα)	NR1H3	Oxysterols	OA, BC, H, HY, AN, CO, C, BS
		Liver X receptor-β (LXRβ)	NR1H2	Oxysterols	BC, CP, H, T, HY, AN, CO, C, BS
	1I	Vitamin D receptor (VDR)	VDR	1α,25-dihydroxybitamin D_3	OA, BS
		Pregnane X receptor (PXR)	NR1I2	Endobiotic and xenobiotics	BC, C, CP
		Constitutive androstane receptor (CAR)	NR1I3	Xenobiotics	OA, BC, HY, C, BS

2	2A	Hepatocyte nuclear factor-4-α (HNF4α)	HNF4A	Fatty acids	BC, C, H, T, HY
		Hepatocyte nuclear factor-4-γ (HNF4γ)	HNF4G	Fatty acids	Low expression
	2B	Retinoid X receptor-α (RXRα)	RXRA	9-cis retinoic acid	OAM, CP, H, HY, CO, C, BS
		Retinoid X receptor-β (RXRβ)	RXRB	9-cis retinoic acid	OA, BC, H, T, HY, AN, CO, C, BS
		Retinoid X receptor-γ (RXRγ)	RXRG	9-cis retinoic acid	OA, BS, CP, H, HY, AN, BS
	2C	Retinoid X receptor-2 (RXR2)	NR2C1	Orphan	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Retinoid X receptor 4 (RXR4)	NR2C2	Orphan	OA, BC, CP, H, T, HY, AN, CO, C, BS
	2E	Tailless homolog orphan receptor (TLX)	NR2E1	Orphan	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Photoreceptor-cell-specific nuclear receptor (PNR)	NR2E3	Orphan	OA, BC, CP, HY, C
	2F	Chicken ovalbumin upstream promoter-transcription factor α (COUP-TFα)	NR2F1	Orphan	OA, BC, T, HY, C, BS
		Chicken ovalbumin upstream promoter-transcription factor β (COUP-TFβ)	NR2F2	Orphan	BC, H, T, HY, CO, C, BS
		Chicken ovalbumin upstream promoter-transcription factor γ (COUP-TFγ)	NR2F6	Orphan	BC, C, HY, T, C
3	3A	Estrogen receptor-α (ERα)	ESR1	Estrogens	OA, BC, H, HY, AN, CO, C, BS
		Estrogen receptor-β (ERβ)	ESR2	Estrogens	OA, BC, HY, AN, CO, BS
	3B	Estrogen-related receptor-α (ERRα)	ESRRA	Orphan	OA, BC, H, HY, CO, C, BS
		Estrogen-related receptor-β (ERRβ)	ESRRB	Orphan	BC, H, HY, CO, C, BS
		Estrogen-related receptor-γ (ERRγ)	ESRRG	Orphan	OA, BC, CP, H, T, HY, AN, CO, C, BS
	3C	Androgen receptor (AR)	AR	Androgens	OA, BC, H, HY, CO, C, BS
		Glucocorticoid receptor (GR)	NR3C1	Glucocorticoids	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Mineralocorticoid receptor (MR)	NR3C2	Mineralocorticoids and glucocorticoids	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Progesterone receptor (PR)	PGR	Progesterone	OA, BC, CP, H, T, HY, AN, CO, C, BS

(continued)

Table 15.11 (continued)

Group	Family	Receptor(s)	Gene	Endogenous ligand(s)	CNS location(s)
4	4A	Nerve growth factor 1B (NGF1-B)	NR4A1	Orphan	OA, BC, CP, H, T, HY, CO, C, BS
		NURR-related factor 1 (NURR1)	NR4A2	Unsaturated fatty acids	OA, BC, CP, H, T, HY, AN, CO, C, BS
		Neuron-derived orphan receptor 1 (NOR-1)	NR4A3	Orphan	OA, BC, CP, H, T, HY, AN, CO, C, BS
5	5A	Steroidogenic factor 1 (SF-1)	NR5A1	Phospholipids	HY
		Liver receptor homolog-1 (LRH-1)	NR5A2	Phospholipids	AN, BS
6	6A	Germ cell nuclear factor (GCNF)	NR6A1	Orphan	BC, H, AN, CO, C, BS

AN arcuate, *BC* brain cortex, *BS* brain stem, *C* cerebellum, *CO* colliculi, *CP* caudate putamen, *H* hippocampus, *HY* hypothalamus, *OA* olfactory area, *T* thalamus

nuclear Factor 4 (HNF4) members. Of these, RXR is of particular importance as it forms heterodimeric complexes with a host of other NRs and is the only Family 2 NR member with a known activating ligand, 9-*cis* retinoic acid.

- *Subgroup 3*—Includes the steroid receptors, which includes the androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), estrogen receptor α (ERα), and closely-related estrogen receptor β (ERβ) members. Cholesterol-derivatives are prominent ligands for this NR subgroup.
- *Subgroup 4*—Includes the orphan nerve growth Factor 1B (NGF-1B), nurr-related receptor factor-1 (NURR1), and neuron-derived orphan Receptor-1 (NOR-1) members. Family 4 NRs are required for neuron development and maintenance.
- *Subgroup* 5—Includes steroidogenic factor 1 (SF-1) and liver receptor homolog-1 (LRH-1). Although generally still classified as orphan receptors, evidence suggests these proteins are regulated by phospholipids. LRH-1 and SF-1 are vital for development and metabolism.
- *Subgroup 6*—Includes only one receptor, the germ cell nuclear factor (GCNF), an orphan receptor that has a critical role in development. This protein remains in its own category due to a critical difference in its LBD; it does not contain an activator function HR.

Nuclear Receptor–Ligand Interactions

NR ligands include a wide variety of small, lipophilic steroids, hormones, vitamins, and fatty acids that either diffuse or are transported across the cell membrane. Of the 48 known human NRs, 24 have known ligands and the remaining 24 are classified as orphan receptors. Ligands bind to the receptor within a ligand-binding pocket, which is largely hydrophobic with polar residues that are critical for NR-ligand interactions. The volume of the ligand-binding pockets differ markedly and correlate, in general, with the size of the ligand, thus providing an additional level of ligand–NR specificity.

Nuclear Receptor–DNA Signaling

Endogenous ligands bind either to cytosolic NRs to initiate receptor dimerization and translocation to the nucleus or bind to nuclear-residing NRs. Regardless, activated monomeric or dimeric NRs bind to sequence-specific cognate DNA response elements commonly located upstream of the transcriptional start site. Transcriptional activity of NRs is further modulated through their association with coregulators. These accessory coregulatory proteins can associate with the NRs prior to or after NR-DNA interaction and can either repress (corepressors) or activate (coactivate) gene transcription. Coactivator proteins include histone acetylation in response to histone acetyltransferase (HAT), other chromatin remodeling proteins (e.g.,

trithorax-group proteins and the nuclear receptor coactivator 1 (NCOA1)), or other stimulatory transcription factors. Alternatively, corepressors can bind directly to the receptor or to the transcriptional machinery at the transcriptional start to repress gene transcription. Examples of corepressors include histone deacetylation in response to HDAC, negative-regulating transcription factors, receptor-interacting protein 140 (RIP140), nuclear receptor corepressor (NCoR1), and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT).

Clinical Summary

Pharmacological treatment of neurological disorders should be tailored to the individual patient. Continued advancement in our understanding of the mechanisms underlying neuroreceptor biology, trafficking, and signaling will leverage the development of improved therapeutics that precisely control different aspects of receptor signaling by any given drug.

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Chapter 16
Pharmacokinetics and Pharmacodynamics

Kathryn E. Qualls

Overview

Pharmacokinetics is commonly defined as the science around the disposition of a drug in the body [1]. This includes absorption, distribution, metabolism, and excretion of a drug. The ability of a drug to produce an effect at the site of action is referred to as *pharmacodynamics. Clinical pharmacokinetics* is a newer term that describes the application of drug pharmacokinetics to the therapeutic management of a patient [2].

Absorption

The two main categories for drug administration can be divided into extravascular and intravascular [3, 4]. The benefit of intravascular administration is that it bypasses the absorption phase. Extravascular administration of drugs can be via different mechanisms, including the following: oral/enteral, subcutaneous, sublingual, intravenous, inhalation, intranasal, topical, and many others.

A key element when looking at absorption is the bioavailability of a drug. *Bioavailability* (*F*) is defined as the percentage of drug that reaches systemic circulation after administration [5].

$$F = \frac{\text{Amount of drug in systemic circulation}}{\text{Amount of drug administered}}$$

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Some extravascular administration routes of a drug have reduced bioavailability when compared to the intravascular administration of the same drug. *First-pass metabolism* decreases the bioavailability of a drug via inactivation in the gastrointestinal tract or metabolism prior to entering the bloodstream [6]. First-pass metabolism explains why with certain drugs the amount given orally/enterally is much higher than the amount given intravenously. Alternative extravascular routes of administration have been created to bypass the first-pass metabolism effect.

Other common factors that affect oral drug absorption include alterations in blood flow to the gastrointestinal tract, removal of parts of the gastrointestinal tract, transit time through the gastrointestinal tract (increased or decreased), and drug–drug and drug–food interactions [3, 5]. Absorption can be purposefully altered when looking at specific drug formulations. Many formulations are made with extended-release mechanisms to release a steady amount of drug over a specific time frame.

Distribution

After a drug reaches the systemic circulation, the next phase is distribution. The *volume of distribution* (V_d) is a measure of the extent of distribution into the tissues of the body [2, 4]. The movement of a drug via distribution is determined by the blood flow to certain tissues and organs within the body.

The distribution of a drug to the central nervous system requires movement through the blood–brain barrier (BBB). The BBB consists of tight cellular junctions that aid in the protection of the central nervous system. Mechanisms by which to cross the BBB include simple diffusion, facilitated diffusion, and active transport [5].

Metabolism

The primary organ for metabolism is the liver. Drugs can be metabolized into active or inactive metabolites. Other organs or tissues that can aid in the metabolism of a drug include the gastrointestinal tract, kidneys, lungs, and brain. Metabolism is broken down into two phases, and the mechanisms for each phase are defined in Table 16.1 [6].

Table 16.1 Phases of metabolism

Phase I metabolism	Phase II metabolism
• Oxidation • Reduction • Hydrolysis	• Glucuronidation • Acetylation • Sulfation

The cytochrome P-450 (CYP450) enzyme system performs much of the phase I metabolism. CYP450 enzymes can be induced or inhibited by a variety of other drugs and produce many drug–drug interactions [5]. The CYP450 system can also be altered by genetic mutations and polymorphisms. Metabolism via the CYP450 system renders drugs to be more water-soluble and gives the ability for easier elimination.

There are two main types of pharmacokinetics exhibited by drugs, and these include first-order kinetics and zero-order kinetics (Figs. 16.1 and 16.2) [6]. *First-order kinetics* is a nonlinear response, and the fraction of the drug removed is constant. *Zero-order kinetics* displays linear response, and the amount of drug eliminated is constant.

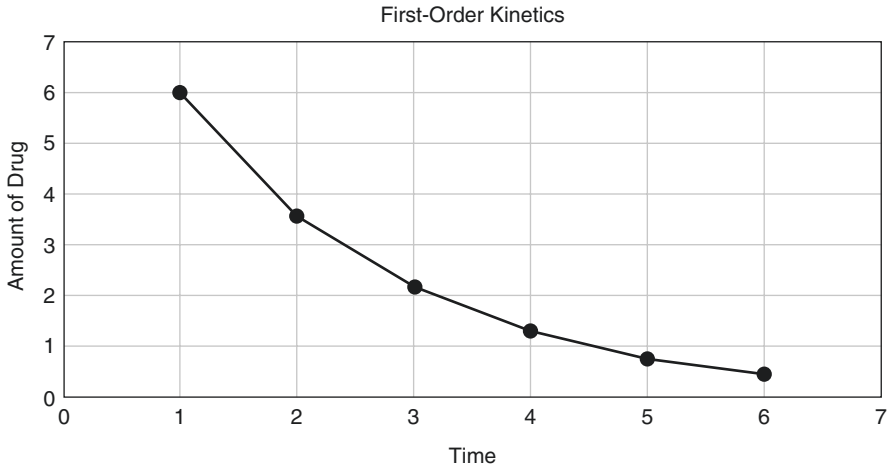

Fig. 16.1 First-order kinetics

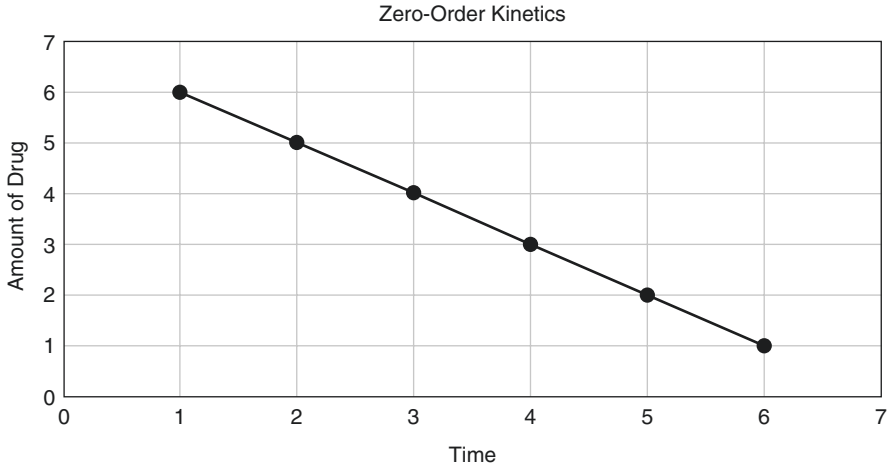

Fig. 16.2 Zero-order kinetics

Elimination

Elimination is the process by which the body removes the drug from circulation. This is typically completed via elimination from the kidneys, liver, and feces [5, 6]. Drug clearance is a measure of metabolism and elimination.

Elimination through the renal system is completed by glomerular and tubular functions. Glomerular filtration is the basis for most drug excretion. Active tubular excretion is an energy-dependent process that requires chemical transporters. Transporters in the proximal tubules of the kidney can be influenced by other drugs in a positive or negative way.

The *half-life* ($T_{1/2}$) of a medication is the time it takes for the drug in the systemic circulation to decrease by 50%. More commonly, the half-life of a drug is used to estimate the time to which a drug reaches a steady-state concentration (C_{ss}) [5, 6]. Most drugs reach a steady-state concentration after five half-lives with continued drug administration. The time to steady-state concentration can be dramatically decreased when giving loading doses of a drug. Examples of this are antiseizure medications, antibiotics, and other medications with longer half-lives.

There are two main types of pharmacokinetics exhibited by drugs, and these include first-order kinetics and zero-order kinetics (Figs. 16.1 and 16.2) [5]. In *first-order kinetics,* the fraction of the drug removed is constant. *Zero-order kinetics* has a constant amount of drugs removed; other names for zero-order kinetics include nonlinear and dose-dependent.

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