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Angelika Böttger · Ute Vothknecht
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Lessons on Caffeine, Cannabis & Co

Plant-derived Drugs and their Interaction with Human Receptors

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Preface

This textbook is based on a lecture we offer for Master of Biology students at the Ludwig-Maximilians-Universität in Munich, Germany. As an interdisciplinary approach between plant and animal cell biology, it covers, on the one hand, cellular signal transduction mechanisms in animals, concentrating on seven-transmembrane receptors (GPCRs) and ion channels. On the other hand, it describes biosynthetic pathways and the role of secondary metabolites in plants. The interplay between these topics is illustrated by our elaboration on prominent plant-derived drugs that constitute potent plant toxins, pharmaceutically used drugs to treat human disease as well as so-called recreational drugs. When we designed the lecture for the Master of Biology, we wanted to teach students how these powerful and often well-known plant-derived drugs interact with molecular and cellular mechanisms in animals, including humans, and how and why plants produce such compounds.

The book therefore starts in the first part with a discussion of the general function of secondary metabolites in plants. Secondary metabolites are not always essential for plant survival, but they play important roles in adapting a plant's life to the microclimate at its location, they help plants in battling with herbivores and pathogens, and they attract pollinators and seed distributors. This part also gives a short historical account on the use of plants as medicines and recreational drugs.

In the second part, we describe two cellular pathways in animals that together provide the great majority of current-day pharmacological molecular targets. These involve G-protein-coupled receptor - and ion channel-signalling. They will at first be dissected into their molecular components and explained in a general way. Then we consider some well-known drugs and toxins (nicotine, morphine, cannabis and many others) and elaborate on their specific target molecules in humans. We explore the role that particular receptors play in normal human physiology and then discuss how the respective drugs interfere with these functions.

In the third part, we look in detail into the plants that produce these compounds. We delve into the history of many drug discoveries and describe specific applications of plant-derived drugs, including some curiosities about their use. We also discuss how plants themselves employ these "bestsellers" from their repertoire of secondary metabolites. Caffeine and nicotine provide two very good examples. However, in many cases, very little is known about the role of specific substances that have become famous or infamous for their effect on humans. In this area a lot of further research is needed to gain an understanding of the part that these compounds play for plants.

All the compounds that we discuss in the first parts of this textbook are produced in plants by highly conserved and tightly regulated biosynthetic pathways. These pathways are described in the fourth part. Secondary metabolites arise from primary metabolism, for instance, amino acid synthesis and the tricarboxylic acid (TCA) cycle. They can also be formed within pathways for the synthesis of structural plant compounds, for instance, lignin, or essential signalling molecules, like hormones. We show

how the specific drugs and toxins, which we have focused on in the first chapters, are produced by secondary biosynthetic pathways in plants. This provides a glance into the rich, colourful and almost unlimited world of secondary metabolites that plants are capable of making.

It is the intention of our lecture and this textbook to teach molecular signalling pathways in animals and biosynthesis pathways in plants that every Master of Biology student should have heard of and to illustrate how these topics are connected. We want to do this in such a way that some immediate everyday relevance becomes obvious. Moreover, while preparing this material, we ourselves realized how ingeniously plants and animals exchange messages. We therefore would like to inspire future biologists and biochemists to take a closer look and study the interactions between the life of plants and animals. This should bring together forthcoming generations of plant and animal biologists and enhance our view of inseparability of all life forms on this earth.

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Contents

I Basics and History of Plant Secondary Metabolites

1 **Plant Secondary Metabolites and Their General Function in Plants** 3
1.1 **General Classification of Secondary Metabolites (Details in Part IV)** 4
1.2 **Overview of Classes of Secondary Metabolites (Details in Part IV)** 5
1.3 **Overview of the Function of Secondary Metabolites in Plants (Details in Part IV)** 6
1.3.1 Adaptation to a Life on Land 8
1.3.2 Attraction (or Deterrent) of Pollinators and Seed Dispersers 8
1.3.3 Defence Against Herbivores, Pathogens and Other Pests 10
1.3.4 Plant-to-Plant Communication 11
1.3.5 Constitutive versus Induced Expression 11
1.3.6 Counterstrategies of the Attacker and Use of Plant-Derived Secondary Metabolites by Other Animals 12
1.3.7 Avoidance of Auto-Toxicity and Premature Toxin Release 13
References 16

2 **Historical and Current Perspective** 19
2.1 **History of Medical Use** 20
2.2 **History of Recreational Use** 23
References 24

II Drugs and Their Human Receptors

3 **GPCRs** 29
3.1 **G-Protein-Coupled Receptors** 30
3.2 **Trimeric G-Proteins** 32
3.3 **G_S, G_{olf} and G_i Targeting Adenylyl Cyclase** 35
3.4 **G_q Targeting Phospholipase C** 38
3.5 **Signal Termination** 39
References 41

4 **Ion Channels** 43
4.1 **Channels and Transporters** 44
4.2 **Membrane Potential and Electrochemical Gradient** 46
4.3 **Voltage-Gated Ion Channels** 47
4.3.1 Voltage-Gated K^+-Channels 47
4.3.2 Voltage-Gated Na^+-Channels 49
4.3.3 Voltage-Gated Ca^{2+}-Channels 50
4.3.4 Neuromuscular Junction 51
4.3.5 VOCs and Neurotransmitter Release 52
4.4 **Extracellular Ligand-Operated Channels: Neurotransmitter Receptors** 53
4.5 **TRP Channels** 54
References 56

5 **GPCRs as Targets for Plant-Derived Drugs** 57
5.1 **Muscarinic Acetylcholine Receptors (MAchR): Muscarine, Atropine and Physostigmine** 58
5.2 **Adrenergic Receptors: Reserpine and Ephedrine** 61
5.3 **Adenosine Receptors: Caffeine** 64
5.4 **Cannabinoid Receptors: Cannabis and Cannabinoids** 68
5.5 **Dopamine Receptors: Neuroleptics and Cocaine** 72
5.6 **5HT2$_A$ Receptors: Psilocybin, Mescaline and Dimethyltryptamine** 74
5.7 **Opioid Receptors: Morphine, Heroin and Salvinorin A** 76
References 80

6 **Ion Channels as Targets for Plant-Derived Drugs** 83
6.1 **Neurotoxin Binding Sites on Voltage-Gated Ion Channels** 84
6.2 **Nicotinic Acetylcholine Receptor (NAchR): Nicotine** 85
6.3 **GABA$_A$ Receptors: Muscimol, Valerenic Acid, Thujone and Benzamidines** 88
6.4 **Glycine Receptor: Strychnine and Tutin** 89
6.5 **Glutamate Receptors: Ibotenic Acid, Kainate and Ketamine** 90
6.6 **TRPV and TRPM8: Heat and Cold, Capsaicin and Menthol** 92
References 94

III Plant-Derived Drugs: Their Function in Plants and Potential Biotechnological Use

7 **Plant-Derived Drugs Affecting GPRCs** 99
7.1 **Muscarinic Acetylcholine Receptors: Atropine, Muscarine and Scopolamine** 100
7.2 **Adrenergic Receptors: Ephedrine and Reserpine** 102
7.3 **Adenosine Receptors: Caffeine** 104
7.4 **Cannabinoid Receptors: Phytocannabinoids** 107
7.5 **Dopamine Receptors: Cocaine and L-DOPA** 110
7.6 **5HT2A Receptors: Ergotamine and Mescaline** 111
7.7 **Opioid Receptor: Morphine/Opiates and Opioids** 112
References 116

8 **Plant-Derived Drugs Affecting Ion Channels** 121
8.1 **Voltage-Gated Ion Channels: Aconitine, Saxitoxin and Ryanodine** 122
8.2 **Nicotinic Acetylcholine Receptors: Nicotine, Coniine, Curare and Anatoxin A** 123
8.3 **GABA$_A$ Receptors: Thujone, Cicutoxin and Anisatin** 127
8.4 **Glycine Receptors: Strychnine and Tutin** 130
8.5 **Ionotropic Glutamate Receptors: Ibogaine, Kainic and Domoic Acid** 131
8.6 **TRPV and TRPM8 Receptors: Capsaicin and Menthol** 133
References 137

IV Biosynthesis of Secondary Metabolites in Plants

9 **Secondary Metabolites in Plants: General Introduction** ... 143
9.1 **Role of Secondary Metabolites for Plant Development** ... 144
9.2 **Regulation of the Biosynthesis of Secondary Metabolites** ... 145
9.3 **General Mechanisms for Biosynthesis** ... 147
References ... 151

10 **Terpenes and Terpenoids** ... 153
10.1 **Introduction** ... 154
10.2 **Monoterpenes (From MEP Pathway, C10, e.g. Menthol, Camphor and Thujone)** ... 157
10.3 **Diterpene (From MEP Pathway, C20, e.g. Salvinorin A and Ryanodine)** ... 161
10.4 **Tetraterpene (From MEP Pathway, C40)** ... 162
10.5 **Sesquiterpenes (From MVA Pathway, C15, e.g. Valerenic Acid, Anisatin and Tutin)** ... 163
10.6 **Sterols and Triterpene (From MVA Pathway, C30)** ... 165
References ... 168

11 **Phenylpropanoids** ... 171
11.1 **Shikimate Pathway (Phenylalanine, Tyrosine and Tryptophan)** ... 172
11.2 **Phenylpropanoids (Derived from Phenylalanine)** ... 174
References ... 177

12 **Alkaloids** ... 179
12.1 **Introduction** ... 181
12.2 **Terpenoid Indole Alkaloids (Derived from Tryptophan, e.g. Ibogaine, Reserpine, Strychnine, Physostigmine, Dimethyltryptamine, Psilocybin and Ergotamine)** ... 181
12.3 **Benzylisoquinoline Alkaloids (Derived from Tyrosine, e.g. Morphine and Curare)** ... 186
12.4 **Phenethylamine Alkaloids (Derived from Tyrosine, e.g. Mescaline)** ... 189
12.5 **Phenylethylamine (Derived from Phenylalanine, e.g. Ephedrine)** ... 189
12.6 **Alkaloids from Condensation of Tyrosine and Phenylalanine** ... 190
12.6.1 Amaryllidaceae (Norbelladine) ... 190
12.6.2 Phenylethylisoquinoline Alkaloids ... 191
12.7 **Pyridine Alkaloids (e.g. Nicotine), Pyrrolidine Alkaloids (e.g. Hygrine) and Tropane Alkaloids (e.g. Atropine, Scopolamine and Cocaine) All Derived from Ornithine and Arginine** ... 191
12.8 **Pyrrolizidine Alkaloids (Derived from Spermidine and Putrescine)** ... 194
12.9 **Purine Alkaloids (Derived from Xanthosine, e.g. Caffeine)** ... 195
12.10 **Piperidine Alkaloids (e.g. Lobeline), Quinolizidine Alkaloids (e.g. Cytisine) and Indolizidine Alkaloids (All Derived from Lysine)** ... 196
12.11 **Steroidal Alkaloids (From Steroids Derived from the MVA Pathway)** ... 197

12.12 **Terpenoid Alkaloids (Derived from the MEP Pathway, e.g. Aconitine)** 198
12.13 **Tropane-Related Alkaloids (Derived from Proline, e.g. Anatoxin A)** 198
12.14 **Isoxazole Alkaloids (e.g. Muscimol)** 198
12.15 **Muscarine** 198
12.16 **Guanidinium Toxins (e.g. Saxitoxin)** 198
12.17 **Kainoids** 199
References 199

13 **Minor Groups of Secondary Metabolites** 205
13.1 **Quinone** 206
13.2 **Polyketide (e.g. Cannabinoids)** 206
13.3 **Fatty Acid-Derived Metabolites** 208
13.3.1 Polyacetylenes (e.g. Cicutoxin) 209
13.3.2 *N*-Acylethanolamines 210
13.3.3 Alkylamides, *N*-acyl amides (e.g. Capsaicin) 210
13.3.4 Conium Alkaloids (Pseudoalkaloids, e.g. Coniine) 211
13.4 **Non-protein Amino Acids** 211
13.5 **Acetylcholine** 212
References 212

Correction to: Plant-Derived Drugs Affecting GPRCs C1

Supplementary Information
Glossary 216

Basics and History of Plant Secondary Metabolites

This part will give a broad overview over the different classes of secondary metabolites that are produced by various plants without giving detailed information about the classification or the biosynthetic pathways of the compounds. The chapter will also explore the general roles that secondary metabolites play in plants such as attraction and deterrence of animals, protection against bacteria and fungi or even allopathy. However, the intrinsic role of most plant-derived drugs is probably the least understood aspect of plant secondary metabolism. This lack of knowledge notwithstanding usage of plants secondary metabolites has a long history. Plant-derived compounds have been exploited in traditional medicine but also as recreational drugs or poisons. So finally, this chapter will look into the history of using and analysing secondary metabolites from the beginning of mankind into modern times with some outlook into future directions of research in this field of plant metabolism that gains more and more interest.

Contents

Chapter 1 **Plant Secondary Metabolites and Their General Function in Plants – 3**

Chapter 2 **Historical and Current Perspective – 19**

Plant Secondary Metabolites and Their General Function in Plants

1.1 General Classification of Secondary Metabolites (Details in Part IV) – 4

1.2 Overview of Classes of Secondary Metabolites (Details in Part IV) – 5

1.3 Overview of the Function of Secondary Metabolites in Plants (Details in Part IV) – 6

1.3.1 Adaptation to a Life on Land – 8

1.3.2 Attraction (or Deterrent) of Pollinators and Seed Dispersers – 8

1.3.3 Defence Against Herbivores, Pathogens and Other Pests – 10

1.3.4 Plant-to-Plant Communication – 11

1.3.5 Constitutive versus Induced Expression – 11

1.3.6 Counterstrategies of the Attacker and Use of Plant-Derived Secondary Metabolites by Other Animals – 12

1.3.7 Avoidance of Auto-Toxicity and Premature Toxin Release – 13

References – 16

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1

What You Will Learn in This Chapter

Primary metabolites are compounds that are associated with essential cellular functions. Therefore, they are very much ubiquitously found in all plants. By contrast, secondary metabolites have much more specific functions. They are often species specific and can be dispensable under many conditions. Nevertheless, the basis of most secondary metabolites are by-products or intermediates of primary metabolism. Secondary metabolites do not generally increase a plant fitness, but in the natural environment, they might be essential for survival and reproduction. They are thus mostly made under controlled conditions for a specific purpose such as defence against pathogens and herbivores, improved tolerance to abiotic stresses, attraction of insects and animals for fertilization and/or seed dispersal or repellence of unwanted feeders.

1.1 General Classification of Secondary Metabolites (Details in Part IV)

The term metabolite comes from the Greek word "μεταβολιτησ" – meaning the "changed" – and comprises intermediates (and end products) of enzymatic reactions that occur as part of biological pathways in living organisms. Metabolites are thus not a defined class of molecules, but the term encompasses compounds of very different chemical forms. There is not even a universally valid definition of this term, and not every substrate and product of an enzymatic reaction is considered a metabolite. The usage is normally restricted to low-molecular-weight compounds and mostly excludes biopolymers such as proteins, polynucleotides and polysaccharides or non-polymeric larger compounds such as lipids. Also, it is often used in a manner restricting it to compounds that have a defined biological role, thereby excluding intermediates of pathways that do not have a function on their own.

More important in the context of this book is the distinction between primary and secondary metabolites. Primary metabolites are compounds that are associated with essential cellular functions, e.g. components of growth and energy metabolism such as basic sugars, amino acids, nucleosides and small organic acids. Functionally, they are directly involved in normal growth, development and reproduction and as such are usually indispensable for the viability of an organism under any condition. Due to the evolutionary conservation of many of these basal cellular functions, primary metabolites mostly show a very broad distribution over a wide range of organisms, i.e. they are considered to be ubiquitous.

Initially, secondary metabolites were defined as "such compounds, which are formed by metabolism but which are no longer used for the formation of new cells" (Sachs 1873; as translated by Hartmann (1996)). This definition was based on the fact that the effect of certain secondary metabolites as human drugs was already known, however, the significance of these compounds for the plants was not yet understood. Only a few years later, a protective function of secondary metabolites for the plant was suggested for the first time (Errera and Durand 1886; Stahl 1888; Kerner von Marilaun 1879). The concept of chemical defence as part of animal-plant interactions was put forward, and it was suggested that phytochemicals play a role in animal attraction and deterrence (Stahl 1888). It should take another 60 years before the inherent biological role of secondary metabolites came back into the focus of plant science (Fraenkel 1959; Hartmann 2008), but the field of plant secondary metabolites has been gaining momentum ever since.

By now it is generally accepted that secondary metabolites are made for a specific intrinsic purpose even if their precise role is only known for a fraction of compounds.

Also, some derivatives of pathways involved in secondary metabolite production are important for plant development as they constitute hormones (ABA, gibberellin, cytokinin, brassinosteroids, strigolactones) or are necessary for photosynthesis, such as the phytoene tail of chlorophyll, ubiquinone, plastoquinone, tocopherol and carotenoids. At this point, the distinction between primary and secondary metabolism becomes blurred. Nevertheless, secondary metabolites are often found to be species specific, and even in those plants where they are very abundant, they might be dispensable under many growth conditions. Therefore, secondary metabolites do not generally increase a plant's fitness, but in the natural plant environment, they might still be essential for survival and reproduction. Consequently, the content of secondary metabolites varies enormously between different species.

1.2 Overview of Classes of Secondary Metabolites (Details in Part IV)

By-products or intermediates of primary metabolism form the basis of most secondary metabolites (Hartmann 1996). Products of the carbon and nitrogen metabolism form the basic structures of the three major classes, the terpenoids (isoprenoids), alkaloids and phenylpropanoids, but also the polyketides, quinones and cyanogenic glycosides (Fig. 1.1). The alkylamides are derivatives of fatty acids, while the glucosinolates derive from sulphur metabolism.

The number of primary metabolites in plants is probably less than 10,000 (Pichersky and Lewinsohn 2011). The total content of plant secondary metabolites has been estimated to be more than 200,000 (Dixon and Strack 2003; Yonekura-Sakakibara and Saito 2009), but it should be kept in mind that (i) the metabolic content of very few plants has ever been studied systematically and (ii) this estimate includes transitory intermediates of metabolic pathways that might not have specific functions, not even as branch points for deviating pathways. However, the number of secondary metabolites vastly exceeds the number of primary ones. Based on a small number of principal molecular scaffolds, plants produce a wide variety of secondary metabolites often with very different biological functions. These variants are due to different sets of enzymes changing the substrate or product range within certain metabolic pathways (Schwab 2003). In parts, this genetic variability resulting in an immense variety of secondary metabolites might be an advantage in dealing with a changing and/or demanding environment. Moreover, the non-essentiality of secondary metabolites for basic cellular functions might have allowed a less stringent selection process on enzymes/genes and resulted in the formation of novel components whose potential could then be exploited by the plant.

The evolution of different synthesis pathways and their specific enzymes was driven by gene duplications and adaptation to the requirements of a specific environment. Furthermore, there was not only gain but also secondary loss of the ability to form certain compounds. Analyses of biosynthesis genes and metabolites have indicated that there are many examples of convergent evolution, meaning that different plants have either independently evolved the ability to make similar or equal compounds (see caffeine) or to make structurally completely different compounds that nevertheless fulfil the same biological function (Pichersky and Lewinsohn 2011).

The presence and distribution of specific secondary metabolites within the plant kingdom, therefore, is neither ubiquitous nor does it follow a clear phylogenetic pattern (Wink

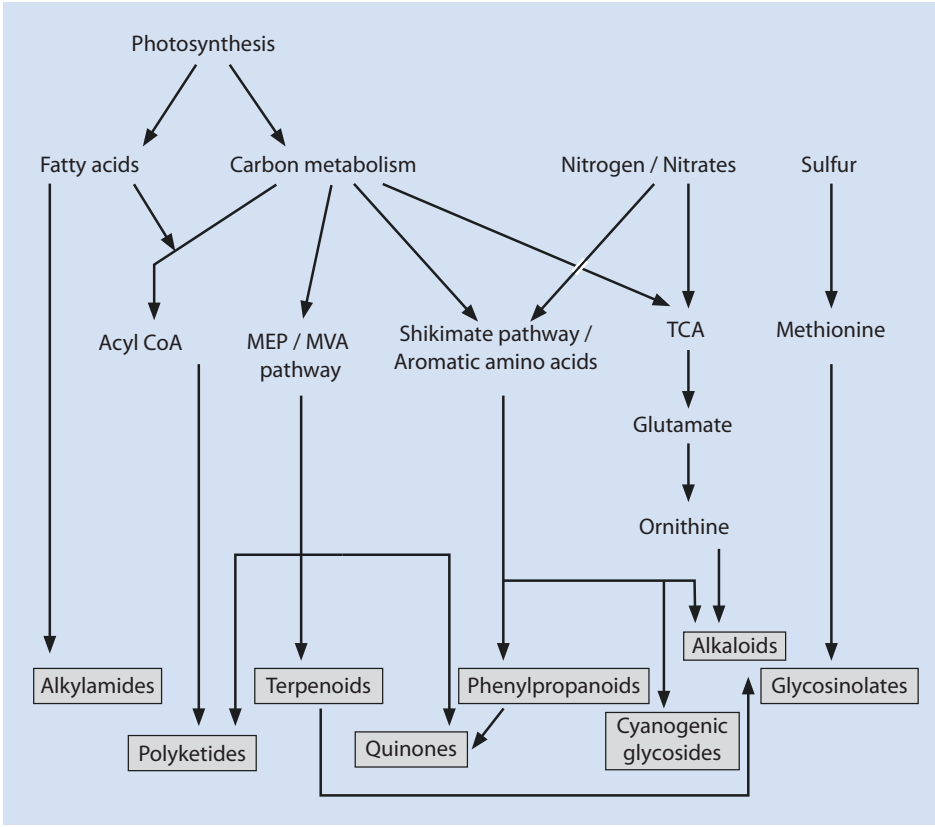

Fig. 1.1 Overview of the principal biosynthetic pathways for plant secondary metabolites. Carbons and to a lesser extent fatty acids derived from photosynthesis are combined with compound from nitrogen and sulphur metabolism

2003; Dahlgren 1980; Zhu et al. 2011). In a large meta-analysis of the distribution of drugs in different kingdoms, Zhu et al. (2011) showed that only 66 out of 740 families of the Viridiplantae contained drug-type secondary metabolites, 61 of which clustered within only 11 groups, orders or clades. A comparison of the *Selaginella* genome with that of seed plants also clearly showed that three gene families closely tied to secondary metabolism underwent a significant expansion in the lycophyte and angiosperm lineages. Two genes known to be involved in the biosynthesis of volatile odorants seemed to be only present in seed plants (Banks et al. 2011), which is in line with the major role of these volatiles in fertilization and seed dispersion.

1.3 Overview of the Function of Secondary Metabolites in Plants (Details in Part IV)

Most secondary metabolites have very complex structures. To synthesize these compounds requires significant resources and elaborate, high-energy-dependent biochemical pathways including many specific enzymes. Therefore, the possession of secondary

metabolites is far from cheap, and the gain of these compounds for the plant must outweigh the costs. Remarkably, despite their long history of medicinal and recreational use, very little is known about the concrete function of many of these metabolites for the plants. Nevertheless, for many compounds their cellular targets in animals are known, and therefore their effect on animals or pathogens can be deduced. Also, in some rare cases, the function of a specific compound for the plant is well studied. Based on this knowledge, a wide range of functions for secondary metabolites can be conceived that cover different aspects of plant life (Fig. 1.2). Most of these are directly connected with the fact that plants are sessile organisms and cannot escape their immediate environment. This means that they have to react to environmental challenges on a cellular level and also have to find ways to spread their progeny over wider areas.

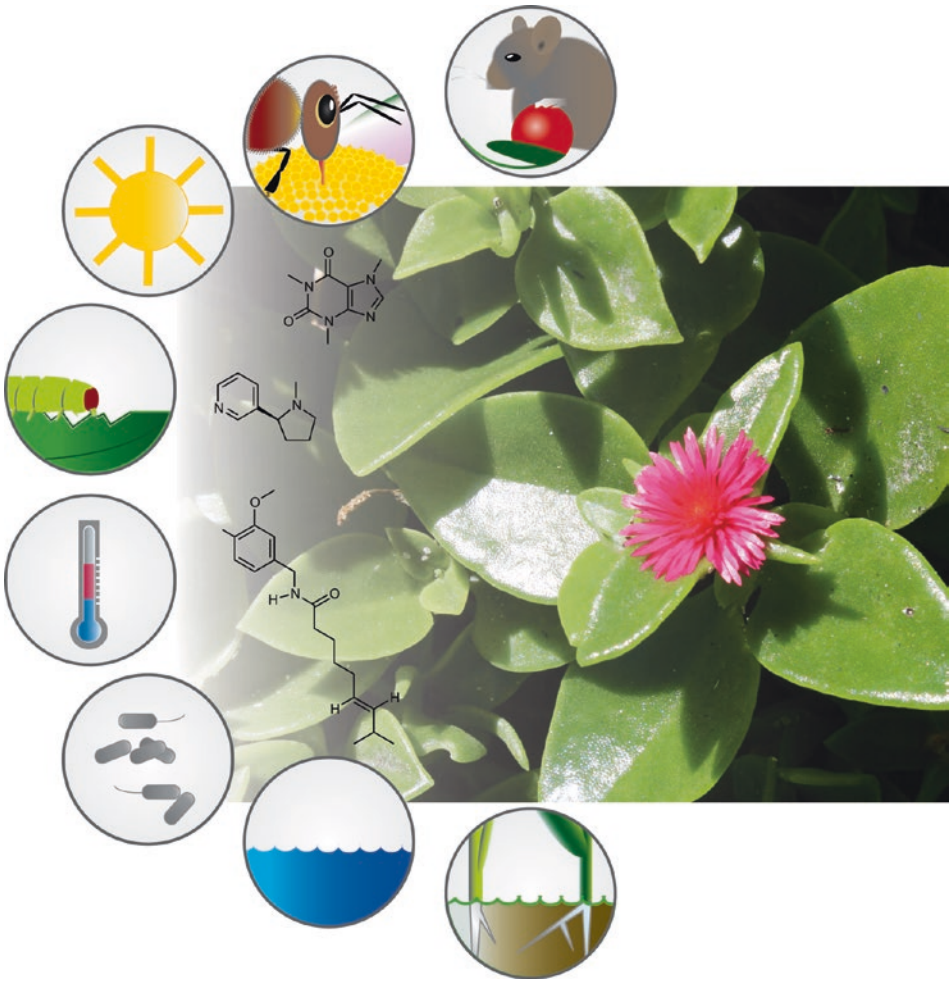

Fig. 1.2 Secondary metabolites play a major role in the plant response to various environmental challenges. Abiotic factors that affect plant growth and development include light, temperature and water availability. Plants also defend themselves against herbivores and pathogens, they attract animals for pollination and seed dispersal and they deter unwanted feeders and fend of competition from other plants

1.3.1 Adaptation to a Life on Land

A wide range of environmental stresses are potentially harmful to plants, and specific protective compounds were developed during evolution. Secondary metabolites such as flavonoids can already be found in algae, but chemical and genomic analyses suggest that the expansion in specialized secondary metabolites started about 500 million years ago with the evolution of land plants (Weng et al. 2012). The transition from a life in the ocean to a conquest of earth's surface was an enormous step that brought with it many new challenges including exposure to UV radiation, lack of structural support, drought stress and attack by newly evolving herbivores and pathogens. Not surprisingly, this step is also marked by a rise of new metabolic pathways to produce compounds helpful in addressing these new threats. These include the formation of phenylpropanoids for UV protection in the early land plants (Lowry et al. 1980), followed by the development of lignins (phenolic compounds) in the vascular plants, which provide the necessary solid scaffold that allows these plants to grow to heights not reached in any older lineages such as the bryophytes. Lignin also formed a material well suited to protect the plant surface from damage by wind or herbivores (Bateman et al. 1998), and both substance classes can already be found in mosses. Cyanogenic glycosides are found from the pteridophytes onwards (Buchanan et al. 2015), and evolutionarily their occurrence coincides closely with the emergence of insects. Alkaloids emerged at about the same time but all in all secondary metabolites are most abundant in flowering plants.

1.3.2 Attraction (or Deterrent) of Pollinators and Seed Dispersers

Animals play an important role for pollination and seed dispersal. Many plants can propagate by self-pollination or rely on wind or other forms of abiotic pollination. However, flowering plants commonly depend on the work of animals to carry pollen from one flower to another for cross-pollination. This allows a wider spreading of their pollen and ensures the new combination of genes that is the hallmark of sexual reproduction. Indeed, it is believed that the success of flowering plants after their first appearance about 200 million years ago is partially due to their efficient system of fertilization. While entomophily (pollination by insects) is probably the most common form of biotic pollination, it can also involve other animals such as birds and bats. During feeding, these animals come in contact with the sexual organs of the plants, thereby transferring pollen from the stamen of one plant to the stigma of another plant. An important feature for the attraction of pollinators are the often elaborate and colourful flowers of angiosperms. However, in addition to pigmentation, also flavours and volatile scents provide a means to attract insects or other animals for fertilization. Last but not the least, a reward system in the form of nectar ensures the ongoing cooperation between the plants and their pollinators.

While the attraction of pollinators is desired by the plant, the nutrient-rich nectar is also a lure for unwanted predators. Nectar robbers partake in a meal without successful pollination. They might reach the nectar via holes that they bite into the base of a flower, or they use the floral opening but without contacting the anthers and stigma. Floral larceny is quite common, but surprisingly very little studied (Irwin et al. 2004). Loss of nectar without pollination can severely affect plant fitness, and therefore plants want to discourage nectar robbers. However, in contrast to the deterrence of herbivores

(see below), defences against nectar robbers should not affect mutualistic pollinators. This can be achieved, when a toxin is targeted towards specific species. For example, the floral nectar of the northern catalpa tree (*Catalpa speciose*) contains iridoid glycosides that make it toxic or unpalatable for nectar thieves such as ants, but it remains edible to legitimate pollinators such as bumble bees and moths (Stephenson 1982). Instead of lacing the nectar, plants also can contain toxins in the petal tissue that affect nectar robbers when they bite through the flower base. It was suggested that this is one role of nicotine, which is found enriched in the basal parts of the corolla (Euler and Baldwin 1996).

Indeed, plant floral nectaries are a nice example for the combined function of primary and secondary metabolites (Heil 2011). The major purpose of nectar is the attraction of pollinators. To this end, its major constituents are primary sugars and free amino acids, providing nutritious carbons and a potential nitrogen source. Attraction of pollinators is aided by volatile organic compounds emitted by the flower. Also, nectar was shown to contain antimicrobial proteins (nectarines) for protection against microbial infections. This is helpful since the opening of the floral nectaries at the base of a flower provides an easy entrance point for microbes. In case of the phytopathogen fire blight, this was shown to be the major entrance point of infection (Buban et al. 2003). Secondary metabolites in the nectar enhance the antimicrobial properties, while their presence in the nectar or the corolla tissue deters nectar robbers.

Box 1.1 Toxic Honey

For humans, the presence of toxic secondary metabolites in nectar can be problematic. They can be transferred into the honey by bees either feeding directly on nectar or on honeydew exudates from sap-sucking insects. Honey poisoning was reported already more than 2000 years ago in Europe and Asia (Gunduz et al. 2008). Basis for the toxic property in this case are grayanotoxins that can be found in *Rhododendron* and other species of the Ericaceae. While rarely lethal they can cause the so-called mad honey disease when grayanotoxin-laced honey is consumed. Various other toxins have been detected in honey including strychnine. Tutin was found in toxic honey after the European honey bee was introduced to New Zealand and started obtaining nectar from the Tutu plant. While honey poisoning mostly occurs accidentally, mad honey has been deliberately harvested in areas such as the Black Sea for a long time, due to its halogenic properties and the belief that it acts as an aphrodisiac.

In a similar fashion, many plants rely on animals for the dispersion of their seeds. Since they are sessile organisms, plants had to develop systems to spread out their progeny over a larger area than their direct surrounding. Seeds of plants such as dandelion, maples or sycamore have parachute- or winglike structures that allow for easy dispersal by wind. Alternatively, seeds can be covered by a nutrient-rich tissue, i.e. fruit, that is eaten by animals. While insects play an important role for pollination, birds, bats and mammals are the most important seed dispersers. They carry the seeds with them until they have moved through the digestive tract. This ensures a wider distribution of the seed and the provision of a fertile environment for their growth after they are discarded with the excrements of the animal. Similar to flowers, also fruits attract animals by colour, flavour and volatile scents. Since it is important that fruits are not eaten before the seeds are mature, colour change from green to red or black is often used to advertise the ripening process to

animals. At the same time, unripe fruits often contain bitter or even toxic components to further deter animals from premature feeding. In rarer cases, plants want to deter unwanted feeders. Consumption of fruits by these animals does not enable successful distribution of seeds, e.g. because they are damaged during digestion. Capsaicin is a well-studied example for the deterrence of unwanted feeders and is discussed in detail in ▶ Chap. 3.

Humans have become an important factor in seed dispersal that differs significantly from those of animals. One important aspect is the high mobility of humans due to technological advances. Many crops but also ornamental plants and trees are nowadays grown in areas very far away from their native range. Initially humans brought these plants with them along trade routes or when they migrated to other countries or even continents. Nowadays, global markets are satisfied by growing important and high-prized plants wherever possible.

Box 1.2 Did Humans Save the Avocado from Extinction?

During the Cenozoic era, when large mammals and other megafauna roamed the earth, many plants also developed large seeds and fruits. However, these large seed dispersers disappeared around 13000 years ago, and yet, fruits such as the avocado retained their large size. While surviving for a long time in small ranges probably aided by the occasional scattering of seeds via unusual disperser, e.g. jaguars, the plants have celebrated their comeback since about 4000–2800 B.C, when its cultivation by humans in Mesoamerica begun. Also other plants that lost their large seed dispersers have faced slow extinction by dwindling numbers but are now rescued by human intervention (Janzen and Martin 1982).

1.3.3 Defence Against Herbivores, Pathogens and Other Pests

Defence against biotic attack is maybe the most important and definitely the best studied role of secondary metabolites. Phytopathogenic viruses, bacteria and fungi infect susceptible or wounded plants, aphids and other insects feed on their sap and herbivores, ranging from small caterpillars to full-size cattle, graze on plants as their food source. Therefore, plants have evolved a variety of chemical defence systems to battle against pathogens and herbivoral predators. Many secondary metabolites have antimicrobial properties. Pathogen-induced defence metabolites are called phytoalexins and include compounds such as isoflavonoids, terpenes, alkaloids and polyacetylenes (Ahuja et al. 2012). Bitterness and toxicity of secondary metabolites are a common way for plants to avoid being eaten or to at least reduce the extent of feeding to a manageable level. Accordingly, animals will either shun a toxic plant completely or will limit its intake.

In order for secondary metabolites to act as effective deterrents for herbivores, they must be recognized by the predator in a way to ensure the avoidance reaction. Bitter-tasting compounds are directly sensed by taste receptors in the mouth. Toxins, however, act on cellular targets in muscles, organs or brains only after they have been internalized. To efficiently deter herbivores before too much damage is done to the plant tissue, a toxin either has to kill very quickly or its presence needs to be perceived by the animal. This might involve learning, in those cases where the toxin is not lethal but the effect is strong enough to make feeding on the plant undesirable. While not very well studied, it is known from *Drosophila* that metabolites such as caffeine and strychnine are recognized by specific

taste receptors in the mouth (Lee et al. 2009, 2015). These receptors belong to the same family of seven-transmembrane gustatory receptors that allow animals to detect different kinds of taste such as bitter, salty, sweet, sour or umami, but they are very specific for the compounds that should be avoided. Thereby, the animals can detect the presence of a toxin early on and avoid continued feeding.

In a more indirect manner, plants have also developed "the enemy of my enemy is my friend" strategies to fight pests and herbivores. It is quite common that plants release volatiles that attract parasitic or predatory enemies of an attacker and help them to identify invested plants (Takabayashi and Dicke 1996). Various species have been shown to attract predatory mites when invested by plant-feeding mites or predatory insects when attacked by larvae. The Southern catalpa tree, for instance, increases the sugar content of its extrafloral nectar to attract ants when attacked by caterpillars of the moth *Ceratomia catalpae* (Ness 2003).

1.3.4 Plant-to-Plant Communication

It has also been suggested that plants use volatile secondary metabolites to communicate with each other in response to abiotic and biotic stress (Karban et al. 2014). These volatiles are often collectively labelled as green leaf volatiles (Dudareva et al. 2013). Brought forward in the early 1980s, the idea of plant-to-plant communication was initially discredited by many scientists and has only been studied thoroughly since the 1990s (Farmer and Ryan 1990). Volatile-mediated communication has now been shown for over 30 different species including trees, shrubs and herbaceous plants, albeit mostly as a response to herbivore attack and under laboratory conditions. By contrast, evidence for the use of volatiles to communicate abiotic stress is rather scarce. Moreover, it should be noted that there is still an ongoing debate, whether plants use these volatiles to explicitly communicate with other plants. Alternatively, or additionally, they could be used to warn potential predators of their defensive status or to quickly distribute information across distances within the context of a single plant, e.g. within the crown of a tree. In this case, other plants can simply "listen" into the communication as long as the compounds are not yet dispersed too much.

1.3.5 Constitutive versus Induced Expression

As mentioned above, the production of secondary metabolites is quite costly for a plant. Resources allocated to their synthesis cannot be used for plant growth or production of seeds. Careful consideration has to be given, whether a compound is synthesized at all times and in all parts of the plant. Therefore, the presence of many secondary metabolites is restricted to tissues that are potential targets of an attack, i.e. only in roots or leaves or floral nectaries. Also, attractants or defence compounds might only be present at a certain time or developmental stage. Depending on the specific environment and grade of occurrence of specific stresses on one hand and the cost of synthesis and storage of a secondary metabolite on the other hand, the plant has to "decide" on a constitutive or induced expression of a specific substance.

If a stress or attack occurs frequently or the speed of synthesis is too slow to be effective before existential damage is done, constitutive expression might be favourable despite the

costs. Typical components of the constitutive defence system are, for example, antimicrobial-acting phenols, lignins and tannins that are commonly found in cell walls and vacuoles (Rehman et al. 2012). Also, cyanogenic glycosides and other glycosides such as saponins are often expressed in a constitutive manner. In line with the complexity of environmental challenges, plants often contain mixtures of different defence metabolites. Thereby, the effectiveness of defence compounds can be increased, and resistance to a specific metabolite is less likely to occur (Wittstock and Gershenzon 2002). With constitutively expressed defence compounds, the economy of resources advocates a preferential allocation to those tissues or parts of a plant that are more prone to herbivory or pathogen attack.

Alternatively, a specific compound can be synthesized only when required, i.e. a defence compound that is produced after an initial attack by a herbivore or a pathogen. In these cases, induced synthesis often has a spatial as well as temporal component. Indeed, combinations of these strategies can be found with a low level of constitutive expression aided by an increased production upon stress or attack. This interplay of constitutive and induced expression is, for example, well studied in tobacco. While tobacco plants always contain a basal level of nicotine – it is indeed the most abundant alkaloid in tobacco leaves – it was shown that biotic attack leads to an increased synthesis (Baldwin et al. 1997) and thereby enhanced protection of the plant.

1.3.6 Counterstrategies of the Attacker and Use of Plant-Derived Secondary Metabolites by Other Animals

Co-evolution of plants and animals has not only produced systems of plant protection. Herbivores have often developed means to overcome toxicity of defence compounds, thereby enabling them to feed on plants that would otherwise be toxic (Foley and Moore 2005). This is less important for specific rare toxins but essential for secondary metabolites that are more common. Even with low toxicity, these might affect an animal when digested in higher amounts or if they accumulate in the body over time. Several different systems have evolved in animals to allow the consumption of toxic plant material (Heckel 2014). Mutations in the cellular target of a toxin that renders it insensitive are the most efficient way to protect an animal from the toxic effects. Also, if active transport is required, uptake of toxic compounds can be eliminated or reduced by changes in the affinity of transporters. Once internalized, metabolic degradation or alteration of the toxin can counter toxicity. The large superfamily of cytochrome P450 enzymes plays an important role in this system. Cytochrome P450 proteins are found abundantly in organs such as the liver and kidney, where they oxidize or hydrolyse toxic compounds to yield a product that can subsequently be conjugated with hydrophilic molecules such as glucuronic acid. This detoxifies the compounds and/or aids in their excretion. Indeed, glycosylation of toxins is a similar strategy that plants use to avoid auto-toxicity (see below). These detoxification systems are costly and often limited to smaller amounts of toxins. Thus, while allowing the herbivore to feed on a specific plant, they still protect the plant from overgrazing. Also, fungi have been shown to metabolize defensive plant saponins by the use of secreted enzymes (Morrissey et al. 2000).

Moreover, once animals have adapted these protective systems, they often use plant-derived metabolites for their own defence against predators or as precursors for pheromonic substances (Wittstock and Gershenzon 2002). For example, monarch but-

terflies have become insensitive to cardenolides and accumulate these compounds to become unpalatable for their predators (Holzinger et al. 1992). While this strategy requires the direct ingestion of the metabolite by feeding on the plant, some species such as *Utetheisa ornatrix* ingest the toxin during their larva state and can pass it on through metamorphosis to the adult moth state and even further to the eggs (Eisner and Eisner 1991).

Box 1.3 Defensive Halitosis
Nicotine is a potent alkaloid found in nightshade plants such as tobacco, which makes these plants or certain parts of the plants poisonous to muscle-moving pests, livestock and humans. The tobacco hornworm, *Manduca sexta*, is a moth whose larvae often feed on the leaves of tobacco or tomato. While the exact mechanism of detoxification is still debated, it appears that nicotine is metabolized via the enzyme cytochrome P450 6B46. However, part of the resulting substance is transported to the haemolymph, reconverted into nicotine and released into the air through spiracles. This mechanism is called defensive halitosis and protects the hornworm from predators such as spiders (Kumar et al. 2014).

Animals also use toxic plant compounds in more subtle ways. Blue tits have been shown to line their nest with parts from aromatic plants such as lavender, curry or mint to protect their offspring from parasites (Petit et al. 2002). Similarly, the leaves of tobacco are used by birds to repel parasites. It was even suggested that city birds use nicotine-containing stubs from smoked cigarette for the same purpose (Suarez-Rodriguez et al. 2013).

1.3.7 Avoidance of Auto-Toxicity and Premature Toxin Release

One severe problem that plants encounter when synthesizing secondary metabolites as defence compounds is auto-toxicity in those cases where these compounds are not only toxic to the attacker but also to the plant itself. A good example is hydrogen cyanide, which is commonly found as a defence compound. Hydrogen cyanide inhibits the mitochondrial cytochrome c oxidase and thereby aerobic respiration. This affects plants as much as animals. Other secondary metabolites interfere with conserved processes in the cell cycle. In all these cases, it has to be ensured that during synthesis and storage, toxic compounds do not come into contact with potential targets within the plant cell. Furthermore, because they are quite costly, unnecessary release of defence metabolites should be avoided. Plants have developed several mechanisms to address these issues.

1. Cellular compartmentalization of biosynthetic pathways: Many biosynthetic pathways for secondary metabolites are separated into different cells or compartments (for more details see ► Chap. 4). Non-toxic initial and intermediate compounds are made in one type of cell, while toxic intermediates and final synthesis steps are restricted to cells/compartments, where a compound toxicity does not affect the plant. This way the plant can ensure that active compounds and their toxic intermediates are prevented from coming into contact with cellular targets in sensitive cells. However, it should be noted that compartmentalization of biosynthetic pathways has also been shown for compounds that, to our knowledge, are not phytotoxic.

2. Storage in protected/safe compartments: Plants have developed several structures that allow for the safe storage of toxic compounds. Common examples for such structures are resin ducts, laticifers, internal glands or glandular trichomes. Often, the same structures allow the easy release of toxic or antimicrobial compounds when the tissue is ruptured upon herbivore feeding or mechanical tissue damage (Wittstock and Gershenzon 2002). While resin ducts are hollow spaces that are filled with resin from surrounding secretory cells, laticifers are elongated living cells that contain secondary metabolites but also defence proteins such as proteinases and chitinases. Internal glands can occur as isolated idioblast or small cell groups, which might contain a central cavity. Glandular or stinging trichomes are external structures covering the surface of leaves or stems. In glandular hairs the secretory substance accumulates in a thin-walled storage cavity above the secretory cells that easily ruptures upon pressure. Stinging trichomes such as the ones covering the common nettle (*Urtica dioica*) are built in such a fashion that the tip breaks easily when touched. The specific build of the calcified cell creates a needle-like end that injects the irritating content into the predator. Plants also sequester hydrophilic phytotoxins in their vacuoles, which are a "safe" compartment within the cellular context.
3. Storage of inactive precursors: Another way to avoid auto-toxicity is the storage of defence compounds as inactive precursors. Many secondary metabolites are found as glycosides, molecules in which sugar (often D-glucose) is bound to a functional group (aglycone), which can be activated by enzymatic removal of the sugar moiety. To prevent early release, enzymes and glycosides are separated into different cells or different compartments (◘ Fig. 1.3). Disruption of the tissue/cell brings enzyme and glycoside in contact with each other, resulting in the release of the active defence compound. Good examples are the glucosinolates (thioglucosides derived from glucose) found in pungent plants of *Brassicales* such as mustard, cabbage or horseradish. Isothiocyanate is released from glucosinolates upon cleavage by a family of enzymes called myrosinases. These enzymes are stored in the vacuole or cytosol of idioblastic so-called "myrosin" cells that are scattered within the plant tissue surrounded by cells harbouring the glucosinolates (Koroleva et al. 2000). Another example are cyanogenic glycosides such as amygdalin found in pits of plants from the rose family, e.g. almonds, cherries, apples or plums (Moller 2010). Cyanogenic glycosides are stored in the vacuole of the same cells that contain the activating glycosidases. Damage of the cell results in mixing of the vacuolar content with the cytoplasma and thus the release of toxic hydrogen cyanide by enzymatic removal of the sugar. Even after take-up of undamaged tissue, the hydrogen cyanide is released by glycosidases in the gut of animals during the digestion process. Sorghum plants have been shown to store the cyanogenic glycoside dhurrin, which makes them resistant to pests such as rootworms (*Diabrotica* spp.). However, the diurnal turnover of dhurrin implies that it might rather function as a source of nitrogen and glucose with its defensive properties as a useful by-product (Adewusi 1990), a role that has been generally suggested for cyanogenic glycosides (Moller 2010).

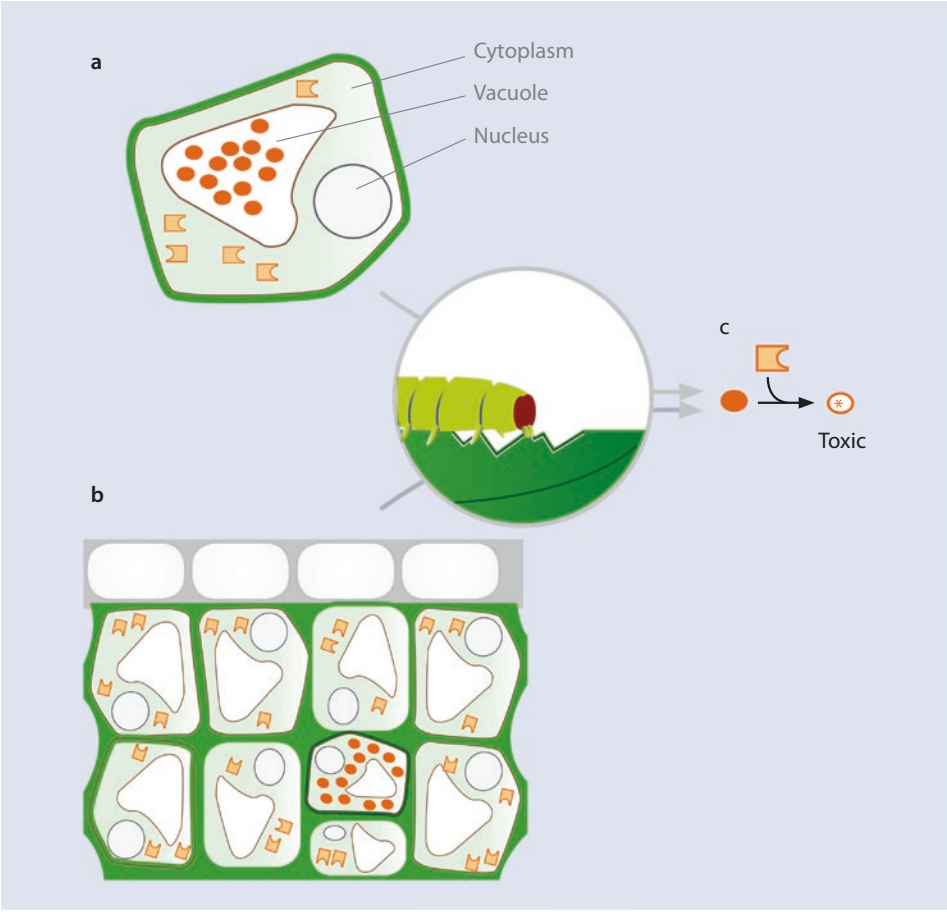

Fig. 1.3 Plants can avoid auto-toxicity of defence compounds by spatial separation between inactive precursors and activating enzymes. **a** Inactive precursors are kept in a different cellular compartment, e.g. vacuole, apart from the activating enzyme. **b** Inactive precursors are stored in specialized cells that are dispersed within the normal tissue. **c** Upon feeding by herbivores, the cells/compartments get destroyed, their content is mixed and the defence compound is produced

Take-Home Message

- Plants are a rich source of secondary metabolites, mostly small metabolic compounds that are not involved in basic cellular functions but provide special traits.
- Important roles of secondary metabolites include (i) protection against harmful environmental conditions, (ii) protection against pathogens and herbivores, (iii) feeding deterrence and (iv) attraction of pollinators and seed dispersers.

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Historical and Current Perspective

2.1 History of Medical Use – 20

2.2 History of Recreational Use – 23

References – 24

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2

What You Will Learn in This Chapter

While many of the secondary metabolites produced by plants make them unpalatable or toxic, the specific capacities of natural plant products have also been exploited by humans for a long time. In its simplest form, they have been used as spices and aroma compounds to give flavour to food. While it is rather easy to imagine this development, it is also obvious that so-called medicinal plants have a long history of use as pharmaceuticals, hallucinogens or painkillers, even though this often requires careful adjustment of the dose to avoid toxic effects. Last but not the least, since the onset of modern science and technical development, plant secondary metabolites and their derivatives have been exploited for technical applications.

2.1 History of Medical Use

Plants are a major nutrient source for humans. Therefore, it has always been important to distinguish edible plants from inedible and toxic ones. Moreover, people have made use of plants for purposes beyond eating since prehistoric times (Wadley et al. 2011). Historical evidence for medicinal and recreational exploitation of plant ingredients dates back to times more than 70,000 years ago. For example, bedding that was found at Sibudu in South Africa was made of Cape laurel (*Cryptocarya woodii*) leaves, a plant still used today in traditional African medicine. There is some evidence that a substance in this aromatic plant is toxic to mosquitos and it was therefore suggested by paleoethnobotanists that this might have been the purpose of its use as bedding material (Wadley et al. 2011).

Written evidence for the medicinal use of plants goes back about 4000 years. These records include text from China, India, Egypt and Sumeria, indicating that medicinal use was already widespread at that time. The use of cannabis, for example, is described in the Hindu scripture Atharva Veda (1200–100 BC), the first Chinese pharmaceutical book (Li 1974) *Shennong Bencaojing* (ca. 100 AD) as well as by Herodot (500 BC). The *Ebers Papyrus* dating to circa 1550 BC is a historic Egyptian medical collection that describes the use of many plants including *Atropa belladonna* (Ebbell 1937). Around 50 AD, a Greek physician, Pedanius Dioscorides, provided a first pharmacopedia *De Materia Medica* of about 600 plants and their medical use for the Roman army, which was in use for about 1500 years. Indeed, many of the plants mentioned in these texts are still used today for herbal medicine or have formed the base for the development of modern drugs.

The advent of modern science was marked by the first books describing specific extraction and distillation methods to obtain medicinal compounds from plants (Brunschwig 1500). Already the first book that described British flora following the Linnaean taxonomy included "the uses as medicines, or as poisons, as food for men, for brutes and for insects, with their applications in oeconomy and in the arts" (William Withering 1776). Withering also discovered common foxglove (*Digitalis purpurea*) as source of the active compound found in multi-herbal medicines for the treatment of heart conditions (Withering 1785) (Fig. 2.1).

The nineteenth century, due to significant developments of chemical methodology, is marked by the isolation and identification of many active plant compounds such as morphine (Sertürner 1805), strychnine (Pelletier and Caventou 1818), caffeine (Runge 1820) or nicotine (Posselt and Reimann 1828). Shortly after, many of these compounds could be synthesized and chemically altered by chemists to improve their function, e.g. acetylsalicylic acid (aspirin). At the same time, the psychoactive, toxic, antimicrobial, analgesic or

Fig. 2.1 Common foxglove (*Digitalis purpurea* © Ute Vothknecht) has long been used to treat various heart conditions due to the presence of the cardioactive steroid glycoside digitoxin

other properties of these substances were tested more systematically, and the exact function of many natural products was determined and often traced back to specific single metabolites. This then allowed the design and synthesis of more specific drugs.

With the advent of molecular biology, the molecular nature of the receptor targets and their interaction with these compounds could be studied. In recent years, the availability of better analytical methods, chemical assays and high-throughput screening platforms has led to a modern form of bioprospecting. Samples of hundreds of plants can be collected, extracted and analysed systematically in a reasonably short time. These methods allow the identification of the active compounds in plants that have been known and used as herbal medicine for ages. Therefore, the collection of knowledge about natural medicine that was gained by men over centuries of trial and error is an import asset in these studies. However, bioprospecting can also be used to identify new, promising compounds for future medicinal applications. Paclitaxel, a *diterpene*, isolated initially from bark of the Pacific yew (*Taxus brevifolia)* is a good example of a drug that was discovered in a random screen for new, medically active compounds against cancer (Wani et al. 1971). Tools offered by genetic engineering have enabled the production of plant secondary metabolites in microorganisms and the targeted alteration of synthesis pathways in order to produce new compounds. The inconspicuous flowering plant periwinkle (*Catharanthus roseus*) produces more than 100 different *terpenoid indole alkaloids*. A point mutation (V214 M) in strictosidine synthase, an important enzyme in the biosynthetic pathway of these compounds, has expanded its substrate specificity so that it now accepts new substrates, leading to the production of novel, unnatural alkaloid compounds (Runguphan and O'Connor 2009). In other cases, however, chemical synthesis or

biosynthesis in microorganisms is so difficult, expensive or inefficient that the active compound is still extracted from the original plant source, i.e. quinine from the *Cinchona* bark. Till today, many prescription drugs (approved or in clinical trials) and even more over-the-counter medications are based on plant extracts (Newman and Cragg 2007). Moreover, worldwide more people depend on traditional herbal medicine than on pharmaceutical drugs making plant-derived secondary metabolites an important issue in the global health system. Consequently, several secondary metabolites are found on the WHO List of Essential Medicines (◘ Table 2.1).

The long-time advantages of herbal medicine are amazing and often underappreciated. For many plants that are used in natural medicine, the active ingredient and/or exact mode of action is not yet discovered. In several cases, it remains unclear, whether the

◘ **Table 2.1** Several plant secondary metabolites or direct derivatives are listed in the WHO List of Essential Medicine. Compounds covered in ► Chaps. 3 and 4 are marked in bold (► http://www.who.int/medicines/publications/essentialmedicines/en/)

Compound	Core or complementary	Function	Plant origin (sole or exemplary)
Artesunate/ dihydroartemisinin[#1]	Core	Antimalarial medicines	*Artemisia annua*
Atropine	**Core**	**Sedation, antidote, mydriatics**	***Atropa belladonna***
Caffeine citrate	**Core**	**Treatment of neonates**	***Coffea arabica***
Codeine	**Core**	**Local anaesthetic**	***Papaver somniferum***
Digoxin	Core	Antiarrhythmic medicine	*Digitalis*
Docetaxel[#2]	Complementary	Cytotoxic and adjuvant medicines	*Taxus brevifolia*
Ephedrine	**Complementary**	**Local anaesthetic**	***Ephedra***
Hyoscine butylbromide[#3]	Core	Palliative care	*Solanaceae* (div.)
Morphine	**Core**	**Preoperative medication and sedation**	***Papaver somniferum***
Paclitaxel	Complementary	Cytotoxic and adjuvant medicines	*Taxus brevifolia*
Quinine	Core	Antimalarial medicines	*Cinchona* (div.)
Salicylic acid	Core	Skin differentiation and proliferation	*Salix alba*
Scopolamine	**Core**	**Palliative care**	***Solanaceae* (div.)**
Vinblastine	Complementary	Cytotoxic and adjuvant medicines	*Catharanthus roseus*

Semisynthetic derivatives of [#1]artemisinin, [#2]paclitaxel and [#3]scopolamine

described function can withstand thorough scientific investigation; nevertheless, the richness of compounds that have already been extracted from plant sources indicates that they should not be easily dismissed. In light of the prospects that modern science is offering with regard to the identification of novel useful substances, it is especially tragic that so much knowledge is getting lost, and many known promising plants are already endangered (Zhu et al. 2011). This is mainly due to the destruction of the natural environment in which many useful medicinal plants or plants with medicinal potential reside.

Also, modern breeding has diminished the content of secondary metabolites in many crops and cultivated plants. To make them more palatable, these plants have been bred to be less toxic, bitter, tart or hot. This way, hydrogen cyanides were removed from cassava, tannins from apples, cucurbitacins from pumpkin squash and cucumber or the bitter-tasting compound from many types of lettuce. While it makes these plants sometimes safer or more often nicer to eat, it goes hand in hand with a reduced content of secondary metabolites. This might even make them less valuable in dietary terms. More importantly it often makes them more susceptible to herbivores and pathogens compared to their "wild" relatives. This requires more extensive use of herbicides and causes some loss of adaptability of the plant to abiotic stress. In years with especially unpredicted or hefty weather conditions, this can result in severe yield losses. Therefore, instead of breeding out the responsible secondary metabolites, attempts are made to mask the bitter taste in order to make some foods more acceptable to consumers (Coupland and Hayes 2014; Drewnowski and Gomez-Carneros 2000). Furthermore, scientists have begun to collect native variants or breed and preserve seeds of wild ancestors of modern crop plants in order to protect the wealth of genetic diversity and useful traits that is of now often even unknown and unexploited (Peres 2016).

2.2 History of Recreational Use

In the same way that plants have been exploited for medicine, they also have a long history in recreational use and/or religious practice. The employment of plant-derived psychoactive drugs is ancient and even now persists in many indigenous cultures. In these cultures, mind-altering drugs are normally used by people with a special role in the society or religion and mostly in specific rituals. However, partaking in mind-altering drugs has by now become a more widespread recreational past-time in many countries. Marijuana (*Cannabis sativa*), opium (*Papaver somniferum*), cocaine (*Erythroxylum coca*), paan (*Areca catechu* L.) and tobacco (*Nicotiana tabacum*) are among the most popular drugs in modern times, but it can be safely assumed that such herbal concoctions have been used in their area of native growth since prehistoric times. Description of the use and effects of drugs such as cannabis or opium dates back as far as the description of herbal medicine, and many of these drugs also have medicinal properties. Knowledge and use of these drugs then spread along the trade routes, and they are now part of a worldwide albeit often illegal market.

A good example for this are the opioids, which are derived from the opium poppy *Papaver somniferum* (Sertürner 1805). Archaeological seed findings in association with human settlements in the wider Mediterranean region indicate that their use dates back to at least 5000 BC (Merlin 2003), even though it can only be speculated whether this was already for psychoactive properties. 2500-year-old findings in south-east Italy support the use of opium poppy in religious cults. The first written record dates back to around 4000 BC, where a Sumerian text refers to the flower as hul gil, "plant of joy", providing a very strong indication for its recreational use.

Box 2.1 The Poppy Goddess

Archaeologist recovered a statue from a subterranean chamber in Gazi, Crete, that is believed to have originated in the late Minoan III time. The trance-like facial expression and three pin-like extensions on its head that resemble poppy capsules lead to the suggestion that his was used in ritual or spiritual ceremonies (Blegen 1936; Marinatos 1937). Finds of paraphernalia that might have been used for opium inhalation strengthen the connection between the "Poppy Goddess" and the use of opium probably to induce a hallucinating state (Kritikos and Papadaki 1967; Merlin 1984).

Opium is also a good example for the difficult interplay between recreational and medicinal application. Opium was already known to ancient Greek and Roman physicians as a powerful pain reliever, and the properties of opium in alleviating depression and pain are also well recorded in ancient texts including Homers *Odyssey*. In the Middle Ages, people started extracting the alkaloids from opium using alcohol. Concoctions such as the original laudanum mixed opium with other ingredients of questionable activity before cleaner, more standardized mixtures were developed. The extraction and subsequent identification of morphine as the principal active ingredient of opium (Sertürner 1805), followed by codeine and thebaine, then allowed the production of cleaner, much more potent drugs for both medicinal purposes and abuse. By the end of the nineteenth century, the synthesis of heroin from morphine then gave rise to an even more potent drug with high addictive potential (Wright 1874). While the use of opium and its derivatives is now outlawed in most countries, the seeds of the opium poppy are still used in cooking and backing. These seeds do not normally contain opium but can become contaminated during processing of seed pods. And while the amount of opium found in the quantities of the seeds used for food purposes is extremely small, it may produce positive drug test results.

Take-Home Message

- Secondary metabolites provide the major basis of herbal medicine, which has been exploited by humans for thousands of years. Probably for as long, the psychoactive properties of plant-derived drugs have been exploited for religious ceremonies and recreational use.
- Preservation of plant diversity together with new methods of bioprospecting will help to retain and further exploit the richness of plant secondary metabolites.

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Drugs and Their Human Receptors

The plant-derived drugs that we want to consider in more detail in this chapter interfere with two important signalling pathways, G protein-coupled receptor signalling (GPCR signalling) and ion channel signalling. These ways of signal transduction are used by our cells to communicate with each other, and they also play important roles in synaptic signalling in the nervous system. We explore the components and general function of these signalling pathways and look how individual endogenous and plant-derived compounds affect them. In most cases, the drugs in question function as receptor agonists or antagonists; sometimes they act on neurotransmitter transporters or as allosteric regulators of ion channel function. Many neurotransmitters, e.g. acetylcholine, bind to several receptors that can be of different nature, including GPCRs and ion channels. We will see that there are very specific drugs able to distinguish different kinds of receptors, while others act on multiple receptors. Cannabis, muscarine and atropine, caffeine, cocaine, opiates and some psychedelics, nicotine, curare, thujone, strychnine and some other plant-derived drugs will be covered, and the endogenous ligands for the respective receptors will be discussed.

Contents

Chapter 3 **GPCRs – 29**

Chapter 4 **Ion Channels – 43**

Chapter 5 **GPCRs as Targets for Plant-Derived Drugs – 57**

Chapter 6 **Ion Channels as Targets for Plant-Derived Drugs – 83**

GPCRs

3.1 G-Protein-Coupled Receptors – 30

3.2 Trimeric G-Proteins – 32

3.3 G_S, G_{olf} and G_i Targeting Adenylyl Cyclase – 35

3.4 G_q Targeting Phospholipase C – 38

3.5 Signal Termination – 39

References – 41

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What You Will Learn in This Chapter

G-protein coupled receptors (GPCRs) are integral membrane proteins that span the membrane seven times. They are coupled to trimeric G-proteins. In this chapter we will discuss the history of their discovery, introduce the concept of second messengers and specifically explain how second messengers arise in response to GPCR-signalling. We will focus on cAMP and phospholipids. Finally we will introduce target enzymes for these second messengers including protein kinase A and protein kinase C.

3

3.1 G-Protein-Coupled Receptors

G-protein-coupled receptors are integral membrane proteins that span the plasma membrane seven times. Due to this characteristic, they are also called "seven-transmembrane receptors" or serpentine receptors (see Fig. 3.1).

In nematodes, over 1000 genes are dedicated to express GPCRs. This number adds up to 5.5% of the *Caenorhabditis elegans* genome. Many of these GPCRs have no known ligand, making them so-called "orphan" receptors. By contrast, in the yeast *Saccharomyces cerevisiae*, only three genes encoding GPCRs are known. These include STE 2 and STE 3, two pheromone receptors important for mating. In plants, such receptors are also rare, and the few serpentine receptors encoded in the plant genomes are not G-protein-coupled (Fredriksson and Schioth 2005).

In humans about 800 genes for GPCRs have been annotated (Civelli 2012). Thus, GPCRs constitute the largest group of membrane proteins encoded in the human genome, and they are targets for the majority of present-day therapeutic drugs. About 400 GPCRs in vertebrates are olfactory receptors dedicated to sensing smell and taste. In most cases the endogenous ligands of olfactory receptors are not known (Tao and Conn 2014). Non-olfactory GPCRs include, to name just a few, rhodopsin in our photoreceptors, adrenergic receptors, acetylcholine receptors, dopamine and serotonin receptors and peptide receptors, e.g. such for opiate peptides and blood pressure regulators like angiotensin and bradykinin. Cannabinoid receptors fall into this molecule class, as do adenosine receptors that are antagonized by caffeine. A recent review states about 360 well-characterized GPCRs with 200 endogenous ligands, whereas 160 of non-olfactory receptors are still "orphans" (Civelli 2012).

Functionally related to the animal GPCRs are light-driven proton pumps that were discovered by Oesterhelt and Stoeckenius in the purple membrane of *Halobacterium salinarum* in 1971 (Oesterhelt and Stoeckenius 1971). Henderson and Unwin described their structure in 1975 as a "simple example of an intrinsic membrane protein" (Henderson and Unwin 1975). Retinal binds to these proton pumps, and, as a prosthetic group, it mediates the establishment of a proton gradient across the membrane of *Halobacterium salinarum*. Upon light absorption, retinal isomerizes from all-trans to 13-cis-retinal, and protons move to the exterior (Oesterhelt and Stoeckenius 1971). Halobacteria, in addition to the proton pumps, also express a chloride pump and two types of sensory rhodopsins that mediate chemotaxis. On the sequence level, these archaeal rhodopsin family members are not related to eukaryotic GPCRs (Ihara et al. 1999). However, their function pre-empts the use of proton gradients in eukaryotic ATPases and the use of retinal as a prosthetic group in animal photoreceptors. Retinal is the aldehyde of retinol, and animals synthesize it from β-carotene (or provitamin A), which is found in carrots and in many other roots, fruits and leaves of plants. It is also the prosthetic group for the animal opsins. With retinal

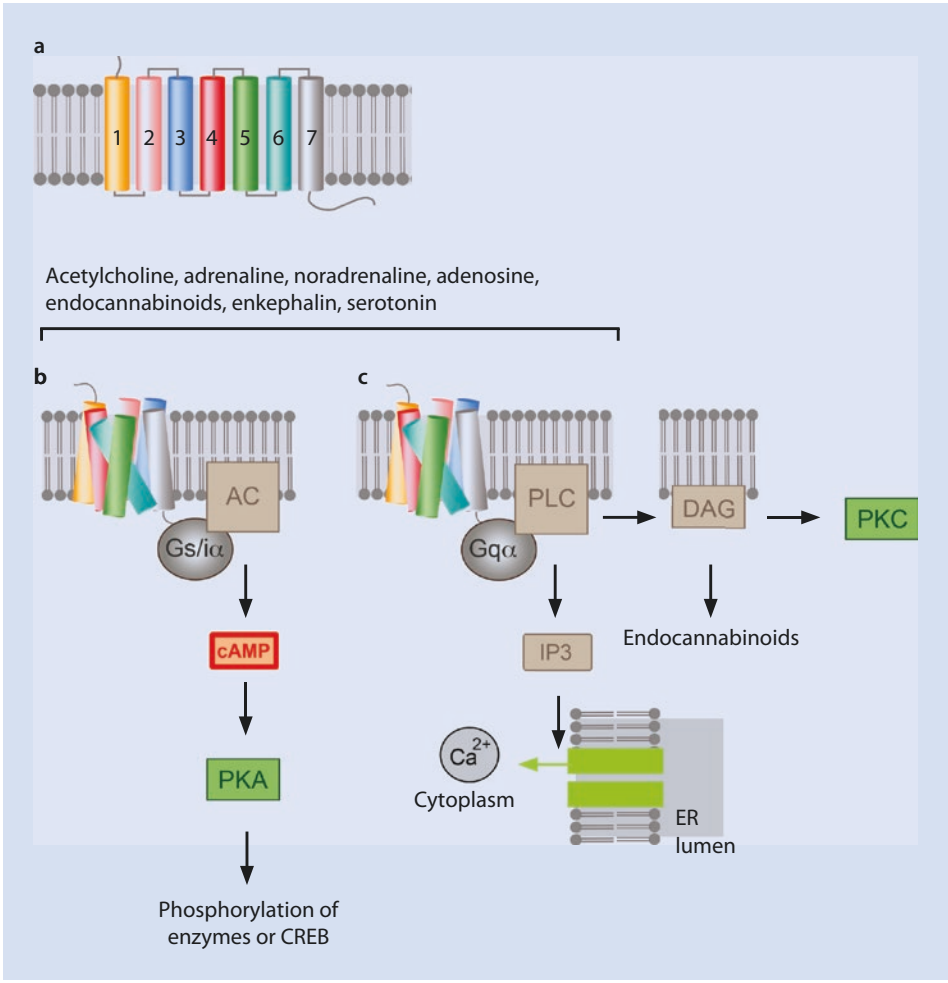

Fig. 3.1 Schematic representation of a G-protein-coupled receptor (GPCR, serpentine or seven-transmembrane domain receptor). **a** Intracellular signalling at GPCRs via G_s/G_i. Adenylyl cyclase (AC) is the target enzyme for G_s/G_i and regulates cAMP levels. **b** Protein kinase A (PKA) is activated by cAMP and in turn phosphorylates substrates including the transcriptional activator cAMP response element binding protein (CREB). **c** Intracellular GPCR signalling via Gq. Phospholipase C (PLC) is the target enzyme for Gq and hydrolyses membrane phosphoinositols producing inositol-3-phosphate (IP3) and diacylglycerol (DAG). DAG remains in the membrane and is a second messenger involved in activation of protein kinase C and a precursor for endocannabinoid synthesis. IP3 opens Ca^{2+}-channels in the membrane of the endoplasmatic reticulum (ER)

bound, opsins form functional photoreceptors, the rhodopsins. Here, upon absorption of one photon, the 11-cis conformation of retinal changes to all-trans, inducing the intracellular signal transduction cascades that allow light perception in photoreceptor cells of the retina. The photoreceptor cells synapse with neurons, and via the optic nerve, all visual information is transmitted to the brain. This is why carrots are good for our eyes.

How are GPCRs in animals coupled with their transducers to evoke cellular responses after ligand binding? The first answer to this question came from test tube assays carried out by Rall and Sutherland in 1962 (Rall and Sutherland 1962). They measured an increase

in cAMP on isolated particles from mammalian myocardium, liver or cerebral cortex after stimulation with catecholamines (epinephrine, also called adrenaline and norepinephrine, also called noradrenaline – these names indicate that these compounds are released from the adrenal gland). In this way, they established a link between hormone action and the enzyme adenylyl cyclase, which produces cAMP from ATP. They inferred that adenylyl cyclase was an allosterically regulated enzyme with a regulatory site somehow responsive to catecholamines and asked whether adenylyl cyclase was the membrane receptor for catecholamines (see ▶ Box 3.1).

Box 3.1 Catecholamine

Catechol*amine*: compound possessing a dihydrobenzol ring (or *catechol* ring) and an *amino* group. They are also called brenzcatecholamines. Adrenaline, noradrenaline and dopamine are naturally occurring catecholamines. Synthetic catecholamines include isoprenaline and dobutamine.

This was considered unlikely after experimental results showing that molecules with vastly different structures could induce cAMP release from fat cell membranes. The tested molecules included the catecholamine adrenaline and very different peptide hormones, such as ACTH (adrenocorticotropic hormone), glucagon and TSH (thyroid-stimulating hormone). It was rather hypothesized that multiple receptors with specificity for their ligands interacted with a common catalytic unit and that this happened at the cell membrane. Thus, the search for a "transducer" was on. This molecule was supposed to couple information from outside the cell via a ligand-bound receptor with the regulation of adenylyl cyclase (Rodbell et al. 1968). In 1971, Rodbell proposed a GTP-regulated protein to be the transducer. The first α-subunit of trimeric G-proteins, which turned out to act as transducer, was isolated in 1980 in the lab of Alfred Gilman (Northup et al. 1980).

Purification of the α- and β-adrenergic receptors followed some years later (Caron et al. 1979). In 1986, the genes encoding the α_2- and β_2-adrenoreceptors were cloned (Dixon et al. 1986). Only then it became clear that they were seven-transmembrane receptors. Further work revealed that they were members of a huge family of GPCRs. In all seven-transmembrane receptors, the N-terminal protein sequences point to the cell exterior; the C-terminal sequences are directed towards the cytoplasm. The seven transmembrane domains are quite conserved between different members of the receptor family. In contrast, the extracellular and intracellular loops are diverse. The extracellular domains are involved in ligand binding, and the intracellular domains are responsible for signal transduction.

3.2 Trimeric G-Proteins

The common "transducers" for all GPCRs are trimeric G-proteins, or GTPases, consisting of α-, β- and γ-subunits. The α-subunit is structurally related to small GTPases or G-proteins such as Ras, Ran and Rab (see ▶ Box 3.2). These G-proteins alter between a GTP-bound and a GDP-bound state. In this way, they work as molecular switches. The critical movement of the switch corresponds to the conformational transformation associated with GTP binding and release. Although the cytoplasm contains much higher concentrations of GTP

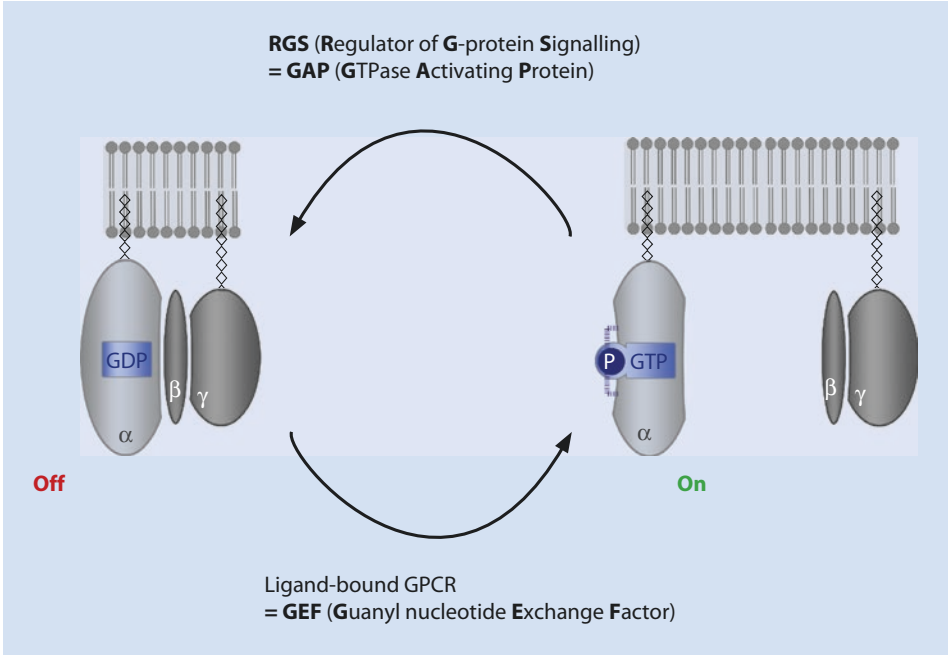

Fig. 3.2 Schematic representation of membrane-anchored trimeric G-proteins and G-protein cycle of α-subunits. "On" state with GTP-bound and dissociated α-subunit, ligand-bound receptor functions as GEF; "off" state with GDP-bound, α-subunit functions as GTPase; GTPase activity is regulated by RGS proteins working as GAPs. Gα-GDP reassembles with Gβ/γ

than GDP, G-proteins, in a nonactivated state, are bound to GDP. This tight binding has to be actively released by GEFs (guanyl nucleotide exchange factors). In the case of GPCR signalling, the ligand-bound receptor usually acts as GEF, leading to GDP release and GTP binding. In this conformation the switch is on (see Fig. 3.2).

Box 3.2 Small G-proteins or small GTPases

Small G-proteins are monomeric *G*TP-binding proteins with low intrinsic GTPase activity (20–40 kDa). GTPase activity can be stimulated by GTPase-activating proteins (GAPs). GTP loading is always assisted by guanyl nucleotide exchange factors (GEFs). Small G-proteins act as molecular switches in many cellular processes including mitogenic signalling (Ras family), cytoskeletal signalling (Rho family), nuclear import/export (Ran family), vesicle transport (Rab and Arf/Sar families) and cell adhesion and migration (Rap family). Large families of GEFs and GAPs exist for each small G-protein, and these are important regulators of the respective cellular functions.

The GTP-bound α-subunit of trimeric G-proteins dissociates from the β/γ-subunits and moves away from the complex to interact with target enzymes. Major target enzymes of activated α-subunits of trimeric G-proteins are adenylyl cyclase, phosphodiesterase and phospholipase C (see Figs. 3.1b and 3.3). In addition, several ion channels are regulated in response to GPCR-activated trimeric G-proteins. Lastly, α-subunits of trimeric G-proteins can activate mitogen-activated kinases (MAP kinases) (Sugden and Clerk 1997).

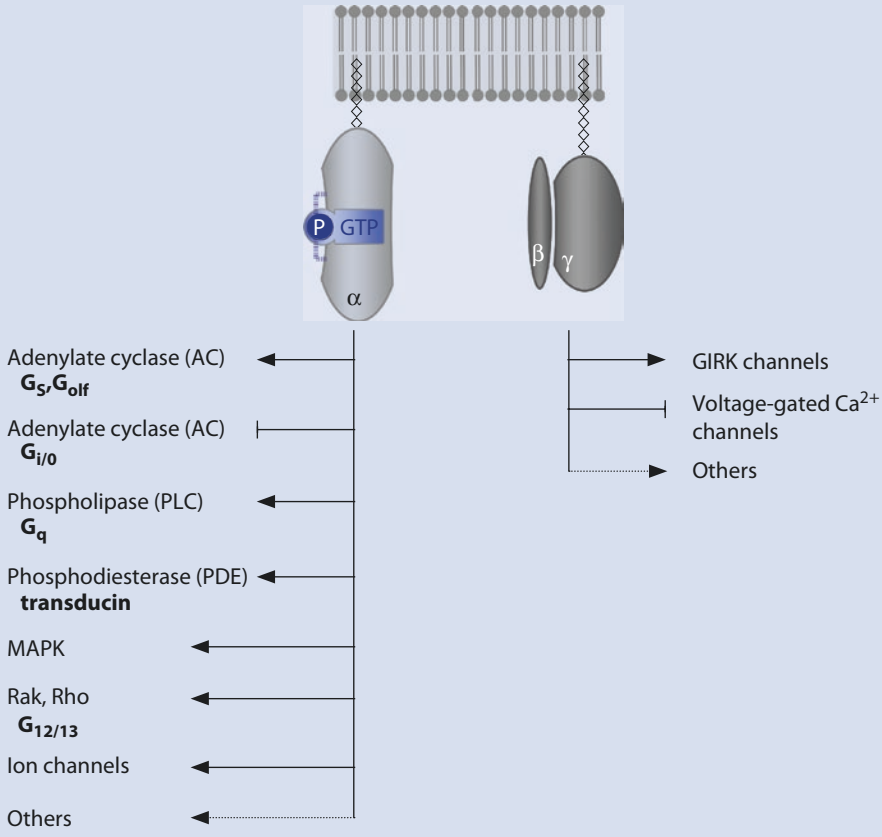

Fig. 3.3 Specific Gα-subunits and Gβ/γ-subunits activate or inhibit distinct target enzymes in the on state of the trimeric G-protein

Box 3.3 MAP kinases

MAP kinases (*m*itogen-*a*ctivated *p*rotein kinases) constitute a family of kinases that are activated through a kinase cascade in response to growth factors (*m*itogens), differentiation signals or others. They phosphorylate certain transcription factors. This leads to gene expression of genes that regulate, e.g. the cell cycle, cell differentiation and cell death. The MAP kinase cascade involves MAP kinase kinases (MAPKK), and these are activated by MAP kinase kinase kinases (MAPKKKs), which can be activated by Ras GTP or upstream kinases including PKC.

Trimeric G-protein α-subunits (Fig. 3.3) are encoded by 17 distinct genes, β-subunits by five and γ-subunits by 12 genes. Trimeric G-proteins comprise:

1. Activators of adenylyl cyclase, i.e. G_S and G_{olf} – the G-protein of olfactory cells mediating smell and taste; these G_S-subunits can also activate ion channels and the growth factor related mitogen-activated kinase cascade (MAP kinases; see ▶ Box 3.3).
2. A diverse group of Gi-proteins inhibiting adenylyl cyclase including G_0, which is present in the CNS.
3. Gq activators of phospholipase, leading to the release of inositol-3-phosphate (IP3) from the phospholipid bilayer.

4. G12/13 regulators of actin cytoskeletal remodelling and migration.
5. Transducin, the G-protein that transduces the signal from activated rhodopsins in the photoreceptor cell. In vertebrates transducin activates a cGMP phosphodiesterase thus initiating the closing of cGMP-gated ion channels of photoreceptor cells and membrane hyperpolarization.

Gβ/γ-subunits have a signalling function of their own. They can, for instance, bind to several types of Ca^{2+}-channels (see below) and inactivate them (De Waard et al. 2005). Moreover, they are known to activate inward rectifying K^+-channels (Kir channels or GIRK channels for those which are activated by G-proteins, see ▶ Chap. 4.2) and have been shown to interact with the MAP kinase pathway, nuclear proteins and the cytoskeleton (Khan et al. 2013) (◘ Fig. 3.3).

There are two well-known pathogenic modifications of G-proteins. These are catalysed by ADP-ribosyltransferases that are part of bacterial toxins, including cholera toxin from *Vibrio cholerae* and pertussis toxin from *Bordetella pertussis*. These enzymes catalyse the transfer of the ADP-ribose element of nicotinamide-adenine dinucleotide to proteins. Cholera toxin targets G_sα-subunits in the intestine and ribosylates an active-site Arg of the GTP-hydrolase. Thereby, the GTPase activity is blocked, rendering the G-protein constitutively active. As a consequence, cAMP levels in intestinal cells are constantly raising causing extreme diarrhoea. Pertussis toxin contains an ADP-ribosyltransferase that targets a cysteine residue at position 4 from the C-terminus of G_i in the lung epithelium. This blocks the interaction of the inhibitory G-protein with the receptor and causes an increase of cAMP in lung epithelial cells resulting in the symptoms of whooping cough. Cholera and pertussis toxins have provided important tools in G-protein research. For example, activated cholera toxin together with ^{32}P-nicotinamide-adenine dinucleotide was used for the first labelling and purifying of Gα from rabbit liver (Northup et al. 1980).

3.3 G_s, G_{olf} and G_i Targeting Adenylyl Cyclase

Adenylyl cyclase (AC) is the enzyme that produces cAMP from ATP. There are ten isoforms, expressed in different human tissues. All are regulated by Gα-subunits; these can be activating (Gsα) or inhibiting (Giα). Some are also activated by the Gβ/γ-dimer that remains after GTP-bound Gα-subunits are dissociated. Thus, the level of cAMP is regulated via AC in response to extracellular signals, the "first messengers" of intracellular communication. cAMP is the "second messenger", produced inside the signal receiving cells and mediating their responses.

The adenylyl cyclases are integral membrane proteins with 12 hydrophobic transmembrane domains. N- and C-termini are directed into the cell interior. The sequences of the transmembrane domains are more variable between species than the highly conserved intracellular loops and the C-terminal sequences. The latter associate with each other to form a functional unit constituting the catalytic domain of the enzyme. The catalytic domain of the AC strongly binds to the activator forskolin, a diterpene of the shrub *Plectranthus barbatus* that has been used for affinity purification of the AC catalytic domains from different species (Hatley et al. 2002) (see ◘ Fig. 3.4).

AC activity and the activity of phosphodiesterases (PDEs), which convert cAMP to AMP and thus antagonize ACs, define the intracellular levels of cAMP. Phosphodiesterases constitute a large protein family encoded by 21 genes in mammals. Through alternative splicing the number of known isoforms reaches ca. 50. These are subdivided into separate

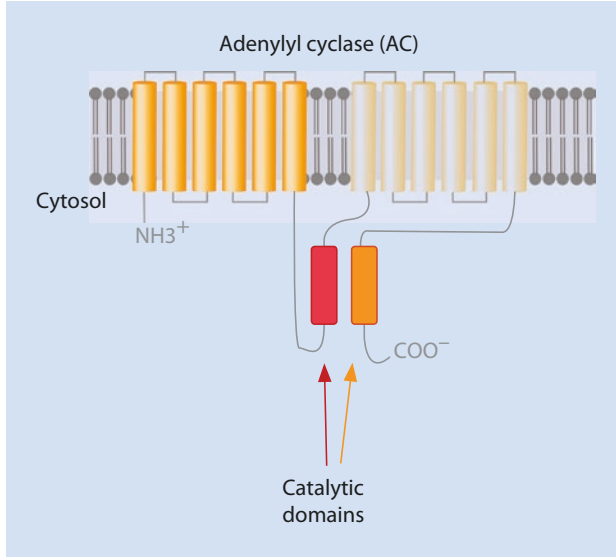

Fig. 3.4 Schematic representation of adenylyl cyclase with intracellular catalytic domains

families, which hydrolyse cyclic nucleotides, and have diverse subcellular localizations and varying affinities for their cyclic nucleotide substrates. Therefore, cAMP that is produced in a cell in response to receptor activation will not be evenly distributed in the cytoplasm. It will rather be restricted to certain subcellular structures. This adds specificity to the cAMP responses (Conti et al. 2014).

cAMP regulates further pathways, including the activity of *p*rotein *k*inase *A* (PKA) (see Fig. 3.5). It is involved in many PKA-dependent processes, such as metabolic pathways, gene regulation and cellular pathways regulating proliferation and apoptosis. cAMP also regulates the conductivity of second messenger-gated ion channels and therefore plays an important role in neurotransmission. Finally, cAMP regulates EPAC-proteins (exchange factor proteins directly activated by cAMP). These are GEFs for small G-proteins including Rap1 and Rap2. Rap proteins partially antagonize mitogenic signalling by growth factors via Ras proteins by inhibiting the MAP kinase kinase kinase (MAPKKK) c-Raf (*r*apidly *a*ccelerated *f*ibrosarcoma). They also have functions in regulating phospholipids (by activating phosphatidylinositol-4, 5-biphosphate 3-kinase (PI-3 kinase), cell adhesion (via cadherin and integrins) and the cytoskeleton (via activation of Rho-proteins) (Zhang et al. 2017).

PKA is ubiquitously expressed in all cells, and numerous molecules, including hormones and neurotransmitters, activate this kinase via GPCR-mediated cAMP production. To obtain specificity in PKA-regulated pathways, its activity has to be controlled on additional levels. This is achieved by its two regulatory subunits. Together with two catalytic subunits, PKA forms a tetramer. The regulatory subunits interact with A-kinase-anchoring proteins (AKAPs), a very large protein family with tightly regulated cellular localization. AKAPs define the localization of PKA holoenzymes in different cellular compartments or structures. Moreover, they function as scaffolds for other signalling molecules. Thus, AKAPs form, within their respective cellular microenvironment, multiprotein complexes with many proteins, including kinases and phosphatases that are responsive to different cellular signalling pathways. In this way PKA activity on its substrates can be precisely related to the respective upstream receptor activation event (Torres-Quesada et al. 2017). When cAMP binds to the regulatory PKA subunits, the catalytic subunits of the enzyme are

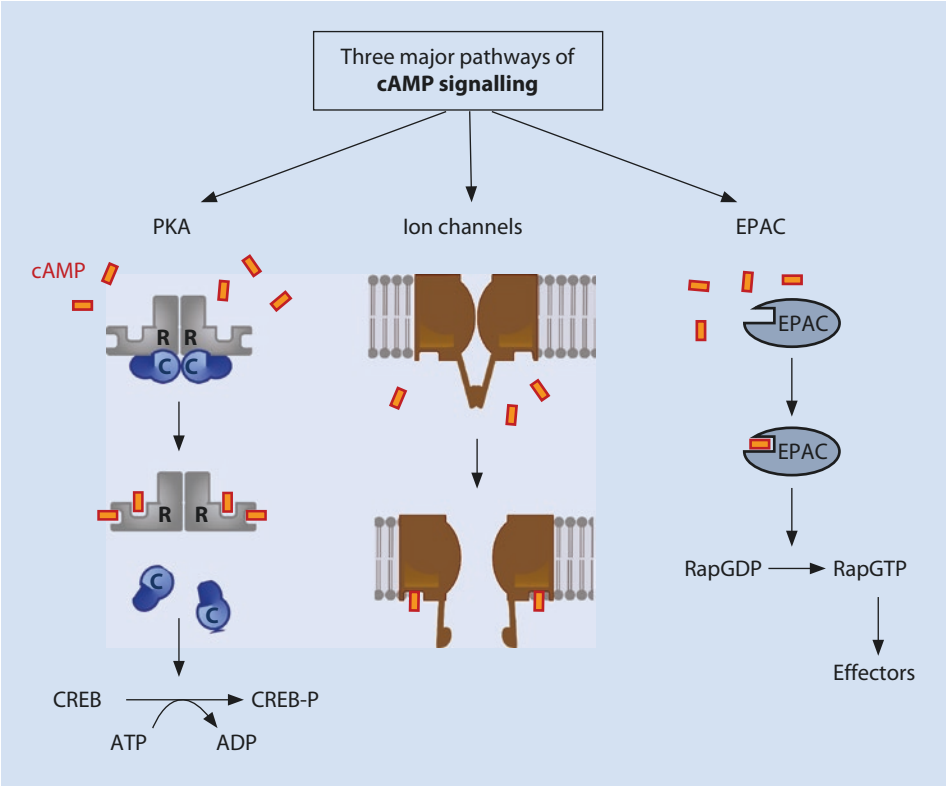

Fig. 3.5 cAMP-signalling pathways comprising activation of protein kinase A (PKA), opening of intracellular ligand-gated ion channels specific for cAMP and binding of EPACs (exchange factor proteins directly activated by cAMP). cAMP-bound EPACs work as exchange factors for small GTPases of the Rap family

released and catalytically active. Due to being anchored close to certain cell compartments, PKA will then phosphorylate target proteins in the surrounding of its anchoring place.

For example, PKA targets the transcription factor CREB in the nucleus. CREB is a member of a family of transcription factors that bind to DNA sequences that are known as "*c*AMP *r*esponse *e*lement (CRE)". CREB stands for CRE *b*inding protein. CREB proteins bind to the palindromic DNA sequence TGACGTCA. Gene activation from this promoter element depends largely on CREB phosphorylation by PKA and thus on cAMP. CREB has a basic leucine zipper DNA-binding domain, which interacts with CRE. In the N-terminal region of CREB sits the kinase interaction domain (KID). This domain contains several serine residues, which can be phosphorylated by kinases. Especially Serine 133 is targeted by PKA. Only when Serine 133 is phosphorylated, CREB interacts with the transcriptional co-activators CBP or p300 and activates transcription (Quinn 2002).

The CREB-dependent pathway of gene transcription is very fast. Some genes are activated within minutes after receptor stimulation. These include genes encoding metabolic enzymes, transcription factors and secretory proteins. Such rapid gene activation is especially important for neuronal rapid response genes, for example, those involved in learning and memory. The involvement of cAMP-mediated transcriptional activation in memory consolidation (long-term memory) has been studied in transgenic mice and is conserved in invertebrates, for instance, in the fruit fly *Drosophila* and in the snail *Aplysia* (Kida 2012). In fact, it was by investigating sensitization to the

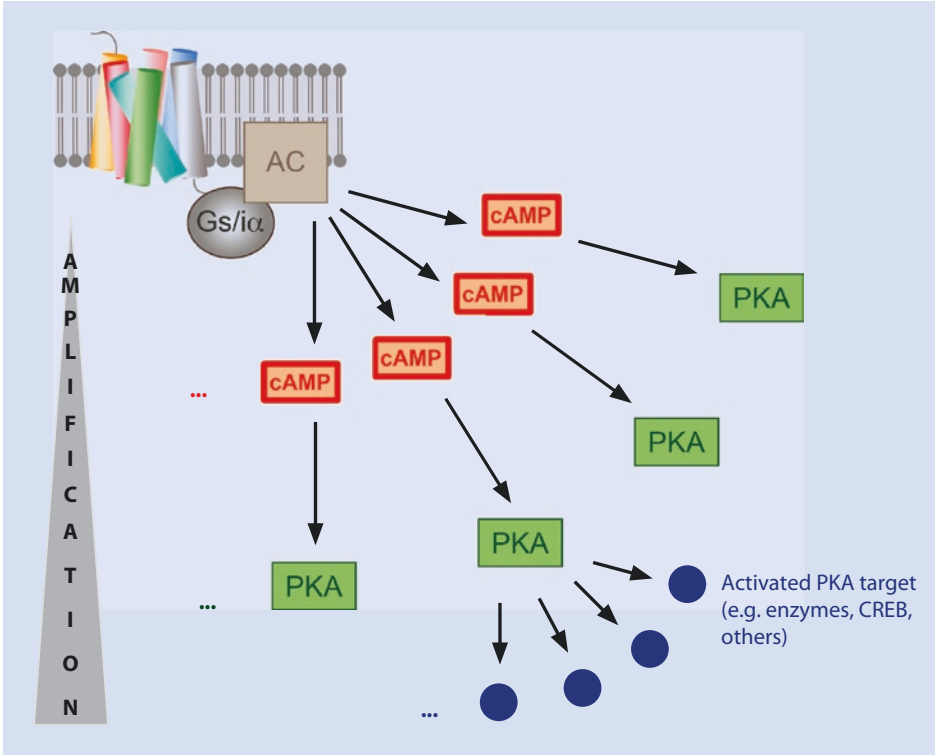

Fig. 3.6 Schematic representation of signal amplification steps after ligand binding to GPCR via G-proteins activating adenylyl cyclase, cAMP production and target phosphorylation by PKA

gill withdrawal behaviour in the mollusc model organism *Aplysia* that the involvement of cAMP and PKA in CREB-dependent gene regulation for the establishment of a long-term memory was originally discovered (reviewed in Kandel et al. (Kandel et al. 2014).

Binding of ligands to a GPCR is the beginning of a large amplification cascade. One ligand-bound receptor molecule can work as GEF for many G-proteins. There is no further amplification when the G-proteins interact with their target enzymes in a 1:1 manner. However, one active AC unit produces large numbers of cAMP. cAMP diffuses into the cytoplasm binding to regulatory subunits of PKA – this slows down the avalanche by a factor of four. However, signal amplification takes off again when catalytic subunits of PKA phosphorylate target enzymes, with each catalytic PKA subunit phosphorylating multiple protein substrate molecules (see Fig. 3.6).

3.4 G_q Targeting Phospholipase C

Phospholipids found on the cytoplasmic side of the cell membrane include phosphoserine, phosphocholine and phosphoinositides. In principle, these phospholipids all participate in signalling; here we concentrate on phosphatidylinositides. In phosphatidylinositides, two glycerol hydroxyl groups are ester bound with two fatty acids and the third hydroxyl with a phosphoinositol. This phosphatidylinositol can be phosphorylated by PI kinases, including PI-3 kinase, PI-4 kinase and PI-5 kinase. It can also be cleaved by phospholipases. Important for GPCR signalling is phospholipase C (PLC), which

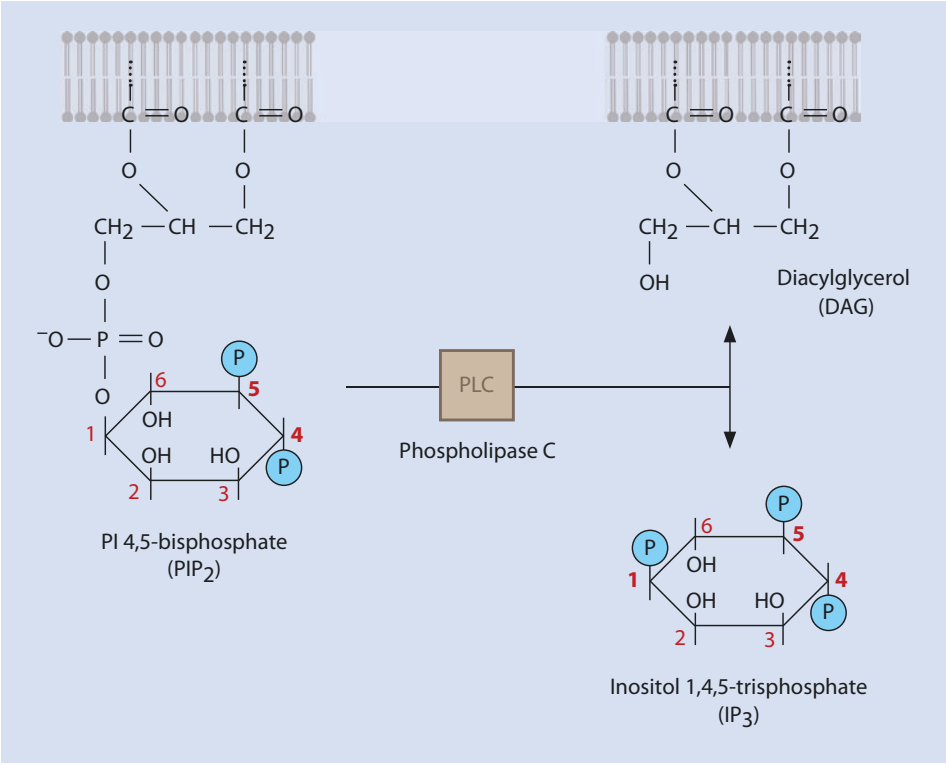

Fig. 3.7 Chemical structure of phoshoinositol 4,5-biphosphate. Phospholipase C hydrolysis of phosphoester bond produces diacylglycerol (DAG) remaining in the membrane and inositol 1,2,5-triphosphate (IP3)

hydrolyses the phosphate ester bond, thus releasing inositol (1,4,5)-triphosphate (IP3). This leaves diacylglycerol (DAG) leftover in the membrane, which serves as second messenger (see Fig. 3.7). IP3 is released into the cytoplasm and binds to IP3-gated Ca^{2+}-channels at the membrane of the endoplasmatic reticulum. These Ca^{2+} signals regulate many cellular responses including proliferation and differentiation processes that can be activated by *p*rotein *k*inase *C* (*PKC*). PKC is itself activated by a combination of second messengers, including Ca^{2+}, phosphatidylserine and DAG. The DAG-binding site can also be triggered by phorbol esters mimicking DAG, e.g. phorbol-12-myristat-13-acetat. This compound has well-documented tumour-promoting properties (Blumberg 1988). Ca^{2+}-signals from the ER also play a role in the regulation of ion channel conductance.

3.5 Signal Termination

GPCR signal termination is a very fast event mediated by receptor phosphorylation and GTP hydrolysis. GPCRs can be phosphorylated at several positions in the intracellular C-terminal region, e.g. by PKA, providing a negative feedback loop. Several more GPCR kinases phosphorylate ligand-bound receptors. The phosphorylated receptor binds to arrestin – another scaffolding protein. As the name suggests, arrestin binding excludes interaction of the receptor with the G-protein. The G-protein "falls off" and this "arrests" signalling. Arrestin can mediate receptor endocytosis by recruiting the adaptor complex

AP2 and clathrin. As a consequence, the receptor can be degraded by endosomal-lysosomal fusion, or it can be recycled by re-fusion of the endosome with the plasma membrane. Arrestin also recruits members of other signalling pathways, e.g. activators of MAP kinase signalling, thus establishing a cellular memory for single signalling events (reviewed in Gurevich and Gurevich (2015)).

GTP hydrolysis is achieved by the use of the intrinsic GTPase activity of Gα-subunits hydrolysing GTP to GDP. In this state, Gα-subunits reassociate with their Gβ/γ-subunits. RGS-proteins, *r*egulators of *G*-protein **s**ignalling, can function as GTPase activity regulators. Together, arrestin binding and regulation of GTPase activity define the signalling parameters of GPCRs in terms of duration and amplitude of the signalling circuit (see Fig. 3.8).

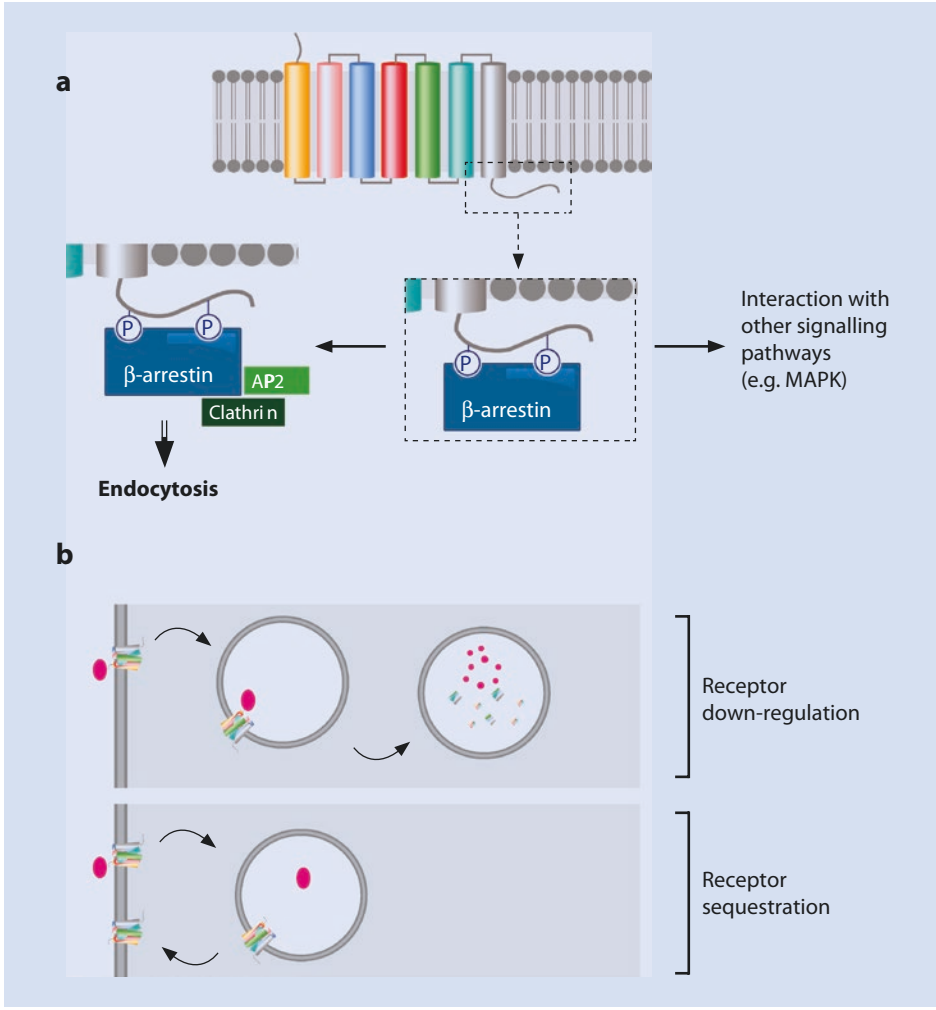

Fig. 3.8 Schematic representation of GPCR-signalling termination showing **a** receptor phosphorylation and arrestin binding. Arrestin activates further signalling or recruits clathrin for receptor endocytosis; **b** endocytosed receptors are recycled (after fusion of the endosome with the plasma membrane) or degraded (after fusion of the endosome with lysoendosomes)

Take-Home Messages

- *7 TM receptors* represent the largest class of transmembrane proteins in animals and are involved in cellular communication by binding of extracellular ligands and transducing this information into the cell. They are also called GPCRs for G-protein-coupled receptors. They function as GEFs for the GDP/GTP binding site of trimeric G-proteins.
- *Trimeric G-proteins* through their Gα-subunits target adenylyl cyclase and phospholipase C leading to the production of second messengers cAMP or IP3 and DAG. Gα and Gβ/γ-subunits additionally target ion channels and the MAP kinase pathway.
- *Second messengers* activate target enzymes including PKA and PKC. cAMP also activates ion channels and regulates cross talk with other signalling pathways.
- *Termination* of GPCR signalling is mediated by arrestin binding and GTP hydrolysis. After arrestin binding, GPCRs are endocytosed and either sorted back to the membrane and reused, or degraded. GTP hydrolysis of Gα leads to reassembly of Gα- and Gβ/γ-subunits for new cycles to start.

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Ion Channels

4.1 Channels and Transporters – 44

4.2 Membrane Potential and Electrochemical Gradient – 46

4.3 Voltage-Gated Ion Channels – 47

4.3.1 Voltage-Gated K^+-Channels – 47

4.3.2 Voltage-Gated Na^+-Channels – 49

4.3.3 Voltage-Gated Ca^{2+}-Channels – 50

4.3.4 Neuromuscular Junction – 51

4.3.5 VOCs and Neurotransmitter Release – 52

4.4 Extracellular Ligand-Operated Channels: Neurotransmitter Receptors – 53

4.5 TRP Channels – 54

References – 56

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What You Will Learn in This Chapter

In this chapter we explain the molecular architecture and function of several ion channels. These include voltage and neurotransmitter gated ion channels as well as channels that are gated by binding of second messengers to intracellular sites of ion channels. You will also be briefly introduced to the large family of "transient receptor potential" (TRP) ion channels. To illustrate the function of ion channels in a physiological context we describe excitation-contraction coupling at the neuromuscular junction in more detail.

4

4.1 Channels and Transporters

Biological membranes are lipid bilayers. They are not permeable for charged molecules, even if those are small, like Ca^{2+}, K^+, Na^+ and Cl^--ions. An exchange of ions between the cell interior and the exterior requires dedicated transport molecules. Therefore, eukaryotic cells are equipped with large numbers of transmembrane proteins that function either as transporters or as ion channels.

Transporters actively move ions through membranes against their concentration gradient. This requires energy, e.g. from ATP hydrolysis. Ion channels, on the other hand, allow passive passage of ions along their concentration gradient. They are inserted into membranes in such a way that they build small pores, which expose a hydrophilic surface at their inside. These channels are regulated in several ways, and they exhibit specificity for specific ions. In the most extreme case, they are almost constitutively open. In this situation, the ion concentration on either side of the membrane will adjust towards a biophysical equilibrium (dependent on the concentration of the ions and their charge as well as the given distribution of all other charged molecules across the membrane).

However, the opening and closing of most ion channels occurs in a regulated manner. Such ion channels are "gated" and usually only open in response to a change in membrane voltage (voltage-gated ion channels), in response to binding of a ligand (ligand-gated ion channels) or after physical stimulation (e.g. mechanically gated ion channels) (see ◘ Fig. 4.1).

Ligands for ion channels can be second messengers (e.g. cAMP, Ca^{2+}) or neurotransmitters (e.g. acetylcholine, glutamate). Many plant-derived drugs target ion channels that are involved in neurotransmission, the conductance of signals from the axon of one neuron to a dendrite of another neuron via a chemical synapse. This also includes neurotransmission from motor neurons to muscle cells at neuromuscular junctions. During this process, changes in voltage, or action potentials, from a presynaptic neuron are converted into a chemical signal at the synapse. The presynaptic neuron releases chemical neurotransmitters into the synaptic cleft. These neurotransmitters then bind to receptors at the postsynaptic membrane of dendrites of the next neuron. This directly opens ion channels on the postsynapse and induces ion influx or efflux according to the ion concentrations on either side of the membrane. In this way, the membrane potential of the postsynaptic membrane changes, converting the chemical signal that the postsynaptic membrane had received into an electrical signal that will be further transmitted within the postsynaptic neuron. In such a way, neurotransmitters "gate", meaning open or close, *li*gand-*g*ated *i*on *c*hannels (*LGIC*s or LICs). They directly evoke a very fast electrophysiological response in the postsynaptic membrane. The major neurotransmitters

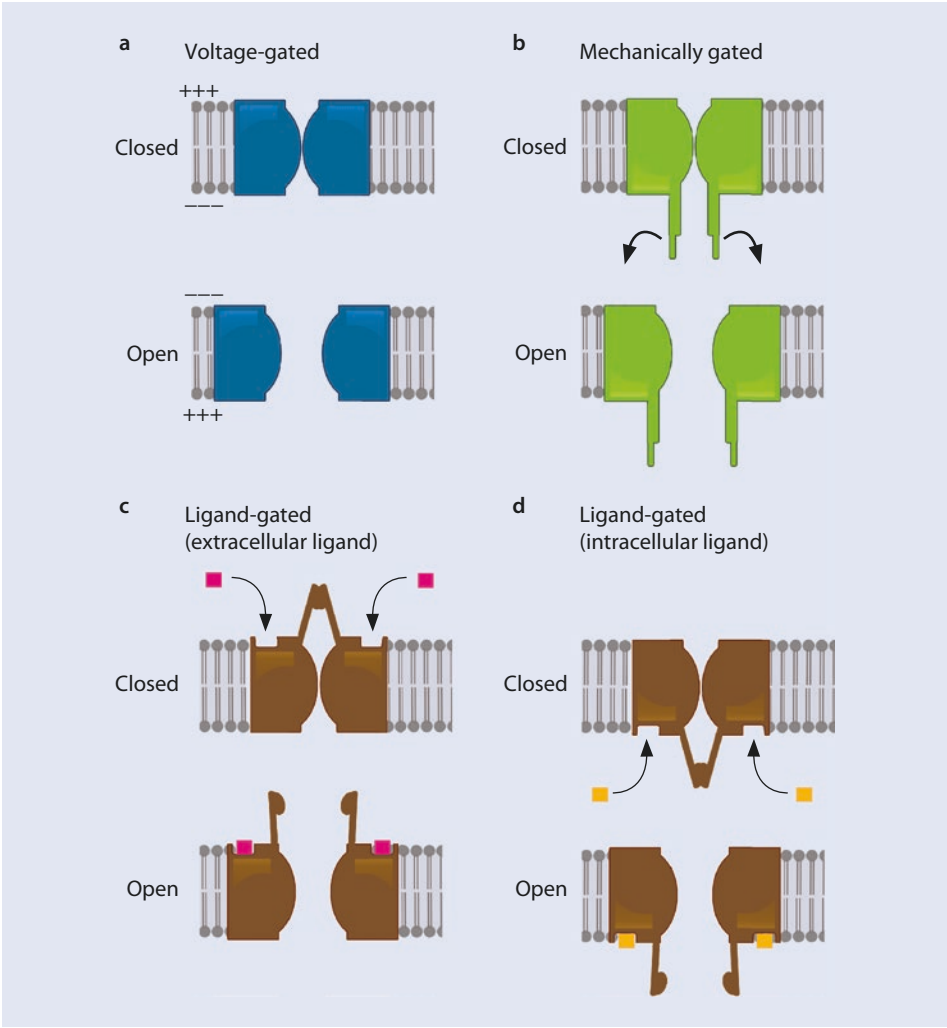


Fig. 4.1 Schematic representation of channel "gating" by **a** voltage, **b** mechanical forces and **c**, **d** ligand binding

in the peripheral nervous system include acetylcholine and noradrenaline. Acetylcholine is also the neurotransmitter at the neuromuscular junction. In the central nervous system, glutamate, γ-aminobutyric acid (GABA) and glycine are the most abundant neurotransmitters.

Alternatively, synaptic release of so-called neuromodulators activates GPCRs. In this case, second messengers are generated inside the cell, and these will then open or close ion channels by interacting with intracellular binding sites on second messenger-activated (SMOCs, channels activated by *s*econd *m*essengers *o*ther than *c*yclic nucleotides) or *c*yclic *n*ucleotide-*a*ctivated ion *c*hannels (CNGCs). GPCR signalling activated by neuromodulators is slower than direct neurotransmitter gating through LGICs, and it additionally

evokes transcriptional, metabolic or behavioural responses in target cells. GPCRs activated by neuromodulators are often also found in presynaptic membranes (Civelli 2012). Interestingly, transmitter molecules may act on LGICs and also on GPCRs as we will see later, for instance, for GABA, glutamate, acetylcholine and serotonin.

Most neurotransmitters and neuromodulators are stored in vesicles at presynaptic axon terminals. Many of these vesicles are already tethered to the membrane and ready to be released. The amount of neurotransmitter available for binding to receptors on the postsynaptic membrane depends on:

1. The frequency of arriving action potentials leading to their release.
2. The retrieval of the neurotransmitter back into the presynaptic neuron.
3. The inclusion of neurotransmitters into neuronal storage granules.
4. The degradation of the neurotransmitter within the synaptic cleft or in the cytoplasm of the neuron.

Vesicle fusion and transmitter release occur after the action potential arrives. A voltage-dependent Ca^{2+}-channel (see later) opens, and Ca^{2+} flows into the cytoplasm. Ca^{2+} -ions provide the trigger for the excitatory machinery to release the content of neurotransmitter vesicles. Most neurotransmitters and neuromodulators will be retrieved after their release. For this task, presynaptic membranes have specific transporters, e.g. the *d*op*a*mine *t*ransporter *DAT*. Cytoplasmic neurotransmitters are transported into storage granules by specific symporters, e.g. the *v*esicular *m*onoamine *t*ransporter *VMAT*. Degradation of synaptic transmitters occurs through enzymes that are secreted into the synaptic cleft. One example is the acetylcholine esterase, which cleaves the neurotransmitter acetylcholine into acetate and choline.

4.2 Membrane Potential and Electrochemical Gradient

The basis for the function of voltage-gated ion channels is the electrochemical gradient across the plasma membrane. Such a gradient exists in all cells of the body, in muscle and gland cells as well as in excitable cells, like nerve cells, where it is used for electrochemical signalling, e.g. the generation of action potentials. The electrochemical gradient is due to differences in the concentration of Na^+, K^+ and Ca^{2+} ions between the cytoplasm and the extracellular space. These differences are maintained in all cells. They are generated by ATP-dependent ion pumps, which move ions across membranes against their concentration gradient and use ATP as their energy source.

The Na^+/K^+-pump, also called $3Na^+/2\ K^+$ATPase, is a large transmembrane protein with an intracellular ATP-binding site. Upon binding of ATP, the transporter opens towards the cell interior and binds three Na^+-ions from the cytoplasm. It then changes conformation and opens towards the outside of the cell, where the Na^+-ions are released. This means that Na^+-ions have passed through the membrane against their concentration gradient of 150 mM (outside) versus 12 mM (inside). ATP is hydrolysed and then the pump, now outwardly open, binds two K^+-ions. As a result, the pump changes conformation and opens towards the inside, releasing K^+ into the cell. This process thus also works against the K^+ concentration gradient of 140 mM inside and 4 mM outside the cell (Skou and Esmann 1992). Similarly, ATP-driven Ca^{2+}-pumps keep a low Ca^{2+} concentration in the cytoplasm by constantly pumping it out of the cell, where the concentration of Ca^{2+} is up to more than 10, 000 times higher. In addition, Ca^{2+}-ions are stored in the endoplasmic

reticulum. Here we find ATP-driven Ca^{2+}-pumps, including the sarco−/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) of muscle cells, which pump Ca^{2+}-ions into the lumen of the ER. Digitoxin, a glycoside from *Digitalis purpurea* blocks the $3Na^+/2\ K^+$ATPase in heart muscle cells, which counts for the effects of this drug on heart function.

Ion channels, in contrast to ATP-driven ion pumps, allow passage of ions according to their concentration gradient. This does not require energy. Some K^+-channels are always, at least partially, open, which induces an outward K^+-current, leaving the inside of the membrane negatively charged in the resting state. On the other hand, Na^+-channels are not constitutively open, making the membrane seemingly impermeable for these ions and allowing a high extracellular Na^+-concentration. This is important for the osmotic balance of the cells. Moreover, opening of gated Na^+-channels leads to influx of Na^+-ions upon a stimulus, making the membrane potential more positive. This is called depolarization. Vice versa, opening of gated Cl^--channels allows influx of negatively charged ions (Cl^--concentration is usually higher outside the cell) – resulting in a decrease of the membrane potential, which is called hyperpolarization. Similarly, opening of K^+-channels increases efflux of positive ions and hyperpolarizes the membrane. An interesting class of K^+-channels are G-protein regulated inwardly rectifying channels (GIRK channels, (Luscher and Slesinger 2010)). They open in response to Gβ/γ-subunits of activated trimeric G-proteins and, under certain conditions, show higher inward current of K^+-ions than outward current. This represents a reversal of the expected K^+-ion flow according to the physiological K^+-ion concentrations outside and inside the membrane. It is caused by intracellular Mg^+-ions and polyamines, which occlude the channel pore for outward K^+-currents. This occurs at the equilibrium membrane potential for K^+-ions of −90 mV. At resting membrane potential, opening of GIRKs increases K^+-conductance leading to hyperpolarization. However, when the extracellular concentration of K^+-ions reaches ca. 20 mM, inward current of K^+-ions outweighs outward current (Bichet et al. 2003; Isomoto et al. 1997).

4.3 Voltage-Gated Ion Channels

Voltage-gated ion channels (VOCs) are closed at resting membrane potential and open when the membrane is depolarized. All voltage-gated ion channels have three important structural components.

1. A transmembrane structure harbours a central pore for the ions to pass.
2. A voltage-sensing helix moves in response to changes in the membrane potential. It is part of a larger voltage-sensing domain spanning the membrane four times.
3. A plug-like structure that closes the channel after ions have moved through.

4.3.1 Voltage-Gated K^+-Channels

Voltage-gated K^+-channels are composed of four channel forming α-subunits and accessory β-subunits, the latter not being involved in ion conductance. K^+-channel α-subunits constitute a protein family with 40 members grouped into 12 classes. They are named K_vα1-12 and produce channels with different properties. In Kvα 1-channels, four subunits of 600–700 amino acids' length, each spanning the membrane six times, make up the pore. Within the same subfamilies, α-subunits can homo- or hetero-oligomerize, and therefore the diversity of K_v-channel oligomers that exist in cells is very high. Some family members

(K_v5, 6, 8 and 9 family members) act as silencers when forming hetero-oligomers with ion-conducting channel forming subunits (Gutman et al. 2005). For each α-subunit, the N- and C-termini are localized in the cytoplasm. In the N-terminal region, the inactivation segment is found. It closes the pore after ion passage. Helices S5 and S6 are similar to helices in non-gated K^+-channels, and they interact directly with the passing ion (Sokolova et al. 2001). Helix S4 is the voltage-sensing helix, and S1, S2 and S3 assist in the opening process. Together they constitute the voltage-sensing domain of the channel (reviewed in Labro and Snyders (2012)) (see Fig. 4.2a). Such voltage-sensing domains are not exclu-

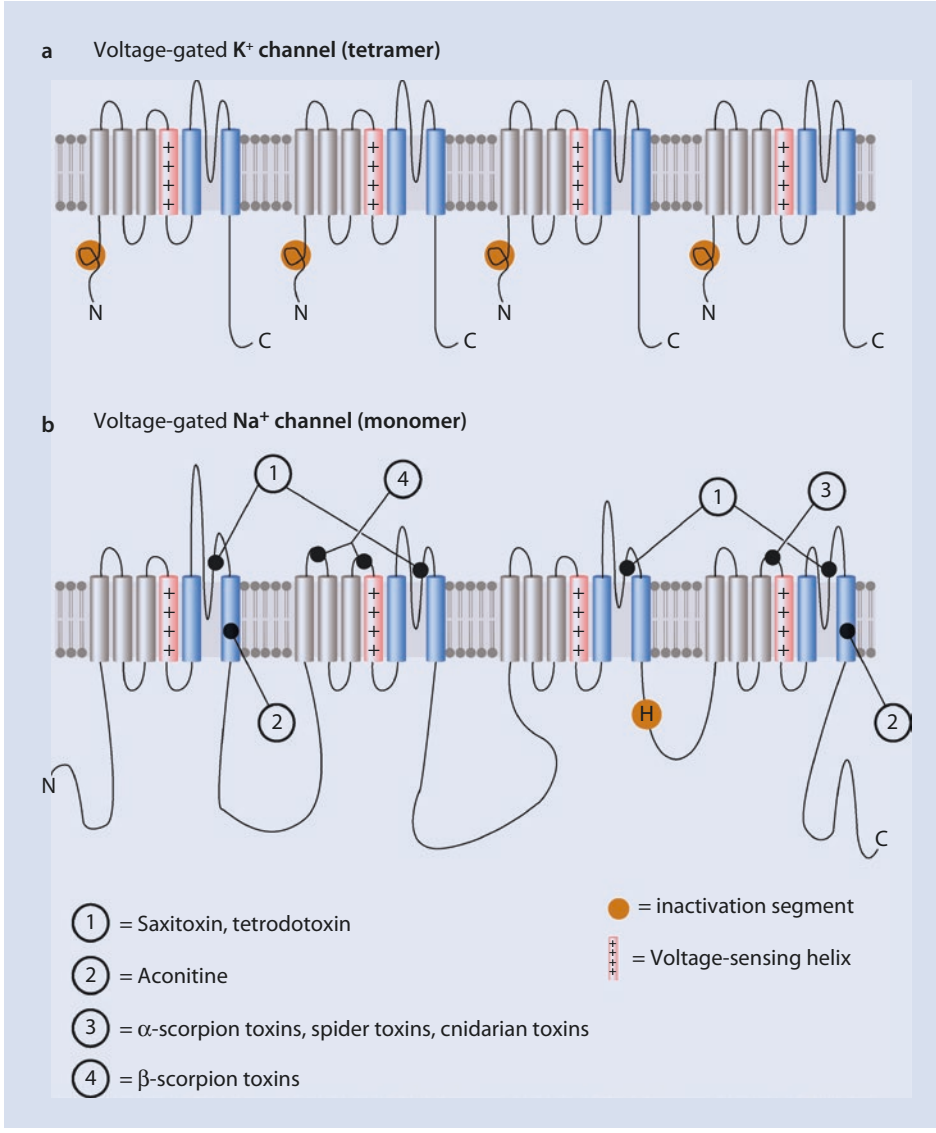


Fig. 4.2 Transmembrane domain structure of voltage-gated K^+ and Na^+-channels indicating the location of voltage-sensing helices and inactivation segment(s). **a** Voltage-gated K^+-channel, **b** voltage-gated Na^+-channel. The binding sites for neurotoxins are indicated. (Modified from Cestele and Catteral (2000))

sive to voltage-gated ion channels, but they have also been found in other proteins that respond to changes in membrane potential, e.g. voltage-sensitive phosphatases. Two recent structural studies have elucidated the movement of helix S4 in response to changes in the membrane potential in two different isolated voltage-sensing domains, one from the *Ciona intestinalis* voltage-sensing phosphatase and one from a voltage-gated potassium channel (Li et al. 2014; Nozaki et al. 2016). In both cases, helix S4, which typically contains positively charged amino acid residues (Lys and Arg), moves towards the exterior side of the membrane in response to depolarization. During movement, it also rotates around its own axis. This change in the position of helix S4 is suggested to induce pore opening in channel proteins.

4.3.2 Voltage-Gated Na^+-Channels

Voltage-gated Na^+-channels are overall similarly structured to voltage-gated K^+-channels. However, instead of being assembled by four independent subunits, they are formed by one large protein that has four repetitive subdomains, which are structurally and functionally equivalent to the monomers of the K^+-channels. Each subdomain is thus composed of six transmembrane helices (S1–S6). Large intracellular loops are formed between the four subdomains. The single inactivation segment for these channels is constituted by a patch of hydrophobic amino acids localized in the third intracellular loop, just behind helix S6 of the third subdomain. The lining of the channels is made by helices S5 and S6 of each subdomain, and their intervening sequences constitute the selectivity pore. These regions show extended sequence similarity with the domains that form the pore in non-gated K^+-channels. Structural investigation of voltage-gated Na^+-channels had been difficult because of the large size of these molecules. Their structure has been deduced by comparison with voltage-gated potassium channels and from functional and mutational analysis of neurotoxin binding, including tetrodotoxin and saxitoxin, which plug the pores of these channels. In this way also the S4 helix has been indicated as voltage sensor in Na^+-channels, and experimental evidence from mutagenesis studies has supported this function (see later) (Catterall 2000). In 2017, a cryo electron microscopic structure of a putative voltage-gated sodium channel from the American cockroach became available (Shen et al. 2017). In this study, the impact of the voltage-dependent conformational change of helix S4 within the voltage-sensing domain on interactions with pore-opening regions of the molecule is demonstrated. They indicate coupling of the voltage-sensing mechanism to the move of pore-forming helices in between adjacent repeats of the channel protein (see Fig. 4.2b).

The proteins described above constitute the α-subunits of Na^+-channels that associate with one or two β-subunits in mammalian cells. The α-subunits are sufficient to form the functional voltage-responsive ion channel, and β-subunits are involved in modulation of channel parameters. In humans, nine Na^+-channel α-subunits are known, named as $Na_v1.1$–$Na_v1.9$ and Na_x. They are expressed abundantly in the central and peripheral nervous system, in dorsal root ganglia, Schwann cells and astrocytes. Some heritable human disorders, so-called channelopathies, are associated with these proteins. These include several kinds of epilepsy, familial hemiplegic migraine, autism spectrum disorder and channelopathy-associated insensitivity to pain. $Na_v1.4$ is specifically expressed in the skeletal muscle, and mutations in this gene are connected with potassium-aggravated myotonia. $Na_v1.5$ is, among other organs, expressed in gastrointestinal smooth muscle cells and associated with irritable bowel syndrome (Huang et al. 2017).

4.3.3 Voltage-Gated Ca^{2+}-Channels

Voltage-gated Ca^{2+}-channels include the CaV1-L-type channels that provide long-lasting Ca^{2+}- currents in the skeletal muscle, heart, neurons and endocrine cells. CaV2-N-type, P/Q-type and R-type channels are involved in synaptic transmission in the nervous system. CaV3 T-type channels have a transient kinetics and include pacemaker channels in sinoatrial nodes as well as channels involved in stimulus-secretion coupling in gland cells, neuronal rhythmicity and the sperm acrosome reaction (Berridge 2012). VOC channels open upon membrane depolarization, resulting in a cytosolic Ca^{2+}-spike. Immediately after Ca^{2+}-entry, the channel is inactivated, ensuring that no more Ca^{2+} can get in and the spike diffuses. After recovery from inactivation, the channel is ready for another opening.

The structure of voltage-gated Ca^{2+}-channels is similar to the structure of voltage-gated Na^{+}-channels described above (see Fig. 4.3a). They consist of multimeric complexes with the channel forming α-subunits surrounded by several accessory subunits involved in regulating channel activity. These include β-, γ- and δ-subunits (see Fig. 4.3b). The α-subunit consists of four subdomains with six transmembrane helices each and large intracellular loops between them. Helices S1–S4 constitute the voltage sensor, and S5/S6

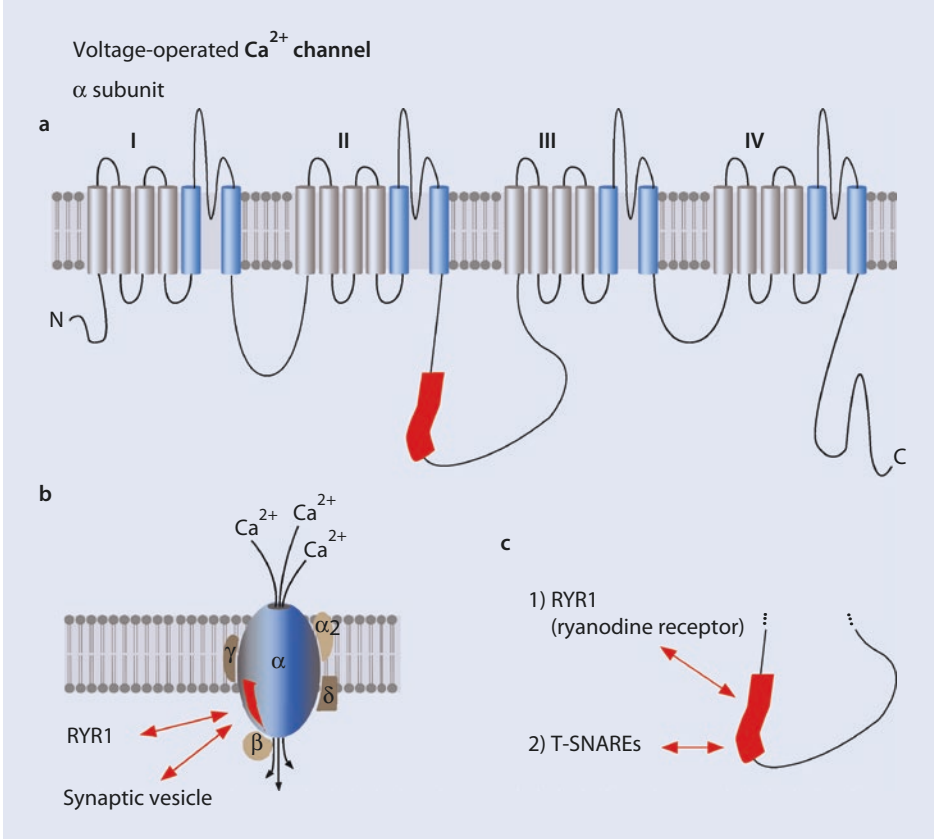


Fig. 4.3 **a** Transmembrane domain structure of voltage-gated Ca^{2+}-channels; **b** subunit composition of functional channel composed of the pore-forming α-subunit and accessory α2, β, γ and δ-subunits. (Modified from Berridge (2012)); **c** interaction domains for SNAREs and ryanodine are indicated

forms the conductance pore and the selectivity filter. The cytoplasmic loops have binding sites for downstream effectors. For example, L-type channels on skeletal muscle cells interact directly with the ryanodine receptors RYR1 through a specific amino acid motif in their cytoplasmic loops between subdomains two and three of the α-subunit. In this way, a depolarization signal at the muscle cell membrane at the neuromuscular junction triggers Ca^{2+}-release from the sarcoplasmic reticulum for muscle contraction. Moreover, neuronal N-type or P/Q-type channels interact directly with SNARE proteins of the exocytosis complex at presynaptic nerve endings. This allows fast relay of action potentials to vesicle fusion and neurotransmitter release (see Fig. 4.3c).

The activity of VOC channels can furthermore be regulated by many signalling molecules, including PKA, Gβ/γ-subunits and Ca^{2+}-ions (Berridge 2012).

4.3.4 Neuromuscular Junction

Ca^{2+}-entry channels on excitable cells are best known from their involvement in regulating muscle contraction and neurotransmitter release. As an example to illustrate the physiological cooperation of different ion channels during synaptic transmission, the neuromuscular junction is described. At this synapse, motor neurons projecting from the spinal cord make contact with muscle fibres to activate muscle contraction. For this purpose, two Ca^{2+}-channels co-operate in the muscle cell, ryanodine receptors (RYR) on the membrane of the sarcoplasmic reticulum (the muscle cell ER) and L-type voltage-gated Ca^{2+}-channels on the plasma membrane of the muscle cell (see Fig. 4.4).

The neurotransmitter conveying the action potential of the excited motor neuron to the postsynaptic membrane of the muscle cell is acetylcholine. Acetylcholine "gates" the *n*icotinic *a*cetyl*ch*oline *r*eceptor (NAchR), an ion channel on the muscle cell. Acetylcholine binding leads to Na^+ and Ca^{2+}-influx into the muscle cell, membrane depolarization and opening of voltage-gated Na^+-channels. This further depolarizes the membrane and eventually results in opening of high voltage-activated L-type Ca^{2+}-channels on the plasma membrane of muscle fibres (L-VOCs). The L-type VOCs can be inhibited with dihydropyridine, a synthetic Ca^{2+}-channel blocker that is pharmacologically used to treat hypertension.

Ca^{2+} is an essential component for muscle contraction. A muscle contracts when actin fibres are moved by myosin, the actin motor protein. In the resting state, actin fibres in skeletal and heart muscle cells are covered with tropomyosin, which is bound to the Ca^{2+}-binding protein troponin. In the Ca^{2+}-free state, tropomyosin filaments therefore block the myosin-binding sites on the actin filaments. When Ca^{2+}-ions are released from the sarcoplasmic reticulum, they bind to troponin allowing tropomyosin filaments to move away from the myosin-binding sites. Myosin is now able to contact and move the actin filaments and the muscle contracts. The responsible Ca^{2+}-channels are Ca^{2+}-gated ryanodine (RYR) receptors on the SR membrane. They open in response to the Ca^{2+}-signal that is created after excitation of the muscle cell (see Fig. 4.4).

RYR receptors have their name because they are sensitive to the plant alkaloid ryanodine, a poison and insecticide from *Ryania speciosa*, a South American member of the *Salicaceae* family. They differ from the previously described plasma membrane voltage-gated Ca^{2+}-channels by their size and their five times higher ion conductance. They are assembled from four monomers, each consisting of ca. 5000 amino acids. This size enables visibility of these receptors in electron microscopy studies. Such images show RYR receptors as electron-dense protrusions in the junctions between transverse tubular invaginations of the plasma membrane and the sarcoplasmic reticulum. Several subtypes are

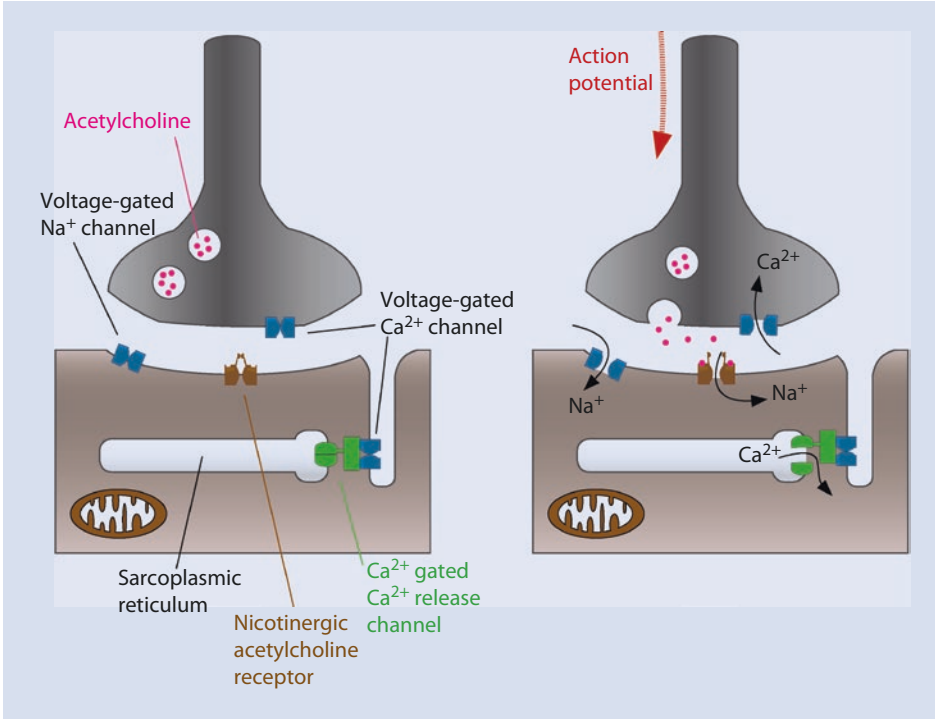


Fig. 4.4 Schematic representation of neuromuscular junction; left-hand panel, resting state; right-hand panel, excited state; upper, presynapses with N-type voltage-gated Ca^{2+}-channels, synaptic vesicles containing acetylcholine; lower, postsynapses (muscle cell membranes) with nicotinic acetylcholine receptor (NAchR); NAchR, acetylcholine; voltage-gated L-type Ca^{2+}-channel, RYR (ryanodine) – channel on sarcoplasmic reticulum membrane

known, including RYR1 on skeletal muscle, RYR2 on the myocardium, and RYR3, which is expressed in many cell types but was first identified in the brain (Zalk et al. 2007). Opening of RYR1 for excitation-contraction coupling in muscle cells is believed to occur by a direct physical interaction of membrane L-VOCs with RYR1 via a RYR site in its second intracellular loop. Ca^{2+}-influx after opening of L-VOC may also contribute, because RYR channels are strongly regulated by Ca^{2+}-ions and open in response to Ca^{2+}-signals. This is well described for RYR2 in the heart. However, high cytoplasmic Ca^{2+}-concentrations can trigger closing of these channels.

There are a number of genetic diseases associated with RYR mutations. These include malignant hypothermia, a deadly rise in body temperature induced in patients with a certain RYR-1 mutation under anesthesia. This mutation corresponds to a mutation in the pig RYR1, which is known to cause porcine stress syndrome. Furthermore, some RYR1 mutations are implicated in congenital myopathies, atypical periodic paralysis and others (Van Petegem 2012).

4.3.5 VOCs and Neurotransmitter Release

The neurotransmitter acetylcholine is stored in synaptic vesicles in the motor neuron and released upon arrival of an action potential. This requires vesicle fusion with the presynaptic membrane and Ca^{2+}-influx mediated by the P/Q-type voltage-gated Ca^{2+}-

channel in the membrane of motor neurons. N-type and R-type VOC channels are similarly built, and all three types are mainly localized in neurons. They have a synprint site (synaptic vesicle interaction region) in the second intracellular loop. It binds SNAREs and thus anchors the channel to the exocytosis machinery involving syntaxin (t-SNARE) and SNAP25 (v-SNARE). Exocytosis is triggered by interaction of the Ca^{2+}-binding protein synaptotagmin with the SNARE complex that is completely preformed and ready to secrete vesicle content into the synaptic cleft. In response to an action potential arriving at the presynapse, the opening of Ca^{2+}-channels provides the necessary Ca^{2+}-sparks. Physical coupling of the channel to the SNARE complex allows fast neurotransmitter release. Mutations in the synprint site of VOCs have been found in patients with human genetic disorders including episodic ataxia and familial hemiplegic migraine (Berridge 2012).

4.4 Extracellular Ligand-Operated Channels: Neurotransmitter Receptors

A large family of ion channels is gated by binding of ligands, e.g. neurotransmitters. This causes either excitatory or inhibitory neurotransmission depending on the ion conductance of the channels. Excitatory receptors are gated by acetylcholine (NAchR), serotonin (5-HT-3-receptor) and glutamate (NMDA, AMPA and kainite receptors) and allow passage of Na^+ and Ca^{2+}-ions into the cell, inducing membrane depolarization. Glycine- and GABA-gated channels are Cl^--channels. Their opening increases the negative charge at the membrane inside the cell, leading to hyperpolarization (as long as the outside concentration of chloride ions is higher than the inside concentration) (see Fig. 4.5).

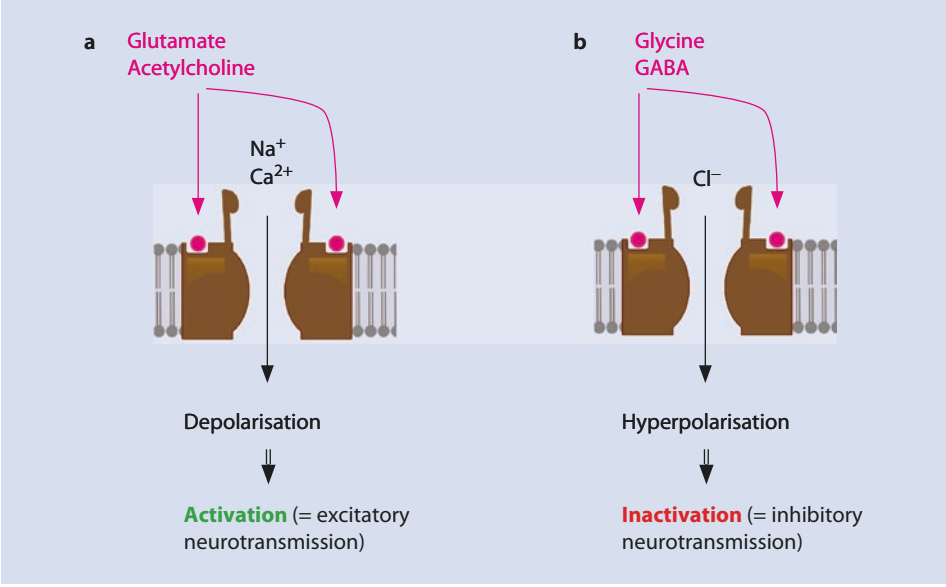


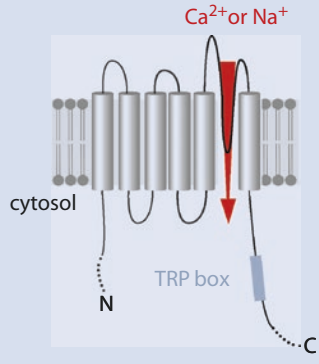
Fig. 4.5 **a** Glutamate-/acetylcholine-gated cation channel; opening induces membrane depolarization and excitatory neurotransmission; **b** glycine-/GABA-gated anion channel; opening induces membrane hyperpolarization and inhibitory neurotransmission

Despite having opposing effects on the membrane potential, the excitatory NAchR and 5-HT3 (serotonin) receptors and the inhibitory glycine and $GABA_A$ receptors belong to the same family of *p*entameric *l*igand-gated *i*on *c*hannels (*pLIC*s). The genes encoding them have evolved from bacterial precursors. However, bacterial pLICs and eukaryotic channels have a different extracellular structure. In eukaryotic channel proteins, the extracellular part is characterized by a highly conserved arrangement of two cysteines forming an intramolecular disulphide bridge. Therefore, they are also called cysteine-loop receptors (Nys et al. 2013).

4.5 TRP Channels

Transient receptor potential ion channels represent a large protein family of channel proteins, encoded by 13 different genes in the worm *Caenorhabditis elegans* and over 30 genes in mammals. They are involved in perception of taste, temperature, pain and mechanical stimuli. The family name is derived from the *Drosophila* TRP channels that mediate visual signalling in fly photoreceptor cells. In contrast to mammalian rhodopsin signalling via activation of transducin and cGMP phosphodiesterase, in flies a G_q protein is coupled to rhodopsin. This activates phospholipase C, which hydrolyses phosphoinositol-4, 5-phosphate PI(4,5)P2. The products DAG and IP3 then, by a still debated biochemical mechanism, lead to opening of TRP channels, which allow Ca^{2+}-influx and membrane depolarization of photoreceptor cells of the fly eye. In a fly mutant with a defect in this TRP channel, the normally stable action potential after light exposure was only "transient"; hence, the name "*t*ransient *r*eceptor *p*otential ion channels" was given.

All TRP channels are permeable for cations, including Na^+, K^+ and Ca^{2+}, with more or less selectivity for the respective ion. They are divided into six subfamilies, including the *c*anonical TRPs (TRP*C*), *v*anilloid TRPs (TRP*V*), *m*elastatin-related TRPs (TRP*M*), polycystins (TRPP), mucolipin TRPs (TRPML) and TRPA, which can sense mechanical stimuli, temperature and pain (Fig. 4.6), (Berridge 2012).



TRPC	(Canonical)
TRPV	(Vanilloid)
TRPM	(Melastatin related)
TRPP	(Polycystins)
TRPML	(Mucolipins)
TRPA	(Mechano, temperature, pain)

Fig. 4.6 Schematic representation of transmembrane domain structure and overview of TRP channel family members; in structure, common TRP box, *light grey*; ion-conducting pore, *red arrow*; diverse N- and C-terminal regions, dotted lines

The function of many of these receptors is not yet known. However, the structure of all members of this family is very similar. They show an arrangement of six transmembrane segments with a pore loop between segments S5 and S6. This is similar to the subdomains of VOCs. In TRP channels, four independent channel proteins oligomerize to form the complete channel. This involves homo-oligomerization but also hetero-oligomerization of members of different subfamilies resulting in a great variety of possible TRP channels. Members of TRPC, TRPV and TRPM families differ in their intracellular N-terminal and C-terminal domains. All contain a so-called TRP box in the intracellular C-terminal part. TRPC and TRPV family members are then characterized by their possession of ankyrin repeats in the intracellular N-terminal region. TRPM family members do not have such ankyrin repeats. Their C-terminal intracellular domains are very large and often have enzymatic domains, e.g. protein kinase or ADP-ribose pyrophosphatase domains (Berridge 2012).

Take-Home Messages

- *Ion channels* allow passage of ions through membranes according to their concentration gradient and without the use of energy. Transporters actively move ions through membranes against their concentration gradient using energy.
- *An electrochemical gradient* exists in all cells of the body. It is maintained by the action of the $3Na^+/2\ K^+$- ATPase establishing an opposite concentration gradient across the membrane for K^+-ions of 140 mM inside versus 4 mM outside the cell and for Na^+ -ions of 12 mM inside versus 150 mM outside the cell. In excitable cells, changes in the membrane potential are used for electrochemical signalling.
- *Voltage-gated ion channels* (VOCs) open or close in response to changes in the membrane potential leading to depolarization or hyperpolarization of the membrane. These channels conduct Na^+-, K^+- or Ca^{2+}-ions, and they harbour a central pore for ion passage, a voltage-sensing helix moving within the membrane according to the membrane potential and a plug that closes the channel after opening.
- For *excitation-contraction coupling* at the neuromuscular junction, Ca^{2+}-entry channels are activated in response to action potentials travelling down the motoneuron. Ca^{2+}-influx into the presynapse allows release of the neurotransmitter acetylcholine (AC). AC binds to NAchRs on the muscle cells, starting membrane depolarization. This finally activates L-type voltage-gated channels on the muscle cell plasma membrane that directly interact with ryanodine receptors on the membrane of the sarcoplasmic reticulum mediating Ca^{2+}release for muscle contraction.
- *Receptor-operated ion channels* are activated by ligands, for instance, neurotransmitters. They include glycine- and GABA-gated Cl^-channels and the nicotinic acetylcholine receptor (NAchR) of the pLIC family and glutamate receptors.
- *TRP channels* represent a large family of channel proteins. They are involved in perception of temperature, taste, pain and mechanical stimuli and conduct cations, including Na^+, K^+ and Ca^{2+}.

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GPCRs as Targets for Plant-Derived Drugs

5.1 Muscarinic Acetylcholine Receptors (MAchR): Muscarine, Atropine and Physostigmine – 58

5.2 Adrenergic Receptors: Reserpine and Ephedrine – 61

5.3 Adenosine Receptors: Caffeine – 64

5.4 Cannabinoid Receptors: Cannabis and Cannabinoids – 68

5.5 Dopamine Receptors: Neuroleptics and Cocaine – 72

5.6 $5HT2_A$ Receptors: Psilocybin, Mescaline and Dimethyltryptamine – 74

5.7 Opioid Receptors: Morphine, Heroin and Salvinorin A – 76

References – 80

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What You Will Learn in This Chapter

In this chapter we will describe the physiological and psychological activities of some prominent plant derived compounds that target components of GPCR-signalling pathways. These include ephedrine and reserpine, muscarine, atropine and physostigmine, caffeine, cannabis, cocaine, morphin and some hallocinogenic drugs.

5.1 Muscarinic Acetylcholine Receptors (MAchR): Muscarine, Atropine and Physostigmine

Acetylcholine is, besides noradrenalin, the major neurotransmitter in the parasympathetic and sympathetic nervous systems. It is discharged at all preganglionic synapses and most postganglionic nerve endings of the parasympathetic nervous system; see ◘ Fig. 5.1. Sympathetic postganglionic neurons connected to perspiratory glands also use acetylcholine. Moreover, it is the neurotransmitter at all neuromuscular junctions connecting neurons with skeletal muscle cells. In the brain, acetylcholine is used in the cholinergic inhibitory neurotransmitter system and as a neuromodulator for neuronal pathways governing plasticity, sensory perceptions upon waking up, sustained attention, promotion of REM sleep and memory.

Acetylcholine activates two different kinds of receptors, GPCRs, agonized by muscarine and called muscarinic acetylcholine receptors (MAchR), and ion channels, agonized by nicotine and called nicotinic acetylcholine receptors (NAchR). In ganglia of both, parasympathetic and sympathetic nerve endings nicotinic receptors are found. These will be discussed later. Postganglionic parasympathetic neurons use muscarinic receptors for neurotransmission. Substances mimicking the action of acetylcholine on postganglionic parasympathetic neurons are therefore parasympathicomimetics. These include the mushroom toxin muscarine after which the receptor is named (see ► Chap. 3). Substances blocking the action of acetylcholine are parasympatholytic. The plant tropane-alkaloid atropine is the most prominent example (see ► Chap. 3).

Moreover, MAchRs are expressed in different brain areas, and their signalling is involved in neuronal networks for processes of attention, learning and memory.

There are five isoforms of MAchRs. Of those, M1, M3 and M5 are coupled to $G_{q/11}$ and phospholipase C (PLC). Thus, their activation induces Ca^{2+} efflux from the endoplasmic reticulum and activation of protein kinase C (PKC). M2 and M4 are coupled to G_i thus inhibiting adenylyl cyclase. The $G_i\beta/\gamma$-subunits activate GIRK channels (inwardly rectifying K^+-channels) and induce hyperpolarization. M2 is localized at the heart and is important for control of heartbeat and rhythm. In pacemaker cells the "funny current" or pacemaker current, which is a result of the opening of "*h*yperpolarization-activated *c*yclic *n*ucleotide-gated cation channels" (HCN), is inhibited by the acetylcholine signalling-induced decrease in cAMP. The opening of GIRK channels makes it harder for the pacemaker cells to be depolarized. Physiologically this is important for maintenance of the regular heartbeat (Kruse et al. 2014); see ◘ Fig. 5.2.

Many synthetic compounds have been developed to block MAchR signalling, e.g. benztropine (trade name Cogentin), which is used to treat symptoms of Parkinson's disease, and so-called long-acting muscarinic receptor antagonists LAMAs like aclidinium, gly-

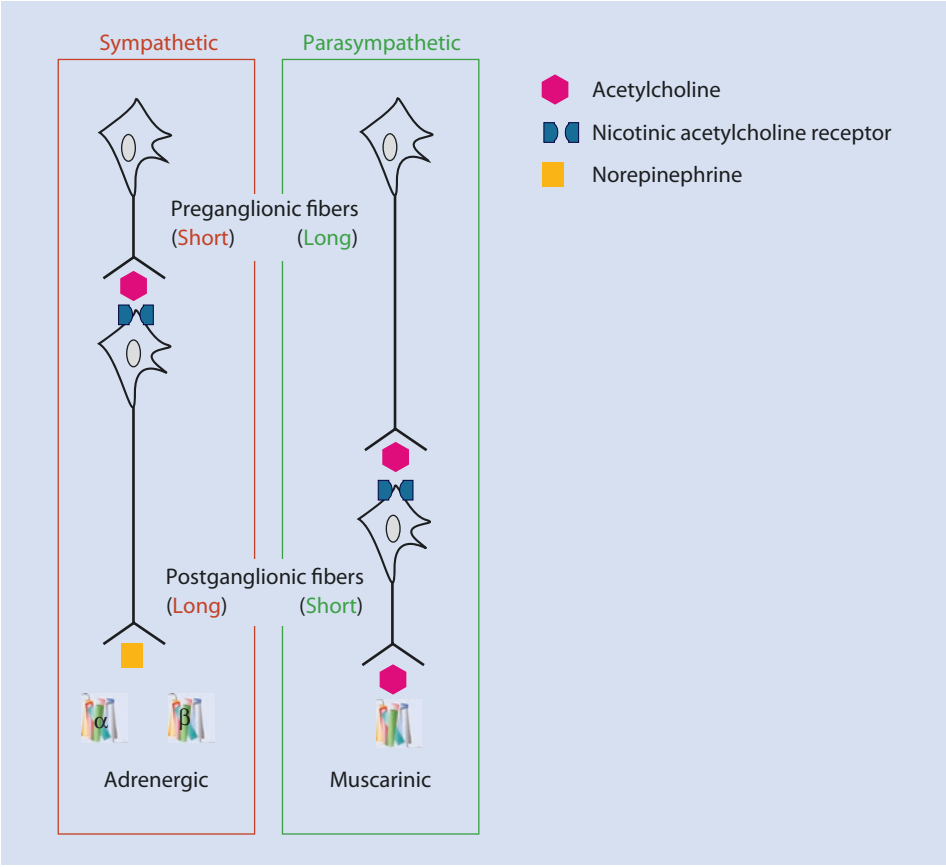

Fig. 5.1 Schematic representation of sympathetic and parasympathetic nervous system with muscarinic and nicotinic acetylcholine receptors and α- and β-adrenergic receptors, acetylcholine and norepinephrine (noradrenaline) from preganglionic and postganglionic fibres as indicated

copyrronium and tiotropium bromide, which have been approved for treating chronic airway disease in Europe. The latter work on M1, M2 and M3 receptors that are present in epithelial cells, inflammatory cells, submucosal gland cells, smooth muscle cells and neurons of tissues in the bronchial tree (Alagha et al. 2014). In addition, muscarinic MAchRs are targets for designing subtype-specific drugs affecting the brain. The receptor subtypes are expressed in different areas; M1, M2 and M4 especially in the cortex and M1, M2, M3 and M4 in the hippocampus. M5 receptors are prominent in the striatum and the ventral tegmental area (Thiele 2013). All receptors are expressed post- and presynaptically. M2/M4 activation on postsynaptic membranes leads to the opening of GIRK channels and the closure of high-voltage-gated Ca^{2+}-channels (including P/Q-, L- and N-type), thus mediating membrane hyperpolarization. On presynaptic membranes, it predominantly closes high-voltage-gated Ca^{2+}-channels and thus decreases neurotransmitter release and

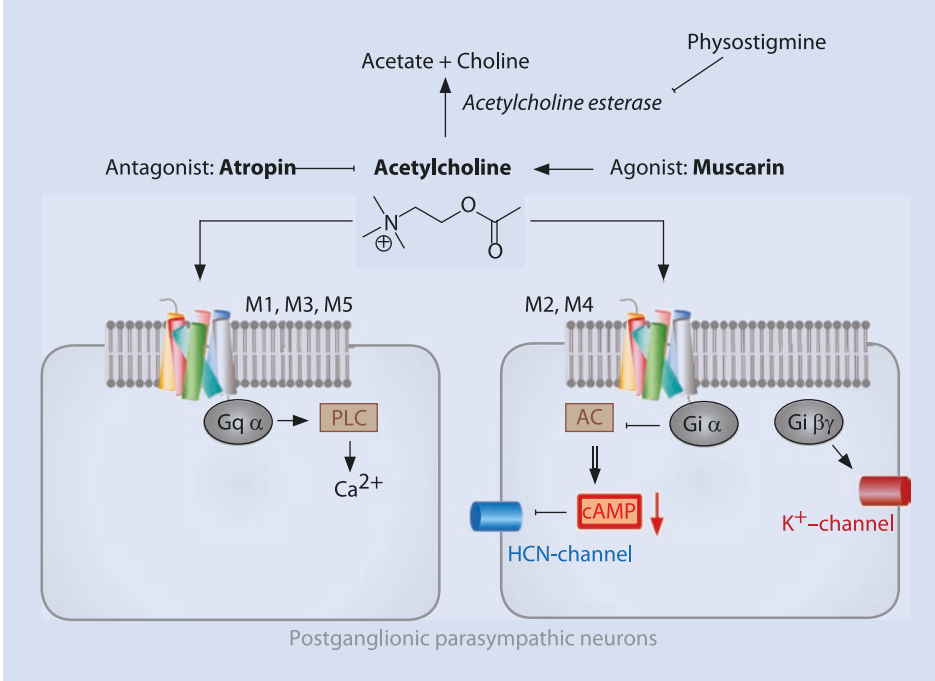

Fig. 5.2 Schematic representation of intracellular signalling pathways at M1, M3 and M5 muscarinic acetylcholine receptors and M2, M4 muscarinic acetylcholine receptors; receptor antagonist atropine, the receptor agonist muscarin and the acetylcholine esterase inhibitor physostigmine are indicated

excitability of synaptic membranes. M1, M3 and M5 receptors also inhibit Ca^{2+}-channels at presynapses; however, at the postsynaptic membrane, they modulate several ion channels via the generation of intracellular Ca^{2+}-signals and the activation of the phospholipid pathway. This leads to closure of several types of K^+-channels and to membrane depolarization (Thiele 2013). Hence, muscarinic acetylcholine signalling in the central nervous system contributes to information processing in different neuronal circuits in a complex manner.

For instance, special attention was given to M1 receptors, by developing specific positive allosteric regulators that have the potential to weaken the cognitive symptoms of Alzheimer's disease. M1 receptor knockout mice show an age-dependent cognitive decline, and mouse models for Alzheimer's disease seem to benefit from such compounds (Melancon et al. 2013). A similar idea is applied to schizophrenia, where M1 and M4 receptor agonists with antipsychotic activity are being developed (Shekhar et al. 2008). The recently solved structures of M2 and M3 receptors have shown that the ligand-binding sites for receptors of both types are deeply buried inside the membrane and that the amino acids directly engaging into ligand binding are almost the same. Therefore, the design of subtype-selective muscarinic receptor ligands might be difficult, and pharmacological research is concentrating on developing allosteric interactors; see ▶ Box 5.1 (Kruse et al. 2013).

Box 5.1 Allosteric Regulator
Allosteric regulators are compounds that target an enzyme or receptor at a site outside the substrate- or ligand-binding pocket. In this way they regulate the activity of the target molecule indirectly. Positive allosteric regulators increase the activity or ligand binding, and negative allosteric regulators diminish it.

Acetylcholine is degraded by acetylcholine esterase. Inhibitors of acetylcholine esterase thus prolong and increase the synaptic transmission by acetylcholine. Physostigmine, an indole alkaloid of the Calabar bean (*Physostigma venenosum*), is a reversible acetylcholine esterase inhibitor. It mainly targets the acetylcholine in postganglionic parasympathetic neurons and thus indirectly acts like muscarine as a parasympathomimetic. Therefore, it can be used to counteract atropine poisoning (Nickalls and Nickalls 1988). There are a number of other plant-derived acetylcholine esterase inhibitors, including galanthamine, which has recently been approved for use in treatment of the symptoms of Alzheimer's disease. Infamously, chemical weapons, such as tabun and sarin, are synthetic cholinesterase inhibitors, which act in a quasi-irreversible fashion. Moreover, a number of acetylcholine esterase inhibitors have been used as pesticides, including organophosphates.

The acetylcholine receptor agonist muscarine is, in contrast to acetylcholine, not degradable by acetylcholine esterase. Therefore, it has a strong and lasting effect, whereby the main symptoms of muscarine poisoning (from mushrooms including *Clitocybe* and *Inocybe* species) concern the parasympathetic nervous system. They include strong contraction of the pupils, heavy perspiration, nausea and others. Muscarine is also neurotoxic, and it can lead to heart paralysis due to the effects on M2 receptors in pacemaker cells as described above. Furthermore, the pyridine alkaloid arecoline is another acetylcholine receptor agonist. Its structure is based on nicotinic acid and it is not specific for MAchRs but also binds to the NAchR.

Atropine, the alkaloid from *Atropa belladonna*, *Datura stramonium* and *Mandragora officinarum*, is an antagonist of MAchRs and is therefore a parasympathicolyticum. This makes it an antidote of muscarine and acetylcholine esterase inhibitors, including the chemicals used in warfare and pesticides mentioned above. Therefore, it is always part of medical emergency gear. Medically, it is used to increase the heart rate in some conditions, dilate pupils, reduce salivary gland secretion (for instance during surgery) and relax intestines. Obviously, agonists of the MAchRs and inhibitors of acetylcholine esterase counteract atropine poisoning. Further plant-derived antagonists of MAchRs include the tropane alkaloids scopolamine and hyoscyamine.

5.2 Adrenergic Receptors: Reserpine and Ephedrine

Adrenergic receptors were the first to be described as GPCRs (see ▶ Chap. 1). Especially the β2-adrenoreceptor has served as a model for the pharmacology of GPCRs. It was the first to be cloned and also the first one to reveal the molecular structure of GPCRs (Dixon et al. 1986; Rasmussen et al. 2007). Adrenergic receptors are present on target organs of the sympathetic nervous system (see ◘ Fig. 5.1). These include smooth muscles of blood vessels and bronchia, the heart, and organs such as the urinary bladder, gastrointestinal

tract, skin, kidney, and brain. They are divided into α- and β-groups, whereby α1, α2, β1, β2 and β3 subgroups are distinguished. A1 receptors are coupled to Gq and α2 receptors are coupled to Gi-proteins. In contrast, all subtypes of β-adrenergic receptors are coupled to Gs, therefore stimulating the adenylyl cyclase activity and increasing the cellular cAMP concentration.

Endogenous ligands of all adrenergic receptors include adrenaline (epinephrine) and noradrenaline (norepinephrine), two hormones released by the adrenal gland in response to acute stress. Both are also produced in certain neurons and function as neurotransmitters, whereby here noradrenaline has a more central position. Chemically they are catecholamines (see ► Box 3.1). They are biogenic amines and are produced from tyrosine, like serotonin and dopamine. One naturally occurring alkaloid that is present in lower fungi is ergotamine. It binds to all catecholamine receptors with high affinity (Zajdel et al. 2015).

Both adrenaline and noradrenaline bind α-receptors with high affinity. At smooth muscle synapses, α1-receptor activation leads to increase of Ca^{2+} ions and therefore muscle contraction. Activation of presynaptic α2-receptors induces negative feedback via Gi-proteins and inhibits muscle contraction. Synthetic α1-receptor antagonists include prazosin (Pfizer trade name Minipress), which is used to treat high blood pressure. The indole alkaloid yohimbine from the West African jungle tree *Corynanthe yohimbe* is a selective α2-receptor antagonist. Therefore, it enhances transmitter release from presynapses without affecting α1-receptors, thus acting as a sympathomimetic (Shannon and Neuman 2000).

B1-receptors are present in the heart where their activation increases power and frequency of heartbeat. B1 signalling leads to increase in cAMP and activation of protein kinase A, which phosphorylates Ca^{2+}-channels of the L-type. This increases Ca^{2+}-influx and enhances release of Ca^{2+} from ryanodine receptors of the sarcoplasmic reticulum resulting in stronger muscle contraction. In addition, G_s-activation by stimulating β1-receptors leads to direct opening of Ca^{2+}-channels in pacemaker cells and in this way increases heart rate.

B2-adrenergic receptors are expressed in smooth muscle cells of the blood and bronchial vessels. The latter are activated by adrenaline from the bloodstream; few are activated by synaptic noradrenaline. This leads to an increase in cAMP via Gs and adenylyl cyclase stimulation. cAMP blocks *m*yosin *l*ight *c*hain *k*inase (*MLCK*). In contrast to striatal muscle, where Ca^{2+}-mediated release of troponin from actin-binding sites on the myosin molecules induces contraction, in smooth muscle cells, MLCK phosphorylates myosin light chains, and this allows myosin to contact actin fibres for contraction. Therefore, β1-receptor activation, by inhibiting MLCK, leads to smooth muscle relaxation on vessels and vessel dilation as well as bronchodilation. The synthetic compound propranolol blocks both types of β-receptors. It is used to treat hypertension; however, a new generation of drugs is more selective for β1-receptors, and these include atenolol and bisoprolol (Fergus et al. 2015; Ogrodowczyk et al. 2016). The natural compound higenamine known for use in traditional Chinese medicine has partial agonistic activity on β-receptors, its pharmacology has recently been reviewed and it seems that the molecular basis for the effects of higenamine is still not completely understood (Zhang et al. 2017). Since 2017, it is also on the list of forbidden substances of the World Anti-Doping Agency.

The hormonal action of adrenaline and noradrenaline after release from the adrenal gland in the presence of strong stress stimuli induces the so-called fight-or-flight response.

This involves increase of glucose release into the blood, degradation of glycogen in the liver and in muscle cells, release of free fatty acids into the blood, increase in blood pressure and heart rate and partial dilation of smooth muscles, especially in the intestines (Catterall 2015). For instance, β-receptor activation and concomitantly activated PKA mediate phosphorylation of glycogen synthase. Phosphorylated glycogen synthase is inactive; therefore, glycogen synthesis is stopped. At the same time, PKA phosphorylates the enzyme phosphorylase kinase, thereby activating it. This enzyme then phosphorylates glycogen phosphorylase, which activates glycogen hydrolysis and glucose-1-phosphate is released. In this way, the availability of glucose in the cell is increased in response to adrenergic receptor activation. Dilation of vessels in skeletal muscles allows better blood supply to muscles. This is also promoted by increased heart rates. In the brain, CREB can be activated by PKA activation, and this is important for long-term potentiation (see ◘ Fig. 3.1). In summary, release of adrenaline and noradrenaline from the adrenal gland induces a complex reaction of the organism to enable it to deal with acute stress.

Noradrenaline as a neurotransmitter is stored in neuronal granules prior to release into the synaptic cleft. It is degraded after release by catechol-O-methyltransferases. Moreover, a *nor*epinephrine *t*ransporter (*NET*, SLC6A2) mediates reuptake of noradrenaline into neurons. This 12-transmembrane domain molecule is dependent on Na^+ and Cl^- ions and belongs to the large family of solute carrier proteins. NET is exclusively expressed in noradrenergic neurons, but it can also transport dopamine back into the cell. Its molecular function and structure are highly related to dopamine transporters (DAT, see ► Sect. 5.5). Such catecholamine transporters regulate the neurotransmitter concentration in the synaptic cleft and thus their availability for signal progression. Drugs targeting NET include the synthetic imipramine, which is used as an antidepressant. It increases noradrenaline signalling in the brainstem in areas involved in regulating sleep-wake rhythm, motivation and focusing attention and alertness.

Another natural compound with activity on adrenergic receptors is ephedrine. It was shown that the four different isoforms of ephedrine (which are the result of the two chiral centres in the molecule) bind to different isoforms of α- and β-adrenergic receptors (Ma et al. 2007; Vansal and Feller 1999). However, pharmacological studies have also suggested that their major targets are norepinephrine transporters (Kobayashi et al. 2003; Rothman et al. 2003). In any case, ephedrines are considered to act as sympathomimetics.

Inside the cytoplasm, neurotransmitters have to be brought into neuronal granules from where they are released into the synaptic cleft in response to excitation. Outside such granules, they will be degraded via oxidation by *m*ono*a*mino*o*xidases (*MAO*). These enzymes catalyse desamination of catecholamine neurotransmitters using H_2O and oxygen. They are located at the outer mitochondrial membrane. Inhibitors of MAO A, which is the main isoform implicated in neurotransmitter degradation, can have antidepressant activity. The synthetic MAO inhibitors iproniazid and moclobemide are examples. The indole alkaloid harmaline is a plant-derived MAO inhibitor. It is present in lianas, especially in the Amazonas Region, and is an ingredient of the hallucinogenic plant mixtures from *Banisteriopsis* known as Ayahuasca (see ► Sect. 5.6).

The indole alkaloid reserpine, which is found in certain species of *Rauvolfia*, on the other hand, targets the "vesicular transporter for monoamine storage" (VMAT, SLC18a). This is another solute carrier family member. It is a proton/neurotransmitter exchanger, and the isoform VMAT2 mediates accumulation of norepinephrine, epinephrine, dopamine, serotonin and histamine in neuronal granules. An ATP-driven proton pump increases the proton concentration in storage granules resulting in a pH of ca. 5.5.

Proton outward flow synchronously allows neurotransmitter inward flow and leads to accumulation of catecholamines inside neuronal storage vesicles. VMAT2 is expressed in all monoaminergic neurons and does not show selectivity for dopamine, noradrenaline or serotonin. Reserpine binds the vesicular transporter with a very low off rate and blocks neurotransmitter transport (Eiden and Weihe 2011). Therefore, it depletes neurons of catecholamine-containing granules that can be released at the synapse and thus works as a sympatholytic. Reserpine has been used in the past to treat high blood pressure and at higher doses some kinds of psychosis (Shamon and Perez 2009). As side effects, due to depletion of dopamine, depression and symptoms of Parkinson's disease are observed. This has been known for a long time (Lemieux et al. 1956).

5.3 Adenosine Receptors: Caffeine

Caffeine exerts a variety of effects in vertebrate as well as invertebrate species. Most commonly those are associated with sleep regulation, increased alertness and activity including locomotor activity and learning and memory. Some of the effects of caffeine are conserved between invertebrate and vertebrate species.

The effects of caffeine on various cellular targets are concentration dependent; see Fig. 5.3. At high concentration, ca 0.5–10 mM, caffeine blocks phosphodiesterases and thus the degradation of cAMP. Its inhibition of phosphodiesterases is made responsible

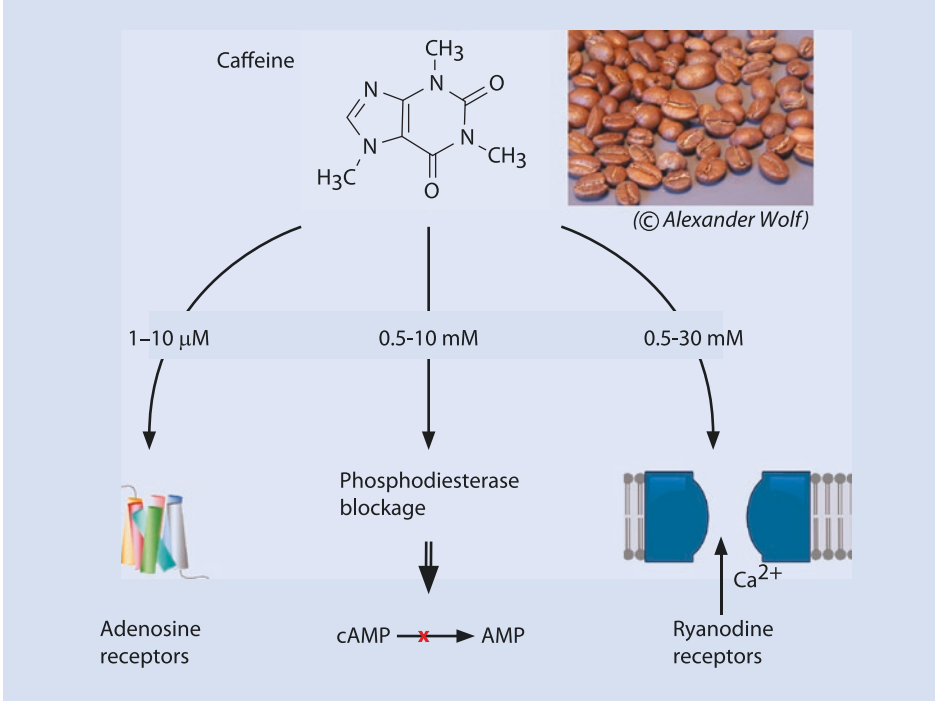

Fig. 5.3 Schematic representation of concentration-dependent activity of caffeine on three cellular targets comprising adenosine receptors, phosphodiesterase and RYR (ryanodine) receptors

for the profound influence that caffeine has on molluscs, various insects and spiders (Mustard 2014). Spiders react with widely irregular spider nets when exposed to caffeine (but also to marijuana, amphetamine and GABA agonists; see ▶ Sect. 7.3 in ▶ Chap. 7, ▶ Box7.1). The reason for this is unclear. However, the expected increase in cAMP levels may have an effect on the spiders' memory. In analogy, as has already been shown 30 years ago, mutations in the *dunce* gene encoding a *Drosophila* phosphodiesterase and thereby also causing high cAMP levels, lead to deficiencies in learning and memory in flies (Davis et al. 1995).

Caffeine was also demonstrated a long time ago to bind to ryanodine receptors in skeletal muscle. Caffeine at concentrations between 0.5 and 30 mM shifted the Ca^{2+}-concentration threshold for channel opening to lower values, making the ryanodine receptor more sensitive (Pessah et al. 1987). Caffeine interactions with the single ryanodine receptor isoform from several invertebrate species have now also been reported (Mustard 2014).

In humans, the main actions of caffeine are attributed to its antagonistic action on adenosine receptors. This is suggested by the low concentration of caffeine between 1 and 10 μM that is needed to inactivate adenosine receptor signalling. In comparison with the effects on phosphodiesterases and ryanodine receptors, this is at least 1000-fold less (Mustard 2014). A metabolic product of caffeine degradation is theophylline, which is also found in plants that produce caffeine, but in lower concentrations. Theophylline, like caffeine, antagonizes adenosine receptors. However, it also has several other clinically relevant targets in cells, e.g. it is an inhibitor of phosphodiesterases (PDE3 and 4) at low concentrations, and it activates histone-deacetylase 2 and inhibits the inflammatory transcription factor NFκB (Barnes 2013).

The endogenous ligand (receptor agonist) for adenosine receptors is adenosine. It is produced in cells from AMP – the degradation product of ATP – by 5'nucleotidase. This reaction is antagonized by adenosine kinase. Extracellular adenosine is produced from ATP, which is secreted as transmitter or co-transmitter and hydrolysed outside the cell. Adenosine transporters let adenosine pass through the membranes of nerve and glia cells, thus establishing the equilibrium between extra- and intracellular adenosine. Under conditions of sufficient energy supply in cells, adenosine kinase activity is high, and therefore the adenosine concentration inside the cell is low, making transporters work inwardly. However, at high-energy demand, when the intracellular ATP levels decrease, activity of adenosine kinase is low, adenosine concentration inside raises and the transporters work outwardly. As a result, more adenosine is found outside the cell and available for activating adenosine receptors. In this way, adenosine receptors signal in an energy-dependent way (◘ Fig. 5.4). Minor changes in steady-state ATP levels in brain cells (normally 5 mM) can therefore lead to relatively major changes in the extracellular adenosine concentration, which is normally low with 30–200 nM (Landolt 2008). Such changes can be induced by hypoxia, exercise, high altitude and exhaustion. Adenosine receptor signalling induces appropriate physiological and psychological responses. These include sleep regulation, the modulation of motor activity through the dopaminergic system and flavour preferences (Huang et al. 2011).

Adenosine also has some psycho-stimulatory effects. These seem to depend on its interaction with the dopaminergic neurotransmitter system (Ferre 2010). Yet, caffeine is neither considered addictive nor classified in any of the schedules of the Controlled Substances Act in US legislation for the Single Convention on Narcotic Drugs of 1961, which is an international treaty for controlling production and supply of specific drugs

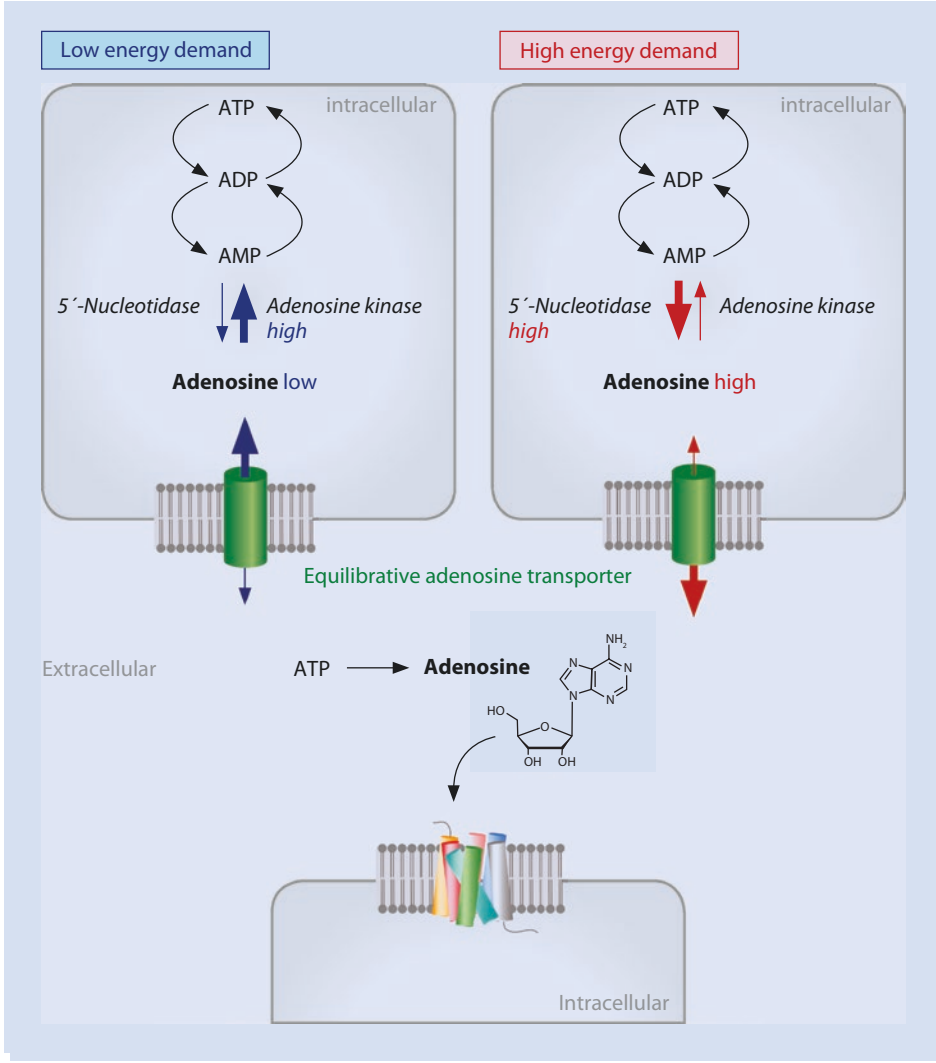

Fig. 5.4 Schematic representation of anabolic and catabolic pathways for adenosine. Direction of the pathways is dependent on cellular energy demand; adenosine concentration is low in cells with high ATP levels (low energy demand), because of high activity of adenosine kinase; equilibrative adenosine transporter is turned into inward direction removing adenosine from extracellular space; adenosine concentration is high in cells with low ATP levels (high cellular energy demand) and turns the equilibrative adenosine transporter into the outward direction. Adenosine accumulation in the extracellular space activates adenosine receptors

(Yeh 2012). A study analysing the activation of brain regions after caffeine consumption shows that caffeine activates brain regions involved in the control of anxiety and vigilance and in cardiovascular regulation. In contrast, brain areas involved in reinforcement and reward were not activated (Nehlig et al. 2010). Nevertheless, symptoms of physical dependence can be experienced after caffeine withdrawal. These include headache, fatigue, decrease in energy and depression of mood (see ▶ Box 5.2).

Box 5.2 Drug Tolerance and Dependence

The US "Controlled Substances Act" regulates drugs, which can be classified within five groups including narcotics, depressants, stimulants, hallucinogens and anabolic steroids. Adverse effects of these drugs include the development of physical and psychological dependence, tolerance, and withdrawal symptoms after cessation of drug intake.

Physical dependence: the body has become adapted to the presence of a drug and responds with physical symptoms when drug intake is stopped.

Tolerance: the effects of the drug are reduced after prolonged intake of a certain amount-to achieve the same effects the dose needs to be increased.

Withdrawal: physical and psychological symptoms appearing when drug intake is abruptly stopped or reduced, these may include increased blood pressure, tremors, confusion, seizures and others, depending on the drug in question.

Psychological dependence: the person is craving for a drug.

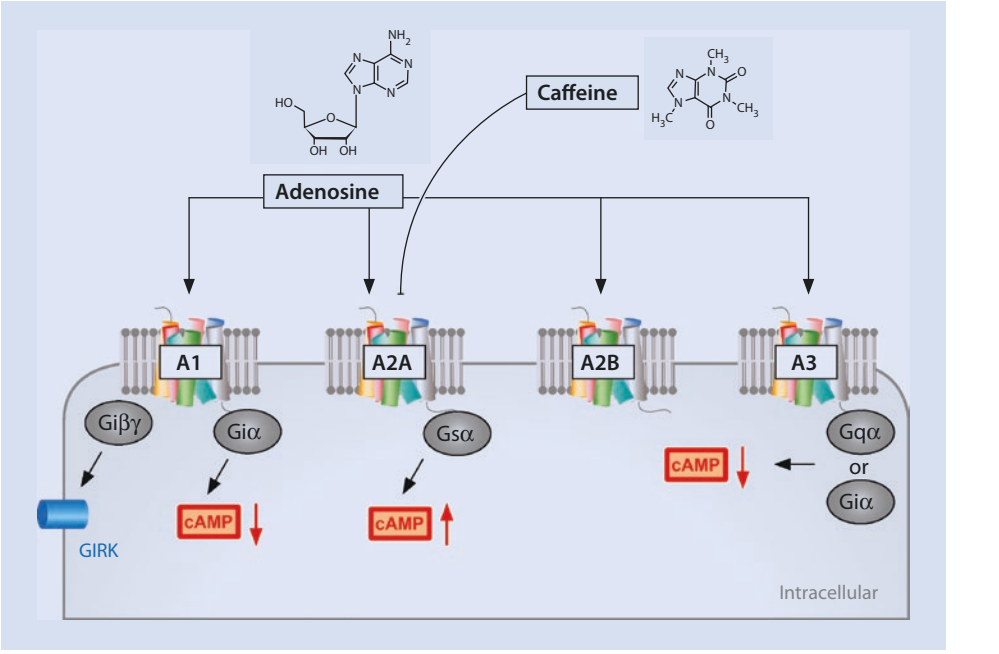

Fig. 5.5 Schematic representation of intracellular signalling pathways at A1, A2A, A2B and A3 adenosine receptors; caffeine mainly blocks A2A-receptor signalling

Several isoforms of adenosine receptors are known, including A_1, A_{2A} and A_{2B} and A_3 receptors. A_1 receptors are coupled with G_{io} thus leading to inhibition of adenylyl cyclase and decrease in cAMP. The $G_i\beta/\gamma$-subunit can be coupled to the activation of GIRK channels, inducing hyperpolarization and a block in neurotransmitter release. A_1 receptors are expressed in the brain cortex, hypothalamus, hippocampus and basal ganglia (Fig. 5.5).

A_{2A} and A_{2B} receptors are coupled to G_s and mediate an increase in cellular cAMP levels. A_{2A} receptors are expressed in the striatum, nucleus accumbens and olfactory bulb of the brain. They co-localize with dopamine receptors in the brain region responsible for motor control. Hetero-oligomers of D_1 and A_{2A} receptors have been reported in

striatal neurons. A_1 and A_{2A} receptors can be tonically activated and are involved in signal transduction for sleep-wake regulation. In addition, pharmacological targeting of these receptors has been implicated in neuroprotection, e.g. after spinal cord injury (Rivera-Oliver and Diaz-Rios 2014).

In mice, A_{2A} receptors have been suggested to be the main target for caffeine. Outside the nervous system, A_{2A} receptors are highly expressed in the spleen, thymus, blood platelets and leucocytes. A_{2A} receptors localized on arteriolar smooth muscle cells mediate vasodilation. In recent years, the A_{2A} receptor has been in the centre of interest for drug development. Adenosine itself is used in clinics to induce coronary artery vasodilation. Regadenoson is the first synthetic A_{2A} receptor agonist approved by the FDA. It is also approved in Switzerland and used as a vasodilator (► www.pharmawiki.ch). Further therapeutic use for A_{2A} receptor agonists is implicated in inflammatory diseases, neuropathic pain and wound healing (de Lera Ruiz et al. 2014). A_{2A} receptor antagonists, conversely, are being tested for treating Parkinson's disease. These are expected to increase motor activity in striatal neurons where adenosine antagonizes dopamine D_2 receptors signalling when they are co-localizing with A_2 receptors.

A_{2B} receptors are widely expressed, but with low abundance, they are the most adenosine-insensitive receptors (only activated by mM concentrations). They are involved in the regulation of hypoxia and inflammation.

A_3 receptors are coupled to G_{io} and G_{q11} – they are expressed at low levels. They are now also considered as a potential therapeutic target because they are up-regulated in rheumatoid diseases and other inflammatory conditions as well as in cancer tissue. Therefore, efforts are directed at designing A_3 receptor-selective drugs (Borea et al. 2015).

5.4 Cannabinoid Receptors: Cannabis and Cannabinoids

The endocannabinoid system comprises two cannabinoid receptors, CB1 and CB2, their endogenous cannabinoid ligands and enzymes involved in synthesis, release, transport and degradation of endogenous cannabinoids.

CB1 receptors are very abundant in the central nervous system. They are found in basal ganglia and in the brain, especially in the cortex and hippocampus and in the cerebellum. They are localized at membranes of presynaptic nerve terminals (Mackie 2005; Nyiri et al. 2005). CB1 is mainly coupled to inhibitory G-proteins (G_i). GTP-bound $G_i\alpha$ subunits inhibit the activity of adenylyl cyclase causing a decrease in cAMP and inactivation of PKA. Moreover, CB1 receptors mediate inhibition of neurotransmitter release from the presynapse by blockage of several types of Ca^{2+} channels (Brown et al. 2004) and activation of G-protein-coupled inwardly rectifying K^+ channels (GIRK). GIRK channels can be activated by the direct action of Gβ/γ subunits released from GTP-bound Gαi/o proteins. Gβ/γ-subunits are known to stabilize the binding of GIRK channels to the phospholipid phosphatidylinositol-4,5-bisphosphate, PIP2. Another well-documented effect of CB1 is the activation of the MAP kinase cascade (mitogen-activated protein kinase). MAP kinases are activated in response to growth factor receptor signalling (e.g. the receptor tyrosine kinase EGF-R). The effect of CB1 receptors on MAP kinases probably involves several mechanisms including a direct interaction of Gβ/γ-subunits with some MAP kinase activators (Dalton et al. 2009). Activated MAP kinases may mediate changes in gene expression as a long-term response to CB1 activation.

CB2, on the other hand, is predominantly localized outside the CNS, e.g. in the spleen. It can be up-regulated during injury and inflammation in sensory neurons and in the

spinal cord. CB2 is coupled to G_i, inactivating adenylyl cyclase. Moreover, it activates MAP kinase signalling in a PKC-dependent manner. It is probably not involved in inactivation of Ca^{2+}-channels or activation of GIRKs (Pertwee 1997).

The endogenous ligands for both receptors, CB1 and CB2, are derivatives of arachidonic acid. The two major endocannabinoids are arachidonoylglycerol (2AG) and arachidonoylethanolamine (AEA, anandamide). 2AG is thought to be responsible for a fast direct CB1 activation. This results in inhibition of presynaptic neurotransmitter release. Anandamide apparently modulates neurotransmission on a slower time scale and may therefore mediate a more tonic signal and long-term depression in this system. It binds to CB1 receptors, but in addition, it was shown that it also activates the TRP channel TRPV1 – also known as vanilloid receptor. In recent years, ion channel regulation, especially involving TRP channels (discussed later), has been identified as an important function of endocannabinoids in addition to their function as CB-receptor ligands (De Petrocellis et al. 2017).

The endocannabinoid system is a thoroughly investigated retrograde signalling system for neurotransmission in the nervous system. Cannabinoid receptors on presynapses are activated by signals from their respective postsynapses, and in response they block secretion of neurotransmitters from the presynapse. The signal that is received by the CB1 receptor is essentially produced in response to postsynaptic activity (Wilson and Nicoll 2001). While neurotransmitters are usually stored in secretory granules and released after membrane depolarization, endocannabinoids are not visibly stored but made "on demand". Thus, they deliver information about postsynaptic activity back to the presynapse. This function of the endocannabinoid system is seen as a paradigm for retrograde signalling of neurons (Ulugol 2014); see Fig. 5.6. The ligands for cannabinoid receptors, the endocannabinoids, are in fact the most ubiquitous endogenous signalling molecules. The change in neurotransmitter release caused by cannabinoid receptor activation is potent, fast and long-lasting.

The cannabinoid receptor ligand 2AG is formed mainly from diacylglycerol (DAG) by the enzyme diacylglycerol lipase (DAGL) (Fig. 5.7a). The stimuli that initiate its production and release include an increase in intracellular Ca^{2+}-concentration, causing the so-called Ca^{2+}-driven endocannabinoid release, and activation of G_q-coupled receptors, causing the so-called basal receptor-driven endocannabinoid release. It is also possible that the postsynaptic cell experiences a combination of both types of stimuli, and this is called Ca^{2+}-assisted receptor-driven endocannabinoid release. Ca^{2+}-signals are a response to depolarization of the postsynaptic membrane as a result of neurotransmitter signalling from the presynapse. Voltage-gated Ca^{2+}-channels are activated and this leads to the production of DAG due to an unknown mechanism. DAG is, as discussed above, also produced by PLC, which can be activated in response to GPCR signalling via activation of G_q. Metabotropic glutamate receptors in the postsynaptic membrane are activated by glutamate neurotransmission from the presynapse. Activation of the MAchR and some others also increases DAG in the membrane.

For degradation, 2AG is oxidized by the enzyme COX-2 (cyclooxygenase) in a two-step reaction leading to prostaglandin E2 glyceryl ester (therefore the enzyme is also called prostaglandin synthase 2). *M*ono*a*cyl*g*lycerol *l*ipase (*MAGL*) and *α,β-h*ydrolase *d*omain containing *ABHD*6 and *ABHD*12 degrade 2AG to arachidonic acid and glycerol. *F*atty *a*cid *a*mide *h*ydrolase (*FAAH*) degrades anandamide to arachidonic acid and ethanolamine. The sap of the fire tree (*Euphorbia tirucalli*), which is used to make latex and oil, contains the MAGL-inhibitor euphol – an anti-inflammatory drug. An overview over further 2AG degradation pathways is shown in Fig. 5.7b, (Ohno-Shosaku et al. 2012).

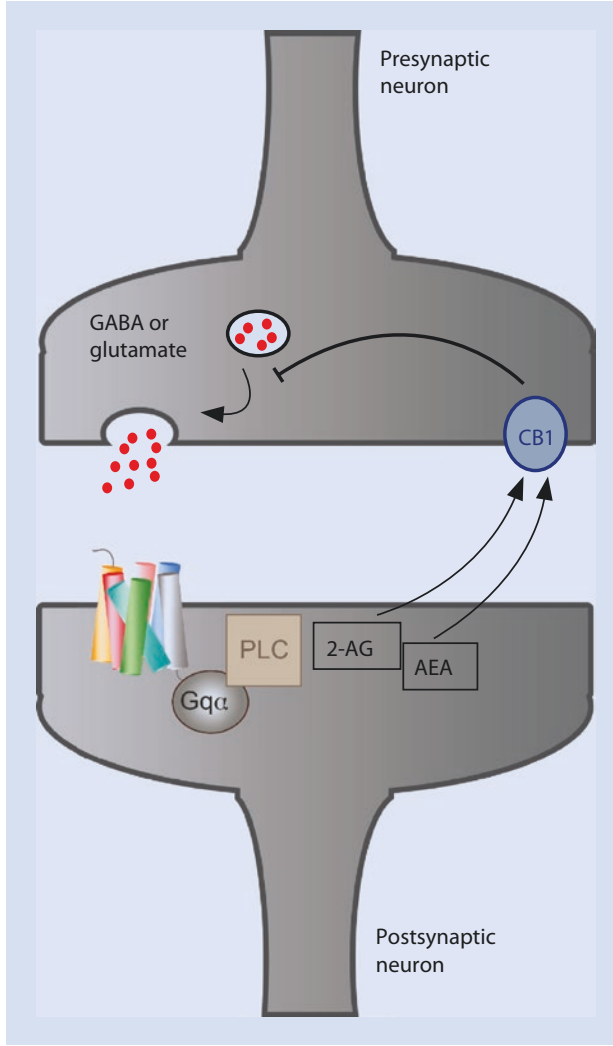

Fig. 5.6 Schematic representation of retrograde signalling by cannabinoid receptor CB1, localized at the presynapse. Receptor activation inhibits presynaptic neurotransmitter release. Endogenous cannabinoids are synthesized in postsynaptic membranes on demand (meaning as a result of neurotransmitters arriving from the presynapse); 2-AG and AEA are produced after stimulation of metabotropic postsynaptic neurotransmitter receptors leading to activation of G_q-proteins and PLC. (Modified from (Ulugöl 2014))

CB1 receptors are the primary target for Δ^9-tetrahydrocannabinol (THC) from the plant *Cannabis sativa*. THC functions as a partial agonist at CB1 receptors. Exposure to THC produces several physiological effects including catalepsy, analgesia, hypolocomotion and hypothermia. It also has psychological effects like heightened sensory awareness, euphoria and hyperphagia and induces impairment of short-term memory (Pertwee 2008). Analgesia is mainly mediated by blocking neurotransmission of the GABA and glycine inhibitory interneurons that, in the absence of pain, keep the break on descending nerve tracts from the brain (in the periaqueductal grey matter; see opiate signalling).

After chronic exposure tolerance to most of the THC effects is produced. However, THC is very lipophilic and appears to accumulate in fatty tissue from where it can be released for a long time after drug withdrawal. Therefore severe symptoms of withdrawal and dependence are hard to demonstrate in laboratory animals (Johnson and Lovinger 2016). Besides endogenous and phytocannabinoids, there are some synthetic cannabi-

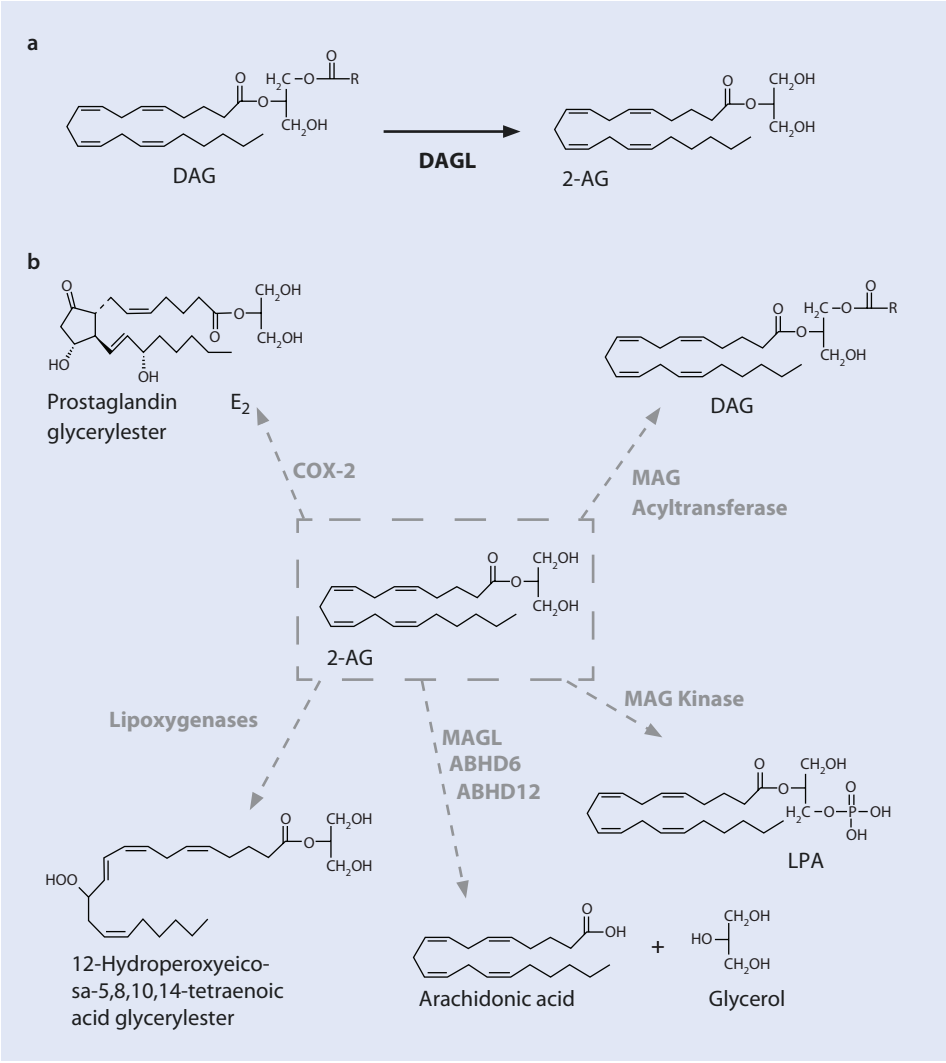

Fig. 5.7 **a** Endocannabinoid 2-arachidonylglycerol (2-AG) is produced from diacylglycerol (DAG) by the enzyme diacylglycerol lipase (DAGL); **b** degradation pathways for the endocannabinoid 2-arachidonylglycerol (2-AG) involving the enzymes cyclooxygenase (COX-2), monoacylglycerol acyltransferases (MAG-acyltransferase), lipoxygenases, monoacylglycerol lipases such as MAGL and α,β-hydrolase domain containing ABHD6 and 12 and monoacylglycerol kinase (MAG kinase)

noids that are clinically used. These include dronabinol and nabilone, synthetic THCs, both of which have been approved for use against chemotherapy-induced emesis in Canada and the USA, nabilone also against anorexia associated with AIDS-related weight loss and diabetic neuropathy. Rimonabant is a synthetic receptor antagonist. It was approved for use in obesity and smoking cessation; however, it had to be withdrawn due to the occurrence of depression. Another component of *Cannabis sativa* plants is cannabidiol. It has low psychoactivity and interacts with CB1 and CB2 receptors but probably has other targets too (Bergamaschi et al. 2011).

5.5 Dopamine Receptors: Neuroleptics and Cocaine

Cocaine does not interact with neurotransmitter receptors directly. It rather blocks the reuptake, mainly of the neurotransmitter dopamine, from the synaptic cleft into the presynaptic cell. Dopamine is often referred to as "happy hormone" (Glückshormon). Deregulation in the dopaminergic pathway is implicated in depression and bipolar disorder. Besides mood, cognition and emotion, dopamine also controls motor activity, and the loss of dopaminergic neurons in the brain is the cause of Parkinson's disease. Dopamine acts via pre- or postsynaptic receptors and generally imposes a slow modulation of fast glutaminergic and GABAergic neurotransmission.

Five dopamine receptor subtypes are expressed in specific neurons and in smooth muscle cells. Of those, D_1 and D_5 are coupled to G_s; thus, activation leads to an increase in intracellular cAMP. By activation of K^+-channels, D_1- and D_2-signalling can inhibit target neurons. Alternatively, D_1- and D_5-signalling can also activate Na^+-channels, causing activation of target neurons. The effect of dopamine therefore depends on the nature of the neurons that are connecting to the respective dopaminergic nerve endings.

D_2, D_3 and D_4 are coupled to G_i, and receptor activation leads to Giα-dependent decrease in cAMP. Giβ/γ activation of K^+ channels leads to a stabilizing of the resting potential in nerve cells. D_2, D_3 and D_4 receptors at postsynaptic sites thus inhibit neurotransmission. D_2 receptors on presynaptic sites induce a decrease in dopamine release via Giβ/γ-mediated inhibition of L/N-type Ca^{2+}-channels (Beaulieu and Gainetdinov 2011).

In the striatum D_1 activation is involved in a pathway facilitating motor activity. D_2 activation is involved in a pathway inhibiting motor activity. Because the activating pathway via D_1 is coupled to Gs and the inhibiting pathway via D_2 is coupled to Gi, dopamine increases cAMP via D_1 in the activating pathway, activating "activation", and decreases cAMP via D_2 in the inactivating pathway, inactivating inhibition. Therefore the overall effect of dopamine in the striatum is motor stimulation. D_1 is expressed widely in the brain including nigrostriatal, mesolimbic and mesocortical areas. It is also expressed outside the brain, for instance, on smooth muscle cells and stimulates dilation of kidney vessels, so more blood goes through the kidney.

D_2 is expressed in specific brain regions and made responsible for symptoms of schizophrenia and nausea. D_3 is expressed in the cerebellum. It is a target for drugs against depression, addiction and Parkinson's disease. Finally, D_4 and D_5 are expressed in some brain regions, including the hippocampus. Dopamine plays a role in the mesolimbic and mesocortical systems of the mesencephalon and is thereby involved in neurotransmission for reward and motivation, cognitive control and emotional response. The positive symptoms of schizophrenia, including hallucinations, delusions and thought disorder, have also been connected to dopaminergic neurotransmission in the mesencephalon. Loss of dopamine signalling leads to anhedonia (loss of motivation and energy) as it can often be observed in Parkinson's disease patients (Boyd and Mailman 2012).

Some drugs, so-called neuroleptica, have been developed to control psychiatric disorders. Of those, the classical neuroleptica haloperidol and clozapine interact with D2-dopamine receptors. Clozapine has some special properties in addition and probably also interacts with other neurotransmitter receptors; however, these are not completely understood yet (Beaulieu and Gainetdinov 2011; Seeman 2014). Apomorphine is an agonist for D_1 receptors and is used against symptoms of Parkinson's disease (Stacy and Silver 2008); see ◘ Fig. 5.8.

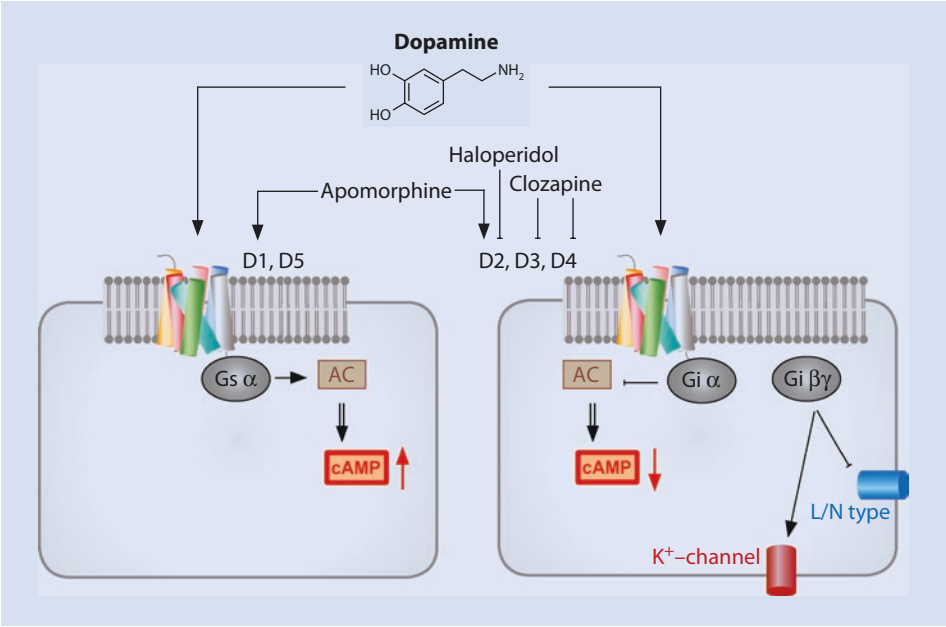

Fig. 5.8 Schematic representation of intracellular signalling pathways on dopamine receptors D_1, D_5 and D_2, D_3, and D_4. Agonist apomorphine on D_1, D_5 and D_2 receptors, antagonists haloperidol on D_2, clozapine on D_2, D_3, and D_4

The target for cocaine is the *dopa*mine *t*ransporter (*DAT*) (Vaughan and Foster 2013); see Fig. 5.9. DAT regulates the availability of dopamine in the brain by translocating dopamine from the synaptic cleft back into the presynaptic neuron. DAT also transports serotonin and noradrenalin back to the presynapse. DAT is a solute carrier transporter and a $2Na^+/1Cl^-/1$ dopamine-symporter (SLC6A3), similar to the noradrenaline transporter NET (see ▶ Sect. 5.4). Like NET, it has 12 membrane spanning transmembrane domains and large regulatory intracellular domains at the N- and C-terminus. The cytoplasmic domains present targets for many post-translational modifications of DAT, including phosphorylation by protein kinase C and ubiquitination of a specific lysine residue, which is regulated by the ubiquitin ligase Parkin. Mutations in the *parkin* gene have been implicated in familial Parkinson's disease. The failure of ubiquitine dependent DAT degradation causes accumulation of the protein and this leads to the death of dopaminergic neurons (Vaughan and Foster 2013).

DAT adapts an inwardly facing or an outwardly facing conformation and this directs the movement of dopamine into the cell or out. Cocaine directly blocks the inward movement of dopamine. In this way, it prevents the reuptake of dopamine after its release into the synaptic cleft. This leads to dopamine "overflow".

Locally applied cocaine works as an anaesthetic. It provided the chemical lead for development of a number of synthetic local anaesthetics, including lidocaine. In the central nervous system, cocaine induces euphoria, changes feelings for starving and thirst, abolishes the need for sleep and increases performance and activity. Side effects can be due to increased sympathetic signalling due to the disturbed noradrenalin uptake and include anxiousness, increase of breathing and heart frequencies and others. Snuffing and

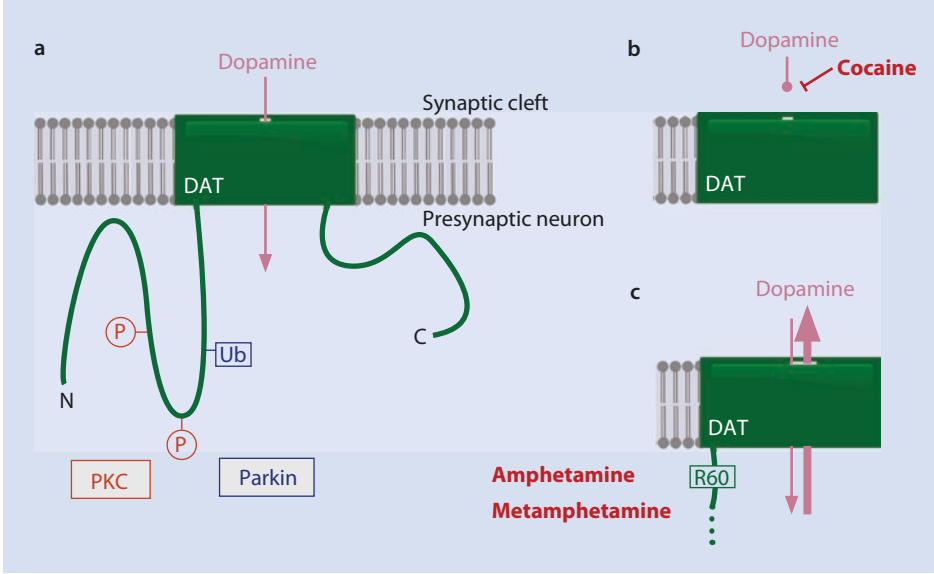

Fig. 5.9 **a** Schematic representation of dopamine transporter molecule DAT in the membrane of presynaptic dopaminergic neurons; **b** cocaine blocks dopamine transport into presynaptic cell preventing neurotransmitter reuptake; binding sites for Parkin and phosphorylation sites for protein kinase C are indicated; **c** amphetamine and methamphetamine bind to R60 at intracellular region of DAT and reverse its activity into outward direction

smoking of cocaine severs mucous membranes of the nose, mouth and throat. Very rarely polymorphisms in the DAT gene have been seen in *a*ttention-*d*eficit *h*yperactivity *d*isorder (*ADHA*) and bipolar disorder patients.

The synthetic drugs amphetamine and methamphetamine also increase the availability of dopamine in the synaptic cleft. In contrast to cocaine, they stabilize the outward conformation of DAPT by binding to a specific arginine residue in its intracellular N-terminal domain (Arg60); see Fig. 5.9b, c. This increases dopamine efflux and thus the availability for this neurotransmitter in the extracellular space. In addition, amphetamine has a long-term impact on the DAT protein level because it increases its endocytosis and also reduces mRNA expression of the DAT gene (Vaughan and Foster 2013).

5.6 $5HT2_A$ Receptors: Psilocybin, Mescaline and Dimethyltryptamine

The term "hallucinogen" was introduced due to the observation that consuming certain compounds caused hallucinations. Other terms for a diversity of drugs with this characteristic are psychotomimetics (mimicking psychosis, Hoffer 1967), phantastica (Lewin 1964), psychedelics or mind manifesting (Oswald 19957) and entheogen (god within) because people often have mystical experiences after the use of such compounds (Ruck 1979).

Non-synthetic hallucinogens include psilocin, which is metabolized from psilocybin. This can be found in certain mushrooms (e.g. *Psilocybe semilanceata*, liberty cap or "magic mushroom"). Psilocin induces somatic (dizziness, nausea, blurred vision), perceptual (altered shapes and colours, sharpened hearing) and psychic effects (hallucinations, distorted sense of time, dreamlike feeling, visual hallucinations and an altered concept of reality). The latter are so-called positive effects of schizophrenia (see ► Box 5.3) and the drug is also used to induce a "schizophrenic" condition in animal models. Similar effects are caused by the synthetic drug LSD. Mescaline is found in the San Pedro or Peyote cactus *Echinopsis pachanoi* which is growing in Peru and Ecuador. DMT (dimethyltryptamine) is one of the chemicals from *Psychotria viridis*, known as Ayahuasca. These compounds all have structural similarity with the neurotransmitter serotonin, and their effects are caused in large parts by their binding to the $5HT_{2A}$ subtype of the serotonin receptor, whereby mescaline only partially agonizes the receptor. It has been known for a very long time that the symptoms seen in people after mescaline uptake are almost identical with symptoms of patients suffering from schizophrenia (Osmond and Smythies 1952). With mescaline, in contrast to psilocybin, therefore also the negative effects of schizophrenia, like social withdrawal and apathy, are induced. Further hallucinogens include ketamine, a neurotoxic NMDA-channel blocker (see ► Sect. 6.5) (Nickalls and Nickalls 1988).

Box 5.3 Schizophrenia

Schizophrenia describes "any of a group of severe mental disorder that have in common such symptoms as hallucinations, delusions, blunted emotions, disordered thinking and withdrawal from reality" (The New Encyclopedia Britannica, 15th edition); positive symptoms of schizophrenia then refer to conditions of excess or distortion of normal function and include hallucinations, delusions and thought disorder; negative symptoms refer to a loss of normal function such as lack of emotion, social withdrawal, loss of motivation and others.

5-hydroxytryptamine (5HT) or serotonin receptors have seven families, ($5HT_{1-7}$). They are all GPCRs with the exception of $5HT_3$, which is a ligand-gated Na^+ and K^+ channel. Many pharmacological and animal studies have indicated that LSD and the plant-derived psychedelic drugs mentioned above target $5HT_{2A}$ (Nichols 2004). This receptor is coupled to Gq. Thus, it mediates activation of phospholipase C leading to production of IP3 and DAG and as a result to opening of IP3 Ca^{2+}-channels in the ER and activation of PKC. In addition it is suggested that $5HT_{2A}$ also mediates activation of phospholipase A, thus stimulating the arachidonic acid pathway, and phospholipase D, releasing second messengers choline and phosphatidic acid (Nichols 2004).

Localization studies have revealed that $5HT_{2A}$ receptors are predominantly postsynaptic. They are mainly found in the neocortex and the thalamus of the brain. They have also been identified at locations further away from synapses and therefore function in neuromodulation. Receptor agonists cause a robust increase in the activity of pyramidal neurons. These are multipolar neurons with large apical dendrites, multiple basal dendrites and a large number of dendritic spines involved in neuroplasticity and cognition. They are excited by glutamate and inhibited by GABA. Because of their dendritic complexity, a

single cell can receive thousands of inputs, both excitatory (majority) and inhibitory. Pyramidal neurons in the prefrontal cortex, for instance receive input from areas that process sensory information. Stimulation of $5HT_{2A}$ receptors in the thalamus and neocortex makes these pyramidal cells hyperexcitable which can override the sensory input. In this way, a sensory and cognitive information overload is perceived, and this might contribute to the symptoms that are experienced with psychedelic drugs.

To add another level of complexity, serotonin and metabotropic glutamate receptors (mGlu), both GPCRs, interact with each other and form hetero-dimers or hetero-oligomers. This affects ligand binding and signal transduction. LSD, for instance, binds to dimers of mGlu2/3 and $5HT_{2A}$. Dysregulation of such receptor complexes has been found in some patients with schizophrenia (Fribourg et al. 2011).

5.7 Opioid Receptors: Morphine, Heroin and Salvinorin A

Opiates are a class of different alkaloids that are present in the opium poppy. These include morphine and codeine. Furthermore, in lower concentrations, noscapine and thebaine are found. There are other substances, including synthetic- and plant-derived drugs that produce morphine-like effects in humans. These substances are referred to as opioids. Accordingly, the receptors that all of these compounds activate are opioid receptors. There are three subtypes of opioid receptors: μ, δ and κ. All are coupled to G-proteins.

The endogenous ligands for opioid receptors are peptides, including the pentapeptides Met-enkephalin and Leu-enkephalin (Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu) (Fig. 5.10). These are synthesized in cells from precursor proteins, e.g. the 35 kDa protein proenkephalin A. Prohormone processing enzymes, such as PC1, PC2 and furin, specifically hydrolyse peptide bonds at basic amino acids and release four Met-enkephalin peptides and one Leu-enkephalin peptide from each proenkephalin A molecule. Proenkephalin B, also called prodynorphin, is a 26 kDa protein and, when cleaved by prohormone processing enzymes, releases Leu-enkephalin peptides and dynorphins, which are extended Leu-enkephalins with 17, 13 or 8 amino acids beginning with an N-terminal Leu-enkephalin sequence. β-Endorphins are extended Met-enkephalins. The precursor is proopiomelanocortin, the C-terminus of which constitutes the 90 amino acid long β-lipotropin. From β-lipotropin, β-endorphin, melanocyte-stimulating hormone (MSH) and γ-lipotropin can be released by proteolytic processing. All opioid peptides are referred to as endorphins. This term is derived from "*end*ogenous" and "m*orphine*" (Pasternak and Pan 2013).

Opioid peptides, like other neuropeptides, are stored in large dense-core vesicles (diameter ca. 20–250 nm). Usually, these coexist in neurons with small clear-core synaptic vesicles (ca. 40–60 nm diameter) containing small-molecule "classical" neurotransmitters (including acetylcholine). Whereas the vesicles with small-molecule neurotransmitters are stored close to the synapse, many of them already tethered to the synaptic membrane to be readily releasable, dense-core vesicles with neuropeptides are also found at extrasynaptic sites. In addition, very often neuropeptides coexist in dense-core vesicles together with biogenic amine transmitters, including catecholamines, serotonin and histamine (Hokfelt 2010). After release, the latter enable fast

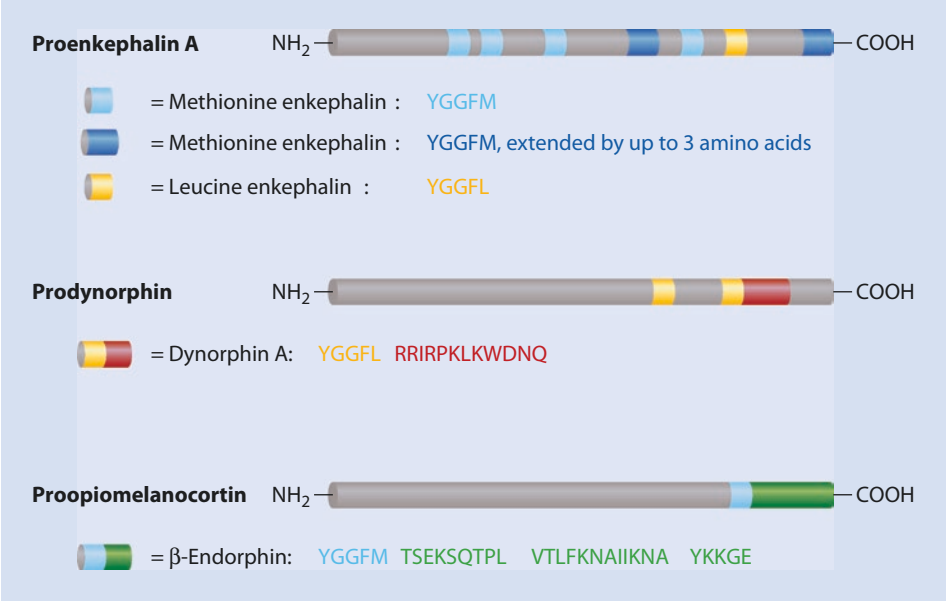

Fig. 5.10 Schematic representation of opioid peptide precursor proteins proenkephalin A, prodynorphin and proopiomelanocortin; positions of endogenous opioid peptides within the precursors are indicated, and the amino acid sequences of Met- and Leu-enkephalins, dynorphin A and β-endorphin are given in single-letter code

neurotransmission, whereas the peptides, including opioid peptides, are co-secreted and act on GPCRs to modulate neurotransmission. The peptidergic neuromodulatory effects are slower and last longer than neurotransmission by classical small-molecule neurotransmitters (Merighi et al. 2011).

Endogenous and plant-derived opioids (such as the opiate alkaloids morphine, codeine and thebaine) bind to opioid receptors. β-Endorphin is a ligand for μ-receptors, enkephalins bind δ-receptors and dynorphins are specific for κ-receptors. Opiates, including morphine, specifically act on the μ-receptors (hence the name). The semisynthetic diacetylmorphine, known as heroin, binds even more efficiently than morphine. Activation of opiate receptors by endogenous or drug derived opioids inhibits release of excitatory neurotransmitters from the presynapse and induces hyperpolarization of postsynaptic neurons.

M-opioid receptors are abundant in neurons involved in pain perception. Conscious pain has both, a sensual component, telling whether and where there is pain, and an emotional one, evaluating the pain. Thus, the neuronal network involved senses, processes, and puts pain signals into a cognitive context. Opioids target pain transmission from the periphery by activating descending nociceptive inhibition pathways. On the other hand, they also modulate the emotional component of pain perception by inhibiting neurotransmission in certain areas of the brain cortex (Pasternak and Pan 2013).

A three-neuron scheme is considered to transmit pain from the periphery to the cortex. This includes the first-order neuron, which receives pain information induced by

tissue damaging mechanical stimuli, thermal stimuli or factors released from damaged or inflamed tissue and transmits it to the dorsal horn of the spinal cord. The second-order neuron forms a synapse with the first one in the spinal cord and transmits information to the thalamus. The third-order neuron then synapses with the second one and transmits information to the somatosensory cortex (Cross 1994). The modulation of pain perception is achieved by descending nerve tracts from the brain (especially the *peri*a*queductal grey matter*, *PAG*) transmitting signals to the spinal cord, mostly ending in spinal interneurons. These interneurons release endogenous opioids, which activate μ-opioid receptors in dorsal horn neurons and thereby inhibit pain transmission. Moreover, descending nerve tracts in the PAG are blocked in the absence of pain signals by GABAergic inhibitory interneurons. In response to ascending pain signals, this block is released by enkephalin signalling. In addition, opiate receptors in dorsal horn neurons are also activated by peripheral mechanical stimuli (like normal and therefore not painful touch) to prevent pain transmission to the thalamus. This is the reason why rubbing injured body parts immediately after the insult partially releases pain. In summary, signalling at opioid receptors very specifically blocks the perception of pain.

Opioid signalling also occurs in certain areas of the brain and induces psychological effects such as euphoria and feelings of pleasure and reward. This is caused by excessive release of dopamine. In addition, opiate receptors are located in the respiratory centre of the brainstem where they are involved in controlling the breathing rate. Overdoses of heroin or morphine can arrest breathing altogether, causing death.

Opioid receptors transduce signals in a cell type-specific manner dependent on post-translational modifications of the intracellular domains of the receptors. Originally it was shown that they couple to pertussis toxin-sensitive G-proteins. These include G_{i1-3} and $G_{oA/B}$, both AC inhibitors. They can also be coupled to pertussis toxin-insensitive G-proteins, including G_q14 and 16, activating PLC and neuronal G_i (Gz) and G_s, which affect AC and the opening of cAMP-dependent Ca^{2+}- and Na^+-channels. Finally, the β/γ-subunits of several G-proteins modulate the activities of a number of enzymes including AC 2,4,7, PLC, MAPK and others (Tso and Wong 2003).

Opioids are two-faced. They belong to the most effective painkillers in the world yet also induce the most severe tolerance and withdrawal symptoms. Responsible for the adverse effects of opioid consumption are molecular and cellular adaptations to repeated stimulation with opioids. In the centre of these adaptations is an enhancement of the AC responsiveness – so-called AC superactivation. This effect is specific for pertussis toxin-sensitive G_i-signalling. The molecular mechanism is not clear yet. Involvement of G_s signalling and Gβ/γ signalling and post-translational modifications of AC and other proteins are discussed.

Interestingly, the selective κ-opioid receptor agonist Salvinorin A from the plant *Salvia divinorum* is investigated as a drug with potential to treat addiction, e.g. for cocaine and morphine (Kivell et al. 2014; Simonson et al. 2015). This is supposed to be due to the interaction of κ-opioid receptors with the dopamine system. In particular, it has been shown that κ-agonists increase the extracellular dopamine concentration by an as yet unclear mechanism (Table 5.1).

Table 5.1 Summary of GPCRs as targets for plant-derived drugs

GPCR	Endogenous ligand	Plant-derived drugs	Major mode of drug action
Muscarinic acetylcholine receptor (MAChR)	Acetylcholine	Muscarine Atropine Physostigmine	Agonist Antagonist Acetylcholine esterase inhibitor
α-, β-adrenergic receptor	Adrenaline/ noradrenaline	Higenamine Ephedrine Reserpine	Partial agonist on β NET inhibitor VMAT2 inhibitor
Adenosine receptor	Adenosine	Caffeine	Antagonist
Cannabinoid receptor	Endocannabinoids (2-AG, AEA)	THC	Partial agonist CB1
Dopamine receptor	Dopamine	Cocaine	DAT inhibitor
$5HT_{2A}$ receptor	Serotonin	Psilocybin DMT Mescaline	Agonist Agonist Partial agonist
Opioid receptor	Enkephalins, β-endorphin	Morphine Salvinorin A	Agonist on μ Agonist on κ

Take-Home Messages

Many *plant-derived drugs* target GPCRs.

- *Muscarine* activates muscarinic acetylcholine receptors (MAchRs), and *atropine* blocks them. Acetylcholine is discharged at all preganglionic synapses and most postganglionic synapses of the parasympathetic nervous system. MAchRs are GPCRs present on postganglionic parasympathetic neurons. As an agonist for these receptors, muscarine is a parasympaticomimetic. In contrast, atropine works as parasympatholyt by antagonizing acetylcholine at the same receptors. Furthermore, inhibitors of acetylcholine esterase, the enzyme degrading acetylcholine, also have parasympaticomimetic actions by increasing the concentration of acetylcholine within the synaptic cleft. They include chemical weapons like tabun, organophosphate pesticides as well as physostigmine.
- *Ephedrine* works as an "indirectly acting sympathomimetic" by targeting the noradrenalin transporter NET. Adrenalin and noradrenaline are neurotransmitters in the nervous system and they are also secreted as hormones into the blood from the adrenal gland. The latter mediates the "fight-or-flight" bodily response to stress. Synthetic adrenergic receptor antagonists are used to treat high blood pressure. *Reserpine* is an inhibitor of the vesicular transporter for monoamines and a sympatholytic because it depletes neuronal granules of noradrenaline.
- *Caffeine* targets several molecules in animals, including phosphodiesterases, therefore decreasing the concentration of cAMP. In humans, the observed effects of caffeine consumption are attributed to its antagonizing adenosine receptors. Adenosine is produced from AMP, the degradation product of ATP. Acting as an

extracellular ligand for adenosine receptors, it signals in an energy-dependent way to induce physiological and psychological responses, such as modulation of motor activity and sleep.

- *Cannabis (THC)* from the plant Cannabis sativa partially agonizes cannabinoid receptors. Cannabinoid receptors are activated by endogenous cannabinoids including arachidonoylglycerol and anandamide, both derivatives of arachidonic acid. They are released from the postsynapse after neurotransmission at both, activating (glutamate) or inhibiting (GABA) synapses. Cannabinoid receptors at the presynapse are activated in response and block secretion of the neurotransmitter. This system comprises a paradigm for retrograde neuronal signalling.
- *Cocaine* and the synthetic drugs amphetamine and methamphetamine inhibit the dopamine transporter DAT. DAT is a solute carrier transporter and a Na^+/dopamine symporter. It regulates the availability of dopamine, serotonin and noradrenalin for synaptic signalling on receptors by transporting it from the synaptic cleft back into the cell. Its inhibition therefore induces a dopamine "overflow" and this may cause euphoria and increased activity and performance. Side effects relate to disturbed uptake of noradrenalin and include increases in breathing and heart frequency.
- *Hallucinogens* include psilocybin, mescaline and the synthetic drug LSD. They act preferably on $5HT2_A$ receptors which are GPCRs. Their activation leads to a robust increase in the activity of pyramidal neurons, which receive input from brain areas processing sensory information. This induces a sensory and cognitive overload.
- *Morphine* and other opioids bind to opioid receptors. Opioid receptors are activated by endogenous peptides including enkephalins. They are involved in modulating pain perception. Morphine is the agonist for the μ-opioid receptors. It has strong analgesic effects. Acting on opioid receptors in certain brain regions, it also causes euphoria and feelings of pleasure and reward. On the downside it induces tolerance, addiction and extremely strong withdrawal symptoms after extended usage.

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Ion Channels as Targets for Plant-Derived Drugs

6.1 Neurotoxin Binding Sites on Voltage-Gated Ion Channels – 84

6.2 Nicotinic Acetylcholine Receptor (NAchR): Nicotine – 85

6.3 $GABA_A$ Receptors: Muscimol, Valerenic Acid, Thujone and Benzamidines – 88

6.4 Glycine Receptor: Strychnine and Tutin – 89

6.5 Glutamate Receptors: Ibotenic Acid, Kainate and Ketamine – 90

6.6 TRPV and TRPM8: Heat and Cold, Capsaicin and Menthol – 92

References – 94

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What You Will Learn in This Chapter

In this chapter we will describe the physiological and psychological actions of prominent plant derived and synthetic compounds that target ion channel signalling. These include neurotoxins binding to voltage gated ion channels as well as drugs and toxins like nicotine, thujone, muscimol and strychnine interacting with neurotransmitter gated ion channels. Synthetic compounds such as benzamidine, ketamine and PCP will be discussed. Finally we will briefly explain the interactions of capsaicin and menthol with specific TRP-channels.

6.1 Neurotoxin Binding Sites on Voltage-Gated Ion Channels

Many neurotoxins exhibit their effect through binding to voltage-gated cation channels. These include a number of animal venoms that are produced by frogs, snails, snakes and spiders.

Pharmacological studies have identified several sites of action for neurotoxins on voltage-gated sodium channels. At extracellular sites (site 1, ◘ Fig. 4.2b), the water-soluble heterocyclic guanidines tetrodotoxin (TTX), a tarantula toxin and saxitoxin (STX) from dinoflagellates and snail-derived peptides, like μ-conotoxin, can dock. This blocks ion conductance. These toxins have been used to identify regions of the channels that are involved in pore opening and to distinguish between different sodium channel isoforms (Cestele and Catterall 2000). It was suggested that bacteria of the genus *Vibrio*, which produce tetrodotoxin and are found in some marine animals, e.g. *Fugu vermicularis*, might be the source of the poison (Noguchi et al. 1986). Saxitoxin is enriched in some mussels and the source of paralytic shellfish poisoning. It is produced by dinoflagellates, which in some conditions grow abundantly in marine regions and cause "red tides". Conotoxins are produced by snails of the genus *Conus*. They comprise a large variety of short peptides of 10–30 amino acids. These are stabilized by disulphide bonds and due to their small size easily penetrate the skin of prey animals.

Some toxins bind especially to the activated state of Na^+-channels and keep it open. This causes persistent activation. They bind to a site on helix S6 of the first and the fourth subdomain of the α-subunits of the channel (site 2) (◘ Fig. 4.2b). Aconitine from plants of the *Aconitum* genus is the most famous toxin in this group. Due to persistent channel activation, aconitine at first works excitatoric on central and peripheral nerves and causes arrhythmia of the heart. Later it leads to paralysis and eventually it causes death.

Pyrethrins, which are found in several plants from the *Asteraceae* family, also target voltage-gated Na^+-channels (Soderlund et al. 2002). Specifically, they change the kinetics of activation and inactivation of the neuronal voltage-dependent Na^+-channel in some arthropodes. However, by exposing large populations of house flies to these compounds for a long time, the so-called knockdown-resistant flies appear, which have amino acid substitutions in the linker sequence between helix S4 and helix S5 of the second subdomain of this channel protein. Interestingly, these sites also differ in their amino acid sequence between mammals and insects. Mammalian Na^+-channels are much less sensitive to pyrethrins counting for the low mammalian toxicity of these compounds. This makes pyrethrins sought after insecticides (Vais et al. 2000).

A-scorpion-, β-scorpion- and some spider-toxins constitute a group of peptide neurotoxins. They contain 60–70 amino acids and bind to extracellular loops of the channel (sites 3 and 4, ◘ Fig. 4.2b). This changes the voltage-dependent gating of the channels and slows down their inactivation. Lipid-soluble toxins, including brevetoxins and ciguatoxins, bind to transmembrane segments of the channel (site 5). They cause a shift in activation gating and lead to repeated firing of neurons. These polyether compounds are produced by dinoflagellates (*Karenia spec* and *Chattonella*) and also contribute to neurotoxic shellfish poisoning during *Karenia* blooms, e.g. in the Gulf of Mexico (Watkins et al. 2008).

VOCs are also affected by a number of animal toxins. The P/Q type channel is sensitive to Ω-agatoxin, a poison of the spider *Agelenopsis* that paralyses the spiders pray (American grass spider). N-type channels are blocked by ω-conotoxin, a poison produced by deepwater snails (*Conus geographicus*). R-type channels are expressed in brain regions responsible for pain transmission and in the amygdala, a brain region involved in fear response. They are sensitive to some tarantula venoms, e.g. SNX-482, a peptide with 41 amino acids from *Hysterocrates gigas*, the giant baboon spider.

The tarantula toxin guangxitoxin (GxTX) belongs to a class of spider venoms comprising small peptides with a characteristic cysteine knot fold that is stabilized by disulphide bridges. They act on voltage-gated Ca^{2+}-, K^+- and Na^+-channels. Their affinity depends on the channel conformation displaying either open or closed states. This varies from 2 nM for the inactive (closed) channel to 200 nM for the activated (open) channel conformation. GxTX specifically targets $K_V2.1$-potassium channels. Thus, fluorescently labelled derivatives of GxTX have been used to report receptor activity of a $K_V2.1$-potassium channel. Fluorescence was measured when the toxin was bound to a closed channel. It decayed when the channel opened as the toxin lost affinity and dissociated. This was reversible, after repolarization of the cell, fluorescence could be recovered (Tilley et al. 2014).

6.2 Nicotinic Acetylcholine Receptor (NAchR): Nicotine

The first ligand-gated ion channel investigated at the molecular level was the NAchR. It belongs to a large family of *p*entameric *l*igand-gated *i*on *c*hannels (*pLIC*s) (Lynagh and Pless 2014). These channel proteins are composed of five identical or homologous subunits. Each subunit has four transmembrane domains. The N-terminal and C-terminal domains are extracellular located. The N-terminus forms a large extracellular domain that is stabilized by a conserved cysteine bridge. Therefore, these receptors are also referred to as *C*ysteine-*l*oop *r*eceptors (*CLR*s), see ◘ Fig. 6.1. When ligands interact with specific extracellular binding sites, the channels open. The extracellular domain provides a large basin, the so-called vestibule. Due to the size of this structure, it is able to take hydrated ions, allowing very fast ion entry. After that the ions have to pass the selectivity filter, which is formed by two of the transmembrane helices (M2 and M4). The movement of ions through this narrow part of the pore requires loss of the hydrate shell. The energy for this process is provided by transient interactions of the passing ions with amino acid residues lining the pore, and with water molecules.

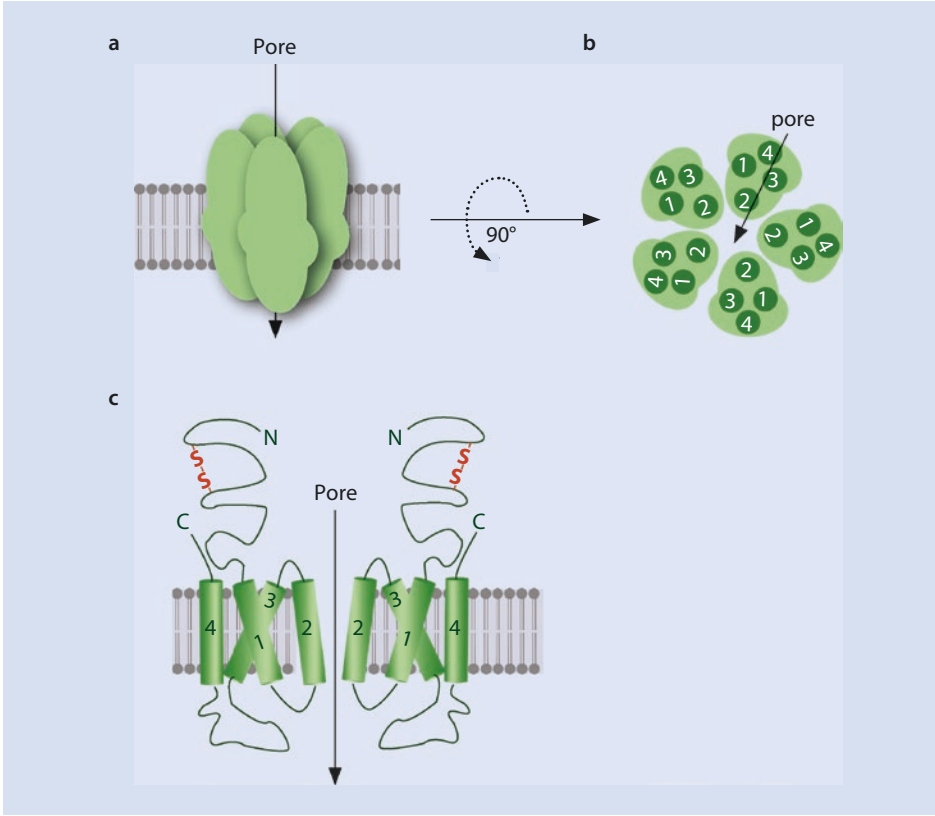

Fig. 6.1 **a** Schematic representation of pentameric ligand-gated ion channel (pLICs) **b** shows how each subunit has four transmembrane domains and **c** indicates highly conserved extracellular domain containing loops stabilized by disulphide bridges, hence the name cysteine loop receptors. These include GABA, glycine, serotonin and nicotinic acetylcholine receptors. (Modified from Berridge (2012))

The NAchR has a molecular mass of 290 kDa. It consists of five subunits arranged around a central pore. Each subunit has four transmembrane domains. By variable combinations of these five subunits, a wide range of different channels is produced. In the muscle type, the embryonic form is composed of two α1-, one β1-, one γ- and one δ-subunit ((α1)2(β1)1(γ)1(δ)1). Adult forms, on the other hand, are composed of two α1-, one β1-, one δ- and one ε-subunit ((α1)2(β1)1(δ)1(ε)1). Some neuronal subtypes are (α4)3(β2)2, (α4)2(β2)3 and (α7)5.

Acetylcholine is the ligand for NAchRs. It gates the flow of positive ions into the muscle cell and induces membrane depolarization. NAchRs play an important role in mediating activation-contraction coupling at the neuromuscular junction. When two acetylcholine molecules bind to the extracellular domain of the receptor, within a few μs' the channel opens and strong Na^+ influx results in an inwards net flow of positive charge. This changes the resting membrane potential of the muscle cell from −85 mV to −60 mV and provides the first step in the cascade of events inducing muscle contraction (see Fig. 4.4). The gating mechanism involves twisting of the second helices of the membrane spanning domains of the five subunits lining the channel. The selectivity pore in this cation

channel is negatively charged. Point mutations in the second helices of the five receptor subdomains change the conductivity of the channel (Lynagh and Pless 2014; Nys et al. 2013).

NAchRs are involved in excitation-secretion coupling at neuronal synapses in the central and peripheral nervous system. For instance, in the carotid body, acetylcholine is released from afferent nerve endings that report oxygen tension. Moreover, acetylcholine is used as a neurotransmitter in the parasympathetic autonomous nervous system (this takes care of the "rest and digest and breed and feed" regulation of the body). In the sympathetic nervous system, acetylcholine is used at preganglionic short nerve endings where it helps to depolarize postganglionic fibres, resulting in adrenalin and noradrenaline receptor activation (taking care of our "fight or flight" response); see ◘ Fig. 4.7.

Presynaptic facilitatory NAchRs modulate neurotransmitter release, e.g. of acetylcholine, glutamate, noradrenalin, dopamine and GABA. Decreased expression of NAchRs has been observed in several disorders including schizophrenia, epilepsy and drug addiction. *Myasthenia gravis*, a usage-dependent muscle weakness, is caused by autoantibodies against the NAchR. There are numerous drugs that target the NAchR (Daly 2005). Some are produced by animals, e.g. bungarotoxin, a snake venom. These compounds antagonise acetylcholine, probably by preventing the twisting of the helices. Bungarotoxin is an 8 kDa peptide with a so-called three-finger fold. By forming intramolecular cysteine bridges, this molecule is extremely stable.

Plant-derived drugs interfering with the NAchR include curare alkaloids from the plant *Strychnos toxifera.* They are competitive antagonists of the receptor and used as an arrow poison. By antagonizing acetylcholine, they cause muscle paralysis. When the diaphragm gets paralysed, the result is a fatal breathing arrest. Poison Hemlock, a common plant found on meadows and fields in Europe and North Africa, contains, amongst other poisonous alkaloids, coniin, a competitive NAchR antagonist. Apparently, this was used to make the "Cup of Hemlock" (Schierlingsbecher) to execute the death penalty on Sokrates.

Nicotine is a competitive agonist for the NAchR, and it has given the receptor its name. Nicotine crosses the blood-brain barrier and reaches the brain cells in 10–20 s. Its elimination half-life is 2 h. Nicotine acts on α3β4 receptors that are present on autonomic ganglia and in the adrenal medulla. By binding to these receptors, it increases their permeability for Na^+ ions and thus increases the excitability of neurons in the brain. At high doses nicotine is toxic for humans, because it then also targets the neuromuscular junction and induces muscle contractions. However, at lower doses it increases neurotransmitter release in the brain, for instance, at dopaminergic neurons, and this explains feelings of euphoria and relaxation that can be experienced through tobacco smoking. Interestingly, tobacco also contains monoamine oxidase inhibitors (enzymes that break down serine, dopamine and norepinephrine, see ► Sect. 5.4). This fact possibly contributes to tobacco addiction.

In the sympathetic nervous system, nicotine stimulates the release of adrenalin. Therefore, an additional stimulator effect on the sympathicus is observed, including increased heart rate, blood pressure and facilitation of memory and attention. The golden chain tree produces the alkaloid cytisine, which has similar effects as nicotine. It has been used in tobacco replacement therapy because it does not appear to be addictive (produced in Bulgaria under the trade name Tabex) (Walker et al. 2014). However, cytisine at appropriate doses stimulates the acetylcholine receptor and desensitizes it for acetylcholine. Therefore, this compound is toxic, and in Germany, the golden chain tree was the "poisonous plant of the year" in 2012 (► https://de.wikipedia.org/wiki/Giftpflanze_des_Jahres).

Some cyanobacteria produce the NAchR agonist anatoxin A. This is also called "very fast death" factor because it kills animals within a few minutes. It has been made responsible for the death of 30,000 flamingos on lake Bogoria in Kenia in the autumn 1999 (Krienitz et al. 2003).

Acetylcholine receptors also have permeability for Ca^{2+}-ions. This is important for some additional functions of these receptors on non-neuronal cells. They are expressed on immune cells, including macrophages. Stimulation then modulates inflammation. On B- and T-cells, the activation process is inhibited by acetylcholine and also by nicotine, which is responsible for immune suppression that can be observed in heavy smokers.

Finally, some prominent toxins acting on acetylcholine-dependent neurotransmission do not work as agonists or antagonist at the receptor. They rather are inhibitors of acetylcholine esterase, the enzyme that degrades acetylcholine after its release into the synaptic cleft. This leads to increased stimulation of the receptor. For example, physostigmine from *Physostigma venenosum* is a reversible acetylcholinesterase inhibitor. It is toxic but is used therapeutically as an antidote to poisoning with receptor antagonists (Proudfoot 2006). Organophosphorus compounds have similar effects. These include the infamous nerve gas Sarin and many insecticides that were used in the past. These have been banned in the 1970s.

6.3 $GABA_A$ Receptors: Muscimol, Valerenic Acid, Thujone and Benzamidines

$GABA_A$ receptors are chloride channels, in contrast to $GABA_B$, which is a GPCR (Bormann 2000). Their activation thus gates influx of negatively charged ions and therefore membrane hyperpolarization and neuronal inhibition. They also belong to the family of pentameric ligand-gated ion channels and have a similar structure as the previously discussed NAchRs. However, there is one important difference. They are anion channels, and therefore their selectivity pores are positively charged. Thus, the electrostatic potential of the pore is positive, and this provides conductivity for anions, in contrast to the negative electrostatic potential of cation channels in this class.

Chloride is the only halogen anion used in biological systems. Therefore, selectivity is only required for exclusion of much larger anions like phosphate, sulphate, bicarbonate or anionic peptides. Accordingly, the selectivity filter does not distinguish between chloride, bromide and iodate.

GABA was discovered in 1960 as an inhibitory neurotransmitter. $GABA_A$ receptors are prominent targets for psychoactive synthetic drugs including barbiturates and benzodiazepines. They are expressed in the brain, spinal cord, basal ganglia, cerebellum (Purkinje cell, motor control) and in the thalamus (sleep control). A variety of drugs bind to different sites on the channels, and their activity is dependent on the precise subunit composition of the pentameric receptors (Lynagh and Pless 2014). 19 different $GABA_A$ – subunits are encoded in the human genome (Sigel and Steinmann 2012). Thus, $GABA_A$ receptor composition is highly diverse. This opens the possibility to design very specific compounds that interfere only with certain subsets of these receptors and thereby to obtain more finely tuned drugs (Sigel and Steinmann 2012). $GABA_{A1}$ to $GABA_{A6}$-receptors, for instance, are composed of α, β and γ-subunits. They are sensitive to benzodiazepines in contrast to $GABA_{A0}$-receptors, which are composed of α, β and δ or ε-subunits and are insensitive to these drugs. Benzodiazepines and barbiturates (derived from valerenic acid) bind the $GABA_A$ receptor at two different positions, and both these sites are not physically overlap-

ping with the binding site of the endogenous receptor agonist GABA (Bormann 2000). They are therefore classified as allosteric modulators. Barbiturate derivatives are employed in anaesthetics, as sedatives and in hypnosis. They have been used as anti-epileptic drugs. Their disadvantages include the induction of dependence due to changes in the function of inter-neuronal AMPA receptors in the ventral tegmentum of the brain. Other important problems are sedative side effects and especially the danger of accidental (or suicidal) overdosing, which is essentially lethal. Marilyn Monroe allegedly was a victim of barbiturates. Barbiturates bind to an allosteric site on GABA channels in the second small intracellular loop. At low doses this increases the action of GABA. However, at higher dose the channel opens even in the absence of GABA (Olsen 2015).

Benzodiazepines, the so-called tranquillizers, replaced barbiturates in the late 1960s of the last century. They also enhance the activity of GABA. The first benzodiazepine synthesized was chlordiazepoxide produced by Roche under the trade name of Librium, and later diazepam was introduced under the trade name of Valium. The East German Valium equivalent was called Faustan.

A true GABA-agonist is muscimol. It binds to the same site as GABA and opens the chloride channel. In this way, it activates the inhibitory centre of the CNS and increases the serotonin concentration. It acts as a mild sedative and has psychoactivity. The prodrug for muscimol is ibotenic acid, which is found in some forest fungi such as *Amanita pantherina* and *Amanita muscaria*. By decarboxylation of ibotenic acid, e.g. when the mushrooms are dried, muscimol is formed. Changes in visual perception (distortion of sizes and shapes) and auditory hallucinations are observed after ingestion of dried *Amanita* fungi. In more sensitive people or at higher doses, dissociative reactions and loss of the ability to communicate are also common (Johnston 2014).

GABA-active plant products include sesquiterpenes, for instance, valerenic acid, a constitute of the essential oil of valerian (*Valeriana officinalis*). Like synthetic barbiturates, it is an allosteric positive modulator of GABA-gated channels (Luger et al. 2015). By contrast, the sesquiterpene anisatin, the polyacetylene cicutoxin and the monoterpene ketone thujone are GABA channel blocker (Hold et al. 2000). Thujone, at high concentrations, can induce epileptic convolutions. Antagonists of GABA signalling can be used as antidotes, e.g. for barbiturate poisoning. These include bicuculline and picrotoxin.

6.4 Glycine Receptor: Strychnine and Tutin

The glycine receptor (GlyR) is another important pentameric chloride channel in spinal cord and brain stem neurons. It is involved in motor control and pain perception and especially in fast inhibitory neurotransmission. Most prominently, it mediates a fast feedback inhibition in motoneurons during activation of muscle contraction (Fig. 6.2). Strychnine, produced by bark and seeds of *Strychnos nux-vomica*, and tutin from plants of the *Coriaria* genus both act as antagonists for glycine and block the feedback inhibition of motoneurons, leading to spastic muscle contractions and convulsions. Eventually, cramping of respiratory muscles can cause death. Thirty to 120 mg of strychnine are deadly to humans. Tetanus toxin works in the same pathway. It blocks SNARE-degradation at these inhibitory synapses and thus inhibits the release of glycine (and GABA) with similar effects on muscle contraction as strychnine. Strychnine, however, in addition to GlyR, binds NAcR and $5HT_3$-ion channels and has little specificity but high affinity for these receptors (Brams et al. 2011).

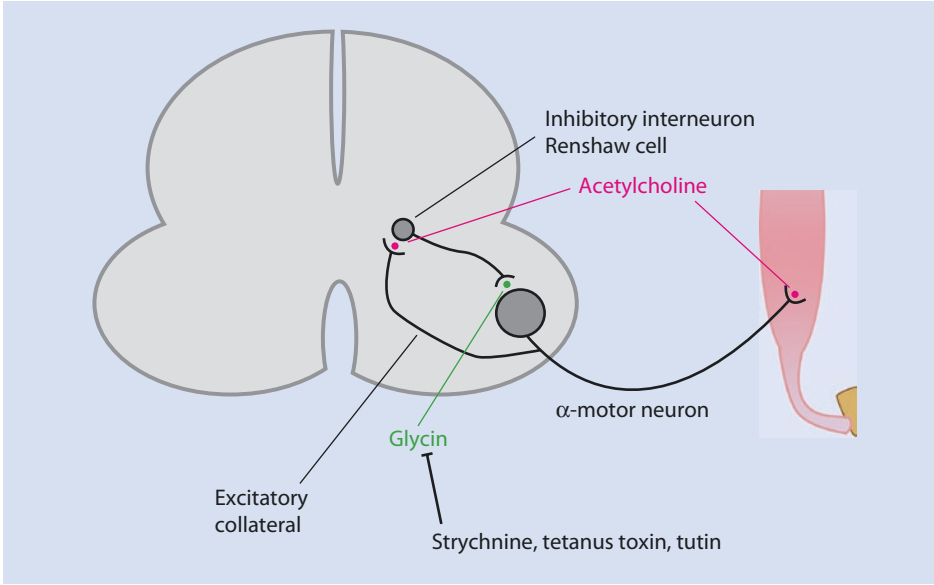

Fig. 6.2 Schematic representation of the circuit controlling the firing rate of α-motor neurons. It shows the α-motor neuron with a collateral branch forming a synapse with an interneuron, the Renshaw cell. The activation of this branch occurs during excitation of the α-motor neuron, and the Renshaw cell responds by releasing the inhibitory neurotransmitter glycine at another synapse with the α-motor neuron. This mediates feedback inhibition for α-motor neuronal excitation. Renshaw cells are located in the ventral horn of the grey matter of the spinal cord. α-motor neurons activate muscle cells by releasing acetylcholine

Cysteine loop pentameric ligand-gated ion channels are apparently also involved in the rapid onset of the symptoms of alcohol ingestion. Ethanol interacts weakly with water-filled cavities in $GABA_{2A}$, glycine, NAchR and $5HT_3$ receptors and stabilizes certain conformations, leading to alterations in neuronal neurotransmission. This was demonstrated in structural studies. In mouse models, especially $GABA_A$ and GlyRs have been indicated in changes in behaviour after alcohol ingestion. Moreover, polymorphisms in genes encoding these receptors are believed to count for the development of alcohol dependence (Trudell et al. 2014).

6.5 Glutamate Receptors: Ibotenic Acid, Kainate and Ketamine

Takashi Hayashi injected glutamate into the grey matter of the brain of dogs and induced epileptic seizures (Takashi Hayashi 1954). This was the basis for the glutamate model of epilepsy; it was thought that epilepsy arises when quantities of glutamate surpass a critical level. Later it was established that glutamate-related amino acids worked excitatory on neurons, and pharmacological studies of *N-m*ethyl-*D-a*spartate (*NMDA*), α-*a*mino-3-hydroxy-5-*m*ethyl-4-isoxazole*p*ropionic *a*cid (*AMPA*) and the natural compound kainate binding to these receptors led to a distinction of ionotropic glutamate receptors into NMDA, AMPA and kainate receptors. Glutamate overload

causes neuronal cell death due to excess Ca^{2+}-influx into neurons. This occurs during spinal cord injury, in Alzheimer and other neurodegenerative diseases, alcohol withdrawal disease and also under conditions after over-rapid benzodiazepine withdrawal.

Glutamate-gated ion channels are primarily located in the brain. The AMPA and kainite receptors are pure ligand-gated cation-channels with glutamate as the endogenous ligand. The third one, the NMDA receptor, is ligand and voltage gated. All glutamate receptors are formed from four subunits GluA1-GluA4. They are heterotetramers with varying composition of these subunits. Each subunit has a large N-terminal extracellular domain responsible for receptor assembly and trafficking. This is followed by the transmembrane helix M4 and a short intracellular loop, which leads to the M2-helix that does not span the membrane completely but is rather buried into the inner membrane leaflet. It is followed by a loop half inside the membrane and half intracellular. This loop connects to helix M3 spanning the membrane and is followed by another large extracellular loop, which contains the ligand-binding site. Then helix M1 and a small intracellular C-terminal domain follow. The glutamate receptors are additionally regulated by auxiliary subunits. Ibotenic acid is an agonist for all three glutamate receptors and for metabotropic glutamate receptors. It causes excitation, agitation and neuronal cell death.

AMPA is a synthetic glutamate derivative and binds AMPA receptors. Ethanol is an antagonist on these receptors! Kainate is a product of seaweed. It is an antagonist of the kainite receptor. It causes neuronal cell death and induces excitotoxic lesions. It is also used as a remedy against parasitic worms. Similarly, the kainite analogon domoic acid, which is produced by algae and causes amnesic shellfish poisoning, has been used as an anthelmintic in Japan (Hanayanagi).

NMDA-receptors are activated by two processes. They need binding of ligands and the release of a Mg^{2+}-ion-block. Therefore, they only open after membrane depolarization, usually caused by glutamate binding to co-expressed AMPA receptors. This change of membrane charge releases the Mg^{2+}-ion. NMDA receptors are therefore "silent" as long as they are expressed alone on neuronal membranes and only become active when AMPA receptors are co-expressed. Moreover, in addition to glutamate, they have obligatory co-agonists, namely, glycine or D-serine. NMDA receptors play a role in synaptic plasticity, learning and memory. In the cholinergic nervous system, NMDA receptor signalling induces acetylcholine release (Traynelis et al. 2010).

The synthetic drug *p*henyl*c*yclohexyl-*p*iperidine (*PCP* or angels dust), originally used as narcotic, is an open channel blocker of the NMDA receptor. Ketamine was synthesized in order to obtain a general anaesthetic with less side effects than PCP. Ketamine turned out to be a non-competitive antagonist of NMDA channels. As a hypnotic, it induces sleep and dissociative anaesthesia, meaning that protective body reflexes are maintained. Ketamine also has psychedelic potential and has been used as a street drug since the mid-1970s. In small doses, it causes hallucinations, "out of body" experiences, euphoria and others. Chronic administration causes loss of short- and long-term memory.

Notably, ketamine produces both positive and negative symptoms of schizophrenia (see ▶ Box 5.3). It is also used to induce a "schizophrenic condition in animals". Therefore, the original hypothesis of schizophrenia being caused by hyperactive dopamine transmission was amended. The "glutamate" hypothesis suggests that a hypofunction of NMDA receptors on cortical and subcortical GABAergic interneurons is the underlying cause of

excessive dopaminergic and glutaminergic signalling in the cortex. This leads to the positive and negative symptoms of schizophrenia (see ▶ Box 5.3) (Cioffi 2013). Moreover, a polymorphism in the NMDA receptor subunit gene GRIN2B was found in association with the disease (Li and He 2007).

6.6 TRPV and TRPM8: Heat and Cold, Capsaicin and Menthol

TRPV receptors can be subdivided into several subfamilies, of which TRPV1 and TRPV2 are chemo- and thermosensors, TRPV4 is functioning as thermo- and osmosensor and TRPV5 and TRPV6 are Ca^{2+} channels in epithelial cells.

TRPV1 channels are localized on peripheral sensory nerve endings, in the brain and in the spinal cord. They are pain receptors because they detect noxious stimuli and, in response, activate afferent sensory neurons. Therefore, they have been extensively studied as novel targets for the development of analgetics. As non-specific cation channels, they conduct Ca^{2+}, K^+ and Na^+-ions. TRPV1 channels mediate signals of higher temperatures, acids and chemicals that we associate with heat and pain (see hot chillies), as well as inflammatory responses that we also experience as heat and pain. Small molecule chemicals interacting with TRPV1 include capsaicin, the active compound of hot chilli peppers. Interestingly, capsaicin is also used as part of creams to reduce pain, for instance, in arthritic disease conditions. This is supposed to be due to its ability to desensitize TRPV1 (Zhang et al. 2008a, b). Further chemicals binding TRPV1 channels are vanillotoxins from tarantula venom. Endogenous TRPV1 agonists are referred to as endovanilloids, and they include the endocannabinoids anandamide and N-arachidonoyl dopamine, and endogenous lipids like N-acetylethanolamines and polyunsaturated fatty acids. As previously described, endocannabinoids are released from cells in an activity-dependent manner. This also counts for derivatives of arachidonic acid, including eicosanoids, which are released after stimulation of nerve endings with mediators of inflammation, such as bradykinin and prostaglandins (Brito et al. 2014).

TRPV1 channels are part of our temperature-sensing system in the body. Temperature sensitive neurons are present in trigeminal ganglia and dorsal root ganglia and convey signals received from peripheral terminals to the CNS. TRPV1 channels respond to increased temperature (>43 °C), which constitutes a moderate heat. Some of the chemical activators of TRPV1 work by shifting down the threshold temperature for channel opening thus inducing pain sensations already at normal body temperature. TRPV2, also found on sensory neurons and in the brain, is activated at ca. 50 °C, a noxious heat. TRPV3, especially found in the skin and tongue, responds to pleasant warmth of 34–40 °C and TRPV4 to 27–40 °C. The latter is expressed in the airway, liver, heart and brain and in the skin. It is also activated by the endocannabinoid anandamide.

In contrast, TRPM8 channels, members of the melastatin-related TRP family, are modulated by temperatures below 25 °C. They are voltage-dependent cold-sensing Ca^{2+} channels. Menthol and eucalyptol work as allosteric regulators on TRPM8 channels. Binding of these compounds shifts the activation temperature for the channel up; therefore the receptor is activated at higher temperatures. Moreover, these compounds also shift the voltage-dependence of TRPM8 to more negative membrane potentials. Together

with other temperature sensitive receptors TRPM8 is expressed in temperature sensitive neurons, in trigeminal ganglia and dorsal root ganglia. Endogenous ligands for TRPM8 include lysophospholipids, which are products of phospholipase A-catalysed hydrolysis of glycerophospholipids (Berridge 2012).

Interestingly, the gene encoding TRPM8 had first been isolated as a transcriptional marker from prostate epithelial cells (Tsavaler et al. 2001). Meanwhile its expression was also demonstrated to be up-regulated in prostate and other types of cancer (Yudin and Rohacs 2012), but its function in cancer is not clear yet.

Take-Home Message

- Aconitine from the plant Aconitum napellus, conotoxins from snails, tarantula venom toxins, saxitoxins from dinoflagellates and bacterial tetrodotoxins interfere with the function of voltage-gated ion channels, and therefore they are strongly neurotoxic.
- Nicotine agonizes acetylcholine at the nicotinic acetylcholine receptor (NAchR). NAchRs are ion channels belonging to the pLIC family of pentameric ligand-gated ion channels. They transmit signals from motoneurons into muscle cells at the neuromuscular junction and between neurons in the central and peripheral nervous system. Antagonists for these receptors include snake venoms including bungarotoxins and plant poisons such as curare and coniine. Nicotine at doses resulting from tobacco smoking increases neurotransmitter release from neurons in the brain, including dopaminergic neurons, explaining euphoric and relaxing sensations due to tobacco consumption. Stimulation of adrenalin release in the sympathetic nervous system is the cause of sympathomimetic effects.
- *Muscimol and Thujone* interact with $GABA_{A/C}$-receptors. GABAergic chloride channels are also members of the pLIC family. By gating influx of negatively charged ions, they induce hyperpolarisation. Muscimol is produced from ibotenic acid found in poisonous fungi and acts antagonistically to GABA, inducing sedation and psychological effects. Thujone is a GABA receptor antagonist. At high doses it induces epileptic convolutions. Synthetic drugs for GABA receptors include barbiturates and benzodiazepines (tranquillizer).
- *Strychnine* is an antagonist for glycine channels, pLICs conducting chloride ions. Such receptors mediate feedback after muscle excitation. Strychnine thus induces spastic muscle contractions. Tetanus toxin, by degrading SNAREs at the same synapses, has a similar effect on muscle contraction.
- Ketamine and PCP are synthetic drugs antagonizing glutamate receptors, more specifically NMDA channels. They cause symptoms of schizophrenia and are hallucinogens.
- *Capsaicin and menthol* interact with TRP-channels that are involved in sensing heat and cold. ◘ Table. 6.1 lists these ion channels, their endogenous ligands and plant derived drugs with their mode of action on each of them.

Table 6.1 Summary of ion channels as targets for plant-derived drugs

Ion channels	Endogenous ligand/ activator	Plant-derived drugs	Major mode of drug action
Nicotinic acetylcholine receptor NAchR	Acetylcholine	Nicotine Cytisine Curare alkaloids Coniine Physostigmine	Agonist Agonist Antagonists Antagonist Acetylcholine esterase Inhibitor
$GABA_A$ receptor	GABA	Muscimol Cicutoxin Valerenic acid Thujone Anistatin Bicuculline	Agonist Antagonist Allosteric positive Allosteric negative Allosteric negative Allosteric negative
Glycine receptor	Glycine	Strychnine Tutin	Antagonist Antagonist
NMDA receptor Kainate receptor AMPA receptor	Glutamate	Ibotenic acid	Antagonist
TRPV1 channel	Voltage heat	Capsaicin	Allosteric positive
TRPM8 channel	Voltage cold	Menthol	Allosteric positive
Voltage-gated Na^+ channel	Voltage	Aconitine	Persistent activation
Ryanodine receptor	Ca^{2+}	Ryanodine	Channel closure

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Plant-Derived Drugs: Their Function in Plants and Potential Biotechnological Use

Plants are a rich source of secondary metabolites, many of which affect important human receptors. Therefore, plants containing these metabolites have long been used for medicinal purposes and as source for recreational drugs. While plants such as poppy harbour only few, closely related compounds of interest, other plants contain whole sets of different metabolites. These are derived from various and sometimes uncommon pathways and they act on a large set of different receptors. Also, many compounds can affect more than one receptor. In this chapter, we describe representative compounds and their main plant sources for the classes of receptors introduced in ► Chap. 2. Thus, in line with ► Chap. 2, cannabis, muscarine and atropine, caffeine, cocaine, opiates, nicotine, curare, thujone, strychnine and several other drugs will be covered in more detail. Natural sources of these drugs from various plants will be described, and in the – unfortunately rare – cases, where the role of these drugs in the natural plant environment is known, this function will be elaborated on.

Contents

Chapter 7 **Plant-Derived Drugs Affecting GPRCs – 99**

Chapter 8 **Plant-Derived Drugs Affecting Ion Channels – 121**

Plant-Derived Drugs Affecting GPRCs

7.1 Muscarinic Acetylcholine Receptors: Atropine, Muscarine and Scopolamine – 100

7.2 Adrenergic Receptors: Ephedrine and Reserpine – 102

7.3 Adenosine Receptors: Caffeine – 104

7.4 Cannabinoid Receptors: Phytocannabinoids – 107

7.5 Dopamine Receptors: Cocaine and L-DOPA – 110

7.6 5HT2A Receptors: Ergotamine and Mescaline – 111

7.7 Opioid Receptor: Morphine/Opiates and Opioids – 112

References – 116

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What You Will Learn in This Chapter

The presence of GPRCs in plants is still a question of debate. While G-protein coupled signalling exists, the signalling cycle is typically not activated by seven transmembrane-spanning receptors. By contrast, many plant secondary metabolites are known to affect human GPRCs. Some are very specific for a single type of receptor; however, many others act on more than one type, albeit with often strongly different affinities.

7.1 Muscarinic Acetylcholine Receptors: Atropine, Muscarine and Scopolamine

In case of the *muscarinic acetylcholine receptor*, the name-giving compound is derived from some poisonous mushrooms, especially of the *Inocybe* and *Citcybe* species, such as the ivory funnel (Malone et al. 1962; Lurie et al. 2009). Muscarine, a quaternary amine, was first isolated from *Amanita muscaria* (Fig. 7.1), even though it is present in this mushroom in very low concentrations (Schmiedeberg and Koppe 1869), and the toxic compound responsible for its toxicity was later shown to be muscimol. Muscarine is an acetylcholine agonist that binds to all muscarinic acetylcholine receptors independent of subtype (Fig. 7.1). It can activate the receptors with a slower but longer-lasting effect

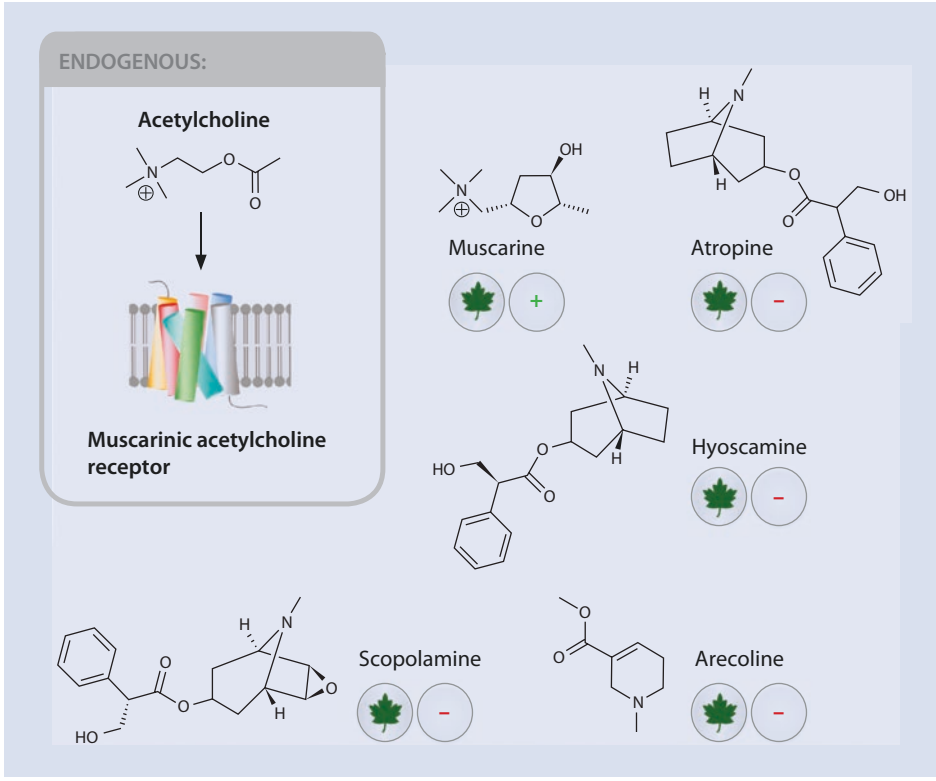


Fig. 7.1 Examples of natural compounds that act as agonists or antagonists of the muscarinic acetylcholine receptor

Fig. 7.2 *Amanita muscaria* and *Atropa belldonna* (© Max Weigend)

compared to the endogenous ligand acetylcholine probably because it is not hydrolysed by acetylcholinesterase (Fraser 1957).

The *muscarinic acetylcholine receptors* are also targeted by plant-derived secondary metabolites (Fig. 7.1). The best known of those is atropine, a *tropane alkaloid* found in plants such as *Atropa belladonna* (deadly nightshade, Fig. 7.2), *Atropa mandragora* (mandrake), *Datura stramonium* (Jimson weed) and other members of the Solanaceae family. Indeed, it was the isolation of atropine and its use in neurological studies that helped to identify acetylcholine as an important neurotransmitter in mammals (Mein 1831).

Atropine has a long history of use in medicine, cosmetics and as a poison (Holzman and Robert 1998). Its name is derived from Atropos, one of the three fates that according to Greek mythology ended the life of each mortal by cutting their life thread (Jenkinson 1986). Theophrastus (370–285 AD) described the use of mandrake for treating wounds or skin infections. It was in connection to the use of mandrake wine that the term anaesthesia is recorded for the first time by Dioscorides (40–90 BC) in his *De Materia Medica*. Cosmetic use is based on its property to dilate the pupils, which in older times was considered a mark of beauty and is the reason for the given species name *belladonna*. The atropine in nightshade extracts inhibits constriction of eye muscles that would reduce pupil size. Allegedly, already Cleopatra made use of extracts from the atropine containing Egyptian henbane (*Hyoscyamus muticus* L.) to dilate her pupils. It is still used to today in eye drops as a mydriatic substance in treatment of amblyopia or uveitis.

More importantly, atropine is on the WHO Model List of Essential Medicine (► www.who.int). As a competitive *muscarinic acetylcholine antagonist,* it is an antidote to muscarine and therefore even twice on that list (see ► Table 1.1). It can also be used as treatment in case of poisoning by organophosphate insecticides or nerve gases such as tabun and sarin (Bajgar 2004). These compounds inhibit the enzyme acetylcholine esterase that degrades acetylcholine in the synaptic cleft and similar to muscarine lead to an increased impact of acetylcholine that is counteracted by atropine.

Chemical analyses showed that all plant parts contain atropine (Kuganathan and Ganeshalingam 2011). Due to its toxic effects, it is likely produced as a deterrent against herbivory. However, despite the high atropine content, the seeds of deathly nightshade and other atropine containing plants are dispersed by animals. Interestingly, this lack of effect of atropine on these animals is not very well studied. What few studies there are

suggest a quick elimination of atropine from the body of these animals, i.e. via high levels of serum tropine esterase activity (Sawin and Glick 1943) or other types of metabolic breakdown in the liver. Activity of the tropine esterase can vary between animals of the same species, but pronounced activity was found in the serum of rats, chicken and rabbits as well as the liver of frogs (Glick and Glaubach 1941). Bees supposedly can also tolerate atropine since it has been found enriched in honey of bees pollinating deadly nightshade plants (Hazslinszky 1956; Islam et al. 2014).

The deadly nightshade and other plants from the Solanaceae family additionally contain the *tropane alkaloids* scopolamine and hyoscyamine, both of which are antagonists of muscarinic acetylcholine receptors. Scopolamine is also on the WHO Model List of Essential Medicine and has been employed in its purified form since its first isolation in 1880 (Ladenburg 1881); however, all of these tropane alkaloids have been used as active ingredients of plant extracts long before. As such, these plants are well described parts of ethnomedicine such as the use of Jimson weed and henbane in Tibet (Ma et al. 2015).

The *pyridine alkaloid* arecoline is a partial agonist of the *muscarinic acetylcholine receptor* and as such has parasympathomimetic effects. It is found in the areca nut (*Areca catechu* L.) and has long been used in Chinese traditional medicine as a remedy against tapeworm or diarrhoea (Liu et al. 2016). The nut is often chewed wrapped in betel (*Piper betle*) and tobacco leaves or mixed with these and other spices thereby creating a blend of active ingredients. It is very popular in Asia, allegedly used by about 600 million people, making it one of the most-used plant-derived drug.

7.2 Adrenergic Receptors: Ephedrine and Reserpine

Ephedrine and the related plant-derived *phenylethylamines* pseudoephedrine, methylephedrine and methyl pseudoephedrine are mixed acting sympathomimetics. They directly interact with α- and β-adrenergic receptors (Fig. 7.3), but they also act indirectly by inhibition of the noradrenaline reuptake, thereby increasing the amount of noradrenaline in the synaptic cleft (Kobayashi et al. 2003; Reith et al. 1997). Ephedrine and pseudoephedrine are found in plants of the *Ephedra* genus such as *Ephedra sinica*. Better known as má huáng (麻黄), this plant has a long use in traditional Chinese medicine. Ephedra extracts are also part of dietary supplements used to promote weight loss and athletic performance (Shekelle et al. 2003). While on the WHO Complementary List of Essential Medicines, there are also concerns regarding severe side effects (Haller and Benowitz 2000; Andraws et al. 2005). The closely related *phenylethylamines,* norephedrine and norpseudoephedrine, are found in Khat (*Catha edulis*, qat), a flowering plant whose leaves have been chewed in its native African and Arabian range in a manner similar to the chewing of coca leaves in South America. Very little is known about the function of these compounds in plants. Allelopathic effects on seed germination have been observed for *Ephedra* extracts, but the molecular base for these properties or its use in the natural environment is completely undetermined.

The *indole alkaloid* reserpine is found in the roots of Indian snakeroot (*Rauvolfia serpentina*), a plant widely distributed in India and the sub-Himalaya, where it has a long history as a medicinal herb. It inhibits the "vesicular transporter for monoamine storage" (VMAT), thereby blocking the uptake of noradrenalin (but also dopamine and serotonin) from the cytoplasm into neuronal storage granules (Schuldiner et al. 1993). By contrast,

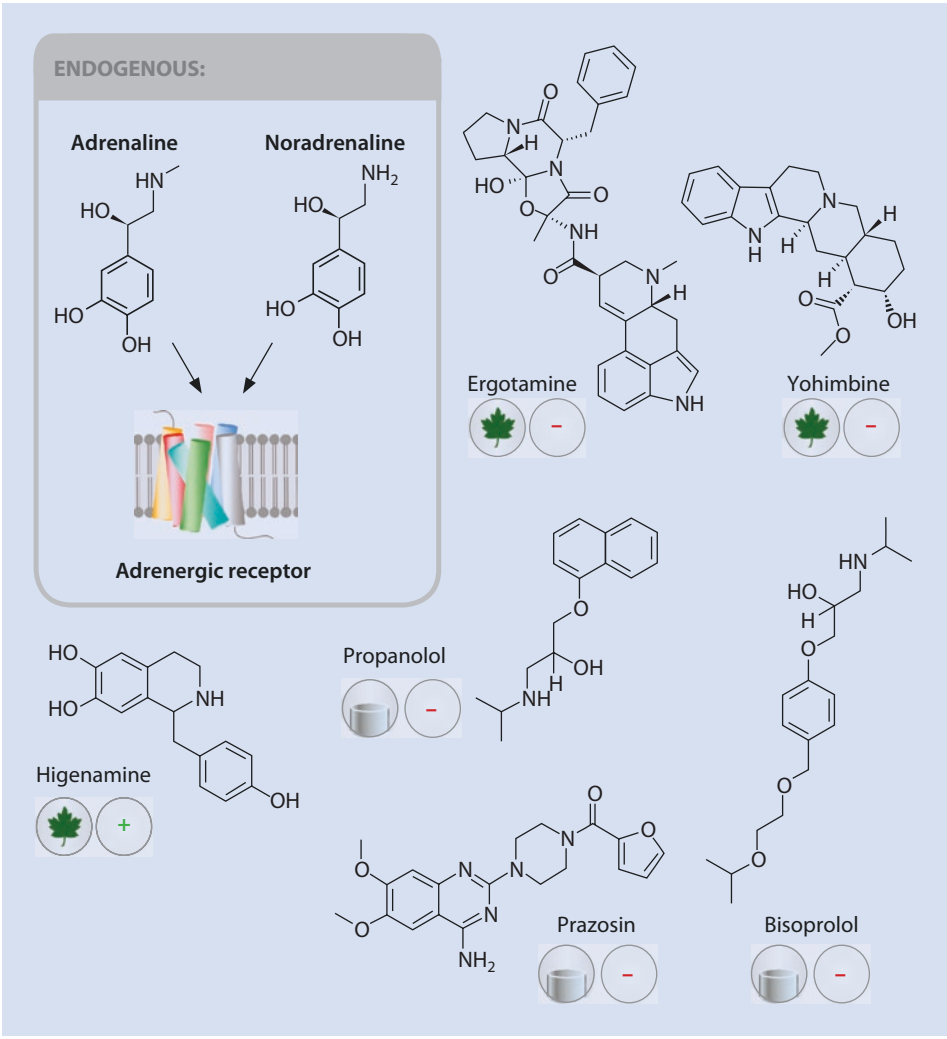


Fig. 7.3 Examples of natural and synthetic compounds that act as agonists or antagonists of the adrenergic receptor

the *indole alkaloid* yohimbine has high affinity for the α2-adreneric receptor. It is found in the bark of the yohimbine evergreen (*Pausinystalia johimbe*), a tree native to West and Central Africa. It has been used in traditional African medicine as a general tonic and as an aphrodisiac. However, extracts of the yohimbine bark are banned as dietary supplement in many countries due to insufficient scientific data and concerns about adverse effects (Aguilar et al. 2013).

Norcoclaurine, also called higenamine, is an antagonist of β2-adrenergic receptors. It is found in plants of several genera including *Nandina domestica* (sacred bamboo), *Aconitum carmichaelii* (Chinese wolfsbane) and *Galium divaricatum* (Lamarck's bedstraw) or the seeds of *Nelumbo nucifera* (Indian lotus), where it accumulates in various organs depending on the plant, including fruits, roots, stems or seeds. Traditional Chinese medicine has used Chinese wolfsbane for treatment of asthma, but positive effects on fat

burning, erectile dysfunction and other maladies have also been described (Zhang et al. 2017; Bai et al. 2008). As with other plant-derived compounds that affect adrenergic receptor function, its role for the plant itself is unknown.

7.3 Adenosine Receptors: Caffeine

Caffeine is probably the psychoactive drug with the highest proportion of usage worldwide (Fredholm et al. 1999). It is a *purine alkaloid* that is most prominently found in the seeds and leafs of coffee plants (Fig. 7.4). Its presence has been shown in over 60 other types of plants including the tea bush, kola nut, yerba mate, guarana berries or the yaupon holly. The formation of caffeine is a case of convergent evolution, which means the biosynthetic pathways to make the compound are different in various plants and have evolved independently (Denoeud et al. 2014; Huang et al. 2016).

The use of leaf extracts from caffeine-containing plants probable dates back many thousand years (Ukers 1922; Murray 2001; Weinberg and Bealer 2001). Clear evidence for the use of coffee as a stimulating agent is found in fifteenth-century Yemen from where it spread to Egypt and North Africa. By the sixteenth century, it had reached the rest of Persia, Turkey and the Middle East, from where the habit of drinking coffee came to Italy and to the rest of Europe. Ultimately, coffee plants were transported by the Dutch to the East Indies and to the Americas. By now coffee trees are grown in over 80 countries and have become one of the most important crops and the second most traded commodity internationally after oil (Mishra and Slater 2012). While coffee is often taken because of its stimulating properties, the extract contains many other substances creating its specific taste and to cater towards a market where the taste but not the stimulating properties is desired, decaffeinated coffee is in demand. Therefore, already in 2003, coffee trees have been manipulated by RNAi-mediated gene silencing of the gene encoding theobromine synthase. These plants show a 70% lower content of caffeine due to reduction in theobromine synthase activity (Ogita et al. 2003).

Caffeine was one of the first secondary metabolites to be isolated in its pure form. It was identified in 1819 by the German chemist Friedlieb Ferdinand Runge, allegedly at the behest of the German poet and scientist Johann Wolfgang von Goethe (Runge 1866).

Fig. 7.4 Cauliflorous flowers and fruits of a coffee plant (© Ute C. Vothknecht)

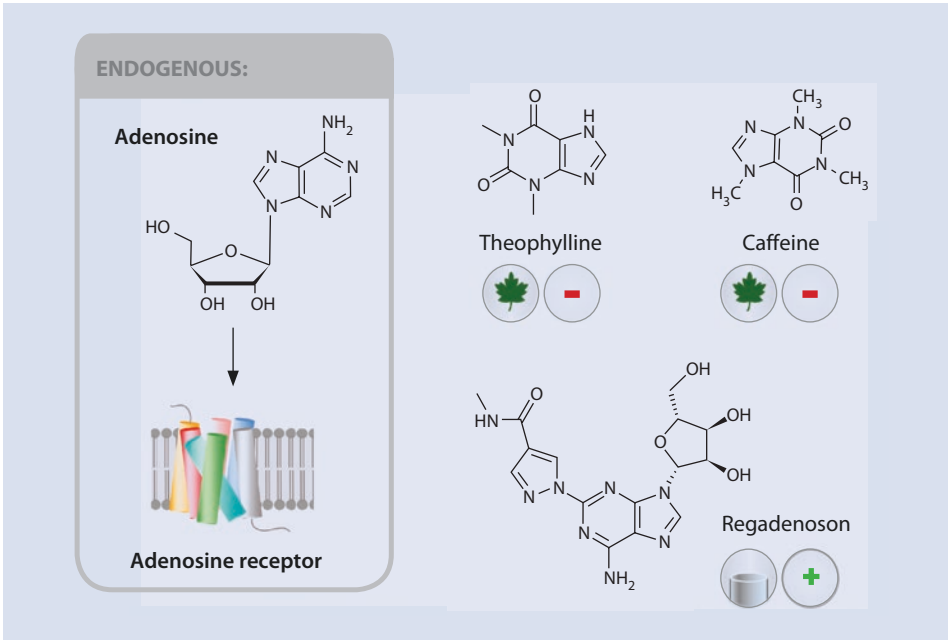


Fig. 7.5 Examples of natural and synthetic compounds that act as agonists or antagonists of the adenosine receptor

Caffeine affects several cellular targets. It is an antagonist for *adenosine receptors*, (Fig. 7.5), and it was suggested that this interaction is the major cause for the stimulating effect, because much lower concentrations are required compared to other cellular targets at least in mammals (Fredholm et al. 1999). Caffeine was also shown to lower the threshold for Ca^{2+} release via the *ryanodine* Ca^{2+} release channel in the sarcoplasmic reticulum, thereby increasing intracellular calcium levels (Kong et al. 2008). In higher concentrations, it also causes inhibition of phosphodiesterase and a subsequent increase in intracellular cyclic adenosine monophosphate levels. This latter action was suggested to be the base for its toxicity to insects (Nathanson 1984), further indicating that caffeine and other *methylxanthines* such as theophylline are used by plants as defence chemicals against herbivory since they act as a natural insecticide.

Box 7.1 Effect of Caffeine on Spiders

The effect of different drugs on the capabilities of spiders to weave their net was analysed by some scientists from NASA (Noever et al. 1995). Based on an earlier study performed by Peters and Witt in 1948, they fed different drugs to spiders and watched them spin their net. No results were published from the 1948 study, and only a few pictures of the NASA experiments are available, but it appears that caffeine had one of the most pronounced effects of all drugs analysed, with a net architecture that was highly disturbed.

Caffeine is also toxic for animals such as spiders (see ▶ Box 7.1) or mussels (Chen and Bayne 1995) as well to humans where the lethal dose is about 10 g for an average-weight adult (Ritchie 1975; Rivenes et al. 1997).

Fig. 7.6 Cauliflorous pods and opened fruit of a cacao plant (© Ute C. Vothknecht)

It should also be noted that caffeine is metabolized in the body to other compounds such as theobromine, theophylline and paraxanthine (Fredholm et al. 1999). All of these are not only metabolization products of caffeine but also found in various plants. Theobromine is the major active ingredient in cacao (*Theobroma cacao*, Fig. 7.6). It has similar properties as caffeine but is much less potent.

Caffeine released from decaying seeds and leaves accumulates in the soil where it causes *allelopathic effects*. It was suggested that this is due to the inhibitory effect of caffeine on mitosis as observed in lettuce (Friedman and Waller 1983) and is one of the growth-limiting problems in intercropped coffee plantations (Bustos et al. 2008). While this effect is important for growers who want to diversify by intercropping coffee with medicinal and aromatic plants, it is unclear whether it plays a role in warding off competition by other plants under natural conditions. In a similar way, the exuding of caffeine from primary roots was suggested to cause an allelopathic effect but more likely is a way to fend off underground attacks by nematodes (Baumann 2006). Caffeine also might have a use in attracting pollinators since it was shown that it can enhance the reward memory of bees (Wright et al. 2013).

In addition to caffeine and related alkaloids, also some *flavones* act on the *adenosine receptor* (Jacobson et al. 2002). These include galangin found in some plants from the ginger family or the *Helichrysum* genus as well as cirsimarin and cirsimaritin, flavonoids from the traditional medicinal plant *Microtea debilis* (Hasrat et al. 1997). Also, *aurones* such as hispidol that are widely distributed in yellow flowering plants and have a role in flower colouring and stress response (Pare et al. 1991) have been identified as adenosine receptor antagonists (Jacobson et al. 2002).

7.4 Cannabinoid Receptors: Phytocannabinoids

Cannabinoid receptors (CB1 and CB2) are regulated in animals by a group of lipid-based ligands called endocannabinoids. However, plant-derived ligands for these receptors were known well before endocannabinoids were identified and even provided the name for this receptor family (Fig. 7.7). Origin of these ligands, now called phytocannabinoids, are plants of the genus *Cannabis*, foremost *Cannabis indica* and *Cannabis sativa* (Fig. 7.8). In contrast to endocannabinoids (which are *N-acylethanolamines*), they are terpeno-phenolic compounds derived from *polyketides* that despite their different chemical origin can bind to and affect the action of cannabinoid receptors due to a structural similarity that fits the ligand-binding site (Thomas et al. 1996). They include the best-known cannabinoids Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD).

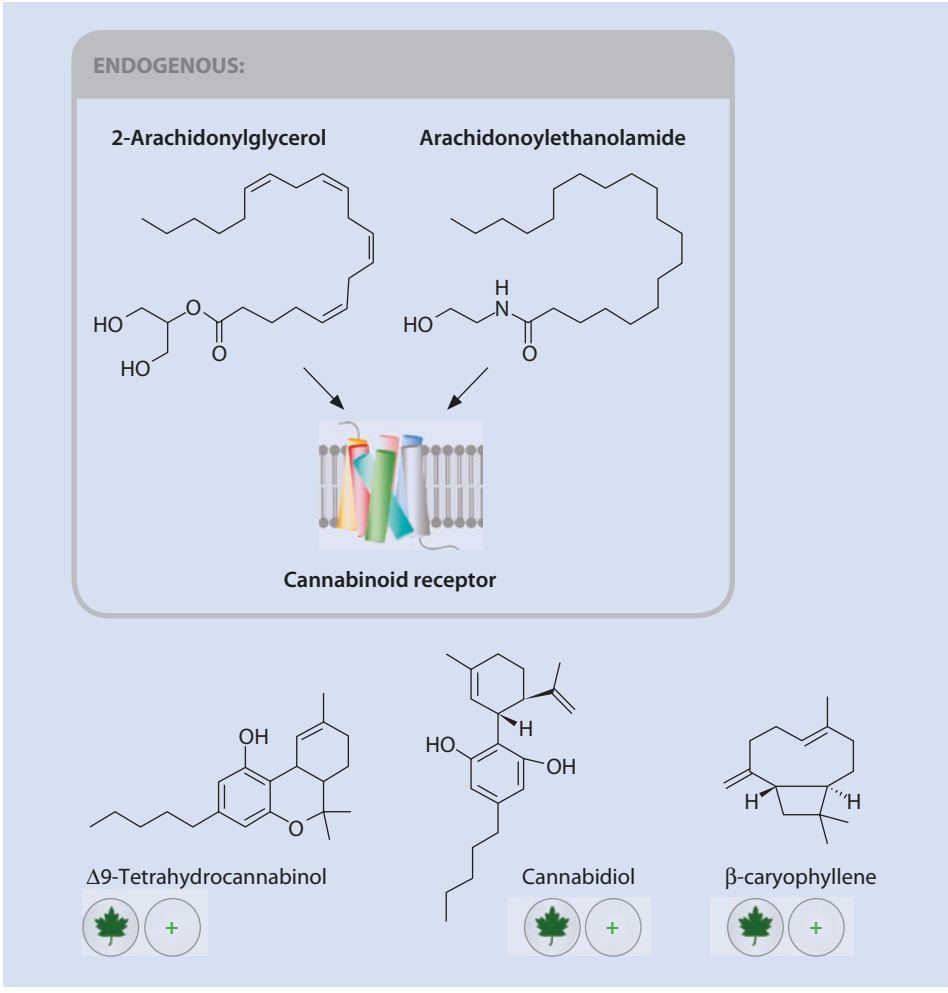


Fig. 7.7 Examples of natural compounds that act as agonists of the cannabinoid receptor

Fig. 7.8 *Cannabis sativa* (© Max Weigend)

The use of these plant-derived compounds to affect the human mind has a long history. Already the Atharvaveda, an old sacred Indian text, and the first Chinese pharmaceutical book describe the medical powers of *Cannabis* (Li 1973). A description by Herodotus (500 BC) of the habit of central Eurasian Scythians to take cannabis steam baths and recent findings of hemp seed pots in a Scythian burial site support the idea that the nomadic tribe brought psychoactive Cannabis strains to Europe where its usage is known throughout the classical Greek and Roman time. By contrast, hemp – a strain of *Cannabis* (*Cannabis sativa* L.) that has low THC content and therefore little to none psychoactivity – has been used for fibre production since over 10,000 years in Europe (see ► Box 7.2). Impressions of plant fibre cordage were found in clay fragment at a burial site in Czechoslovakia dating back to 26,900 BC, making it one of the oldest human artefacts (Pringle 1997; Fleming and Clarke 1998).

Box 7.2 Short History of Hemp Usage

Historical records indicate that hemp has also been used since ancient times to prepare paper for writing in Asia (about 100 BC in China and third century AD in India) and Europe (since the sixth century). It was often used in combination with other fibres. Allegedly, the "Magna Carta" was written on hemp paper as well as an early copy of the first Gutenberg Bible and two original drafts of the "Declaration of Independence". The latter was supposedly due to Benjamin Franklin having owned a hemp paper mill in the Americas, but neither is supported by clean-cut historical evidence. Other famous writings allegedly pinned on hemp paper include "The King James Bible" (the seventeenth century), the works of Mark Twain and Lewis Carroll's *Alice in Wonderland*.

THC, the principal active ingredient of cannabis, was only isolated in 1964 (Gaoni and Mechoulam 1964). By now it is known that plants of the *Cannabis* genus contain over 100 compounds that are characterized as cannabinoids based on their structure (Aizpurua-Olaizola et al. 2016); however, CBD and THC are the two cannabinoids usually produced in greatest abundance. Of these two, only THC has psychoactive properties. The ratio of CBD:THC varies in different species of *Cannabis* but does not change much throughout the life of a plant. Most other known phytocannabinoids do not have psychoactive properties but some can enhance the effect of THC, while other such as CBD can even counteract it. Indeed, also THC is normally present in the cannabis plant as its acidic precursor THCA (tetrahydrocannabinolic acid) that has no psychoactive properties. In order to work on the cannabinoid receptors, it needs to be decarboxylated to THC by heat or acidification, which explains the usual intake form for the drug by smoking or digestion. Some cannabinoids can also bind to other receptors, i.e. cannabigerol, which is a potent α2-adrenoreceptor agonist and a moderate 5-HT1A receptor antagonist (Cascio et al. 2010).

The *Cannabis* plant is dioecious, and cannabinoids are produced mainly in the bud of female plants, where they are concentrated in a viscous resin that is secreted by *glandular trichomes*. Very little substantial evidence has so far been obtained that would shed light onto the function of cannabinoids in the female bud. As with many secondary metabolites, it is believed that it plays a role in self-defence, e.g. against herbivores. Cannabinoids also seem to have antibacterial properties, but these have mainly been investigated with the idea for use as new antibiotics (Appendino et al. 2008; Radwan et al. 2009). For THC, high UV-B absorption properties have been described, and it could thus act as a sunscreen to protect the plant from UV damage (Pate 1983; Lydon et al. 1987).

Novel phytocannabinoids that bind to the *CBS2 receptor* but are not psychoactive have been identified in recent years in other plants (Gertsch et al. 2010). These include *N-alkylamides* such as N-isobutylamide from *Echinacea* spp., which bind peripheral CB2 receptors (Gertsch et al. 2006). The CB2 receptor is also targeted by the dietary cannabinoid β-caryophyllene, a *bicyclic sesquiterpene* commonly found in plant such as cloves, rosemary or hops but also in cannabis (Gertsch et al. 2008). Its presence could contribute to the described anti-inflammatory and analgesic effects of clove oil. As for the plants, it was also shown that β-caryophyllene is part of the root exudate emitted by certain maize plants when attacked by larvae of coleopteran pests such as the Western corn rootworm (*Diabrotica virgifera)*. Here it acts as an attractant for the entomopathogenic nematode *Heterorhabditis megidis*, a natural enemy of the rootworm, which helps the plants to fend of the attack (Rasmann et al. 2005). While this system is present in the maize ancestor line as well as European breeding varieties, it was outbred in American maize lines resulting in their higher susceptibility to *D. virgifera* infection (Kollner et al. 2008). ß-caryophyllene is also found in the volatiles released by maize leafs in response to attack by lepidopteran larvae like *Spodoptera littoralis*, where it helps to attract parasitic wasps.

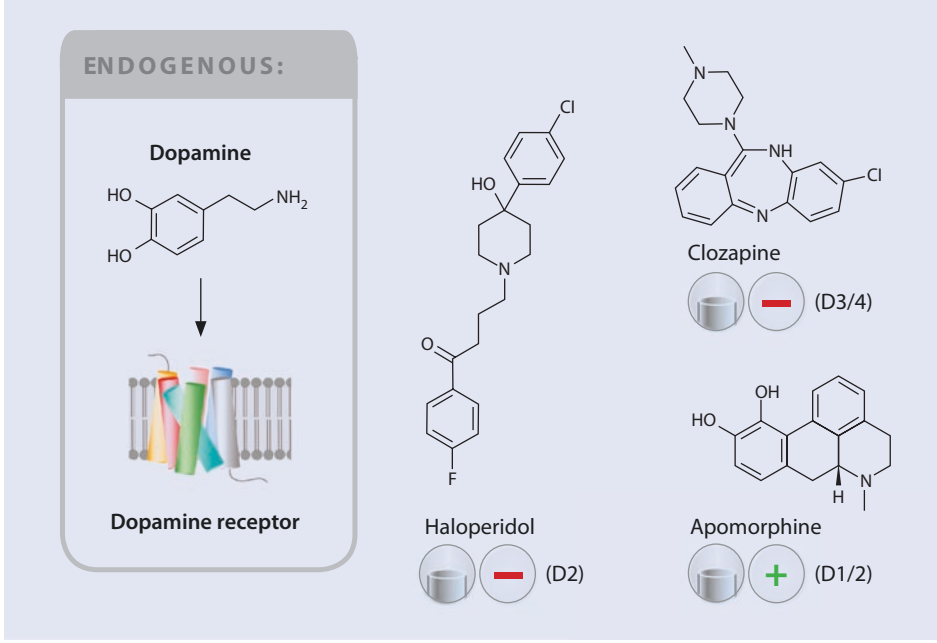
Catechins are *phenylpropanoids* ubiquitously present in vascular plants, and selected tea catechins were found to have weak affinity for *CB2* and moderated affinities for *CB1 receptors* (Korte et al. 2010). Falcarinol, a *polyacetylene* from plants of the Apiaceae family such as carrots, acts selectively on CB1 (Leonti et al. 2010). Other secondary metabolites do not bind CB-receptors but interfere with signalling indirectly. These include *fatty acid derivatives* such as the N-acylethanolamines, N-linoleoylethanolamide and N-oleoylethanolamide found in chocolate (*Theobroma cacao* L.) and many other plants or palmitoylethanolamide that inhibits anandamide breakdown (Di Marzo et al. 1998; Maurelli et al. 1995; Di Tomaso et al. 1996).

7.5 Dopamine Receptors: Cocaine and L-DOPA

As presented above (see ▶ Sect. 5.5 in Chap. 5), dopamine has a very important function for the reward system in the human brain, and therefore all drugs that enhance dopamine signalling may have an exhilarating effect on humans. There is no secondary metabolite known to directly interact with the dopamine receptor (◘ Fig. 7.9), but compounds such as cocaine can enrich dopamine concentration by inhibition of the *dopamine transporter* (Ritz et al. 1987). These inhibitors also affect the transporters for serotonin and norepinephrine leading to multiple effects.

Cocaine is a *tropane alkaloid* exclusively found in coca plants (*Erythroxylum* spp.). While found in the leaves of many species of *Erythroxylum*, larger amounts of cocaine are only present in two cultured varieties. Cocaine concentration can vary with environmental conditions and age, but synthesis seems to occur throughout the lifetime of the plant (Rivier 1981). The most likely role of cocaine is protection against pathogens and herbivores since coca plants in the field are unusually pest free with herbivore attack observed only rarely (Plowman and Weil 1979). It was shown that spraying tomato plants with cocaine caused rearing, tremors and walk-off behaviour in *Manduca sexta* larvae. Higher concentrations led to the death of the larvae after 24–48 h (Nathanson et al. 1993). Cocaine furthermore affected the hatching of larvae if eggs were exposed, and it was also shown to be effective against mosquito larvae.

Legumes plants such as velvet bean (*Mucuna pruriens*) or broad bean (*Vicia faba*) were shown to contain L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of adrenalin, noradrenalin and dopamine. It occurs in plants as an intermediate in the synthesis of



◘ **Fig. 7.9** Examples of synthetic compounds that act as agonists and antagonists of the dopamine receptor

alkaloids such as melanin or catecholamine and some plants enrich L-DOPA in their leaves and bean pods (Sathiyanarayanan and Arulmozhi 2007).

L-DOPA can be converted by DOPA carboxylase to dopamine and thus increase dopamine concentrations in the central and the peripheral nervous system. This appears to be part of a defence system since velvet beans seem to be well protected against attacks by small mammals and insects. It was shown that larvae of the Southern armyworm show an increased mortality when fed with either velvet seeds or L-DOPA (Rehr et al. 1973) indicating that this compound is directly involved in the protection. By contrast, the macro algae *Ulvaria obscura* seems to use dopamine itself as an anti-herbivore to reduce consumption by snails and isopods (Van Alstyne et al. 2014). As observed before, some animal can overcome these defences and even use them for their own advantages. In case of L-DOPA, the pea aphid *Acyrthosiphon pisum* can feed on broad beans without detrimental effects. Even more, it sequesters L-DOPA and even passes it on to the next generation. Zhang and co-workers (2015) further suggested that it might be used to improve wound healing and to protect against UV-A damage. Similar to caffeine it was suggested that plants might use L-DOPA for allelopathy (Nishihara et al. 2004; Soares et al. 2012) since they extrude the compound from the roots into the soil where it reaches high enough concentrations to become inhibitory for plant growth.

7.6 5HT2A Receptors: Ergotamine and Mescaline

The *5HT2A receptor* agonists psilocybin and ergotamine come from mushrooms and not plants. The alkaloid psilocybin is one of the major psychoactive substances of "magic mushrooms" such as members of the genus *Psilocybe* (Hofmann et al. 1959). Ergotamine, which also binds dopamine receptors and adrenergic receptors with high affinity, is a derivative of ergoline and part of the ergot family of alkaloids found in lower fungi (Schardl et al. 2006). It is best known as the causal agent of ergotism, a poisoning that occurs in humans and other mammals who consume rye grains contaminated with the fungus *Claviceps purpurea*. Medicinal usage of ergot fungus began in the sixteenth century to hasten childbirth (or induce abortion), yet dosage uncertainties discouraged the use. It has also been employed to prevent post-partum haemorrhage (Lonitzer, 1593).

Ergotamine was first isolated from the ergot fungus by Arthur Stoll at Sandoz in 1918, and it was in connection with his work on ergotamine that the German chemist Albert Hofmann synthesized LSD in 1938 (Hofmann 1980). Ergotamine is not restricted to rye grain since infection of grass seeds with endophytic fungi producing mycotoxins is quite common (Czarnoleski et al. 2010). Ergotamine is also found in the seeds of certain dicotyledonous plants, and it is believed that in all cases it is of fungal origin (Steiner et al. 2006). It is further believed that the formation of ergotamine and other mycotoxins might be part of a mutual benefit situation, where the compound made by fungi protects the seeds from pathogen infection and herbivores, while the fungus obtains nutrients from the plant.

A plant-derived secondary metabolite known to interact with the 5HT2A receptor is the *phenethylamine alkaloid* mescaline (Fig. 7.10), which is found in several members of the Cactaceae (Poisson 1960; Crosby and McLaughlin 1973). Buttons from peyote cacti (*Lophophora williamsii*) and mescal bean (*Sophora secundiflora*) are used by native North Americans to brew a hallucinating drink for spiritual purposes and archaeoethnobotany evidence suggest that this habit might have been around for at least 5500 years (El-Seedi et al. 2005; Bruhn et al. 2002). Mescaline is a *nonselective serotonin receptor agonist*, inter-

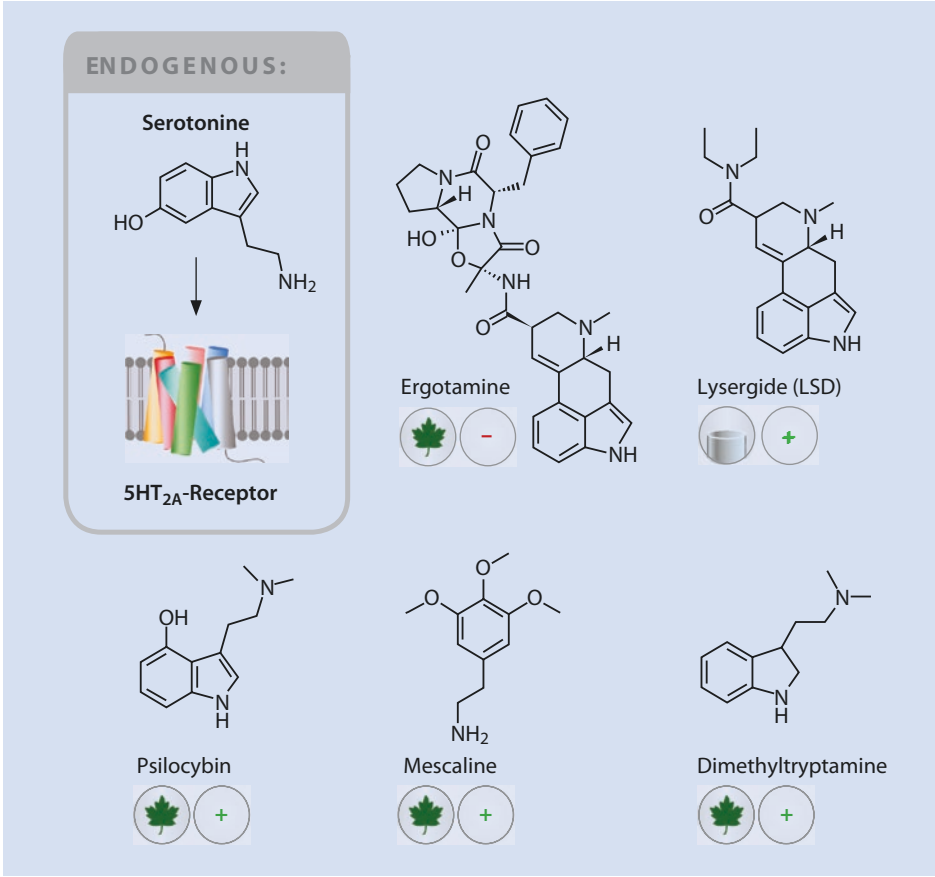


Fig. 7.10 Examples of natural and synthetic compounds that act as agonists and antagonists of the dopamine receptor

acting with all of the 5HT receptors, but experimental evidence indicates that its hallucinogenic properties stem from its interaction with the 5HT2a receptor (Appel and Callahan 1989). Very little abuse is observed with mescaline use likely due to bitter taste, nausea induction and low potency (3000 times less potent then LSD). Nothing is known to date about the role of mescaline in the plant itself.

7.7 Opioid Receptor: Morphine/Opiates and Opioids

The best-known plant-derived secondary metabolites that affect *opioid receptors* are the psychoactive ingredients of opium poppy, morphine and codeine (Weid et al. 2004). While opioids is a term to describe all substances that produce morphine-like effects by interaction with the opioid receptors (Fig. 7.11) independent of their origin, opiates is used as a generic term solely for the different alkaloids present in the opium poppy. While morphine is the main *opiate* found in opium poppy, it was shown that this plant contains >30 different alkaloids, including further important pharmacologically active alkaloids such as noscapine and thebaine (paramorphine).

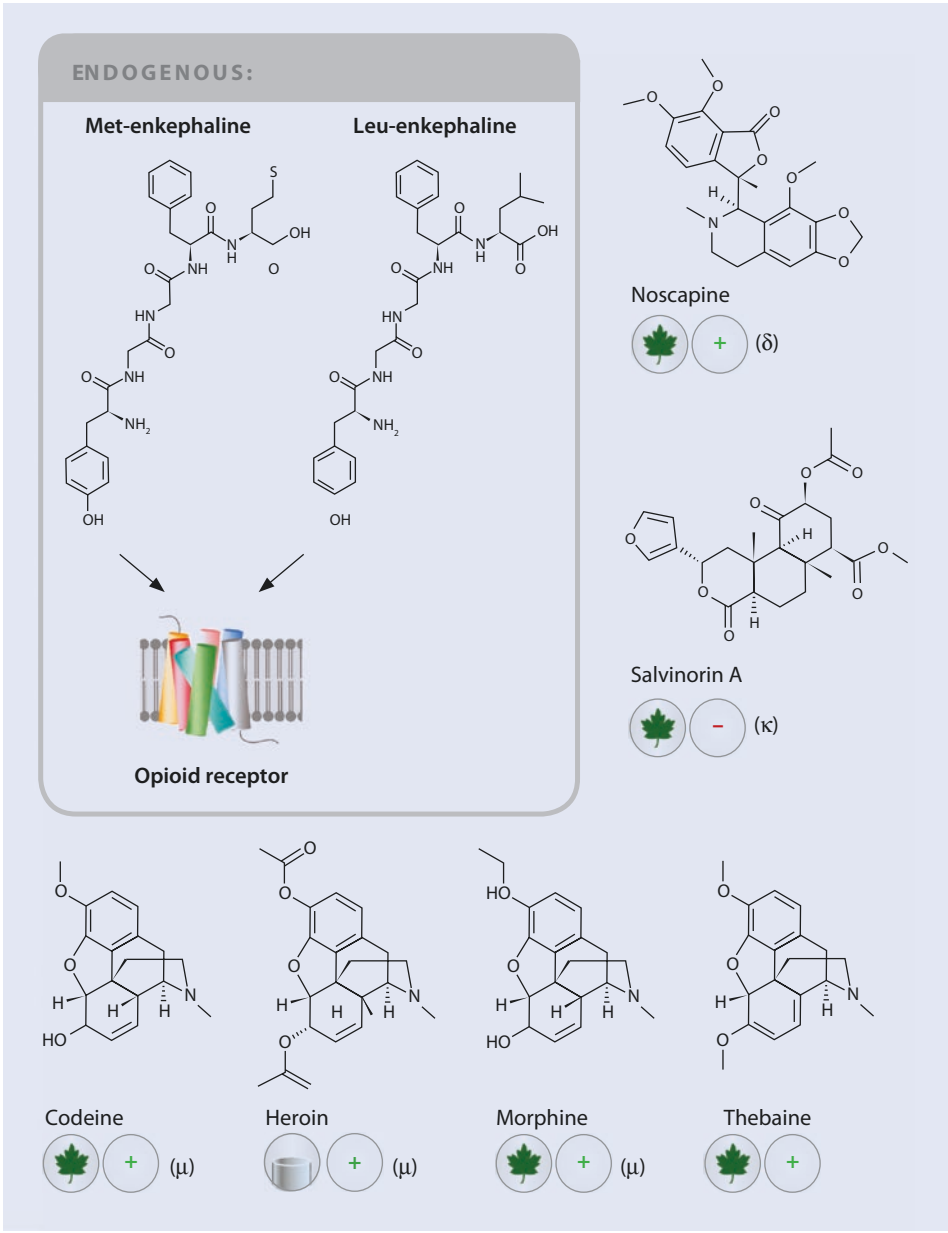


Fig. 7.11 Examples of natural and synthetic compounds that act as agonists and antagonists of the opioid receptor

The pain-relieving properties of opium poppy (*Papaver somniferum*, Fig. 7.12) have been known for millennia. Theriac is a universal remedy dating back to ancient times, and recipes for this remedy can be found in medical and pharmacological books up to the nineteenth century. Although theriac is much older, it was allegedly the ancient Greek that added opium to the set of its ingredients (see ▶ Box 7.3). Even now, opium poppy is the main commercial source for medically used morphine, codeine and their semisynthetic derivatives.

Fig. 7.12 Papaver somniferum flower and pod (© Max Weigend)

Box 7.3 Recipe for Theriac, an ancient universial remedy

A recipe for theriac from the *Pharmacopoea Germanica* (1882):

- 1 Teil Opium
- 3 Teile spanischen Wein
- 6 Teile Angelikawurzel
- 4 Teile Radix Serpentariae (Wurzel der Virginenhohlwurzel, *Aristolochia serpentaria*)
- 2 Teile Baldrianwurzel
- 2 Teile Meerzwiebel
- 2 Teile Zitwerwurzel
- 9 Teile Zimt
- 1 Teil Grüner Kardamom
- 1 Teil Myrrhe
- 1 Teil Eisenvitriol
- 72 Teile Honig

Morphine was also the first alkaloid to have ever being isolated when in 1804 the German chemist Friedrich Sertürner succeeded with its extraction. Due to its medicinal properties, he called it "morphium" in honour of Morpheus, the Greek god of dreams (Serturner 1805).

Opiates are not present throughout all parts of the poppy plant, but they rather accumulate in the latex, the cytoplasm of specialized internal secretory cells called *laticifers* that are closely associated with vascular bundles (Kutchan et al. 1985). Opiate synthesis is an excellent example for cellular compartmentalization of synthesis pathways (see ▶ Chap. 1). Immunolocalization of different enzymes involved in morphine biosynthesis together with shotgun proteomics data indicates that early synthesis steps up to thebaine take place in sieve elements of the phloem (Onoyovwe et al. 2013; Weid et al. 2004). While the synthesis of morphine and codeine can be finalized in the sieve cell, the later stages predominantly take place in adjoining laticifers, where the compounds are then stored.

The function of morphine (and other opiates) for the poppy plant is not well understood. It is not even clear whether the psychoactive properties of morphine are part of a defence mechanism. It is known that after morphium is released from the laticifers upon mechanical damage, it undergoes oxidation and dimerization to bismorphine (Morimoto et al. 2001). Bismorphine can cross-link pectin, thereby making it more stable against hydrolysis, indicating a role of morphine in protection against mechanical damage.

Opiate content can vary in different cultivars of the opium poppy but also between different species of the *Papaveraceae*. Species such as *P. bracteatum*, which have a low morphine and codeine content, are used for the isolation of thebaine, a precursor for the manufacture of semisynthetic and synthetic opioids (Vincent et al. 1977). Targeted mutations such as in the *thebaine oripavine poppy 1* (*top1*) variety, which infer a block of morphine biosynthesis, lead to the accumulation of thebaine and oripavine, but not morphine or codeine (Millgate et al. 2004) and are also used for biotechnological applications.

Opioids of different chemical origin can be found in other plants. For example, salvinorin A, a highly oxidized *diterpenoid*, is the main active psychotropic compound in *Salvia divinorum* (*Ortega et al.* 1982). Sage of the diviners, also called seer's sage, is endemic to a relatively small region in the mountainous north-eastern corner of the Mexican state of Oaxaca and has a long history of use as an entheogen by Mazatec shamans (Valdés et al. 1987). Salvinorin A acts as a κ-opioid receptor agonist and is the first known non-alkaloid compound acting on this receptor (Roth et al. 2002). *Salvia divinorum* synthesizes and excretes salvinorin A via *glandular trichomes* that are distributed over much of the plant's exterior suggesting that they serve a protective function, but as with morphine, the exact role of this compound for the plant is not known.

Take-Home Messages

- Classical GPRCs do not seem to exist in plants, but many plant-derived drugs target *GPCRs* in animals and humans.
- Opioids such as *morphine* and *salvinorin A* are the best described secondary metabolites acting on the *opiate receptor*. The function for the plant is not known, but protection against mechanical damage could be one role of morphine.
- Several phytocannabinoids affect the human *cannabinoid receptors*, the best known being *THC*. In addition to antimicrobial activity, it might also provide protection from UV damage.
- *Atropine, scopolamine and acorine* are plant-derived drugs that target *muscarinic acetylcholine receptors*. Some animals are insensitive to atropine poisoning, including bees, which can accumulate in their honey when they pollinate *Atropa belladonna*.
- *Caffeine,* an antagonist of the adrenic receptor, is probably the plant-derived psychoactive drug with the widest use worldwide. It has multiple other cellular targets including the *ryanodine receptor* and the enzyme *phosphodiesterase*. While toxic to many animals, it can improve the reward memory of bees, thereby increasing pollination probability. In addition, caffeine released in the soil causes allelopathic effects that might help the coffee plant to compete with other plants.
- No plant-derived drug is known to directly target the *dopamine receptor,* but compounds such as *cocaine* affect dopamine signalling by inhibiting the dopamine transporter. In the field, coca plants are mostly pest free indicating that a likely role of cocaine is protection against herbivores and pathogen.
- *Mescaline* is the only plant-derived drug known to target the *5HT2A receptor* since the best-known compound, ergotamine, is made by fungi-infecting the plants. However, nothing is known about the role of mescaline for the plant.

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Plant-Derived Drugs Affecting Ion Channels

8.1 Voltage-Gated Ion Channels: Aconitine, Saxitoxin and Ryanodine – 122

8.2 Nicotinic Acetylcholine Receptors: Nicotine, Coniine, Curare and Anatoxin A – 123

8.3 $GABA_A$ Receptors: Thujone, Cicutoxin and Anisatin – 127

8.4 Glycine Receptors: Strychnine and Tutin – 130

8.5 Ionotropic Glutamate Receptors: Ibogaine, Kainic and Domoic Acid – 131

8.6 TRPV and TRPM8 Receptors: Capsaicin and Menthol – 133

References – 137

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What You Will Learn in This Chapter

Compared to GPCRs, the set of plant-derived compounds that target ion channels appears much more limited. For voltage-gated channels, most known toxins are derived from animals such as snails, spiders and snakes. Also, many ligand-gated channels are targeted by few to none known plant-derived drugs. Nevertheless, ion channels are the target of some of the most potent plant poisons and most commonly used plant-derived drugs.

8.1 Voltage-Gated Ion Channels: Aconitine, Saxitoxin and Ryanodine

While there are several non-plant toxins that affect voltage-gated ion channels, the number of plant secondary metabolites known to inhibit these channels is rather small. However, one of the most potent plant poisons, the *terpenoid alkaloid* aconitine, targets *voltage-gated Na^+-channels*. It is the main toxin found in plants of the *Aconitum* genus (Fig. 8.1), commonly known as devil's helmet, monkshood or wolf's bane. *Aconitum* species are found all around the Northern Hemisphere, and their use for medicinal purposes has been described in Europe, China and Tibet (Chan 2009; Ma et al. 2015). They are also valued as ornamental plants. As one of the most toxic plants found in Europe, extracts of *Aconitum* have been used in many famous poisonings (Bock and Norris 2016), and it was allegedly employed in ancient Rome to execute death penalties. By contrast, the role of aconitine for the plant is not well understood. It is present throughout the plant, and it presence in leaves could help the plant to fend of herbivores such as dear. The high content in the roots and root tubers (Chan 2009) might further indicate a role in protecting the plant against fungi causing root rot or against underground attacks by nematodes.

Pyrethrins are *monoterpenes* found in several plants from the *Asteracea* family such as *Chrysanthemum cinerariaefolium (mums)*, *Calendula officinalis* or *Tagetes erecta* (Kudakasseril and Staba 1988; Ramirez et al. 2013). They are less known but probalby used for as long as aconitine in herbal medicine. Originally obtained from dried and crushed flower heads of *C. cinerariaefolium*, the practice of mum powders as natural insecticides has a long history.

Fig. 8.1 *Aconitum lycotonum* leaves and flowers (© Max Weigend)

Used in Europe at least since the 1900s, the use in Persia dates back much longer (McLaughlin 1973). The action of pyrethrins on *voltage-gated Na^+-channels* is well described; however, an additional action against *voltage-gated Ca^{2+}* and *Cl^- channels* has also been suggested (Soderlund et al. 2002). Pyrethrins have a high arthropod toxicity (also for fish) but much lower toxicity to mammals and birds (Soderlun and Lee 2001; Vais et al. 2000; Mauck and Olson 1976) indicating that they are used as feeding deterrent against arthropod herbivores. However, antifungal and antibacterial activity was also reported (Ramirez et al. 2013). The pyrethrin precursor chrysanthemic acid is synthesized in the apical cell of glandular trichomes that cover the flower achenes even though only sesquiterpene lactones are found in the trichomes of mature achenes. Instead, chrysanthemic acid is transported to the pericarp of the fruit, where, after esterification to pyrethrin, it accumulates outside the cells within the intercellular space. From there, the pyrethrins are taken up by the embryo, where they are believed to protect the seedling against insects and fungi (Ramirez et al. 2012).

Voltage-gated Na^+-channels are also the target of the *guanidium toxin* saxitoxin and its analogues alkaloids neosaxitoxin and gonyautoxin (Cembella 1998). Well known as paralytic shellfish toxins, they are not classical plant-derived secondary metabolites. However, they are also not produced by the shellfish but can accumulate in these organisms via feeding on marine dinoflagellates such as *Alexandrium catenella*, *A. tamarense excavatum* or *Pyrodinium bahamense*. It is also produced by a number of freshwater cyanobacteria (Kellmann et al. 2008; Wiese et al. 2010).

By contrast, the *polycyclic diterpene* ryanodine is the name-giving compound that reacts with the *ryanodine receptors*, a group of *Ca^{2+}-induced Ca^{2+}-release channels* in muscle tissue. It is found in *Ryania speciosa*, a South American species from the *Salicaceae* (Rogers et al. 1948). It affects the channel by locking it either in the half-open or closed state depending on its concentration (Thomas and Williams 2012). Historically, extracts from *Ryania* plants have been used in South and Central America as arrowhead poisons (Santulli and Marks 2015). It was also used as a pesticide and insecticide, the latter indicating that fending of insect larva might be the functional role of this toxin for the plant.

8.2 Nicotinic Acetylcholine Receptors: Nicotine, Coniine, Curare and Anatoxin A

As described in ▶ Chap. 2, a nicotinic agonist is a drug that mimics the action of acetylcholine at *nicotinic acetylcholine receptors* (◘ Fig. 8.2). Plants contain a number of nicotinic agonist, foremost the name-giving *pyridine alkaloid* nicotine that is found in several plants of the *Solanaceae* but is best known as the main alkaloid from tobacco. The *Nicotiana* genus consist of a number of species (◘ Fig. 8.3) found in the Americas, Australia, South-West Africa and the South Pacific, which occur both as herbaceous plants as well as scrubs, and many of which are poisonous to livestock and humans. *N. tabacum* is a perennial herbaceous plant that is found exclusively in cultivation. It originated in the highland of the Andes, probably Bolivia or northern Argentina, and is believed to be a hybrid of two older species, *N. sylvestris* and *N. tomentosiformis* (Murad et al. 2002).

Nicotine is toxic to mammals in high doses and it has even been used as poison (see ▶ Box 8.1). In insects, small doses result in reduced activity and paralysis (Kennedy and Wightman 2011; Wolf and Heberlein 2003), and therefore, extracts of tobacco leaves were a popular pest control method up to the beginning of the twentieth century. While

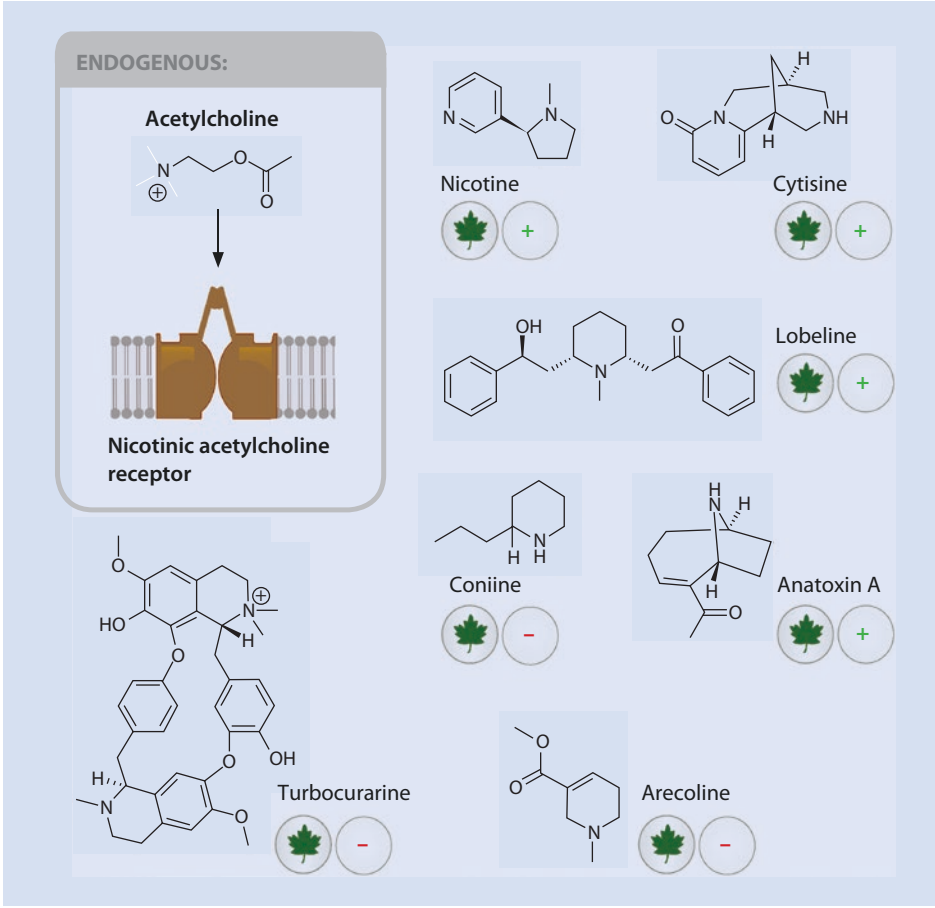

Fig. 8.2 Examples of natural compounds that act as agonists and antagonists of the nicotinic acetylcholine receptor

nicotine itself is no longer a pesticide of choice, nicotine analogues such as imidacloprid are currently widely used for these purposes. *N. rustica*, known in South America as mapacho and in Vietnam as thuoc lao (thuốc lào), is an even more potent variety of tobacco that contains several times more nicotine than *N. tabacum* and is thus used to produce organic pesticides.

Interestingly, nicotine is synthesized in the roots and then gets transported to the aerial parts of the tobacco plant where it accumulates in leave tissue. While it is present to a certain extent at all times, wild species of tobacco increase the synthesis of nicotine after leaf wounding (Baldwin 1988). It acts as an herbicide leading pests, such as caterpillars, to abandon wounded leaves.

However, in the ongoing arms race between plants and their enemies, several tobacco-adapted species have become insensitive to nicotine, e.g. *Manduca sexta*, the tobacco hornworm (Wink and Theile 2002). It was shown that insensitivity is not caused by a change in the nicotine binding site on the nicotinic acetylcholine receptor. Moreover, these species are also insensitive to alkaloids affecting other receptors. Thus, it is believed that in these caterpillars toxic alkaloids are quickly excreted and/or convert to harmless

Fig. 8.3 Flowering *Nicotiana benthamiana* (© Ute C. Vothknecht)

Box 8.1 Nicotine and the onset of modern forensics

In 1851, Hippolyte Visart de Bocarmé allegedly poisoned his brother-in-law, Gustave Fougnies, with an extract of tobacco leaves that he mixed into Fougnies' dinner. The toxic properties of nicotine were already known, and Visart de Bocarmé had tested his concoction on cats and birds. He then used the extract for the murder believing that the substance could not be detected in human tissue. However, the Belgian chemist Jean Stas proofed that Fougnies was poisoned by developing a method to extract the alkaloid from the tissue (Wennig 2009) so that it could be identified. While improved, fundamentally the same analytical method to extract nicotine and other alkaloids from biological specimen is still employed today

metabolites and thus detoxified. Moreover, these species can use nicotine for their own defence (see ► Box 8.2).

In addition to nicotine, there are several more plant-derived secondary metabolites of different chemical nature known to work as nicotinic agonists, making the nicotinic acetylcholine receptor a hot spot of secondary metabolite action. Because of the wealth of substances, only those that have a long-time use and/or are reasonably well studied are described.

Cytisine is a *tricyclic quinolizidine alkaloid* found in plants such as golden rain (*Laburnum*). Because of the similarity in structure to nicotine, it has comparable pharmacological effects. It has been used to assist with smoking cessation (Walker et al. 2014), and leaves of this tree can also be used as tobacco substitutes.

Another well-described plant toxin that affects the nicotinic acetylcholine receptor is the *pseudoalkaloid* coniine and the closely related γ-coniceine, conhydrine, psuedo-

Box 8.2 Defensive halitosis

Several insects have learned to use plant-derived secondary metabolites for their own defences. Caterpillars of the tobacco hornworm transport part of the nicotine taken up by chewing on tobacco leaves or its non-toxic derivatives from the midgut to the haemolymph. From there, nicotine is released into the air through spiracles in their flanks to ward of predators such as the native wolf spider. These caterpillars get eaten when they are fed on nicotine-deficient mutant tobacco (Kumar et al. 2014). However, this defence system seems to come with a cost. Worms feeding on nicotine-deficient tobacco grow bigger and faster than those feeding on plants with normal nicotine levels, indicating that the worms are still partially affected by the drug

conhydrine and N-methylconiine. They are found in plants such as poison hemlock (*Conium maculatum*), and the equivalent to about six to eight fresh leaves or an even smaller dose of seeds or root is deadly. It is most famously known for the death of Greek philosopher Socrates 399 BC (Dayan 2009), and it was also suggested by recent research that Cleopatra might have chosen a mixture of hemlock, wolfsbane and opium to kill herself (Mebs and Schäfer 2008). So, while already a well-known poison in ancient times, it was still a preferred toxin of choice in medieval times at least in literature. For example, Shakespeare referred to hemlock in *The Life of Henry the Fifth* and *Macbeth*. There has been some use as external medication; however, the high toxicity of the plant has favoured its use as a poison (Vetter 2004). Coniine is also found in the nectar of the yellow pitcher plant, *Sarracenia flava*, and it was suggested that the plants use it to stun trapped insects. If so, it would make the yellow pitcher plants especially devious since it attracts insects with its nectar and pollen, then poisons and finally eats them (Mody et al. 1976).

Methyllycaconitine is a *diterpene alkaloid* from *Delphinium* (larkspur), perennial plants that are found throughout the Northern Hemisphere. It is toxic to mammals and has been the cause of livestock poisoning in North America. However, its use as an insecticide was already described by Plinus, the Elder (Jennings et al. 1986). It was shown to be especially toxic for insect due to its high affinity for the insect nicotinic acetylcholine receptor indicating that insectoid herbivores might be the primary target of the compound.

Plant- and insect-derived alkaloids that target the nicotinic acetylcholine receptor (and other ligand-activated channels) also have long been used by tribal hunters in South and Middle America to poison their arrows and darts. The term curare is used for arrow toxins based on different mixtures of plant extracts. In 1895, Rudolf Boehm, a German pharmacist, classified three variants of curare (Boehm 1897). Bamboo or tube curare contains d-tubocurarine, a *benzylisoquinoline alkaloid*, as the major active ingredient. The plant extracts are mostly made from bark of the South American climbing plant *Chondrodendron tomentosum* (King 1948), but the active ingredient is also found in other plants from the genus *Chondrodendron* as well as plants from the closely related genera *Curarea*, *Caryomene*, *Cissampelos* and *Strychnos* such as *Strychnos toxifera* (Philippe et al. 2004). Pot curare contains mainly protocurarine, protocurine and protocuridine, while the major active ingredient of calabash curare is C-curarine I (Battersby and Hodson 1965).

During their travels into the "New World", German scientist Alexander von Humbold and his French college Aimé Bonpland were the first Europeans that were shown the

traditional methods of preparing curare from plants by natives (Bonpland et al. 1814). While curare kills animals that were wounded by poisoned arrows very quickly, the prey is safe to eat due to very low resorption of tubocurarine and other curare alkaloids in the gastrointestinal tract. This property of curare was already described by Humboldt, who tested it by drinking the plant extract unharmed. Nevertheless, it should take more than 100 years before curare became a muscle relaxant during surgery in Western medicine (Czarnowski et al. 2007).

The *Strychnos nux-vomica* tree also belongs to the genus *Strychnos*, and it is the source of strychnine, a *terpenoid indole alkaloid* that targets both, the nicotinic acetylcholine receptor and the ionotropic glycine receptor and which is described in the context of the latter in ▶ Sect. 8.4.

Some cyanobacteria produce the nicotinic acetylcholine receptor agonist anatoxin A. This *tropane-related alkaloid* is also known as the "very fast death factor" due to its strong neurotoxicity (Tufariello et al. 1984). It is produced by a number of species of cyanobacteria and can poison drinking water. It was only discovered in the 1960s when the poisoning of cattle by water from the Saskatchewan Lake in Canada was investigated. Since its discovery, it was determined as the cause for multiple death of cattle or other animals such as dogs (Edwards et al. 1992). The largest death toll so far ascribed at least in part to the action of anatoxin A was the death of about 30,000 flamingos on lake Bogoria in Kenia in the autumn of 1999 (Krienitz et al. 2003). Anatoxin A is only one of the cyanobacteria-derived substances that make toxic harmful algae blooms so dangerous, but it is believed to be the major cause of neurotoxic blooms (Rapala et al. 1993). Prevention of grazing was suggested as one potential role of these algae toxins; however, allopathy could be another factor. Indeed, it was shown that anatoxin A synthesis is increased in the presence of the green alga *Chlamydomonas reinhardtii*, and it can inhibit the mobility of this organism (Holland and Kinnear 2013). However, very little is generally known about the natural role of cyanobacterial toxins.

8.3 $GABA_A$ Receptors: Thujone, Cicutoxin and Anisatin

The best known secondary metabolites affecting $GABA_A$ receptors again come from mushrooms (◘ Fig. 8.4). Muscimol, a *isoxazole alkaloid*, is the actual psychoactive compound that is responsible for the effect of *Amanita muscaria* intoxication. Ibotenic acid is also found in *Amanita muscaria* and related species of mushroom but only serves as a prodrug to muscimol. However, since it is decarboxylated to muscimol upon digestion, it has the same effect during human consumption of these mushrooms.

GABA-active plant products include *sesquiterpenes* such as valerenic acid found in valerian (*Valeriana officinalis)* or *monoterpene ketones* like camphene found in *Cinnamomum camphora*. A more infamous plant-derived antagonist of the $GABA_A$ receptor is the monoterpene ketone thujone found in plants such as thuja, Nootka cypress, mugwort, oregano, common sage, tansy or wormwood. Thujone has a menthol odour and is best known as a component of Absinthe (see ▶ Box 8.3), which contains extracts of the grand wormwood, *Artemisia absinthium*. Thujone is considered as an addictive psychoactive drug and its use in food and drinks is therefore limited by law. In the European Union, up to 35 mg/kg thujone is permitted for alcoholic beverages prepared from *Artemisia* species with stronger limits for food and non-alcoholic beverages. Similar restrictions

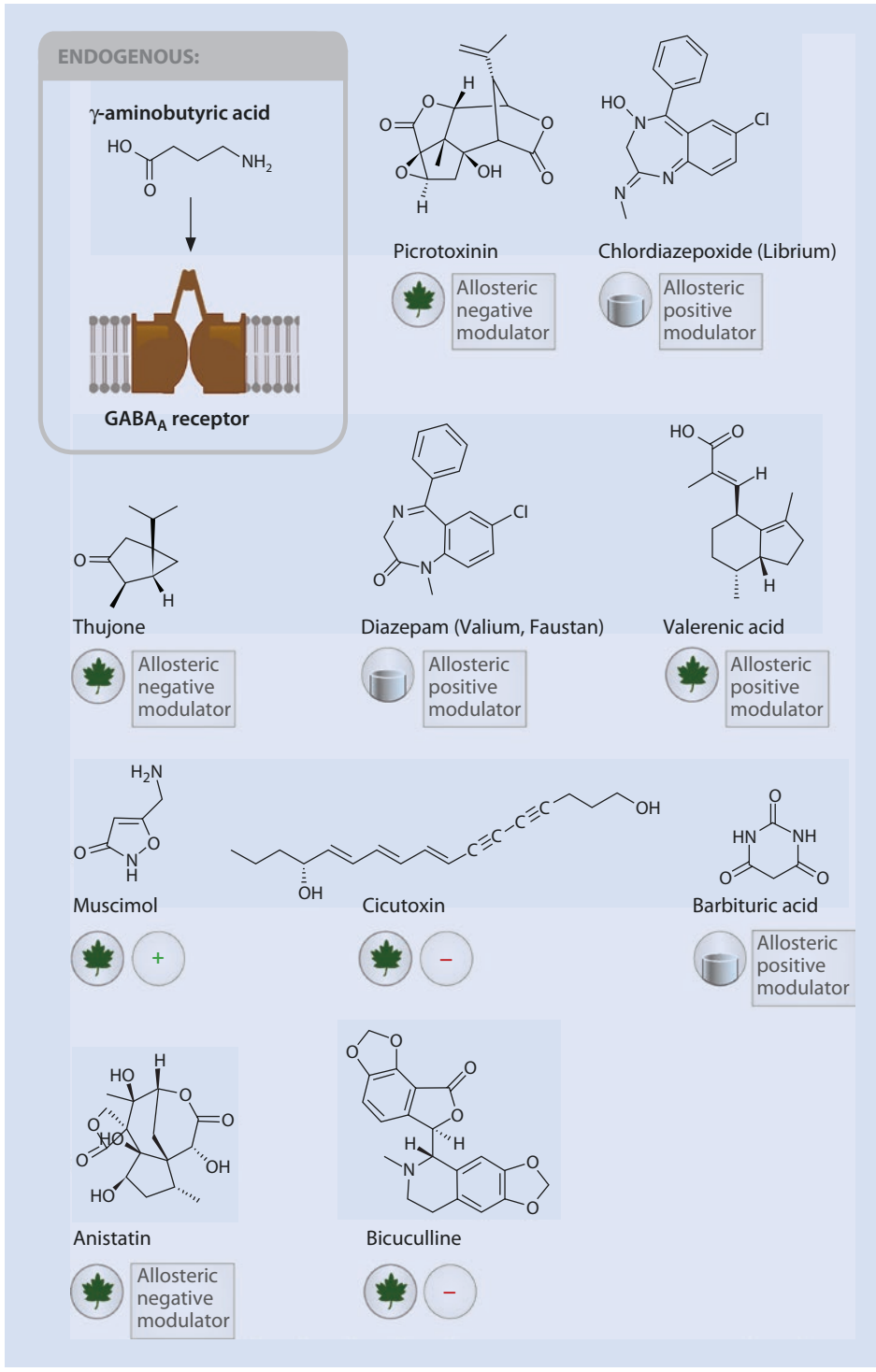

Fig. 8.4 Examples of natural compounds that act as agonists and antagonists as well as positive and negative allosteric modulators of the GABA$_A$ receptor

Box 8.3 La fée verte

Absinthe is an anise-flavoured spirit made from extracts of *Artemisia absinthium* (for thujone content) and *Artemisia pontica* (for colouring) together with other herbs such as green anise and sweet fennel. In Europe, absinthe was forbidden from 1915 onwards because it was labelled as a dangerously addictive drug, but its psychoactive properties (apart from that of the alcohol) seem to have been exaggerated. Indeed, the symptoms of the so-called absinthism are probably cause by chronic alcoholism since the high percentage of alcohol in absinthe would cause effects long before thujone or other psychoactive compound present in the spirit could become a factor

apply for beverages and food products from plants such as mint, thyme or rosemary, which also contains thujone in their oils. It is therefore also a common component of herbal medicine.

As with many other secondary metabolites, the function of thujone in plants is not well understood. A role in protection against insect herbivores and pathogens can be suggested from its human use as moth repellent, antimicrobial compound and for embalming. Upon feeding by herbivores such as antelopes, sagebrush elicits volatiles into the surrounding air and experimental exposure to these volatiles created an increased resistance of exposed plants to herbivore attacks (Karban et al. 2006). GC-MS analysis showed that these volatiles are highly variable between individuals but are generally dominated by either camphene or thujone (Karban et al. 2014). It was suggested that these volatiles might be used to communicate the attack to other individuals in order to reduce herbivory but it remains unclear whether such a communication between plants indeed exist.

A noncompetitive GABA antagonist of different chemical nature is cicutoxin, a *polyacetylene* found in the highly toxic water hemlock (*Cicuta*) and hemlock water dropwort (*Oenanthe*) (Schep et al. 2009). Poisoning is often accidental since roots of plants from the genus *Cicuta*, all of which contain cicutoxin, are often mistaken for roots of edible plants such as parsnip, wild carrot or wild ginseng. Such accidents can be severe, because the root is the most toxic part of the plant, especially in early spring. Toxicity depends on various factors, such as seasonal variation, temperature, geographical location or soil conditions, and the high toxicity of roots could indicate a role in preventing underground attacks by nematodes.

In a similar manner, toxic star anise species can be the cause of accidental poisoning when the plant is mistaken for the non-toxic Chinese star anise (*Illicium verum*) that is used to prepare spicy tea. Causative agent in this case is the *sesquiterpene* anisatin, another noncompetitive GABA antagonist that can cause tonic-clonic seizures (Kakemoto et al. 1999).

Picrotoxin, another $GABA_A$ receptor active *sesquiterpene*, is found primarily in the fruit of the climbing plant *Anamirta cocculus*, which also contains quaternary alkaloids such as berberine. The crushed seeds of the *Anamirta* plant are an effective pediculicide (anti-lice) and have a traditional use as a pesticide. In the nineteenth century, hard multum, a preparation made from *A. cocculus*, was even suggested by some brewers as an addition to malt liquors to strengthen their intoxicating properties.

8.4 Glycine Receptors: Strychnine and Tutin

Strychnine is a plant-derived *terpenoid indole alkaloid* affecting both the *nicotinic adrenaline* and the *ionotropic glycine receptor* (Fig. 8.5). It has a special place in the long list of alkaloid-based secondary metabolites due to its infamous toxic efficiency and a complex structure that has been difficult to solve. It was isolated for the first time in 1818, but the structure was only determined over a hundred years later. Strychnine poisoning is characterized by an initial increase in awareness, followed by unrest, fear and shortness of breath. In the final stage, uncontrolled activity of spinal cord neurons results in cataleptic attacks on the muscles of the back, neck and jaws until the victim dies from suffocation caused by cramping of the respiratory musculature or from exhaustion (Smith 1990). During the whole time, the victim remains conscious, making it an especially brutal death. It was the first alkaloid identified in plants of the genus *Strychnos* together with its slightly less toxic derivative brucine (Pelletier and Caventou 1819).

Strychnine containing trees of the *Strychnos nux-vomica*, *Strychnos ignatii* (St. ignatius bean) and *Strychnos tiente* (Upas tree) variety are found in Southern Asia, Northern Australia, Africa and America. Similar to curare, *Strychnos* extracts have been used as arrow poison by tribal hunters in Asia and Africa. Since the fifteenth century, *Strychnos* seeds were brought to Europe and strychnine containing powder was obtained by crushing of dried ripe seeds of *Strychnos nux-vomica* and later also *Strychnos ignatii*. After its isolation in 1818, the purified substance became widely available and was sold in pharmacies. The use of strychnine as herbal medicine for stomach irritation and fortifier is well described in pharmaceutical collections of the sixteenth century (Eiden 2003). Strychnine was utilized for medicinal purposes until the 1970s, and it is still a much-regarded compound in homoeopathic medicine, where it should be non-toxic at the suggested dilution. It was also used in baits to kill feral mammals, including wild dogs, foxes and rodents, but is now banned in many countries, or its usage is severely regulated and limited (Smith 1990).

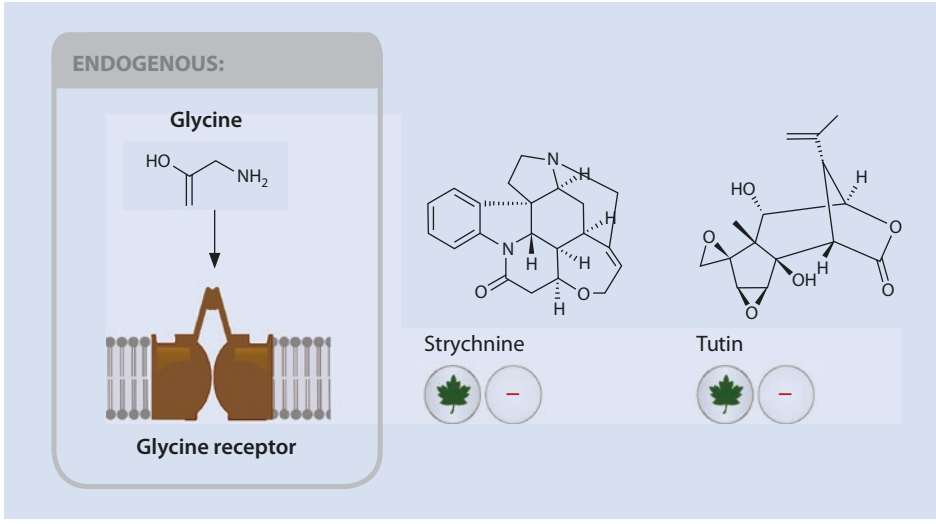

Fig. 8.5 Examples of natural compounds that act as antagonists of the glycine receptor

Strychnine is found in the bark and the seeds of *Strychnos* plants. Very little is known on the natural function of strychnine for the plant; however, several possibilities can be deduced from its properties. Strychnine has a very bitter taste, making it a good feeding deterrent. Indeed, it was shown that larvae of the butterfly *Pieris rapae* responded negatively to strychnine in feeding experiments (Zhou et al. 2009). Moreover, the quick onset of negative effects on the central nervous system caused by strychnine could efficiently protect plants from herbivory.

Despite its toxic properties, strychnine was used early on as a performance enhancement drug (see ▶ Box 8.4). In 1917, Lashley reported on a scientific study showing that rats injected with strychnine navigated mazes faster and with a reduced error rate (Lashley 1917). However, it should be noted that already a few years later, these results were put into question (Miles 1929). A number of subsequent studies have provided varying results, many of them supporting a learning enhancement effect of strychnine (McGaugh and Roozendaal 2009). Long before these experiments, the general population had discovered strychnine as a pick-me-up and would take it as a stronger version of coffee. Even today, strychnine is used as an athletic performance enhancer (the bronze medallist in weightlifting of the 2016 Olympics lost his medal for strychnine doping), and it is therefore on the prohibited list of the World Anti-Doping Agency.

Box 8.4 Strychnine and the 1904 Olympics

The use of strychnine as a performance enhancement drug became famous due to the 1904 Olympic marathon. Taking place in very hot weather and without the drinking stations that are common today, the race took its toll on the 32 participants. The winner, Thomas Hicks, received several injections of strychnine during the race (together with a great amount of brandy), and albeit being able to finish, he was severely dehydrated and weak. While his trainer believed that the strychnine was responsible for the victory, nowadays scientists are much more doubtful. On a side note, Thomas Higgins had initially come in second, but the erstwhile winner of the race, Frederick Lorz, was later disqualified for cheating, since he had driven part of the race by car

Another, much less famous plant-derived secondary metabolite targeting the *glycine receptor* is tutin, a *sesquiterpenoid* and one of the two main toxic compounds found in species of the *Coriaria* genus, such as the New Zealand Tutu plant (Fuentealba et al. 2007). While *Coriaria* extracts are being used in Chinese traditional medicine to treat mental diseases, it might also lead to seizures.

Tutin has similar properties as strychnine and most likely the same function for the plant; however, only little research has been done on the *Coriaria* toxins compared to strychnine. Similar to atropine it has been found in honey of bees feeding honeydew exudate from the sap-sucking passion vine hopper and is therefore often described as a honey toxin (Islam et al. 2014).

8.5 Ionotropic Glutamate Receptors: Ibogaine, Kainic and Domoic Acid

Ibogaine is a *terpenoid indole alkaloid* that acts as an antagonist on the *NMDA type* of *ionotropic glutamate receptors* (Popik et al. 1995) (◘ Fig. 8.6). However, it should be noted that affinity for a number of other receptors, i.e. κ-opoid, 5HT and nicotine acetylcholine

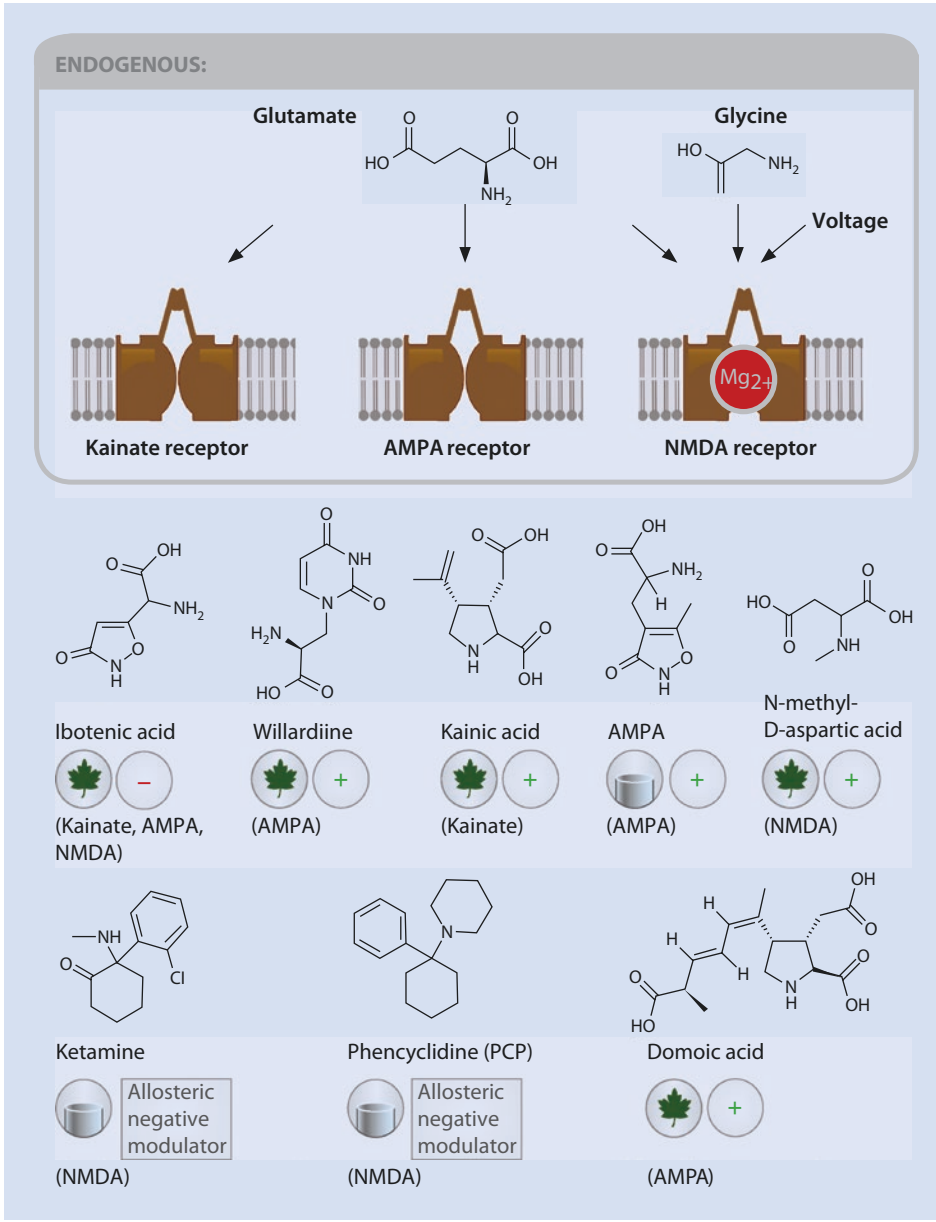

Fig. 8.6 Examples of natural and synthetic compounds that act as agonists and antagonists of ionotropic glutamate receptors

receptors, has been described (Zubaran 2000). It is found in the bark of plants such as *Tabernanthe iboga*, *Voacanga africana* and *Tabernaemontana undulata* and was first described in Western medicine in 1874 as an aphrodisiac and nerve system stimulant. Since the 1930s, ibogaine was sold in France in the form of Lambarène, an extract of the *Tabernanthe manii* plant (Zubaran 2000). It became a popular performance-enhancing substance among athletes but is now found on the list of forbidden substances from the

International Olympic Committee. It was later generally banned in the USA and Europe due to its psychedelic properties and because it is believed to cause addiction (Brown 2013). Nevertheless, it was investigated – and is still used in alternative medicine – as a treatment for addiction to many drugs such as heroin, cocaine and methamphetamine. It was also used to treat depression and post-traumatic stress disorder (Zubaran 2000).

Kainic and domoic acids are *non-protenogenic amino acids* acting on *ionotropic glutamate receptors* (Olney et al. 1974; Mos 2001). They are named after the Japanese word for the red algae from where they were isolated and have long been used in Japan as an anthelminthic to expel parasitic worm. Kainic acid, found in certain red algae, is an agonist of the *kainic type of ionotropic glutamate receptors*. While stimulating in small doses, large doses of kainic acid produce immediate neuronal death by excitotoxic lesion. Domoic acid is found in red algae as well as diatoms of the genus *Pseudonitzschia* and is an antagonist of the *AMPA and kainic acid type of ionotropic glutamate receptors*. It can accumulate in marine organisms such as mollusks and other shellfish that feed on phytoplankton and is the causal agent of amnesic shellfish poisoning and certain harmful algal blooms. Interestingly, diatoms grown in axenic culture have significantly reduced domoic acid production, which is enhanced when marine bacteria are reintroduced into the culture, indicating a correlation between the presence of bacteria and domoic acid synthesis (Bates et al. 1995).

8.6 TRPV and TRPM8 Receptors: Capsaicin and Menthol

That plant metabolites act on TRP receptors that humans and animals use to detect taste is maybe not very surprising. They can entice animals to eat fruits and thereby spread the seeds at a larger distance from the mother plant and/or provide a fertile environment for their growth after they are discarded with the excrements of the animal. Many of these compounds, e.g. sugars or amino acids, are primary metabolites and are therefore not covered here. By contrast, secondary metabolites in fruits are often a means to deter animals either to avoid premature consumption of unripe fruits and seeds or to prevent consumption by unwanted feeders. If the aim is to avoid premature consumption, the non-ripe fruits lack enticing substances and also often advertise their premature status by their colour. The animals learn that these fruits are not yet mature and are discouraged from eating them. Prevention of consumption by unwanted feeders only works if the compound that acts as deterrent is not recognized by those animals that should eat the fruit/seed for dispersion.

In humans and many animals, TRPV1 channels mediate signals associated with heat and pain. They are therefore a perfect target for plants metabolites meant to discourage feeding. The best-known plant-derived compound reacting with this receptor is capsaicin, an *alkylamide* found in some plants of the *Solanaceae* family, such as hot chilli peppers (*C. frutescens*). Because it binds to the *TRPV1 receptor*, capsaicin creates the sensation of burning when it comes in contact with mucous membranes and is therefore an irritant for animals and humans consuming chillies. In small quantities, it is used as a spice; however, larger quantities cause severe irritation and also affect sensitive skin or eye tissue. The genus *Capsicum* originated from South America, and it was suggested that chilli peppers were first cultivated around 6100 years ago in the lowland rainforests of Western Ecuador (Perry et al. 2007). From there, they were brought to Europe, Africa and Asia, where they are used as spice but also quickly found entrance in ethnic medicine (Meghvansi et al.

Fig. 8.7 Chili pepper (© Ute C. Vothknecht)

2010). For example, the use of Naga chilli (*C. chinense* Jacq.) is well described in India as herbal medicine to treat ailments such as tooth ache or muscle pain. Until today, capsaicin is used as an analgesic in many ointments. It was suggested that its function as pain reliever is caused by desensitization of the TRPV1 receptor by constant activation. This might also explain other uses of capsaicin as treatment for certain inflammatory diseases, since malfunction of the TRPV1 receptors is believed to be the cause for these afflictions. Capsaicin is also on the Olympic list of forbidden substances for horses. This is due to the effect of hypersensitization of the TRPV1 receptor, which increases the animals loathing of touching the obstacles and is thus considered a doping offence. It can also be a doping offence due to its pain-relieving properties.

So how does the plant profit from the presence of such a pain-inducing substance? Chillies are low-growing scrubs (Fig. 8.7) and their fruits can be reached by both small mammals as well as birds. Capsaicin and other capsaicinoids are most abundant in the chilli fruit but not in leaves or roots. Within the fruit, small amounts are present in the pericarp and the seeds, while the major part is found in the placenta interlocular septum, where the compound is produced (Hall et al. 1987). It accumulates in droplets under the surface of the cuticle and is easily released into the fruits interior upon slight pressure, where it then covers the seeds from the outside. Capsaicin begins to accumulate early during fruit development and increases with age of the fruit. Therefore, it is not a specific deterrent for premature feeding. Instead, capsaicin seems to act as a deterrent for certain unwanted feeders, in this case small mammals such as the cactus mouse or packrats. By contrast, birds such as the curve-billed thrashers will still feed on the fruits (Tewksbury and Nabhan 2001). Though birds do possess TRPV1 channels that react to heat, these channels do not react to capsaicinoids due to a single point mutation in the binding site. Therefore, the capsaicin-induced firing of the receptor at lower temperatures does not happen (Jordt and Julius 2002). Supposedly, this mutation is an

adaptation to the higher body temperatures of birds. So, why can this be beneficial to the plant? *C. chacoense* is a chilli plant with fruits similar to hot chilli pepper but without capsaicin. Experiments showed that consumption of their non-pungent seeds by small mammals abolished germination because they are destroyed when they are chewed by their molar teeth. By contrast, seeds consumed by birds remained unharmed providing an ideal system for seed dispersal (Tewksbury and Nabhan 2001). This way, the presence of capsaicin in the fruits creates a positive selection of wanted feeders. This is also taken advantage of in industrial applications, where capsaicin is used as an additive to bird feeds in order to deter rodents. Interestingly, it was also shown that capsaicin content of a fruit is not only genetically determined, resulting in different capsaicin content of different pepper varieties, but is also dependent on plant growth conditions (Harvell and Bosland 1997). Environmental conditions such as drought seem to boost capsaicin biosynthesis indicating that there might be more to capsaicin then just its effect as a feeding deterrent.

Capsaicin, similar to caffeine and nicotine, is a good example for the impact of humans on the global spreading of plants. In contrast to the native small mammals, human covet the pungent taste of chillies, which resulted in the widespread distribution and the breeding of new variants all over the world.

While capsaicinoids are exclusively found in chilli peppers, other plant-derived secondary metabolites also target the TRPV1 receptor (Premkumar 2014). These include campher, thymol, vanillin, piperine, eugenol and also cannabidiol; however, most of them have a rather low degree of "hotness" as measured by the Scoville scale (number of times an ethanol extract of a substance has to be diluted to lose its pungency). For example, piperine, a TRPV1 agonist from black pepper, is about 100 times less potent than capsaicin. By contrast, the most infamous TRPV1 agonists are resiniferatoxin and tinyatoxin, *diterpenes* (of the daphnane family) that are found in the latex of *Euphorbia resinifera* and *Euphorbia poissonii*. Resiniferatoxin has an estimated rating on the Scoville scale of 16 billion and thereby 1000 times the score of pure capsaicin. In contrast to capsaicin, resiniferatoxin is found in the latex of the plant indicating that it rather acts as a deterrent against herbivory.

In contrast to TRPV1, TRPM8 channels are normally activated by cold. Similar to the action of capsaicin on TRPV1, some plant-derived compounds can bind to the TRPM8 channel and trigger a cooling sensation even under elevated temperatures when eaten, inhaled or applied to the skin (Kamatou et al. 2013). Such compounds include the *monoterpenes* menthol, the active ingredient found in plants of the *Mentha* genus (Fig. 8.8) such as wild mint (*Mentha arvensis*), horse mint (*Mentha longifolia*) or peppermint (*Mentha x piperita*), as well as eucalyptol (1,8-cineol), the major constituent of eucalyptus oil. These plants are used as ingredients in food or drinks but also as part of medicinal remedies, e.g. to relief skin irritations or minor pains. Menthol is also used as an additive to cigarettes; however, many countries have started to ban this practice since menthol is believed to exacerbate smoking behaviour. Several modes of action might be coming together for menthol to increase the addictive potential of smoking (Wickham 2015). Due to its activation of the cold-receptor TRPM8, menthol seems to be able to suppress smoking-induced irritation of the mouth, throat and lungs as well as natural defence reactions such as coughing that usually would become effective as involuntary resistance against the inhalation of fumes. Furthermore, menthol is a negative allosteric modulator of *nicotinic acetylcholine receptors* and also seems to affect nicotine metabolism thereby increasing nicotine reinforcement.

Fig. 8.8 Flowering horse mint (© Ute C. Vothknecht)

Menthol also has analgesic properties; however, in contrast to capsaicin, they are not related to its interaction with the TRPV1 receptor but because it also activates κ-opioid receptors (Galeotti et al. 2002). Mint oil can also be used as a natural pesticide, but this is rather due to its pulegone and menthone content, both of which have been identified as the major cause for its insecticidal and genotoxic properties, respectively (Franzios et al. 1997).

So, what is the role of these TRPM8 agonists for the plants? It has been suggested that as part of herbivore-induced plant volatiles, menthol can help to attract natural enemies of insect herbivores. Furthermore, it was shown recently for Arabidopsis that menthol was part of those volatiles that that were emitted upon *Lipaphis erysimi* Kaltenbach infestation. In this case, these volatiles did not attract an enemy of the aphid but instead increased the performance of the entomopathogenic fungus *Lecanicillium lecanii* (Lin et al. 2017), thereby helping the plant to fight off the herbivore attack.

Take-Home Messages

- In contrast to GPCRs, much fewer plant secondary metabolites are known to affect ion channels.
- *Aconite* is one of the few plant-derived compounds affecting *voltage-gated channels*; however, it is one the most potent plant poisons. *Saxitoxin* and closely related paralytic shellfish toxins are produced by certain marine diatoms and freshwater cyanobacteria.
- The *nicotinic acetylcholine receptor* is a hot spot of secondary metabolite action. Plant-derived drugs affecting this receptor include one of the most common recreational drugs, *nicotine*. Nicotine acts as a natural herbicide against pest and pathogens attacking tobacco leaves. It is also used by nicotine insensitive caterpillars as a protection against predators.

- The *nicotinic acetylcholine receptor* is also targeted by potent toxin such as coniine, tubocurarine (curare) or the cyanobacterial made anatoxin A.
- The *$GABA_{A/C}$ receptor* is the target of many active compounds found in herbs such as thuja, sage, oregano, valerian or ginger but also toxins such as cicutoxin or anisatin.
- Only two compounds affecting the *glycine receptor* are well described. *Strychnine* has long been used as a pesticide as well as gruesome poison, while *tutin* is rather known due to its presence in toxic honey.
- Human TRPV1 (heat) and TRPM8 (cold) receptors are activated by many compounds found in spices or herbs. They can be used as feeding deterrents, i.e. *capsaicin*, or to attract insects.

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Biosynthesis of Secondary Metabolites in Plants

Secondary metabolites are in principle characterized as such because their presence is not essential to the plants' life; it rather is an optimization and adaptation to environmental conditions. The primary metabolism provides the carbon backbones for secondary metabolites, usually via glycolysis or Calvin cycle, whereas the nitrogen groups are mostly derived from amino acids. Tailoring enzymes are responsible for the diversity of the compounds. Pathways can take place in different compartments of a cell, and storage often occurs in specialized cell types such as in glandular trichomes, resin ducts or laticifers. The biosynthetic pathways are tightly regulated to balance growth versus defence.

The major secondary plant products are terpenes or terpenoids, phenylpropanoids and alkaloids. Terpenoids are produced from isoprene units (C5), either in the cytosol (MVA pathway) or in the plastid (MEP pathway). Phenylpropanoids are characterized by the phenol ring derived from phenylalanine. Alkaloids are nitrogen-containing compounds where the nitrogen is usually part of the heterocyclic ring. Precursors for alkaloids are often amino acids.

Contents

Chapter 9 **Secondary Metabolites in Plants: General Introduction – 143**

Chapter 10 **Terpenes and Terpenoids – 153**

Chapter 11 Phenylpropanoids – 171

Chapter 12 Alkaloids – 179

Chapter 13 Minor Groups of Secondary Metabolites – 205

Secondary Metabolites in Plants: General Introduction

9.1 Role of Secondary Metabolites for Plant Development – 144

9.2 Regulation of the Biosynthesis of Secondary Metabolites – 145

9.3 General Mechanisms for Biosynthesis – 147

References – 151

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What You Will Learn in This Chapter

Plants usually contain many different secondary metabolites, but some species contain very specific groups of secondary metabolites. The amount of the compounds and the kind of compounds vary between different cells, tissues and developmental stages and can be influenced by external stressors. This means that the enzymatic pathways have to be tightly controlled. To synthetize secondary metabolites, plants use products from the primary metabolism as building blocks. The metabolites are further fused and modified by different processes to lead to the variations observed in nature.

9.1 Role of Secondary Metabolites for Plant Development

Secondary metabolites are in principle characterized as such because their presence is not essential for a plant's life. Rather they help plants to optimize growth and to adapt to the changing environmental conditions. This explains also the huge variation of secondary metabolites between different organs and under different conditions. Furthermore, the ability to synthetize specific secondary metabolites has been selected for during evolution when such compounds are important for specific needs. Therefore, some compounds are very specific for a narrow group of species. The adaptation of plants to life on land during evolution (terrestrialization) was accompanied by a large increase in the production of secondary (or specialized) metabolites, as protection against UV light and loss of water became important, as well as the attraction of pollinators.

Nevertheless, some derivatives of these so-called secondary pathways are essential for development as they constitute hormones (such as abscisic acid, gibberellin, cytokinin, brassinosteroid, strigolactone) or are necessary for photosynthesis, like chlorophyll (which contains a phytoene-derived tail), ubiqionone, plastoquinone, tocopherol and carotenoids (all derivatives from the isoprene pathway). Additionally, these compounds are required for the mechanical stability of plants, for instance, lignin (a phenylpropanoid). Other components, such as suberin (a phenylpropanoid), are important to avoid water loss through cell walls. Therefore, the discrimination between pathways that are essential or not essential for plants' life is blurry. In plants, many of the secondary metabolic pathways are located in plastids. This provides these organelles with an important function for adaptation and defence in addition to their essential role for photosynthesis and fatty acid biosynthesis.

Metabolic pathways for secondary metabolites are usually no constitutively expressed, as this would be a waste of energy if the compounds were not needed for survival. Furthermore, compounds can be very selectively produced in specific tissues and/or organs (flowers, green tissues, seeds or roots) according to their function. For example, secondary metabolites to attract pollinators will be produced in floral tissues, whereas compounds important for defence mechanisms are often accumulating either in root or in leaf tissues, depending on the predator that is targeted. In several cases, it has also been shown that compounds can be produced in tissues that are distant from the place of release, which indicates the involvement of a transport mechanism. Especially for toxic compounds, the expression of the genes coding for the necessary enzymes can be limited to individual tissues or cells, such as to the glandular trichomes and to cells close to the phloem or to the pericycle of the root (Fig. 9.1).

Equally important to the spacial regulation of metabolic pathways is the regulation in time, as some components are needed at different stages of development. One example is the expression of genes for such pathways in developing fruits, where either the unripe

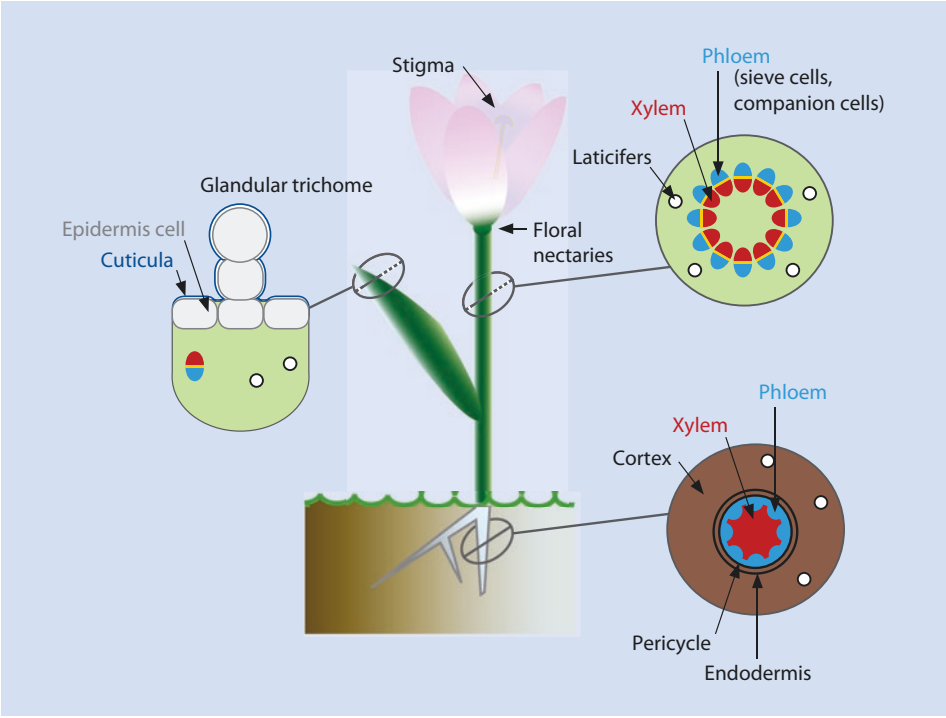

Fig. 9.1 Distribution of secondary metabolites in the different tissues of plants. Secondary metabolites can be found in all above-ground (aerial) organs, such as stems, leaves and flowers, but also in underground organs (roots). Often the place of production is not the place of storage; therefore, the components have to be transported from one tissue to the next. Long-distance transport often takes place in the phloem, which consists of companion cells that are important for the loading of the sieve cells, within which assimilates and metabolites are transported. In the stem, the vascular bundle consisting of phloem and xylem is distributed in the parenchyma. Laticifers are also embedded in the parenchyma. Leaf epidermis cells are covered with a wax layer, the cuticula, and can be modified into trichomes, some of which can accumulate essential oils. Volatiles can be airborne from glandular trichomes or from floral tissues. In the root, the vascular bundle is surrounded by the pericycle and the endodermis. The ground tissue around these tissues is called cortex

fruit is protected from predators by the secondary metabolites or the ripe fruit contains metabolites to attract distributors (Jaakola 2013; Rodriguez et al. 2013).

9.2 Regulation of the Biosynthesis of Secondary Metabolites

Stresses such as drought, extreme temperatures, salinity and high light or UV light modulate the responses that lead to the accumulation of secondary metabolites. However, defence mechanisms against herbivores and other predators seem to constitute the strongest inducers of secondary metabolite production. For their regulation, the hormone jasmonate (JA) plays a pivotal role (Memelink 2009; Zhou and Memelink 2016). Upon contact with pathogens, the endogenous JA levels rise. Furthermore, JA is described as a volatile that can also act on neighbouring plants or more distant plant parts without using internal transport pathways, for instance, through the phloem. JA, by registering the presence or absence of specific stresses, is one of the factors that allow balancing defence sys-

tems and growth. This is important, because both processes compete for the same resources (growth-defence trade-off), and thus investing into secondary metabolism always means that the allocated energy and resources (carbon and nitrogen) might be lacking for other reactions (Huot et al. 2014; Havko et al. 2016). In many cases, a close co-evolution of plants with pollinators or predators has been observed.

The active form of jasmonate, JA-Ile, is perceived in the cell by the SCF^{COI1} complex. COI1 is an E3 ubiquitin ligase which, upon binding of JA-Ile (usually when the levels are elevated), ubiquitinylates the JASMONATE ZIM DOMAIN (JAZ) repressors thereby targeting them for degradation by the 26S proteasome. This releases transcription factors, such as MYC2, which then regulate JA-dependent genes together with other transcription factors. In this way the transcription of many genes responsible for the biosynthesis of secondary metabolites is controlled (Patra et al. 2013; Gimenez-Ibanez et al. 2015; Chezem and Clay 2016; Zhou and Memelink 2016). JA has been shown to induce the biosynthesis of terpenes such as sesquiterpenes, phenylpropanoids and alkaloids. Examples are the biosyntheses of nicotine in tobacco (Dewey and Xie 2013) and terpenoid indole alkaloids in *Catharanthus roseus* (Zhang et al. 2018). Additionally, JA has been shown to increase trichrome density on newly formed leaves of *Arabidopsis* and tomato (Traw and Bergelson 2003; Boughton et al. 2005; Qi et al. 2011). This shows that not only the production of enzymes of the biosynthetic pathways but also the availability of storage places is regulated.

In addition to JA, other hormones are also involved in the regulation of secondary metabolites. These include especially cytokinins and ethylene. Cytokinin, for example, acts on downstream enzymatic steps of the indole alkaloid biosynthesis pathway (Papon et al. 2005). Ethylene can induce the formation of traumatic resin ducts in conifers (Hudgins and Franceschi 2004).

The roles of light in modulating secondary metabolites can be manifold; one of them is the entrainment of the circadian clock. The ability to anticipate the different times of the day provides a tool to produce secondary metabolites at times when they are needed and thereby to reduce the costs of overproduction. Protective compounds against UV light are only needed during daytime, and defence mechanisms are expressed according to the lifestyle of predators (Kim et al. 2011). Cabbage loopers (*Trichoplusia ni*), for example, display rhythmic feeding behaviour, and plants that are in the same entrainment are less attacked by this herbivore compared to out-of-phase plants (Goodspeed et al. 2012). Even fruits and vegetables that have already been harvested are still reacting to the daily cycles of light and darkness and accumulate secondary metabolites accordingly (Liu et al. 2015). Diurnal emission patterns of volatiles to attract specific pollinators have also been observed (Fenske and Imaizumi 2016).

In recent years not only many transcription factors responsible for the developmental and spatiotemporal modulation of gene expression have been identified (Yamada and Sato 2013; Kant et al. 2015), but also additional regulatory mechanisms such as regulation via miRNAs have been reported. miRNAs, for example, target genes encoding for enzymes important for benzylisoquinoline alkaloid biosynthesis (Boke et al. 2015). In addition to the transcriptional modulation of biosynthetic genes, post-translational regulatory mechanisms have been described. These include regulation of enzyme stability and modification by phosphatases/kinases. Often key enzymes of a pathway are regulated in this way, for instance, the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase that catalyses crucial regulatory steps of the mevalonic acid pathway (Leivar et al. 2011; Doblas et al. 2013) and the phenylalanine ammonia-lyase leading to the phenolpropanoids (Zhang and Liu 2015). Feedback mechanisms that limit the production of secondary

plant metabolites are also in place. All these mechanisms allow a tight control of the accumulation of secondary metabolites to balance strategies of survival with growth and development.

9.3 General Mechanisms for Biosynthesis

Some general mechanisms can be observed in all secondary pathways (Fig. 9.2a). In all pathways, some enzymes catalyse the committed step for a pathway. These enzymes are usually highly regulated by feedback mechanisms and post-translational modifications (Anarat-Cappillino and Sattely 2014). The plant primary metabolism provides the carbon backbones for the secondary metabolism, usually via glycolysis, Calvin cycle or TCA cycle (Fig. 1.1). The nitrogen groups are mostly derived from amino acids. The most important starter molecules for the basic building blocks, which form the backbone of secondary metabolites, are therefore acetyl-CoA, shikimate, mevalonic acid, 1-deoxyxylulose 5-phosphate and fatty acids. Condensation reactions between compounds or the addition of groups enlarges these molecules (Dewick 2002). Several kinds of groups can be added to the carbon backbone. These include one-carbon groups (C1), which are methyl groups usually donated by S-adenosylmethionine (SAM), leading to methylation or alkylation reactions. Two-carbon units are supplied by acetyl-CoA, whereas malonyl-CoA supplies C3 units. C5 carbon groups can be added as isoprene units (prenylation). For forming carbon-carbon bonds, several condensation reactions occur; the aldol reaction is the addition of enolates to aldehydes or ketones, and the Claisen reaction is the addition of enolates to esters (Fig. 9.2b, c). Both begin with a deprotonation. The difference is that within the Claisen reaction, an elimination step is needed to generate a β-keto ester.

Nitrogen is incorporated either via aminotransferases (or transaminases), which need pyridoxal-5′-phosphate (PLP) as a cofactor, or via condensation with amino acids. To form these carbon-nitrogen bonds also different condensation reactions can occur. When amines attack the electrophilic carbon atoms of aldehydes and ketones, a C=O double bond is replaced by a C=N double bond, forming an imine (Schiff base). The Mannich reaction converts a primary or secondary amine and two carbonyl compounds into a β-amino carbonyl compound (Mannich base) (Fig. 9.2d).

Alkanes, which are saturated hydrocarbons, can be transformed into unsaturated compounds that contain double or triple bonds (alkenes or alkynes), by desaturases that remove two hydrogen atoms. Unsaturated compounds are usually more reactive and promote cyclization. Many secondary metabolites undergo several steps of cyclization. One way to achieve this is via the Diels-Alder reaction, a cycloaddition, where a conjugated diene and a substituted alkene form a cyclic olefin (Fig. 9.2e). The Pictet-Spengler reaction converts an amine and an aldehyde or ketone to a protonated imine intermediate, which then undergoes cyclization (Fig. 9.2f).

Once the backbone or scaffold is formed, the metabolite complexity and diversity arise especially from oxygenation/hydroxylation reactions catalysed by oxygenases such as cytochrome P450 monooxygenases (Cyt P450) and 2-oxoglutarate-dependent dioxygenases (2OGDs) (Fig. 9.3). Cyt P450s are heme-containing proteins, which are anchored by their N-terminus to the cytoplasmic side of the endoplasmic reticulum (Barnaba et al. 2017; Groves 2015). In contrast, 2OGDs are non-heme proteins that localize in the cytosol as soluble proteins. They have iron Fe (II) as a cofactor and require 2-oxoglutarate (2OG) and molecular oxygen as co-substrates. These so-called tailoring enzymes are not equally distributed between all species; nevertheless, many genes encoding for these enzymes have

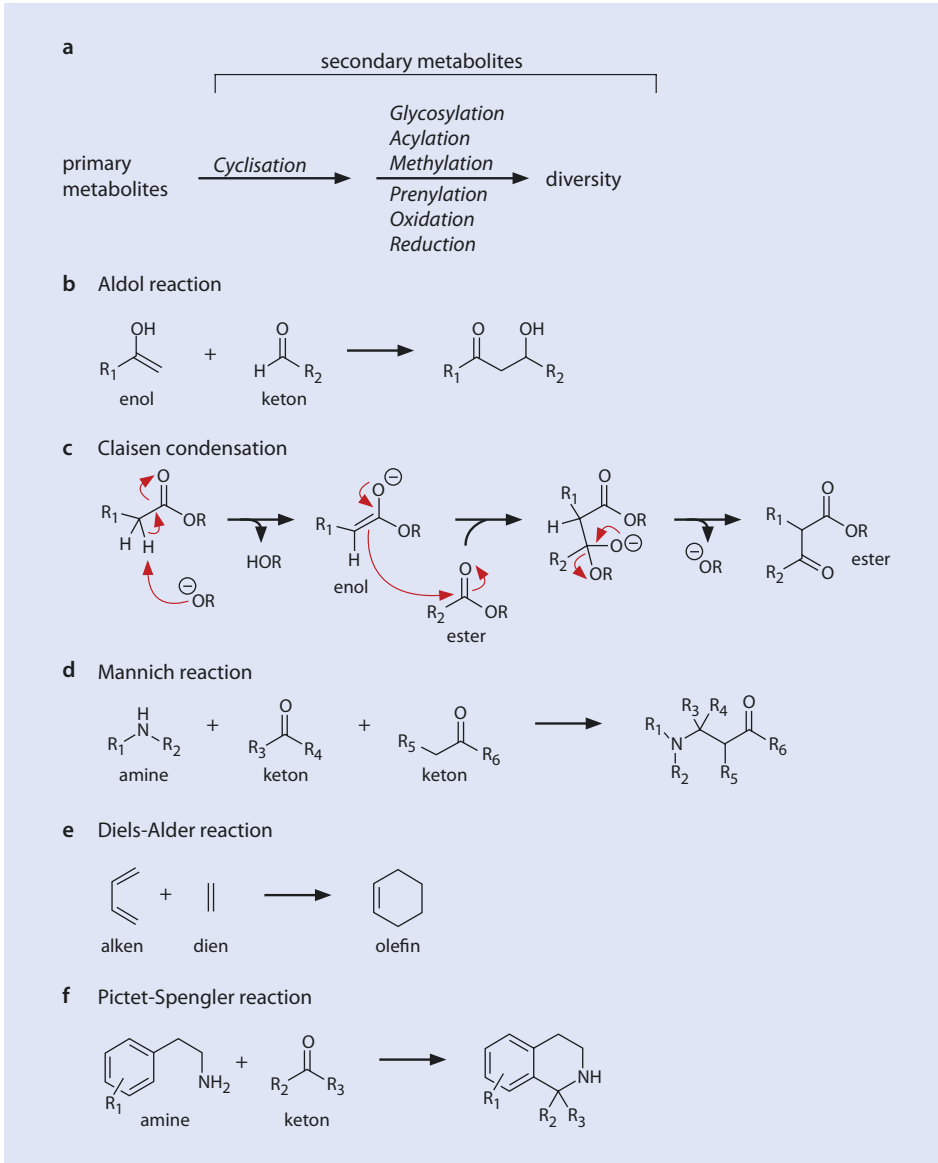

Fig. 9.2 General mechanisms observed in secondary pathways. Secondary metabolites are derived from primary metabolites and are modified with the help of different enzymes to generate the diversity observed in plants (see also ▶ Glossary) **a**. Forming carbon-carbon bonds can occur in serval ways. Often the condensation reactions are aldol reactions **b** or the Claisen condensation **c**. Carbon-nitrogen bonds can be formed via the Mannich reaction **d**. Cyclization can be achieved via the Diels-Alder reaction **e** or the Pictet-Spengler reaction **f**

been identified so far. In plants up to 1% of all genes probably code for Cyt P450 enzymes, indicating the variation of modifications that can be performed (Mizutani and Ohta 2010; Nelson and Werck-Reichhart 2011). Metabolons, an "assembly line" of enzymes, where enzymes cluster together working like a conveyor belt, have been suggested to increase a

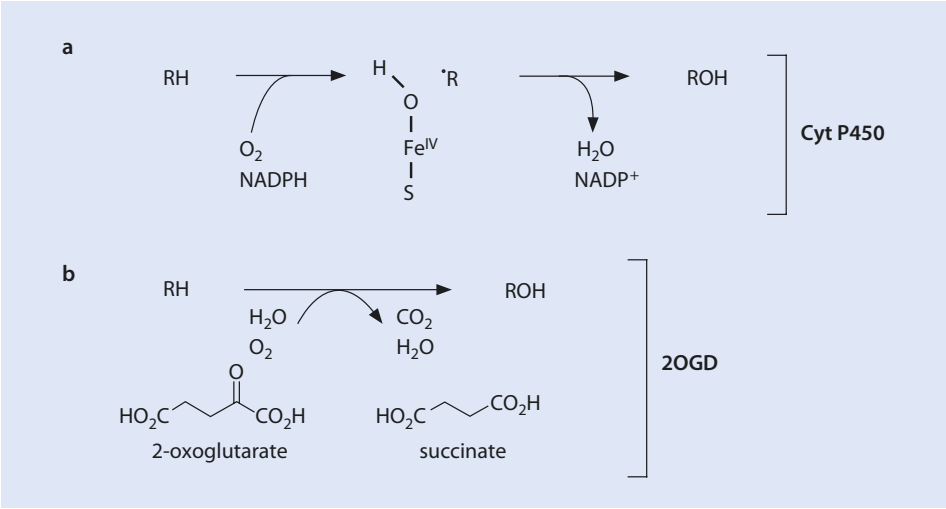

Fig. 9.3 Oxygenation and hydroxylation reactions are catalysed by oxygenases such as cytochrome P450 monooxygenases (Cyt P450) **a** and 2-oxoglutarate-dependent dioxygenases (2OGDs) **b**

pathway's efficiency. Here the anchoring of Cyt P450 enzymes to the ER membrane can serve as a clustering point (Bassard et al. 2017). Such multienzyme complexes allow the direct transfer of an intermediate from one enzyme to another and reduce the dissipation of intermediates into the cytoplasm thus avoiding possible toxic effects.

In the last modification steps, groups can be added to the compounds (acylation, methylation, prenylation) that derive from different pathways, but also reductions and the removal of carbon (decarboxylation), nitrogen and oxygen units are possible (Fig. 9.2a). These modifications are most important for the complexity of these compounds and are often very specific for species or tissues. Furthermore, they can affect the solubility of compounds and therefore the localization of the end product in the cell. Lipophilic compounds will accumulate in membranes, vesicles or dead cells, whereas hydrophilic compounds are often stored in the vacuole. The addition of sugar moieties to the compounds (glycosylation of the aglycon) changes their solubility, stability and biological activity. Especially the solubility of the compound in water is enhanced by glycosylation (e.g. saponins), which can lead to the storage of a compound in its inactive stage in the vacuole. Additionally, the addition of sugar can modulate the toxicity allowing the plant to store a less toxic compound. To combine a sugar with a compound, the sugar moiety is usually activated by UTP to form a uridine diphosphosugar (UDP-sugar). This facilitates a nucleophilic attack, as the nucleotidyl group acts as a leaving group. Finally, most biosynthetic reactions are catalysed by enzymes that can distinguish between the steric properties of their substrates. This also results in different stereoisomers, allowing that (S) and (R) versions of a compound exist. These isomers can have completely different binding affinities for the effector proteins or the receptors they bind to.

Most secondary metabolites can be classified into the three major groups, terpenoids, phenylpropanoids and alkaloids, but also some additional smaller groups exist. Many of these compounds are present in plants that we use for drugs or for teas or consume as food. Several of them or their intermediates can act as phytotoxins or neurotoxins (Table 9.1) (Rietjens et al. 2005). Details are discussed below.

Table 9.1 Important drugs or toxins for which interactions with human receptors or channels have been demonstrated. The respective compound class they belong to is mentioned

Drug or toxin	Compound class
Menthol Thujone	Monoterpenes (MEP pathway)
Ryanodine Salvinorin A	Diterpenes (MEP pathway)
ß-Carotene	Tetraterpenes (MEP pathway)
Tutin Valerenic acid	Sesquiterpenes (MVA pathway)
Digitoxin	Triterpenes (MVA pathway)
Dimethyltryptamine Ergotamine Physostigmine Psilocybin Reserpine Strychnine	Terpenoid Indole alkaloids (derived from tryptophan)
Bicuculline Codeine Curare Higenamine Morphine Thebaine	Benzylisoquinoline alkaloids (derived from tyrosine)
Mescaline	Phenethylamine alkaloids (derived from tyrosine)
Ephedrine	Phenylethylamines (derived from phenylalanine)
Nicotine	Pyridine alkaloids (derived from ornithine and arginine)
Atropine Cocaine Scopolamine	Tropane alkaloids (derived from ornithine and arginine)
Caffeine	Purine alkaloids (derive from xanthosine)
Lobeline	Piperidine alkaloids (derived from lysine)
Cytisine	Quinolizidine alkaloids (derived from lysine)
Aconitine	Terpenoid alkaloids (derived from MEP pathway)
Anatoxin A	Tropane-related alkaloids (derived from proline)
Ibotenic acid Muscimol	Isoxazole alkaloids
Muscarine	Mushroom alkaloids
Coniine	Pseudo-alkaloids
Tetrahydrocannabinol (THC)	Polyketides
Catechin Epicatechin Resveratrol	Phenylpropanoids
Capsaicin	Alkylamides

Take-Home Messages

- Building blocks for secondary metabolites are mainly derived from photosynthesis, glycolysis, TCA cycle and amino acids.
- Once the scaffold is formed, tailoring enzymes modify the structures by adding or removing of groups, oxidation reaction, desaturation and cyclization. Often also glucosylation occurs.
- Plants usually contain many different secondary metabolites, but some species contain very specific groups of secondary metabolites.
- The mixture of compounds varies between different cells, tissues and developmental stages and can be influenced by external stressors.
- Jasmonic acid is a major integrator to balance growth-defence trade-off.

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Terpenes and Terpenoids

10.1 Introduction – 154

10.2 Monoterpenes (From MEP Pathway, C10, e.g. Menthol, Camphor and Thujone) – 157

10.3 Diterpene (From MEP Pathway, C20, e.g. Salvinorin A and Ryanodine) – 161

10.4 Tetraterpene (From MEP Pathway, C40) – 162

10.5 Sesquiterpenes (From MVA Pathway, C15, e.g. Valerenic Acid, Anisatin and Tutin) – 163

10.6 Sterols and Triterpene (From MVA Pathway, C30) – 165

References – 168

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What You Will Learn in This Chapter

Terpenes and their derivatives, the terpenoids, are synthetized from a five C atom isoprene unit. These units are added to each other to give rise to compounds of different complexities. The synthesis takes place either in the cytosol (MVA pathway) or within the plastids (MEP pathway). Monoterpenes, diterpenes and tetraterpenes are products from the MEP pathway, whereas sesquiterpenes, sterols and triterpenes are derivatives from the MVA pathway. Tailoring enzymes leads to the variations observed. End products are important for plant growth such as hormones (gibberellin and brassinosteroids) or carotenoids. They can be simpler structures, which are sometimes also volatile (such as menthol or ß-caryophyllene) or more complex, even polycyclic (such as ryanodine or stigmasterol).

10.1 Introduction

The building units of terpenes are isoprene units (Dewick 2002; Vranova et al. 2012). These precursors with five carbon atoms (C5) are present in the cell as diphosphates, either isopentenyl pyrophosphate (IPP) or its allylic isomer dimethylallyl pyrophosphate (DMAPP). Their fusion is mostly head-to-tail (a 1,4 link) but can also be head-to-head (a 1,1 link) or tail-to-tail (a 4,4 link) and leads to the formation of terpenes of different length (Fig. 10.1). These structures can be further modified at the methyl groups or by adding oxygen atoms and are then called terpenoids or isoprenoids. It is estimated that between 20,000 and 40,000 different structures are present in plants.

Two main pathways exist for the formation of DMAPP and IPP, the mevalonate pathway (MVA) and a mevalonate-independent pathway called MEP (after the first component 2-C-methyl-D-erythritol 4-phosphate) or DOXP pathway (after 1-deoxy-D-xylulose 5-phosphate). The MVA pathway can be found in all organisms, and in plants, it takes place mainly in the cytosol, whereas the MEP pathway resides in plastids (Tholl 2015). Therefore, the MEP pathway and its end products are specific for plants but can also be found in some bacteria. Due to the lack of the MEP pathway in humans, enzymes of this pathway are good targets for the treatment of human pathogens. One example is fosmidomycin, which acts on the DXP reductoisomerase of bacteria and *Plasmodium falciparum* and is used against infections and malaria (Rodriguez-Concepcion 2004).

In animals, the MVA pathway produces components such as cholesterol, dolichol and ubiquinone, which are important for membrane integrity and electron transport. In plants, six enzymes are needed for the MVA pathway (Fig. 10.2). The first steps are the condensation of two acetyl-CoA via acetoacetyl-CoA thiolase to form acetoacetyl-CoA and the addition of another acetyl-CoA by the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase to form HMG-CoA. The HMG-CoA reductase then synthetizes MVA, one of the control steps of the pathway. This enzyme consists of an endoplasmic reticulum (ER) membrane anchor and a conserved cytosolic catalytic domain. It is tightly regulated via phosphorylation and protein stability, stressing the importance of this pathway (Leivar et al. 2011; Doblas et al. 2013). Two phosphorylation steps are needed to produce mevalonate 5-pyrophosphate (MVAPP) and the diphosphomevalonate decarboxylase catalyzes the conversion of MVAPP to IPP. Components of the MVA pathway are distributed between the cytosol and peroxisomes, especially the enzymes for the two last steps could be localized to the peroxisome, while the first phosphorylation step is cytosolic (Pulido et al. 2012).

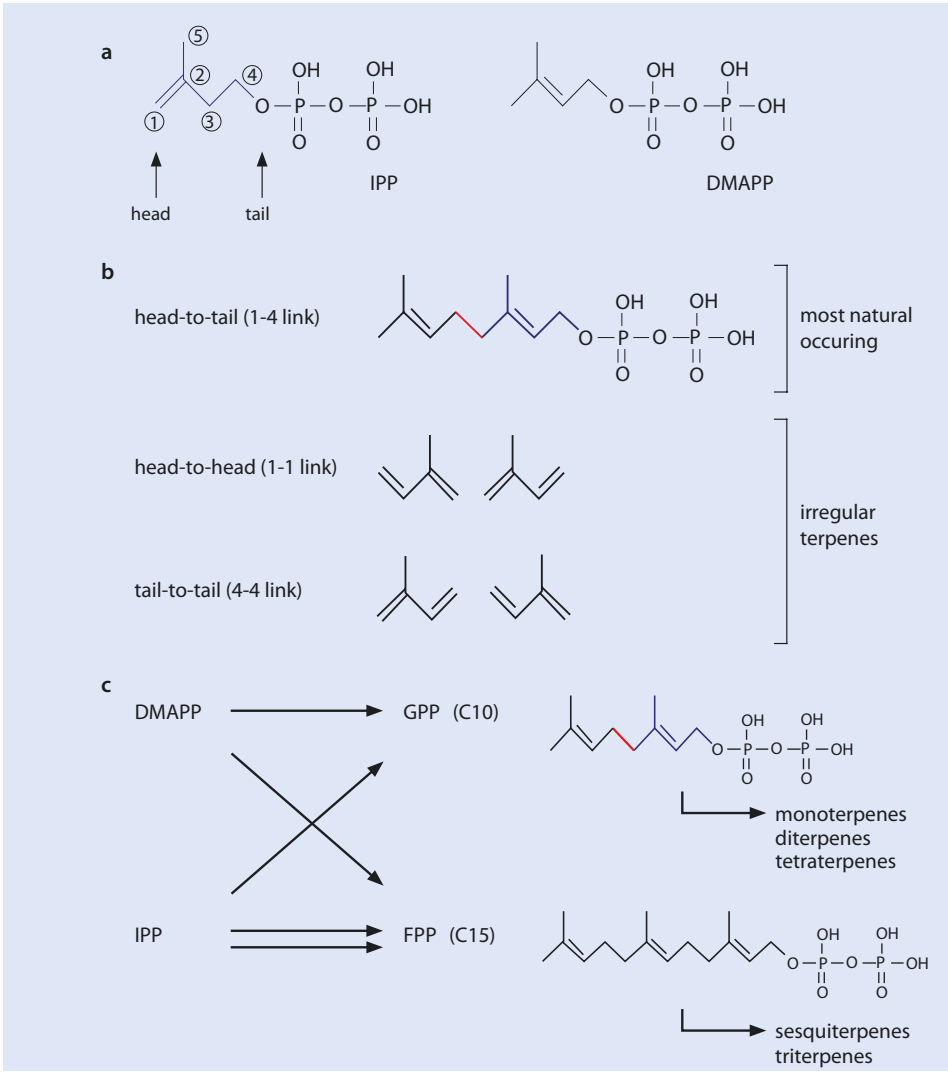

Fig. 10.1 The building units of terpenes are isoprene units. Isopentenyl pyrophosphate (IPP) and its allylic isomer dimethylallyl pyrophosphate (DMAPP) are the precursors of all terpenes. They have five carbon atoms (C5) and are present as reactive pyrophosphates (PP) **a**. They fuse mostly head-to-tail (1,4 link) but can also be head-to-head (1,1 link) or tail-to-tail (4,4 link; irregular terpenes) **b**. These fusion lead to the formation of terpenes of different length, the first product being geranyl pyrophosphate (GPP, C10) and farnesyl pyrophosphate (FPP, C15) **c**

The MEP pathway needs seven enzymes to produce IPP, starting from glyceraldehyde 3-phosphate and pyruvate (Fig. 10.2). The first step is mediated by 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and leads to the formation of 1-deoxy-D-xylulose 5-phosphate (DXP), followed by the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) step yielding 2-C-methyl-D-erythritol 4-phosphate (MEP). Four additional steps are needed to produce 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-pyrophosphate (HMBPP),

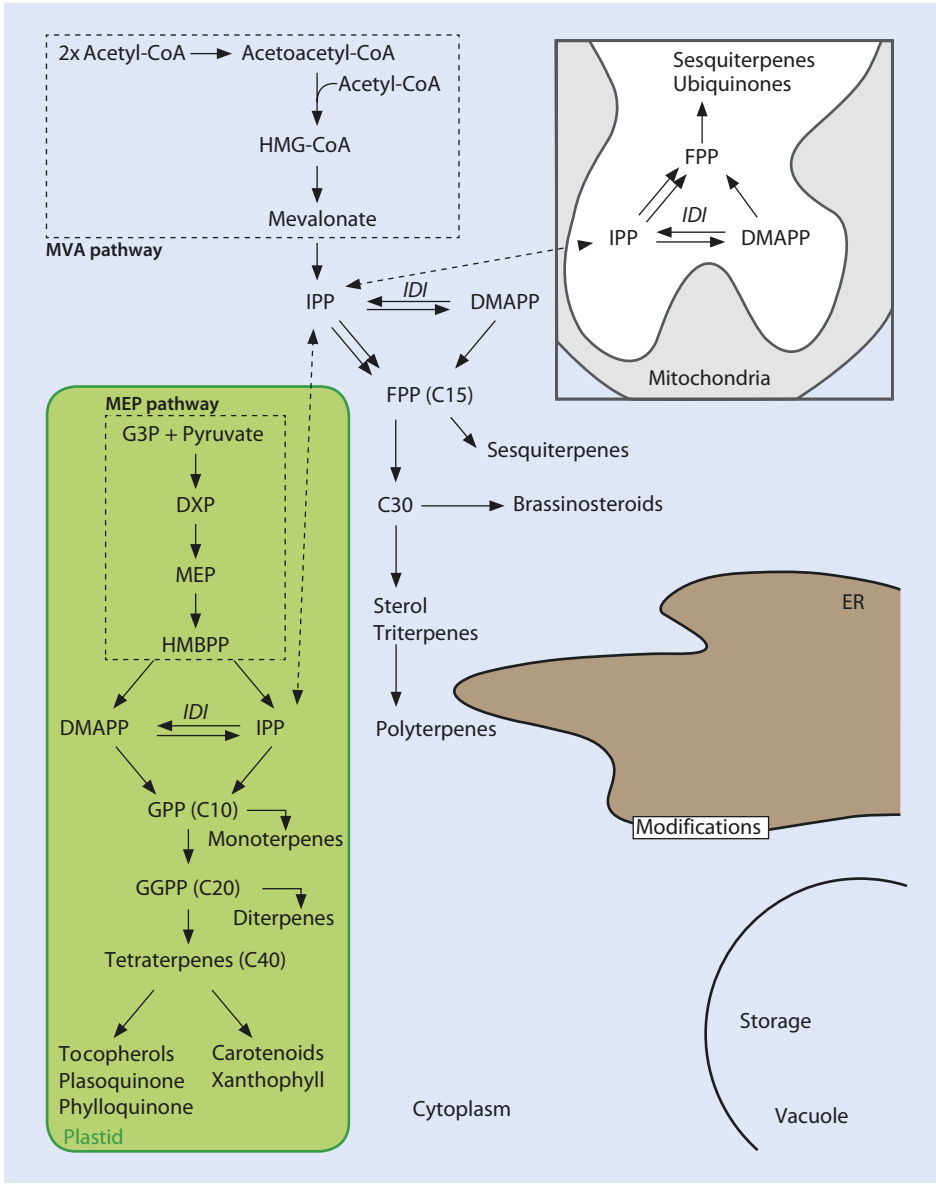

Fig. 10.2 Schematic overview of the biosynthetic pathways and their compartmentalization leading to terpenoids in plants. Several subcellular compartments are involved in the synthesis of terpenoids, especially plastids, mitochondria and the cytoplasm. The mainly cytosolic compartmentalized mevalonate (MVA) pathway and plastid localized mevalonate-independent (MEP) pathway both produce the C5 precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). There is an exchange between these compartments, mainly via isopentenyl pyrophosphate (IPP). The enzymes and end products are different between the MEP and MVA pathways. The C5 units are condensed to generate C10 (geranyl pyrophosphate, GPP), C15 (farnesyl pyrophosphate, FPP), C20 (geranylgeranyl pyrophosphate, GGPP), C30 and C40 precursors. At the ER, many enzymes reside that modify the basic structures (e.g. Cyt P450), and terpenoids are often stored in the vacuole. Enzymatic steps are represented by arrows, one arrow can account for multiple enzymatic steps. Additional abbreviations: *HMG-CoA* Hydroxymethylglutaryl-coenzyme A, *G3P* glyceraldehyde-3-phosphate, *DXP* 1-deoxy-D-xylulose 5-phosphate, *MEP* 2-*C*-methyl-D-erythritol 4-phosphate, *HBMPP* 1-hydroxy-2-methyl-2(*E*)-butenyl 4-pyrophosphate, *IDI* isopentenyl diphosphate isomerase

which is further converted into IPP and DMAPP by the HMBPP reductase. All the enzymes of the MEP pathway are localized in the plastid stroma.

The MVA and MEP pathways in the cytosol and plastids are not completely separated since there is exchange via their common intermediates, especially IPP. IPP can also be transferred to the peroxisomes and mitochondria. The isopentenyl diphosphate isomerase (IDI) mediates the equilibrium and supply of IPP and DMAPP (Berthelot et al. 2012). This enzyme appears to localize in the peroxisomes, mitochondria and plastids, although alternative splice forms have also been found in the cytoplasm (Phillips et al. 2008; Clastre et al. 2011; Guirimand et al. 2012). Besides the difference in the biosynthesis of IPP, there is also a difference in the first condensation step between the MVA and the MEP pathways (◘ Fig. 10.2). Head-to-tail condensation of one DMAPP with one IPP molecule in plastids results in geranyl pyrophosphate (GPP; C10, with 10 carbon atoms; ◘ Fig. 10.1c) formation, the precursor of monoterpenes, and is catalyzed by the GPP synthase in the MEP pathway. For the MVA pathway, the first condensation product is farnesyl pyrophosphate (FPP; C15, ◘ Fig. 10.1c), which is a sequential head-to-tail condensation of two IPPs and one DMAPP molecule by the FPP synthase (FPS) in the cytosol or in mitochondria. Knockout of all FPS genes is lethal for *Arabidopsis*, and embryo development is arrested at the pre-globular stage, demonstrating that this pathway is essential (Closa et al. 2010). In the next condensation step, two GPP or FPP fuse, leading to mainly C30 bodies in the cytoplasm and C20 and C40 bodies in the plastids. Polyprenols comprising C50–C130 bodies are also present in plants; natural rubber can contain more than 10,000 1,4-linked isoprene units.

The primary products of condensation are linear compounds. These are not only precursors for further modifications but also have specific roles in plants. Nevertheless, they also undergo alterations such as reduction, oxidation and side-chain modifications, which lead to terpenoid hydrocarbons, alcohols, ethers, aldehydes, ketones or carboxylic acids and their esters (Tholl 2015). Derivatization often leads to cyclization. Cyt P450 enzymes are involved in many of these steps, which take place mainly in the ER or cytosol. End products from the different compartments vary: in the cytosol mainly sequiterpens (C15), sterols (C30), triterpenes (C30) and polyterpens (>30) are produced, while in the mitochondria especially the coenzyme Q_{10} essential for electron transport is produced and also diterpenes and sesquiterpenes. In plastids, monoterpenes (C10), diterpenes (C20) and tetraterpenes (C40) are synthesized, but many of the end products are then transferred into the cytoplasm.

Many plants produce terpenes in specialized cells or tissues such as the glandular trichomes or the epithelial cells that surround the resin ducts of conifers. This defense strategy allows concentrating terpenoids in areas most likely to be targeted by a predatory organism, such as the surface of leaves (glandular trichomes), in resin ducts or laticifers (see ► Sect. 1.3.7 in ► Chap. 1).

10.2 Monoterpenes (From MEP Pathway, C10, e.g. Menthol, Camphor and Thujone)

Combining two C5 units leads to the most basic terpene, which is the linear monoterpene precursor geranyl pyrophosphate (GPP, C10). This compound is then further processed by monoterpene synthases/cyclases. These mostly belong to the enzyme group of terpene synthases (TPS) (see ► Box 10.1).

Box 10.1 Terpene Synthases (TPS)

Terpene synthases are the class of enzymes with a divalent metal ion cofactor that produce several different terpene end products (C5, C10, C15 and C20 terpenes). One enzyme can hereby generate multiple products from one substrate, but they can also use different substrates, which increase the variation (Tholl 2006; Chen et al. 2011; Pazouki and Niinemets 2016). The first step of the reaction usually involves ionization of the substrate by cleaving off the diphosphate group and the formation of a carbocation. This can lead to cyclization before the reaction is terminated via deprotonation.

Whereas in the moss *Physcomitrella patens* only one *TPS* gene is present, in higher plants over 100 *TPS* genes can be found. Examples are the isoprene synthase, the monoterpene synthases (such as limonene synthase, copalyl diphosphate synthase, pinene synthase, myrcene synthase, ent-kaurene synthase, linalool synthase, sesquiterpene synthases and diterpene synthases).

Monoterpenoids often constitute part of the so-called essential oils. Essential oils (see ► Box 10.2) are defined as non-water-soluble compounds, which usually contain volatile aroma compounds from plants.

The first step in monoterpene formation is the dephosphorylation and ionization of GPP to a geranyl carbocation (► Fig. 10.3). Acyclic monoterpenes are then derived by deprotonation. Those include geraniol (odour of roses, lemon grass and geranium), its isomeric form linalool (in many herbs such as mint, laurels, cinnamon and rosewood and citrus fruits, often acting as olfactory cues for pollinators), myrcene (in bay, thyme, parsley, cardamom and hops) and ß-ocimene (odour of lavender and basil). Many of these compounds can be found in peltate glands from which they are easily released as volatiles upon contact.

Box 10.2 Essential Oils

The term "essential oils" was probably coined in the sixteenth century by alchemists as "*Quinta essential*" (fifth element, universal ether). They occur often in the plant families Alliaceae, Apiaceae, Asteraceae, Lamiaceae, Myrtaceae, Poaceae and Rutaceae and are responsible for the scents of plants especially spices and herbs (Dhifi et al. 2016). This is due to the volatile compounds within the essential oils that are characterized by a high vapor pressure. They are usually stored in secretory glands on leaves (e.g. eucalyptus, mint, thyme, rosemary, sage, basil, marjoram, pine, cypress) and flowers (e.g. orange, lavender, hops, chamomile, clove flower bud), but they are also be found in rhizomes (e.g. ginger), seeds (e.g. coriander, cardamom, pistachios, nutmeg, pepper), fruits (e.g. fennel, anise, citrus, apple) and in wood (e.g. cinnamon, sandalwood, rosewood, balsam fir tree, camphor). They are extracted for industrial (e.g. fragrances) and medicinal purposes by steam distillation, as they are insoluble in water. Essential oils are mixtures of monoterpene and sesquiterpene group but also phenylpropanoids.

In plants, essential oils are important for communication between plants, to attract pollinating insects and to repel predators. Due to their insect repellent and insecticidal activity they are also discussed as biopesticides (Regnault-Roger et al. 2012; Pavela and Benelli 2016).

For the cyclization of monoterpenes, the limonene synthase (a TPS) is the most important enzyme. From the geranyl cation, linalyl pyrophosphate (LPP) is formed, which lacks the trans-double bond that inhibits rotation and thereby cyclization. LPP stays bound to the enzyme. The removal of the pyrophosphate group leads to the linalyl cation, which is then formed into an α-terpinyl cation by cyclization. The α-terpinyl cation can undergo various steps of deprotonation, cyclization and ring closure leading to different cyclic end products (◘ Fig. 10.3). Deprotonation stabilizes the compound and leads to structures

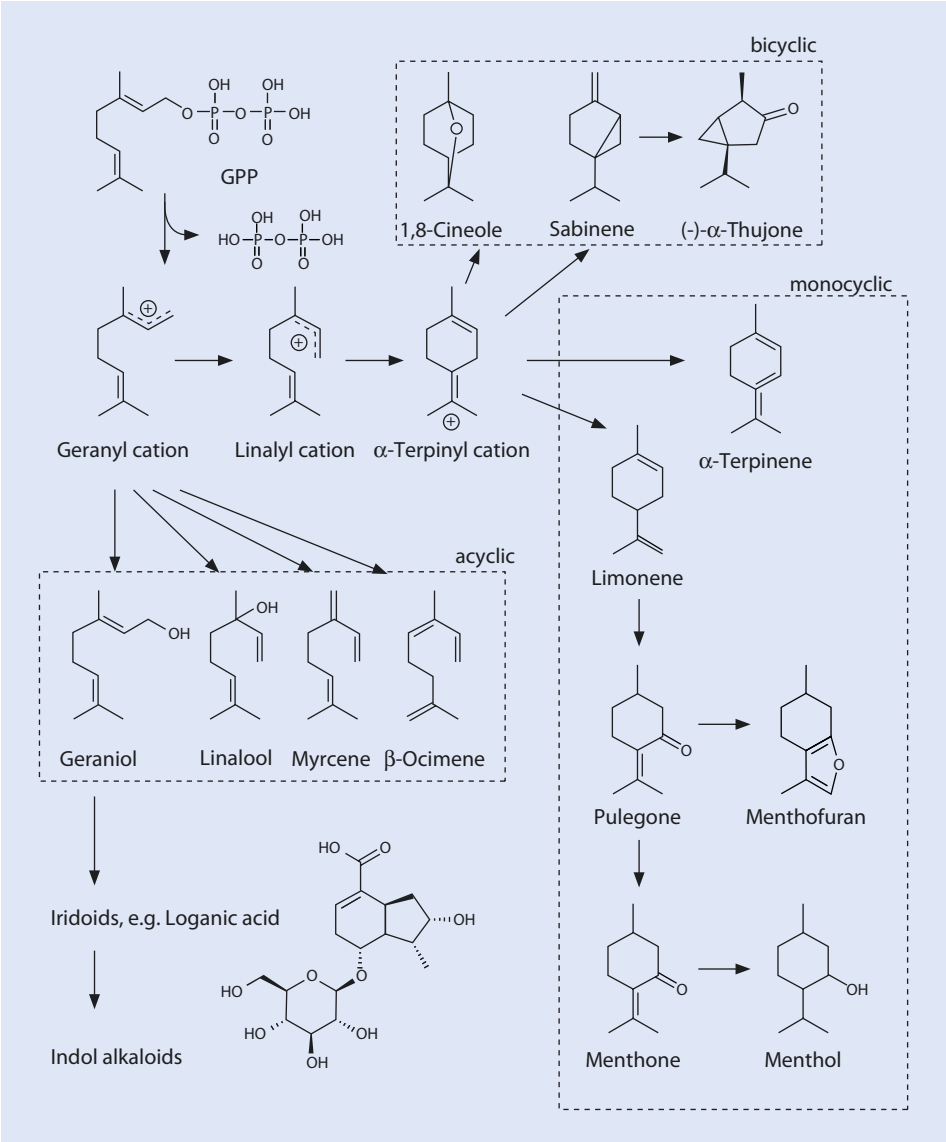

Fig. 10.3 Schematic overview of the biosynthetic pathways and representative members of various monoterpene subfamilies. Abbreviations: *GPP* geranyl pyrophosphate

with one ring (monocyclic, olefins). Among the monocyclic monoterpenes are limonene (odour constituent of citrus), α-terpinene (in cardamom and marjoram) and phellandrene (in eucalyptus, resin of the balsam fir tree, *Abies balsamea,* known as Canada balsam).

Limonene is also an important precursor for the formation of pulegone. The enzyme pulegone reductase then forms menthone from pulegone. Menthol is the oxidized form of menthone (Ahkami et al. 2015). Menthofuran is a side product from pulegone, mainly produced under high light intensities but unwanted in the production of menthol. The main producer of menthol and its derivatives is mint (*Mentha* spp.), making this plant an

important source of essential oils for the flavour, fragrance and aromatherapy industries. Due to its binding to the cold-sensing ion channel TRPM8 menthol can trigger a cooling sensation (Kamatou et al. 2013). The enzymatic reactions occur in specialized anatomical structures of the epidermis called glandular trichomes. The limonene synthase of *Mentha* × *piperita* has been localized only to the leucoplasts (colourless plastids) of gland secretory cells, indicating that the essential oil production occurs within these cells. However, the succeeding steps of monoterpene modification appear to occur outside of the leucoplasts in mint (Turner et al. 1999).

Additional ring closure via the terpinyl cation leads to bicyclic compounds such as careen (in pine, rosemary and cedar), camphene (in turpentine, cypress, camphor, ginger, valerian), 1,8-cineole (in eucalyptus, bay leaves, sage), pinene (in pine resin, pines, spruces and firs, fruits of *Pistacia terebinthus*), sabinene (in *Quercus ilex, Picea abies,* black pepper, nutmeg), fenchol (in rosemary, basil, hops) and camphor. Camphor is biosynthetically produced in plants by way of borneol (Croteau et al. 1981). Besides being present in sage, camphor oil can be isolated by distillation from the wood of the camphor tree (*Cinnamomum camphora,* Lauraceae) or is found in camphor basil (*Ocimum kilimandscharicum*, Lamiaceae). It has served as a fumigant against the Black Death and as an embalming fluid (Chen et al. 2013). These days it is used as an insect repellent and as fragrance, which can induce cold and warm sensations, although it can be toxic at higher dosage. Pinene is discussed as a cannabis antidote as it forms the biosynthetic base for CB2 ligands and has been suggested to act as an acetylcholinesterase inhibitor aiding memory (Russo 2011). Sabinene can further be oxidized to form α-thujone (in *Thuja, Juniperus*, sage, thyme, rosemary), which is known for its presence in the absinthe drink and which acts as a GABA antagonist. All these aforementioned primary products can then be further modified by secondary reactions such as hydroxylation, peroxidation, methylation, acylation, glycosylation or cleavage (often by Cyt P450 enzymes), which increases the diversity of these compounds.

Iridoids, characterized by their cyclopentane (C5) ring fused with a pyran (C6) ring, are formed via a different cyclization step. Geraniol is first oxidized and then undergoes cyclization by the iridoid synthase (De Luca et al. 2014; Ilc et al. 2016). Iridoids, such as aucubin (in *Plantago*) and amarogentin (in gentian, *Gentiana lutea*), are usually stabilized by a sugar moiety. This sugar moiety also allows storage of a less toxic compound. Amarogentin is known for its bitter taste. The iridoid loganic acid (which is also fused to a glucose moiety) is converted to loganine and further secologanin, the last step being an oxidative cleavage of the C5 ring. Secologanin reacts with tryptamine forming strictosidine, leading to indole alkaloids (see ► Sect. 12.2 in ► Chap. 12).

The fusion of two molecules of dimethylallyl pyrophosphate (DMAPP) occurs via c1′–2–3 cyclopropanation by the enzyme chrysanthemyl diphosphate synthase yielding chrysanthemyl pyrophosphate (Yang et al. 2014). Chrysanthemyl pyrophosphate is further converted into chrysanthemol by hydrolysis of the diphosphate moiety, is then oxidized to chrysanthemic acid and further esterified to pyrethric acid. The acids are esterified with one of three alcohols (pyrethrolone, cinerolone or jasmolone), which are probably derived from jasmonate precursors through the oxylipin pathway. This yields the different forms of pyrethroids. The chrysanthemyl diphosphate synthase can be found in glandular trichomes, whereas the later synthesis steps take place in the pericarp. Pyrethrins can be extracted from dried pyrethrum flowers (*Chrysanthemum; Tanacetum cinerariifolium*) and constitute a well-known insecticide, which affects the Na^+-channels of nerve cells especially in insects.

10.3 Diterpene (From MEP Pathway, C20, e.g. Salvinorin A and Ryanodine)

The fusion of two GPPs leads to the formation of geranylgeranyl pyrophosphate (GGPP), the first diterpene with 20 carbons (Fig. 10.4). In contrast to the monoterpenes, diterpens are usually not volatiles due to their larger size. The acrylic phytyl diphosphate, derived from GGPP, is the precursor for tocopherol, phylloquinone and phytol and the side chain of chlorophyll (Zi et al. 2014). All these substances are important for photosynthesis in chloroplasts. Nevertheless, many diterpenoids are produced in leucoplasts.

GGPP, analog to monoterpenes, can by cyclized and rearranged into different forms (Zi et al. 2014). Class I diterpene synthases remove the allylic diphosphate ester bond

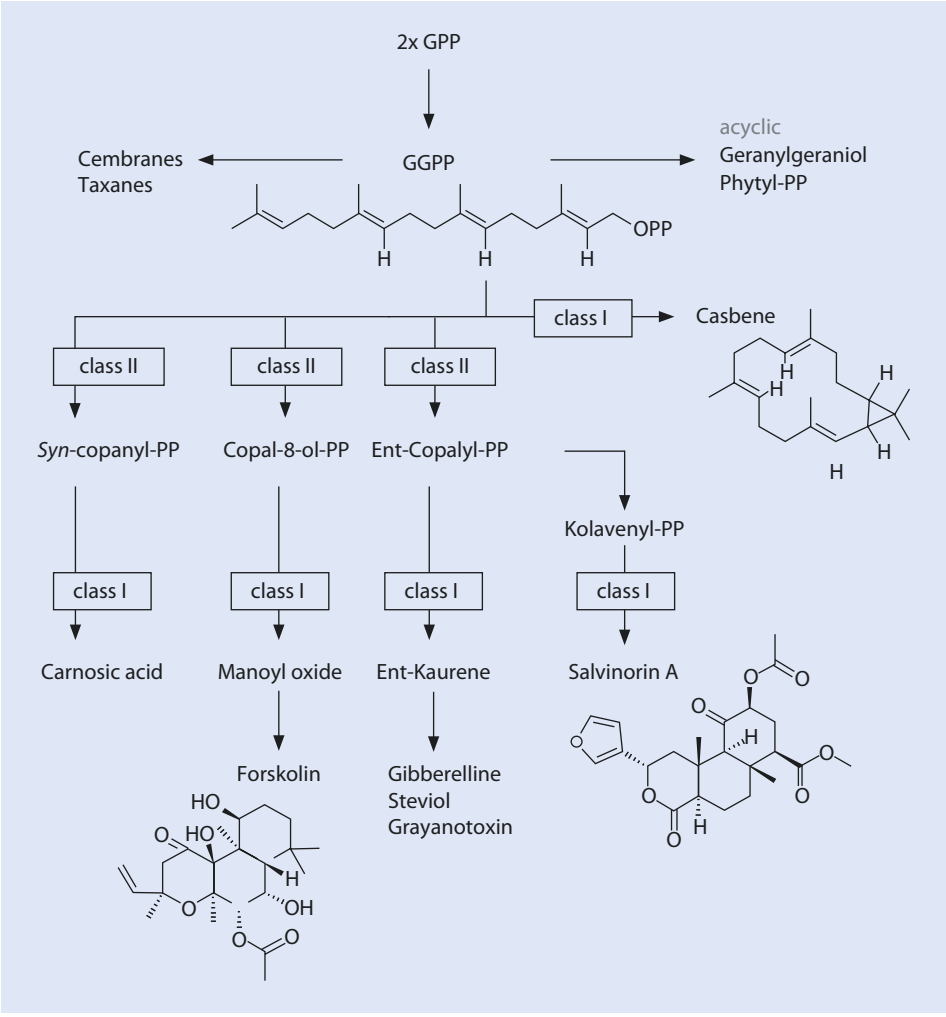

Fig. 10.4 Schematic overview of the biosynthetic pathways and representative members of various diterpene subfamilies. Diterpene synthetases belong to either the class I or the class II type. Abbreviations: *GPP* geranyl pyrophosphate, *GGPP* geranylgeranyl pyrophosphate

present in GGPP whereas class II diterpene cyclases leave this bond intact to be modified by subsequently acting class I diterpene synthases. Both belong to the enzymatic group of TPS (▶ Box. 10.1). In the most common pathway GGPP gives rise to the bicyclic ent-copalyl pyrophosphate via a class II cyclase which forms the bicyclic labdane carbon skeleton (Peters 2010). A type I cyclase closes the third ring leading to ent-kaurene, which is a precursor for the hormone gibberellin.

Other components derived from ent-kaurene are *steviol*, an intermediate for the biosynthesis of the sweetener stevioside (which is a glycosylated derivative) found in *Stevia rebaudiana*; cafestol and kahweol, which are found in coffee, adding to the effect of caffeine; and kauralexins, which are phytoalexins from maize. Furthermore, diterpene resin acids are characterized by tricyclic parent skeletons and are major components of the defense system of pine trees, often accumulating in the resin ducts of the phloem and cortex. In addition, grayanotoxins, toxins found in rhododendrons and other Ericaceae, are suggested to derive from ent-kaurene. The toxins can also be found in the nectar, which when used by honey bees can lead to contamination of the honey ("mad honey") (see ▶ Box 1.1). Grayanotoxins can bind to the group II receptor site in voltage-gated sodium channels within the cell (see ◘ Fig. 4.2b). Forskolin also has a labdane skeleton formed via Copal 8-ol pyrophosphate from the intermediate 13*R*-manoyl oxide. This compound is found in *Plectranthus barbatus* (Syn. *Coleus forskohlii*, Lamiaceae), where it accumulates in the root cork. It has been shown to activate the enzyme adenylyl cyclase and thereby increases intracellular levels of cAMP in humans.

Salvinorin A biosynthesis in *Salvia divinorum* (Lamiaceae), a κ-opiate receptor agonist, is not completely understood but seems to happen via the formation of (-)-kolavenyl diphosphate (Chen et al. 2017). Salvinorin A is a highly oxidized diterpene that is also methylated and acetylated. Peltate glandular trichomes were identified as the major site of salvinorin A accumulation in *S. divinorum* (Siebert 2004).

Derivatives of class I diterpene synthases are, for example, casbene, the antifungal component in castor bean (*Ricinus communis*). Bicyclic diterpenes can be further changed into polycyclic diterpenes, such as the pentacyclic ryanodine (from *Ryania speciosa*, Salicaceae), which interacts with the ryanodine receptor. Others are the tetracyclic phorbol ester present in the latex of many Euphorbiaceae and Thymelaeaceae, which mimics the action of diacylglycerol, and resiniferatoxin, which is present in the latex of *Euphorbia resinifera* (Euphorbiaceae) and hyperactivates the transient vanilloid receptor 1 (TRPV1). Other diterpenoids are formed by macrocyclic-forming steps leading to cembranes and taxanes. Taxol, sold as paclitaxel, is a known anticancer medication.

The diterpene synthases mostly produce olefins, which are highly hydrophobic. Introduction of oxygen increases the solubility and adds hydrogen-bonding potential, a step catalyzed by Cyt P450 enzymes. Some of the compounds, especially if they act as defense compounds, are produced in glandular trichomes such as cembratrieneols in tobacco or carnosol and carnosic acid in rosemary and sage, which seems to have antioxidative and antimicrobial properties.

10.4 Tetraterpene (From MEP Pathway, C40)

Tetraterpenes are produced by the fusion of two GGPP, usually via head-to-head condensation (Ruiz-Sola and Rodriguez-Concepcion 2012; Nisar et al. 2015). This leads to the formation of phytoene by the phytoensynthase. The next step leads to the formation of

lycopene. This is a branch point from which two different cyclases can modify the compound. One branch leads to the α-carotenes, which results in the formation of lutein, whereas the other branch leads to the β-carotenes, resulting in the formation of zeaxanthin, violaxanthin and neoxanthin. Due to the high amount of double bonds, many of these substances are colourful, notably the orange-red coloured carotenoids. Most of the enzymes are attached to the envelope membranes of the chloroplasts. Carotenes and xanthophylls (containing in contrast to carotenes oxygen atoms in form of hydroxyl groups and/or epoxide bridges) are mainly known for their protective role in photosynthesis. β-carotin (or β-carotene) is also degraded into retinal and further to retinol, better known as Vitamin A. Vitamin A is an essential vitamin for humans and animals because they cannot produce β-carotin and need to take it up with the diet. β-carotin accumulates in many fruits, vegetables and fungi, which can be recognized by their orange colour, especially carrots (*Daucus carota*, Apiaceae). Additionally, carotenoid cleavage dioxygenases produce carotenoid cleavage products that have been shown to be important for the formation of hormones such as abscisic acid (ABA) and strigolactones (McQuinn et al. 2015; Hou et al. 2016). Additionally these enzymes can produce cleavage products such as bixin (from Annatto seeds), crocin and safranal in crocus (*Crocus sativus*, Iridaceae; important for taste, colour and smell of saffron), or mycorradicin, which is responsible for colouring of arbuscular mycorrhizal roots.

10.5 Sesquiterpenes (From MVA Pathway, C15, e.g. Valerenic Acid, Anisatin and Tutin)

The first condensation product of sesquiterpenes in the cytosol consists of three isoprene units and is called farnesyl pyrophosphate. It is the result of sequential head-to-tail condensations of two IPP and one DMAPP molecule catalyzed by farnesyl pyrophosphate (FPP) synthase (Fig. 10.5). Sesquiterpenoids are upregulated upon defense and emitted from vegetative tissues in response to herbivore feeding and function as an indirect defense by attracting natural enemies of herbivores. On the other hand, they can be volatile constituents of floral odours that attract pollinators (▶ Box. 10.2).

The sesquiterpene pathway is based on TPS-dependent reactions and works along the same mechanisms shown for monoterpenes (▶ Box. 10.1). Deprotonation of the farnesylation leads to (*E*)-β-farnesene, or there is an additional isomerization via the tertiary diphosphate intermediate nerolidyl diphosphate (Fig. 10.5). The two main acyclic forms are farnesene (green apple odour, aphid alarm pheromone) and nerolidol (in citrus, ginger, jasmine, lavender and lemon grass; woody aroma and reminiscent of fresh bark).

Caulerpenyne, a highly toxic acetylated linear sesquiterpenoid, is found in large amounts in the green algae *Caulerpa taxifolia* (Brunelli et al. 2000; Jung et al. 2002). This is a tropical seaweed and an invasive species in the Mediterranean Sea. Each alga is a single, supersized cell up to several meters in length. When this cell is damaged, caulerpin is metabolized by an enzyme to produce oxytoxin 2, which immediately cross-links proteins forming a gummy gel inside the cell that can plug the break in just 30 s and hardens into protective scar tissue.

The farnesylation is cyclized to yield two major monocyclic forms, which are humulane and bisabolane. Humulane has as an intermediate, the humulyl cation, giving rise to the derivatives humulene and E-β-caryophyllene present in *Syzygium aromaticum*

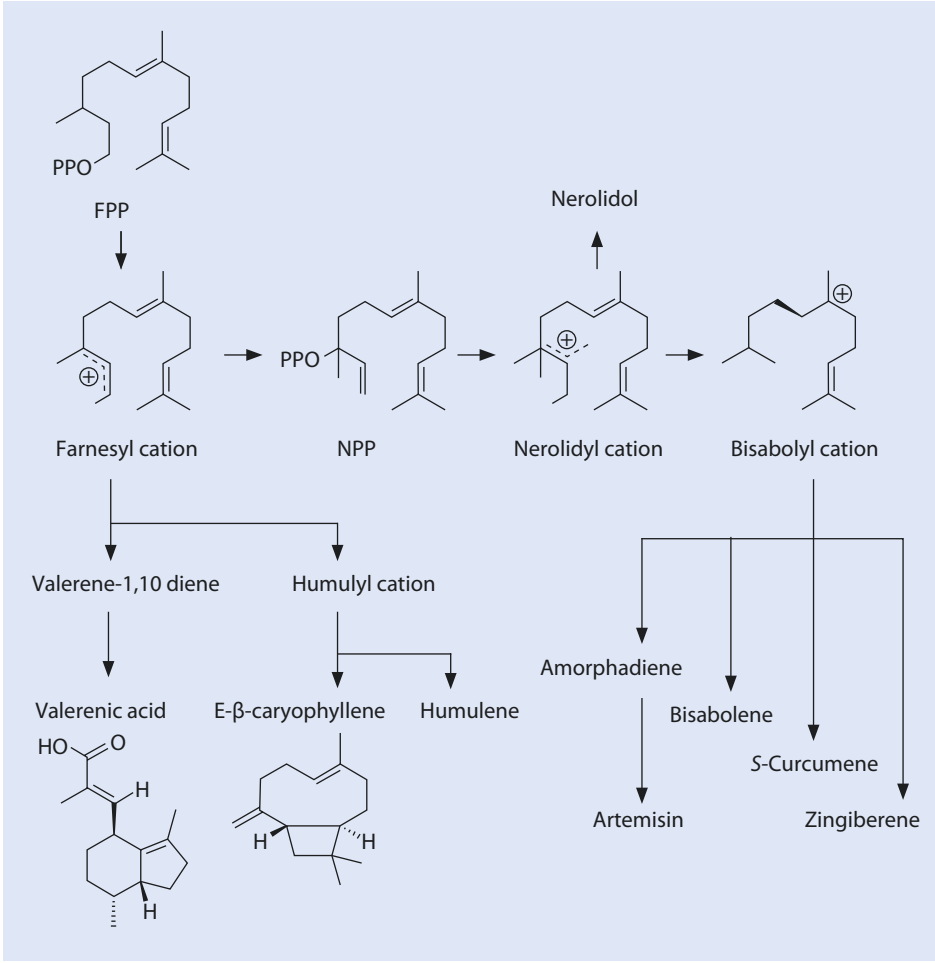

Fig. 10.5 Schematic overview of the biosynthetic pathways and representative members of various sesquiterpene subfamilies. Abbreviations: *FPP* farnesyl pyrophosphate, *NPP* Nerolidyl pyrophosphate

(cloves, Myrtaceae), *Cannabis sativa* (Cannabaceae), *Rosmarinus officinalis* (rosemary, Lamiaceae), *Piper nigrum* (black pepper, Piperaceae) and hops (*Humulus lupulus*, Cannabaceae). β-caryophyllene is emitted from plants, e.g. from maize roots that are attacked by insects. This in turn attracts nematodes that prey on the attacking insect larvae (Rasmann and Turlings 2016). β-caryophyllene is also an agonist of cannabinoid receptor type-2 (CB2) in humans, which perhaps contributes to the calming effect of cloves on toothache (Gertsch et al. 2010; Alberti et al. 2017).

Cyclization via the nerolidyl cation leads to the bisabolyl cation, which gives rise to the stereoisomers zingiberene and *S*-curcumene (in *Zingiber officinale,* Zingiberaceae, ginger; repellant to white flies). Other products of this pathway are bisabolene, which is part of the essential oil from chamomile (*Matricaria recutita L.,* Asteraceae). The bisabolyl cation can be further cyclized to the bicyclic amorpha-4,11-diene. This is an important bicyclic intermediate for the formation of artemisinin, an antimalaria drug. For the isolation of artemisinin and its characterization as a remedy against the disease,

the Chinese scientist Youyou Tu won the Nobel Prize in 2015. Artemisinin is produced in the glandular trichomes of *Artemisia annua* (Asteraceae). Cyt P450 enzymes catalyse the additional modifications, but the last step, the formation of an unusual peroxide bridge, is a photooxidation.

The bicyclic valerena-1,10 diene is the starting product for the synthesis of valerenal and valerenic acid, the latter being the active component in Valerian extracted from the roots of of *Valeriana officinalis* (Ricigliano et al. 2016). The bicyclic picrotoxane is modified into the tricyclic picrotoxin, an equimolar mixture of picrotoxinin and picrotin (found in the fruit of the *Anamirta cocculus*, Menispermaceae). Picrotoxin binds GABA-gated ion channels. Several other plant-derived sesquiterpenes can cause convulsions or other symptoms reminiscent of GABA-gated channel blockade. This is the case with tutin (from *Coriaria arborea*, Coriariaceae), anisatin (from star anise, *Illicium floridanum*, Schisandraceae) and jiadifenolide (from *Illicium jiadifengpi*, Schisandraceae). Many of these products contain lactone motifs and show similarity with GABA (◘ Fig. 8.4). In New Zealand, honey poisoning with tutin was observed (► Box 1.1). This was pinpointed to bees that fed on exudates from an insect that itself had been feeding on *Coriaria sp.* bushes (Larsen et al. 2015).

Englerin A (from *Phyllanthus engleri*, Phyllanthaceae), another compound with a polycyclic terpene skeleton, is a selective activator of TRPC4 and TRPC5 calcium channels, and this activation of transient receptor potential canonical (TRPC) calcium channels selectively kills renal cancer cells (Carson et al. 2015).

10.6 Sterols and Triterpene (From MVA Pathway, C30)

Two FPP are fused (tail-to-tail) by the squalene synthase to form squalene, which is further oxidized to 2,3-oxidosqualene (Thimmappa et al. 2014; Valitova et al. 2016). 2,3-Oxidosqualene is the precursor for sterols and triterpenes and a substrate for several oxidosqualene cyclases.

To form sterols 2,3-oxidosqualene is cyclized to lanosterol (in fungi and animals) or cycloartenol (in plants) via the chair-boat-chair conformation and the protosteryl cation (◘ Fig. 10.6). Lanosterol gives rise to cholesterol, whereas cycloartenol gives rise to phytosterols such as β-sitosterol, stigmasterol and the plant hormone brassinosteroid. Because this backbone is very similar to cholesterol, these compounds can displace low-density lipoprotein (LDL) cholesterol in the human intestine. In some plants, cholesterol formation is also possible, starting from cycloartenol, which is converted into cycloartanol and further into cholesterol. Additionally, in Solanaceae and *Euphorbia* lanosterol was detected, which might contribute to cholesterol biosynthesis. Cholesterol is also a precursor for steroidal alkaloids (see ► Sect. 12.11 in ► Chap. 12).

Stigmasterol is present in many plant fats, which are enriched in various vegetables such as seeds (e.g. rape seeds), soy bean and nuts. Stigmasterol is also present in milk. Pasteurization inactivates stigmasterol, a fact that led to its discovery as the Wulzen factor (antistiffness factor). It had been suggested that stigmasterol could lessen symptoms of arthritis, and this would have asked for avoiding pasteurized milk. Stigmasterol can be used as a precursor in the manufacture of semisynthetic progesterone and vitamin D3.

β-Sitosterol is found in vegetable oil, nuts and avocados. Another derivative of 2,3-oxidosqualene is cucurbitadienol and further cucurbitacins from pumpkin, squash or

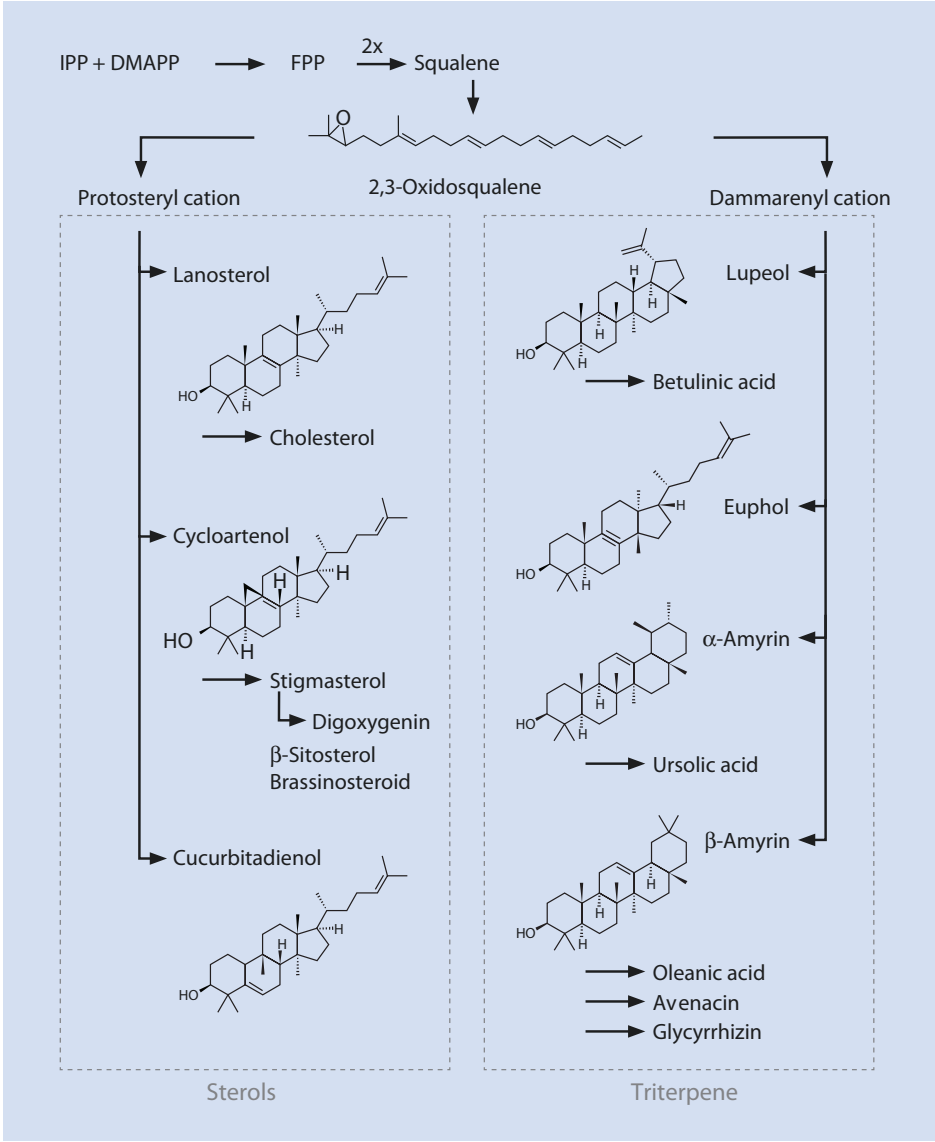

Fig. 10.6 Schematic overview of the biosynthetic and representative members of sterols and triterpene subfamilies. Abbreviations: *IPP* isopentenyl diphosphate, *DMAPP* dimethylallyl diphosphate, *FPP* farnesyl pyrophosphate

cucumber. These substances have been bred out from commercially available varieties, as they taste very bitter and are toxic.

In *Digitalis sp.* (foxgloves, Plantaginaceae) stigmasterol can be converted into pregnenolone and further to cardenolides such as progesterone, which can be converted in several steps into digoxigenin and further to digitoxin and lanatoside by adding sugar moieties. These are also called cardiac glycosides because they control the heart rhythm

(Lindemann 2015; Gurel et al. 2017). Another toxic cardiac glycoside is oleandrin, which is found in laticifers especially of oleander (*Nerium oleander*, Apocynaceae).

Triterpenes are folded into the chair-chair-chair conformation prior to cyclization, leading to a dammarenyl carbocation intermediate (◘ Fig. 10.6). The triterpene synthases catalyse the synthesis of tricyclic, tetracyclic and pentacyclic molecules by concerted reaction steps of single enzymes. As in the previous groups, Cyt P450 enzymes are essential in modifying the scaffolds further to give rise to more elaborate molecules (Ghosh 2017). Triterpenes are often found in plants in a glycosylated form, the so-called saponins. Glycosylation increases the polarity and therefore the water solubility of the triterpenes, which are per se hydrophobic. These amphiphilic properties of the compounds lead to the formation of foam in water, which explains the name saponin (soap). Furthermore, they allow the molecules to insert themselves into membranes, often due to interaction with cholesterols (Lorent et al. 2014). This perturbs the membranes and induces tensions leading to membrane perforation. When acting on red blood cells, which contain high amounts of cholesterol, this can lead to haemolysis. Saponins can also influence the micelle formation between sterols and bile acids, which is necessary for sterol absorption in the intestine, thereby interfering with cholesterol uptake. Saponins are defensive secondary metabolites that allow plants to cope with unfavourable environmental conditions (storing and conserving water, resisting predators and surviving severe weather conditions).

The dammarenyl carbocation can also give rise to protopanaxadiol and further the saponin ginsenoside (from the root of ginseng (*Panax ginseng*, Araliaceae)). Ginsenosides activate apoptosis in animals by triggering fast-mediated cell death through interference with membrane lipid rafts. Moreover, their structures are similar to steroid hormones, and they have been indicated as agonists for multiple steroidal receptors in mammalians (Kennedy and Scholey 2003; Park et al. 2017). Other prominent triterpenes are amyrin and lupeol. Lupeol gives rise to the potential anticancer agent betulinic acid, found in the bark of several trees including birch. α-Amyrin gives rise to ursolic acid, which is found in peels of fruits such as apples and could be acting on the growth-controlling pathways in mammalians. β-Amyrin is transformed into oleanic acid, which possibly has anti-cancer properties, and the saponines avenacin and glycyrrhizin. Avenacin is an antifungal component in the oat root (*Avena sativa,* Poaceae) and glycyrrhizin is the sweet-tasting constituent of liquorice (in *Glycyrrhiza glabra*, Fabaceae) that accumulates in root and stolon. From the sap of the fire tree (*Euphorbia tirucalli*, Euphorbiaceae), the tetracyclic euphol, an anti-inflammatory drug, can be extracted (Dutra et al. 2012).

Take-Home Messages

- Terpenes and terpenoids are synthetized by two different pathways, the cytosolic MVA pathway and the plastid MEP pathway.
- Terpene synthetases are involved in many enzymatic steps of biosynthesis.
- Monoterpenes include menthol and thujone and provide the precursors for indole alkaloids.
- Diterpenes include salvinorin A, ryanodine and resiniferatoxin.
- Sesquiterpenes include ß-caryophyllene and GABA antagonists such as picrotoxin and tutin.
- Especially monoterpenes and sesquiterpenes can be extracted as essential oils.

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Phenylpropanoids

11.1 Shikimate Pathway (Phenylalanine, Tyrosine and Tryptophan) – 172

11.2 Phenylpropanoids (Derived from Phenylalanine) – 174

References – 177

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What You Will Learn in This Chapter

Aromatic amino acids are produced via the shikimate pathway in the plastids, which are the precursors of phenylpropanoids. Phenylpropanoids contribute to the taste of many plant-derived food, but their interaction with the human body is in many cases not well documented. Many of these compounds act as precursors for alkaloids.

11.1 Shikimate Pathway (Phenylalanine, Tyrosine and Tryptophan)

More than 8000 aromatic metabolites of the phenylpropanoid pathway have been identified in plants. They have various functions in plant growth, development, plant-environment interactions and protection from biotic and abiotic stresses (Fraser and Chapple 2011; Quideau et al. 2011; Cheynier et al. 2013; Brunetti et al. 2015). The phenylpropanoid pathway assimilates about 30–40% of the organic carbon on earth, most of it as the lignin polymer in the cell walls.

Phenylalanine is the most important entry point into this pathway, itself being synthesized by the so-called the shikimate pathway (◘ Fig. 11.1). The shikimate pathway is localized in plastids and therefore absent in animals (Tzin and Galili 2010; Tohge et al. 2013). The starting points are phosphoenolpyruvate (from the glycolysis) and erythrose 4-phosphate (from the pentose phosphate pathway). The first intermediate is 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP). The DAHP synthase is the first committing enzyme of the shikimate pathway; as a key enzyme, it regulates the metabolic flux into this pathway (Tzin et al. 2012). The next major intermediates are shikimate and shikimate 3-phosphate. The 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase and the chorismate synthase catalyse the synthesis of chorismate. EPSP is known to be the target of the herbicide glyphosate (Roundup) (Schonbrunn et al. 2001). Dehydroshikimate leads to gallic acid and further to gallotannins. Chorismate serves as a common precursor for the synthesis of ubiquinone via hydroxybenzoate, phylloquinone (Vitamin K1) via isochorismate, and tetrahydrofolate (Vitamin B9) via aminobenzoate.

The three aromatic amino acids phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Trp) are products of this pathway. As they are produced in plastids and humans and animals cannot produce them themselves, they have to be taken up with food ("essential amino acids"). Anthranilate is the main precursor for tryptophan. Prephenate leads to the biosynthesis of tyrosine and phenylalanine mainly via arogenate but also via phenylpyruvate to form phenylalanine or hydroxyphenylpyruvate to form tyrosine. At this stage, the primary metabolism is ending, and the secondary metabolism is beginning.

Tryptophan is a precursor for alkaloids via *tryptamine* (indole alkaloids, see ▶ Sect. 12.2 in ▶ Chap. 12), for auxin (IAA), for camalexin and for glucosinolates. Tyrosine can lead to the formation of cyanogenic glucosides (such as dhurrin), dopa, betalains, tocopherols (vitamin E) and plastoquinone (via 4-hydroxyphenylpyruvate). Tyrosine can also be deaminated to tyramine, which is a precursor for suberin, hydroxycinnamate amide and the isoquinoline alkaloids (see ▶ Sect. 12.3 in ▶ Chap. 12). Although tyrosine can interchange with phenylalanine, phenylalanine is thought to be the main pathway to most secondary metabolites. Extensive regulation and feed-back control regulates the abundancy of the three amino acids and their flow into the downstream metabolic pathways (Tzin and Galili 2010; Tohge et al. 2013).

Phenylalanine is transferred from the plastid to the cytosol where it undergoes further reactions (◘ Fig. 11.2). The committing enzyme for phenylpropanoid biosynthesis is the

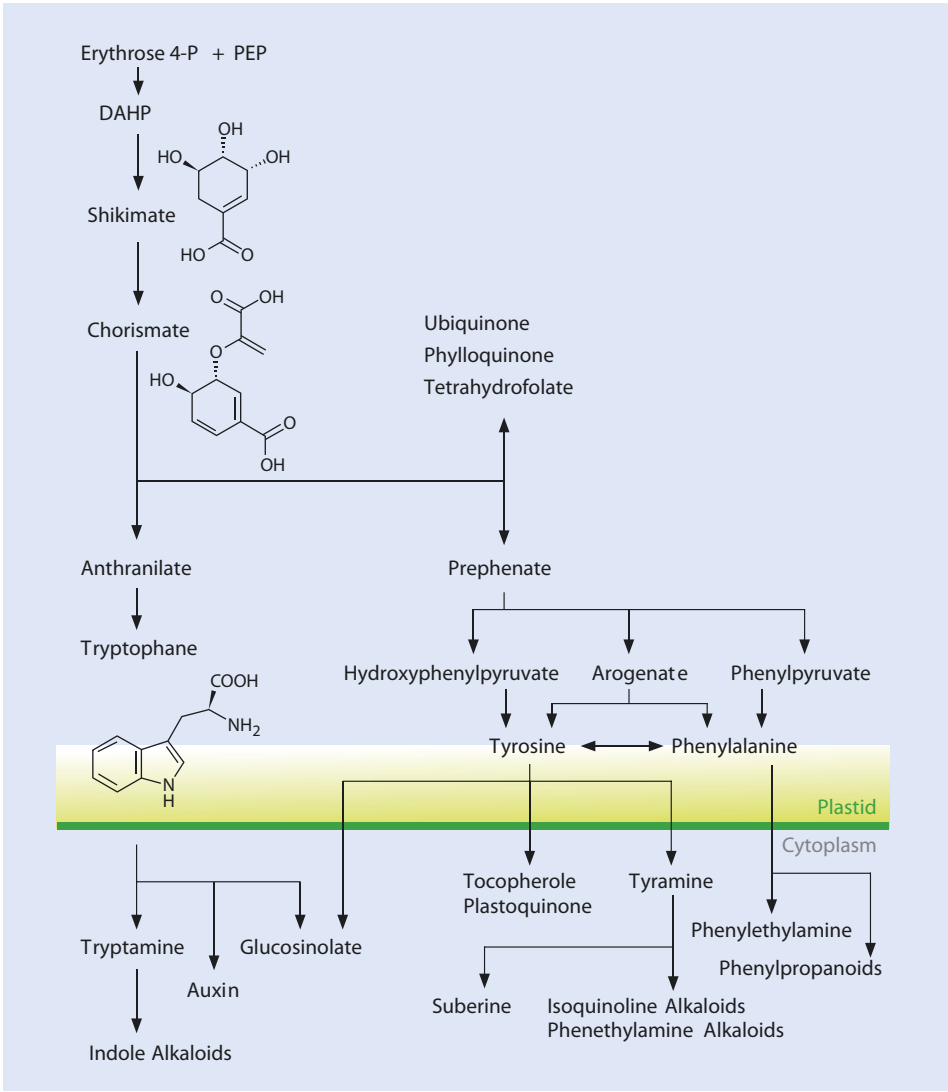

Fig. 11.1 The shikimate pathway and the major classes of secondary metabolites derived from the aromatic amino acids phenylalanine, tyrosine and tryptophan. The shikimate pathway converts PEP (phosphoenolpyruvate) and erythrose 4-phosphate into chorismate. The biosynthesis of the aromatic amino acids takes place in the plastid, whereas the subsequent biosynthesis of secondary metabolites is localized mainly in the cytoplasm. Besides phenylpropanoids, several alkaloids are derived from this pathway. Abbreviations: *DAHP* 3-deoxy-D-arabino-heptulosonate 7-phosphate

phenylalanine ammonia lyase (PAL) that releases the ammonium group (which is reassimilated) leading to trans-cinnamic acid (Zhang and Liu 2015). This enzyme is found to be associated with the ER membrane. PAL activity has been found in higher plants, in some fungi and a few bacteria, but not in animals. Cinnamic acid is a precursor of benzoic acid, another pathway leading to the synthesis of salicylic acid. Cinnamic acid is catalysed by cinnamate 4-hydroxylase, a microsomal monooxygenase containing a heme cofactor, into p-coumaric acid and with the help of the 4-coumarate:CoA ligase (4CL) further to

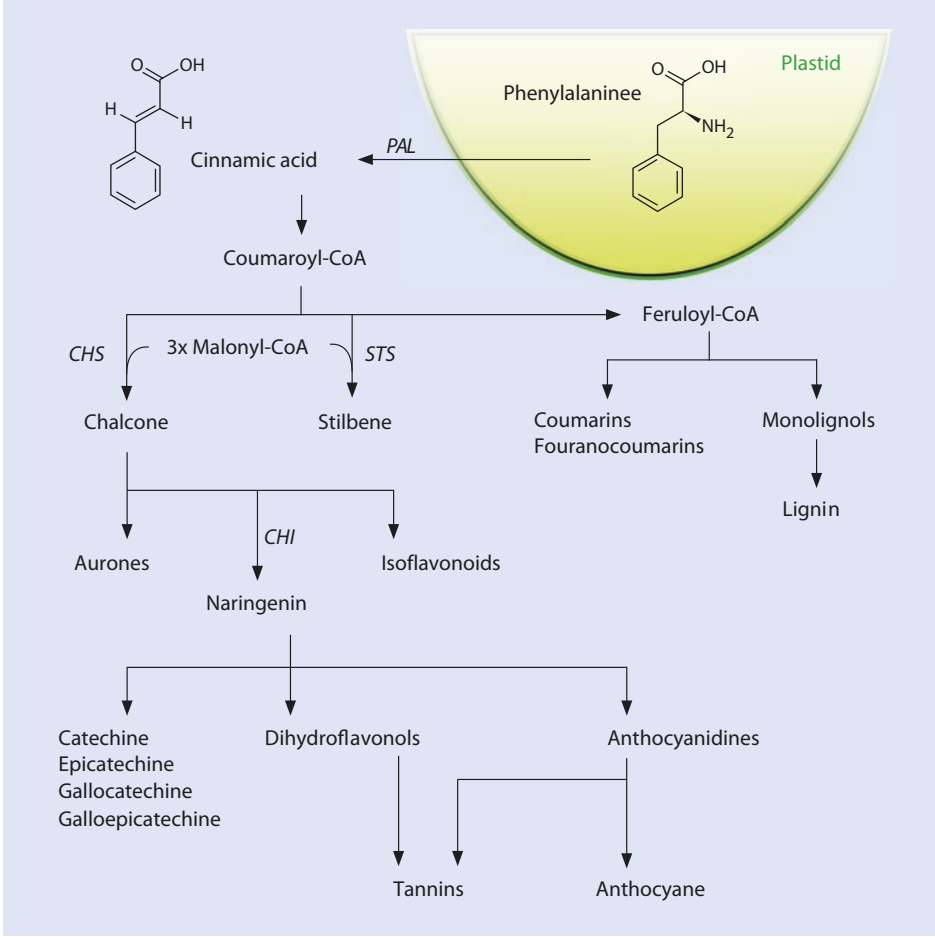

Fig. 11.2 Schematic overview of the phenylpropanoid biosynthetic pathway and representative members of various subfamilies. Phenylalanine is exported from the plastid into the cytoplasm where most of the following steps take place. Important enzymes in the pathway are *PAL* phenylalanine ammonia lyase, *CHS* chalcone synthase, *STS* stilbene synthase, and *CHI* chalcone isomerase

coumaroyl-CoA. These enzymes seem to act in a multienzyme complex anchored to the endoplasmic reticulum membrane ("metabolon"). The activated coumaroyl-CoA is the main precursor of many pathways, the major end products being lignins, lignans, suberin, flavonoids, stilbenes and coumarins. As not many phenylpropanoids are known to interact with human receptors, only the most important steps of this biosynthetic pathway are described here (Kennedy 2014, 2015; Jiang et al. 2016).

11.2 Phenylpropanoids (Derived from Phenylalanine)

Phenylpropanoid compounds are usually further modified by methylation, hydroxylation and acetylation. These modifications can enhance the volatility of scent compounds (Schwab et al. 2008; Baldwin 2010; Colquhoun and Clark 2011; Maffei et al. 2011;

Muhlemann et al. 2014). Therefore, some phenylpropanoids are part of essential oils in plants (see ► Box 10.2). Many phenylpropanoids can also chelate metal ions, which leads to their aggregation and changes in colour or function. Furthermore, glycosylation by UDP-glycosyltransferases (UGTs) enhances the diversity of phenylpropanoids but also influences their compartmentalization, biological activity, solubility, stability and toxicity (Le Roy et al. 2016). Many phenylpropanoids are toxic and unstable molecules and so rarely accumulate in their aglycone form in plant cells. The alteration of natural compounds with acyl moieties is catalysed by acyltransferases (AT) and generates additional variants. The interaction of the hydroxyl groups of phenolics with the p-electrons of the benzene ring allows many phenolic compounds to generate relatively long-lived free radicals able to interfere with oxidation processes. Phenolics that possess two ortho-positioned hydroxyl groups are very good antioxidants (de Cassia da Silveira et al. 2014; Carvalho et al. 2015).

The addition of 3-malonyl-CoA to coumaroyl-CoA catalysed by the chalcone synthase (CHS) leads to chalcone. CHS, a polyketide synthase, provides the first committed step in flavonoid biosynthesis by catalysing the sequential decarboxylative addition of three acetate units from malonyl-CoA to p-coumaroyl-CoA (Austin and Noel 2003; Falcone Ferreyra et al. 2012). In the same active site, CHS then forms chalcone via intramolecular cyclization and aromatization of the linear phenylpropanoid tetraketide. The plants utilize flavonoids for such diverse purposes as antimicrobial defence, flower pigmentation, UV photoprotection, pollen fertility and interaction with the environment.

Chalcones can also provide the basis for the synthesis of aurones, such as hispidol, which is a selective adenosine receptor antagonist. The chalcone isomerase (CHI) modifies chalcone to naringenin (bitter taste of grapefruit), a flavonoid. Flavonoids are subclassified into several families including flavonol, flavone, flavanone, flavan-3-ol, isoflavone and anthocyanidin according to the structure of and the modifications to the aromatic rings. In the group of isoflavone, one class are the rotenones, which are found almost exclusively in legumes (Papilionaceae, Mimosaceae, Caesalpiniaceae and Fabaceae). Rotenone was first isolated from the Peruvian derris root (*Lonchocarpus*, Fabaceae) and is used as fish poison but is also a naturally occurring insecticide. Isoflavones are otherwise known from soy, especially genistein, which is an oestrogen receptor modulator. The flavone galangin is found in some plants from the ginger family and the *Helichrysum* genus, whereas cirsimarin and its aglycone cirsimaritin are flavones from *Microtea debilis* (Caryophyllales).

The flavanone 3-β hydroxylase (F3H) can further modify naringenin to dihydroflavonols such as dihydrokaempferol, dihydroquercetin and dihydromyricetin. Dihydroflavonols are the precursors for catechin and epicatechin, which are formed via leucocyanidin, but also the precursors for gallocatechin and galloepicatechin, which are formed via leucodelfinidin. Catechins and epicatechin are odourless white powders, which can be found in high quantities in cocoa (*Theobroma cacao,* Malvaceae), tea (*Camellia sinensis,* Theaceae) and grapes (*Vitis vinifera*, Vitaceae). These secondary metabolites are induced by stress end elevated UV light, resulting in higher levels if the tea plants are grown in high altitudes. Whereas green teas contain high levels of catechins, the fermentation to yield black tea destroys catechins. Thereby they are enzymatically oxidized yielding a complex mixture of oxidation products, including theaflavins and thearubigins (Tanaka et al. 2009). Dihydroflavonols can be transformed into flavonols, such as quercetin or kaempferol. They are present in a wide variety of fruits and vegetables and are potent antioxidants that serve to protect the plant from reactive oxygen species (ROS).

Condensed tannins (proanthocyanidins) are formed via the condensation of two derivatives of dihydroflavonones, Flavan-3,4-diols and anthocyanidins. The resulting high molecular weight multimers can complex with carbohydrates and proteins. Tannins are able to precipitate and denature proteins, which is where their name is derived from as tannins from wood were used to tan animal hides into leather. Tannins are found in many plants and their products including tea, red wine and fruits such as cranberries and apples, where they contribute to the bitter, astringent taste.

Anthocyanidins are also the precursors for anthocyanins. Anthocyanin pigments are transported to and accumulate in vacuoles, where the low pH leads to conjugation or complexation (with metals, malonic acid or other flavones) and results in compounds with enhanced or modified colours to yield pink, red, purple or blue pigmentation. This pigmentation can best be seen in many flower petals (Grotewold 2006; Petroni and Tonelli 2011).

Addition of 3-malonyl-CoA to coumaroyl-CoA with the help of the stilbene synthase (STS) leads to the formation of stilbene and further stilbenoids. Due to their structure, which is similar to human hormones, they are also called phytoestrogens. Resveratrol is the most widespread stilbene, found especially in grapes (red wine), giant knotweed, peanuts and mulberries. Many of its derivatives are also present in plants (e.g. rhaponticin from rhubarb).

p-Coumaroyl-CoA and p-coumaric acid are the precursors of coumarins and furanocoumarins (Bourgaud et al. 2006). The committing enzyme in their synthesis is hydroxycinnamoyl transferase (HCT), which leads to the formation of caffeoyl-CoA and further feruloyl-CoA to yield scopoletin and esculatin. The third typical coumarin, umbelliferone, is synthetized via 2,4-dihydroxycinnamoyl-CoA. Coumarins, characterized by their vanilla-like or fresh hay-like odour, are found in many plant species, especially Fabaceae and Lauraceae (tonka bean, *Dipteryx odorata;* cinnamon, *Cinnamomum sp.)*. Coumarin is produced during wilting by enzymes, which split off sugar residues. During the process of spoiling of sweet clover, the natural coumarins are converted into toxic dicumarol. Dicumarol blocks blood clotting by inhibiting the enzyme required for the synthesis of prothrombin and in addition antagonizes vitamin K. This can lead to internal bleeding in animals (e.g. sweet clover disease in mammals). Due to these properties, dicumarol is also used in rat poison (e.g. warfarin). Moreover, clinically it serves as an anticoagulant for protection from thrombosis and heart attack (e.g. Marcumar).

Dihydroisocoumarin-derivatives such as phyllodulcin, a high-intensity sweetener, are extracted from hydrangea leaves (*Hydrangea macrophylla var. thunbergii*). Phyllodulcin interacts with the human sweet taste receptor, a G protein-coupled receptor (GPCRs). A prenyltransferase uses umbelliferone, a coumarin mostly found in Apiaceae, as a substrate, which leads to the formation of furanocoumarins. Linear furocoumarins such as psoralen, xanthotoxin, bergapten and isopimpinellin are mainly found in Apiaceae, Moraceae, Rutaceae and Leguminosae, and the angular dihydro-furanocoumarins, such as angelicin, sphondin, and pimpinellin, are confined to the Apiaceae and Leguminosae (Bourgaud et al. 2006). Bergamottin (5-geranoxy-psoralen) from grapefruit has been shown to interfere with drug metabolism by inactivating intestinal Cyt P450 enzymes (Girennavar et al. 2007). Furocoumarin can also intercalate between the base pairs of the DNA, and after UVA radiation, covalent complexes are formed (Gasparro 1996). Therefore, eating furocoumarins, which are, for example, present in celery, can lead to phototoxic reactions in the skin after exposure to sun.

Feruloyl-CoA is also the precursor for the three main monolignols, coniferyl alcohol, sinapyl alcohol and coumaryl alcohol, which are synthesized in the cytosol with added glucose moieties to make them water soluble and to reduce their toxicity. The glucosides are transported through the cell membrane to the apoplast or stored in the vacuole (Dima et al. 2015). Phenylpropenes such as chavicol, eugenol, methyleugenol and estragole are derived from coniferyl alcohols via coniferyl acetate and often found in trichomes (peltate glands) as part of the essential oils (Gang et al. 2001) (see ▶ Box 10.2). By oxidative dimerization of coniferyl alcohols, lignans are formed. Secoisolariciresinol and matairesinol, lignans from the seeds of linum (flaxseed), are digested in the intestine to enterolactone and enterodiol, which have been proven to be efficient ligands for the G-protein-coupled oestrogen receptor (GPER) (Ren et al. 2016). Podophyllotoxin (PTOX) is isolated from roots of *Podophyllum sp.* and binds to tubulin thereby inhibiting the formation of microtubuli. This attribute makes it valuable for chemotherapy. The main flux of this pathway is from the monolignols to lignin. To form lignin the alcohols form multimers in the apoplast. Lignins are 20–30% of a plant's tissue, leading to the fortification of cell walls, especially of the xylem. Extensive incorporation of lignin leads to the death of the cells.

Take Home Message

- Phenylpropanoids are synthetized via aromatic amino acids (Shikimate pathway). Many of them act as precursors for alkaloids.
- Phenylpropanoids are important for plants (stability, pollinators and protection against biotic and abiotic stresses), but few have been shown to interact with human receptors. One example is hispidol, which is a selective adenosine receptor antagonist.

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Alkaloids

12.1 Introduction – 181

12.2 Terpenoid Indole Alkaloids (Derived from Tryptophan, e.g. Ibogaine, Reserpine, Strychnine, Physostigmine, Dimethyltryptamine, Psilocybin and Ergotamine) – 181

12.3 Benzylisoquinoline Alkaloids (Derived from Tyrosine, e.g. Morphine and Curare) – 186

12.4 Phenethylamine Alkaloids (Derived from Tyrosine, e.g. Mescaline) – 189

12.5 Phenylethylamine (Derived from Phenylalanine, e.g. Ephedrine) – 189

12.6 Alkaloids from Condensation of Tyrosine and Phenylalanine – 190

12.6.1 Amaryllidaceae (Norbelladine) – 190

12.6.2 Phenylethylisoquinoline Alkaloids – 191

12.7 Pyridine Alkaloids (e.g. Nicotine), Pyrrolidine Alkaloids (e.g. Hygrine) and Tropane Alkaloids (e.g. Atropine, Scopolamine and Cocaine) All Derived from Ornithine and Arginine – 191

12.8 Pyrrolizidine Alkaloids (Derived from Spermidine and Putrescine) – 194

12.9 Purine Alkaloids (Derived from Xanthosine, e.g. Caffeine) – 195

12.10 Piperidine Alkaloids (e.g. Lobeline), Quinolizidine Alkaloids (e.g. Cytisine) and Indolizidine Alkaloids (All Derived from Lysine) – 196

A. Böttger et al., *Lessons on Caffeine, Cannabis & Co*, Learning Materials in Biosciences,
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12.11 Steroidal Alkaloids (From Steroids Derived from the MVA Pathway) – 197

12.12 Terpenoid Alkaloids (Derived from the MEP Pathway, e.g. Aconitine) – 198

12.13 Tropane-Related Alkaloids (Derived from Proline, e.g. Anatoxin A) – 198

12.14 Isoxazole Alkaloids (e.g. Muscimol) – 198

12.15 Muscarine – 198

12.16 Guanidinium Toxins (e.g. Saxitoxin) – 198

12.17 Kainoids – 199

References – 199

What You Will Learn in This Chapter

Alkaloids are characterized by containing a nitrogen atom, usually within a heterocyclic ring. Many are derived from aromatic amino acids, but for some also other amino acids, ornithine, spermidine, xanthosine and the terpenoids are the basic building blocks. Many well-known compounds such as caffeine, nicotine and atropine fall into this group. The major groups are terpenoid indole alkaloids and benzylisoquinoline alkaloids, but also in many other pathways, toxic or psychedelic alkaloids can be synthesized. The biosynthesis pathway is often spread over different cell types and is very compartmentalized within the cells to avoid toxicity for the plant.

12.1 Introduction

Alkaloids are a diverse group of nitrogen-containing compounds (Facchini 2001; Herbert 2003). Usually this nitrogen atom is in an oxidative state within a heterocyclic ring. Precursors are usually amino acids, and their entire carbon skeleton, except the carboxylic acid of the amino acid, is incorporated into the final alkaloid in the case of quinoline alkaloids, isoquinoline alkaloids and tropane alkaloids (◘ Fig. 12.1). Other alkaloids incorporate the nitrogen atom from the amino acid via transamination and the remaining carbon skeleton is derived from a non-amino acid source (such as acetate, shikimate, terpenoid or steroid origin). This is the case for purine alkaloids, terpenoid alkaloids and steroidal alkaloids. Pseudo alkaloids or not heterocyclic alkaloids are alkaloids in which the nitrogen is positioned outside of the ring system.

Alkaloid biosynthesis requires highly stereo-, substrate- and cell-specific enzymes only present in about 20% of plant species. Among them are Apocynaceae, Fabaceae, Papaveraceae, Rubiaceae and Solanaceae. Alkaloids are also found in bacteria and fungi (e.g. psilocin, muscarine, ergot). Several animals contain alkaloids, which they accumulate most often through their diet. Among them are insects and frogs, feeding on insects, especially the poisonous frogs from the group of poison arrow frog (Dendrobatidae). Over 12,000 alkaloids are estimated to exist, many of them are known for their pharmaceutical actions. Alkaloids are often cytotoxic even for the plant that produces them; therefore, they often accumulate in specific cell types such as idioblasts and laticifers. In contrast to terpenes and phenylpropanoids, alkaloids are usually not found in secretory glands and have not shown to be synthetized in glands.

Several classification of alkaloids exist, usually, as here presented, they are organized into groups according their biosynthetic precursor or carbon skeletal structure.

12.2 Terpenoid Indole Alkaloids (Derived from Tryptophan, e.g. Ibogaine, Reserpine, Strychnine, Physostigmine, Dimethyltryptamine, Psilocybin and Ergotamine)

Terpenoid indole alkaloids can found mainly in Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae. Especially *Catharanthus roseus, Rauwolfia serpentine* and *Camptotheca acuminate* have been analysed according to the secondary metabolites they produce. Tryptophan forms the basis for indole alkaloids. The initial steps are the decarboxylation of tryptophan to tryptamine by the tryptophan decarboxylase, a pyridoxal dependent enzyme, followed by the condensation of tryptamine with secologanin, an iridoid terpene

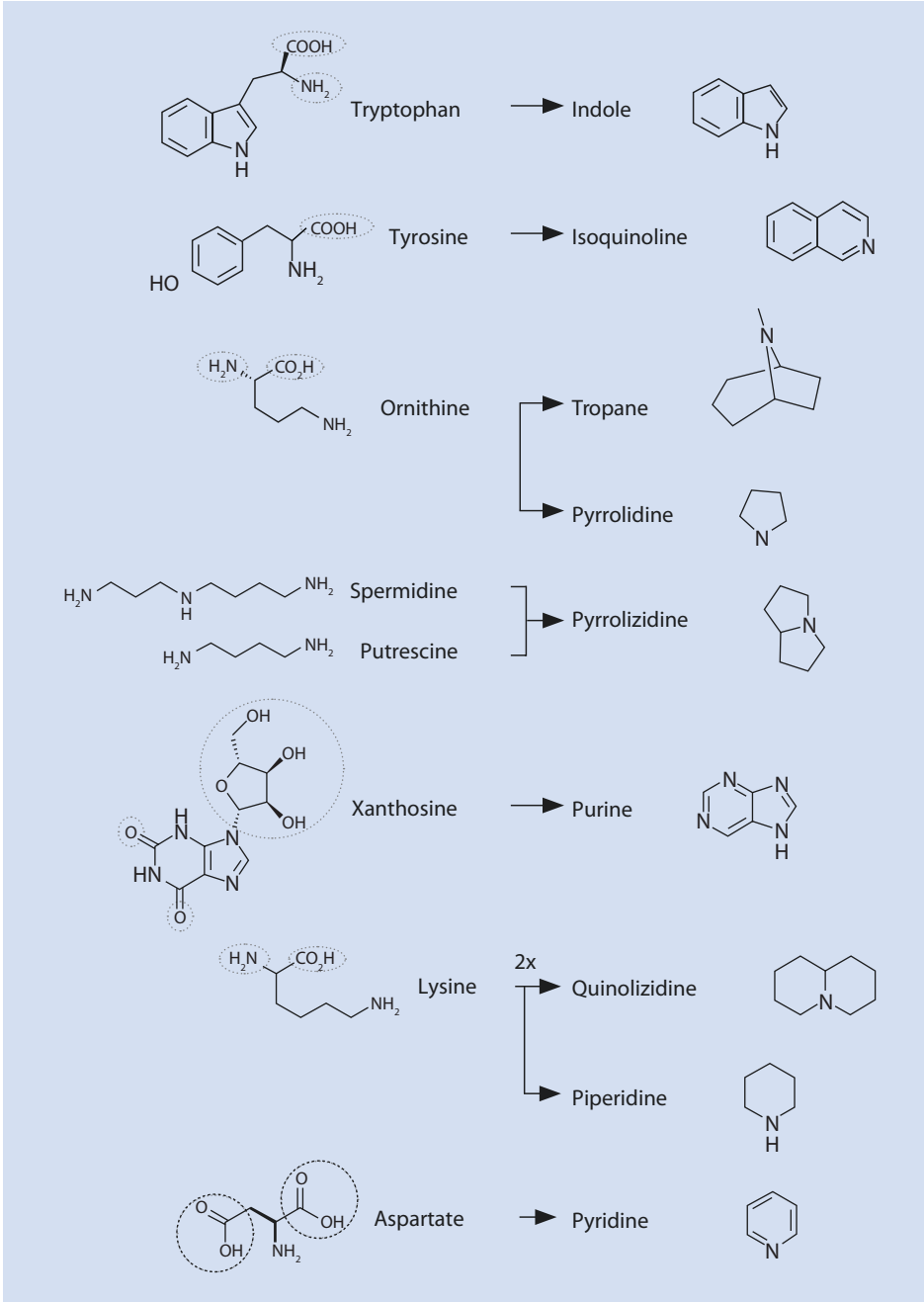

Fig. 12.1 Schematic overview of the main alkaloid backbones and their respective precursors. The structures of the precursors used to form the alkaloids are marked bold. Chains that are removed are circled

derived from geraniol (monoterpene, MEP pathway; see ▶ Sect. 10.2 in ▶ Chap. 10) (De Luca et al. 2014; Ilc et al. 2016). This results in strictosidine glucoside (Fig. 12.2). The condensation is a Pictet-Spengler-type reaction and is mediated by the secologanin

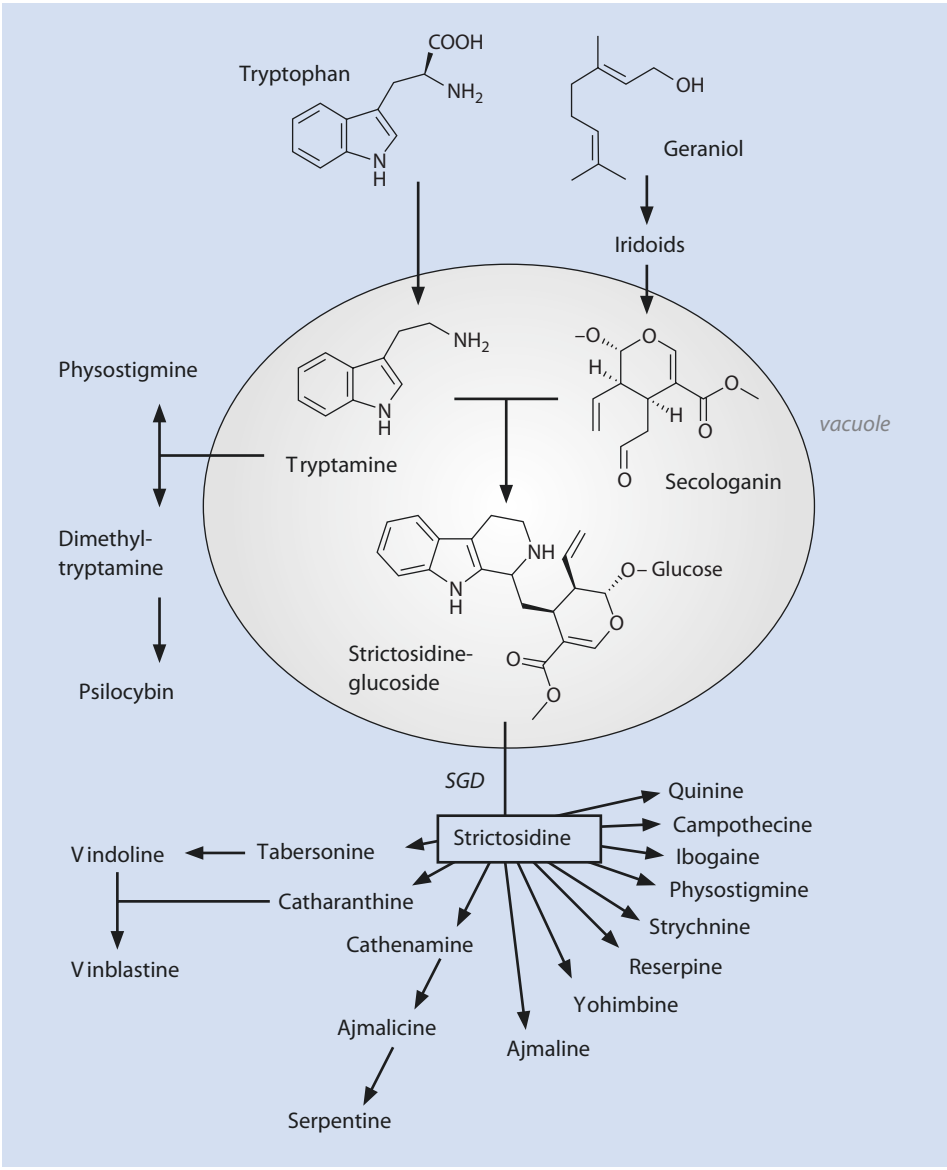

Fig. 12.2 Schematic overview of the biosynthetic pathway of terpenoid indole alkaloids and representative members of various subfamilies. The entry to the pathway is through the biosynthesis of strictosidine. Precursors are the aromatic amino acid tryptophan (► Chap. 11) and secologanin, an iridoid derived from the MEP pathway (monoterpene; ► Sect. 10.1 in ► Chap. 10). The condensation takes place in the vacuole. The committing step is the removal of the glucose moiety from strictosidine glucoside by the strictosidine β-D-glucosidase (SGD)

synthase (Fig. 9.2f). This enzyme is localized within the vacuole of leaf epidermis cells, which means that both, tryptamine and secologanin have to be imported into the vacuole. In contrast, the MEP pathway genes of *Catharanthus roseus* were shown to be preferentially expressed in internal phloem-associated parenchyma (IPAP) cells of aerial (above ground) organs.

Strictosidine β-D-glucosidase (SGD) is the next important enzyme generating the strictosidine aglycone (Stockigt and Panjikar 2007). From *Catharanthus roseus,* it is known that SGD accumulates in highly stable supramolecular aggregates within the nucleus (Guirimand et al. 2011) or as a soluble enzyme that associates with the cytoplasmic face of the endoplasmic reticulum (Stevens et al. 1993). This means that strictosidine, the aglycone, is formed outside of the vacuole. The aglycone is toxic for the cell as it can lead to protein cross-linking and precipitation; therefore the sequestration of the glycosylated form in the vacuole is a protective measure. However, if the tonoplast is disrupted by herbivoral or microorganismal attack, the vacuolar pool of strictosidine glucoside is exposed to the glucosidase, leading to a massive production of the toxic aglycone, a defence mechanism. Under normal conditions, upon hydrolysis of the glycoside one ring opens, leading to the exposure of an aldehyde group (Barleben et al. 2007). This destabilizes the aglycone, and it is rapidly processed by a three-enzyme system to generate the strychnos alkaloid scaffold (Tatsis et al. 2017).

One branch downstream of strictosidine, mainly in *Catharanthus*, proceeds via Diels-Alder reactions (see Fig. 9.2e) through tabersonine (named the *Aspidosperma* type), leading to vindoline. Another branch leads to catharanthine (the *Iboga* type). Coupling of the monomeric alkaloids catharanthine and vindoline leads to the bisindoles vinblastine and vincristine, which both interfere with microtubule dynamics and are therefore used for chemotherapy, especially in leukaemia. Catharanthine is secreted to the waxy cuticle, where it acts as an insect poison and inhibits fungal growth on the surface of the leaf. In animals, it has been shown to act as an inhibitor of voltage-operated calcium channels. In contrast, vindoline is transported to laticifers and idioblasts and distributed within the mesophyll of leaves (Guirimand et al. 2011; Courdavault et al. 2014; Zhu et al. 2015).

In addition to the fact that in *Catharanthus roseus* the vindoline pathway is spread over several cell types, internal phloem parenchyma of aerial organs (geraniol 10-hydroxylase), epidermis of aerial organs, the apical meristem of roots (e.g. secologanin synthase) and laticifers and idioblasts of leaves and stems, the enzymes are also localized in different subcellular compartments (vacuole, cytoplasm, ER). This demonstrates the necessity of spatial separation to avoid toxicity and to provide adequate transport mechanisms.

Another iboga type alkaloid is ibogaine from the bark of African shrubs such as *Tabernanthe iboga, Voacanga africana* and *Tabernaemontana undulata* (Apocynaceae). Ibogaine has psychedelic activity and has been used in the treatment of substance dependence (Brown 2013). After indigestion ibogaine is modified in the gut of animals resulting in 12-hydroxyibogamine, a 5HT analogue, which can serve as a selective serotonin reuptake inhibitor. Noribogaine is also extracted from *Tabernanthe iboga* with slightly different activities in comparison with ibogaine.

A third pathway, mainly in *Rauwolfia,* leads to cathenamine (also called the *Corynanthe* type) and further to vinorine (cytotoxic), ajmalicine (raubasine), ajmaline and serpentine. Ajmaline is an antiarrhythmic agent, whereas ajmalicine is used to treat high blood pressure, acting as an adrenergic antagonist. Serpentine possesses antihistamine activity and is used in the treatment of snakebites.

Other pathways lead to yohimbine in Rubiaceae, an antagonist of the adrenoreceptor and acting as an aphrodisiac, and to reserpine in *Rauwolfia*, a substance blocking the "vesicular transporter for monoamine storage" (VMAT, SLC18a) irreversibly, thereby depleting neurons of norepinephrine, epinephrine, dopamine, serotonin and histamine containing neuronal granules. Reserpine therefore acts as a sympatholytic agent (see ▶ Sect. 5.2). In *Strychnos nux-vomica* (Loganiaceae) strychnine and the less toxic brucine (a dimethoxy analogue) are formed. The non-tryptamine portion of these compounds is

constructed from an iridoid-derived C9 unit and two further carbons supplied from acetate. In *Camptotheca acuminata* the topoisomerase inhibitor and anticancer agent camptothecin is produced. *Rauwolfia* species have high levels of reserpine, ajmaline, ajmalicine, deserpidine, rescinnamine and yohimbine and have therefore been used for the treatment of several illnesses (hypertension, snakebites, fever, cancer, insanity) in several traditional medicines. *Vinca* (periwinkle) is another source of many indole alkaloids, such as vinblastine, vincristine, vindesine and vinorelbine. *Catharanthus* plants (also periwinkle), contain at least 70 different alkaloids in their sap; some of those are very similar to Vinca alkaloids, such as vincristine and vinblastine.

Strictosidine is also the precursor to a group called quinoline alkaloids, which includes the antimalaria drug quinine (from *Cinchona,* Rubiacaea*)*. Here the indole core structure is rearranged into a quinoline system.

When tryptamine is not fused with the terpene group, non-isoprenoid indole alkaloids are formed. Structurally, serotonin (5-hydroxytryptamine)**,** an important neurotransmitter, also belongs to this chemical group as it is derived from tryptophan. This explains why several indole alkaloids can bind to serotonin receptors as they have a similar structure. Phytoserotonin has also been identified in several plant species, including legumes, pineapple, bananas, tomatoes, walnuts and hickory. It acts as a laxative in the intestine of animals and thus, when fruits of plants are eaten, promotes quick expelling of seeds from the digestive tract. Serotonin is also found in the spines of stinging nettles, causing the pain sensation associated with touching these plants.

Phytoserotonin has a similar structure as the plant hormone auxin, and therefore it can act as auxin inhibitor and is involved in regulating development (Pelagio-Flores et al. 2011). Other non-isoprenoid indole alkaloids are harmane, harmine and harmaline. They have been identified in harmal (*Peganum harmala)* and act as monoamine oxidase inhibitors or induce tremor in mice (harmaline). The calabar bean (*Physostigma venenosum,* Fabaceae) contains physostigmine (also known as eserine), which blocks the degradation of the neurotransmitter acetylcholine by inhibiting the acetylcholine esterase (see ► Sect. 5.1). Physostigmine is synthesized by tryptamine methylation, followed by formation of a heterocyclic ring system. The anti-acetylcholine esterase activity is due to the carbamate side chain rather than the ring system.

Gramine or donaxine, a methylated tryptophan, has been found in several grasses (Gramineae, Poaceae) such as *Hordeum*, *Arundo* and *Phalaris* and is toxic for many organisms. Kynurenic acid, another oxidative derivative of tryptophan, can be found in several plants such as dandelion (*Taraxacum officinale*), common nettle (*Urtica dioica*) and potatoes (*Solanum tuberosum*) and is discussed to inhibit the glutamate and the nicotinic acetylcholine receptors (Turski et al. 2011; Turski et al. 2012; Zgrajka et al. 2013).

Tryptamine can be methylated to dimethyltryptamine (DMT), a psychedelic drug that can be extracted from plants such as *Mimosa tenuiflora* (Fabaceae), *Diplopterys cabrerana* (Malpighiaceae) and *Psychotria viridis* (Rubiaceae) but is also found in toads (*Rhinella marina*). Due to its structural similarity with serotonin, it most likely also acts on $5HT_{2A}$-receptors along with other psychedelics (see ► Sect. 5.6).

Psychedelic indole alkaloids are also present in fungi. For example, magic mushrooms (such as *Psilocybe*) contain derivatives of tryptamine and DMT, such as psilocybin and psilocin (acting on the $5HT_{2A}$-receptor). Several toad species (of the *Bufo* genus) are known to contain indole alkaloids, such as bufotenin, a degradation product of psilocybin and isomer of psilocin. Bufotenin can also be isolated from some Fabaceae and acts on the $5HT_{2A}$ receptor. Infection of grass and grains by parasitic fungi of the genus *Claviceps* or other filamentous fungi leads to dark dense sclerotia, which harbour ergot alkaloids.

Ingestion of these infected grains causes poisoning in humans and animals by inducing convulsions and hallucinations ("St. Anthony's Fire" or "ergotism"). Ergot alkaloids have been associated with historical events of mass hysteria such as dancing epidemics in the medieval ages, the plague of Holy Fire (because of the burning sensations in the extremities), the Great Fear at the beginning of the French Revolution and the Salem Witch Trials. However, scientific evidence for this connection is lacking. These fungi can also colonize plants such as Convolvulaceae, and this infection is even seed-transmitted. This way some species belonging to the Convolvulaceae, in particular *Ipomoea violacea* and *Turbina corymbosa*, contain high amounts of ergolines, especially in their seeds. Symbiosis with poisonous fungi protects the host plant from herbivores, while the fungi benefit from nutrition provided by the plant.

Ergot alkaloid biosynthesis starts with the prenylation of L-tryptophan by dimethyl allyl pyrophosphate (DMAPP; an isoprene unit) to yield 4-(γ,γ-dimethylallyl) tryptophan (DMAT). Intramolecular cyclization over several steps leads to chanoclavine-I, a tetracyclic ring system. The oxidized form, chanoclavine-I-aldehyde, is the last common precursor of all classes of ergot alkaloids. In a next step, lysergic acid is formed which, after sequential addition of amino acids such as alanine, proline or phenylalanine, forms ergotamine. This involves formation of peptide bonds and resembles the non-ribosomal biosynthesis pathway for peptides with ATP-mediated activation of amino acids by forming of thioester linkages prior to their transfer to lysergic acid. Simpler derivatives of lysergic acid require the formation of amides, for example, ergine from *Rivea* and *Ipomoea* species. Ergometrine from *Claviceps purpurea* is the amide formed of lysergic acid with 2-aminopropanol.

12.3 Benzylisoquinoline Alkaloids (Derived from Tyrosine, e.g. Morphine and Curare)

Tyrosine forms the basis for the synthesis of berberine and other benzylisoquinoline alkaloids (Liscombe and Facchini 2008; Hagel and Facchini 2013). These alkaloids are most common among the order Ranunculales, specifically the Papaveraceae, Ranunculaceae, Berberidaceae and Menispermaceae families. Papaver somniferum (opium poppy) is one of the most extensively investigated species in this respect (Weid et al. 2004; Ziegler et al. 2009; Beaudoin and Facchini 2014).

Benzylisoquinoline alkaloid biosynthesis begins with two tyrosines: one is converted into tyramine via decarboxylation and further to dopamine (see ▶ Sect. 12.4), whereas the other one is converted to 4-hydroxyphenylacetaldehyd (◘ Fig. 12.3). Both fuse in a Pictet-Spengler-type reaction to render (*S*)-Norcoclaurine (Luk et al. 2007). Dopamine is the precursor for the isoquinoline moiety, whereas 4-hydroxyphenylacetaldehyde is incorporated as the benzyl component. (*S*)-Norcoclaurine is changed into (*S*)-coclaurine, further to (*S*)-*N*-methylcoclaurine and to the intermediate (*S*)-reticuline. It is being discussed whether the methyl groups of reticuline act as protecting groups by reducing the possible coupling modes available during the oxidative coupling process, and these groups are then removed towards the end of the synthetic sequence.

(*S*)-Norcoclaurine, also called higenamine, which is enriched in the fruit of *Nandina domestica* (sacred bamboo, Berberidaceae), in the roots of *Aconitum sp.* (monkshood or wolf's bane, Ranunculaceae), *Tinospora crispa* (Menispermaceae) and the seeds of *Nelumbo nucifera* (lotus, Nelumbonaceae), is used as dietary additive for fat burning but is on the prohibition list of the World Anti-Doping Agency (Zhang et al. 2017).

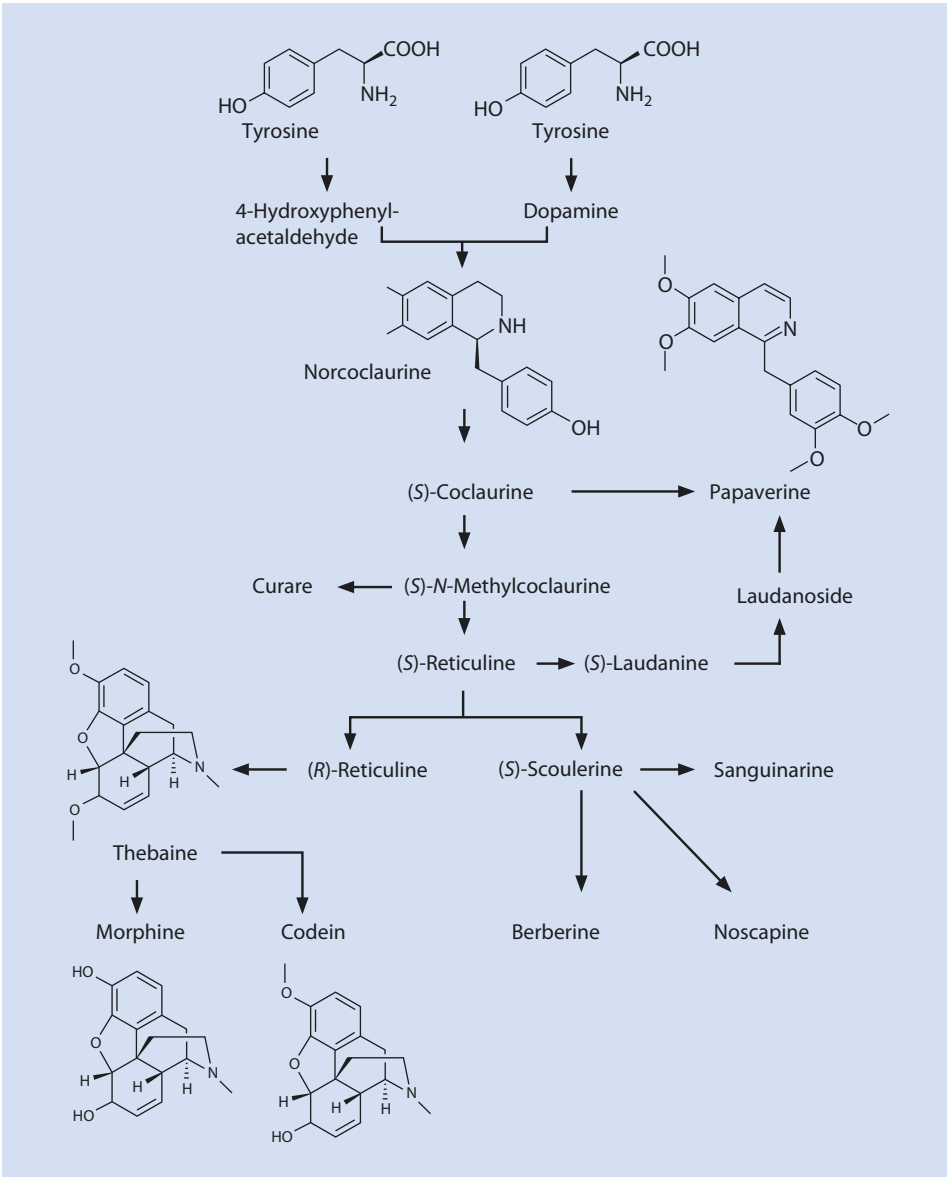

Fig. 12.3 Schematic overview of the biosynthetic pathway of benzylisoquinoline alkaloids and representative members of various subfamilies

The coupling of an (*R*)- and (*S*)-*N*-methylcoclaurine leads to *d*-tubocurarine, the main toxic ingredient of one kind of curare, used as arrow poison. Curare stands for a mix of substances extracted from poisonous plants such as *Strychnos toxifera* (Loganiaceae, from bark scrapings) and from several species of the Menispermaceae (such as *Chondrodendron tomentosum, Curarea sp*., *Caryomene sp*. and *Cissampelos sp*.). Curare acts on the nicotinic acetylcholine receptor (NAchR, see ▶ Sect. 6.2).

After (*S*)-reticuline two main pathways can be distinguished, either via (*S*)-scoulerine or via (*R*)-reticuline.

(*S*)-scoulerine is produced by the berberine bridge enzyme, which requires molecular oxygen as oxidant and releases H_2O_2 as by-product to form the characteristic berberine bridge. The extra carbon atom is supplied from S-adenosylmethionine via an *N*-methyl group. (*S*)-scoulerine can lead to the formation of sanguinarine via (*S*)-cheilanthifoline and (*S*)-stylopine or to the formation of compounds such as berberine and noscapine via tetrahydrocolumbamine and (*S*)-canadine. Sanguinarine accumulates mainly in Papaveraceae, Rutaceae and Fumariaceae. It can be extracted from the rhizome of *Sanguinaria canadensis* (bloodroot, Papaveraceae) and has been shown to intercalate with DNA (Croaker et al. 2016). Berberine, isolated from *Berberis vulgaris* and other plants, is an antiseptic and modulates neurotransmitters. Due to the strong yellow colour of berberine, *Berberis* species were used to dye wool, leather and wood. Noscapine, isolated from the latex in poppy, is used as a cough-suppressing medication. Sanguinarine is not normally present in the latex but accumulates in roots; whereas the last steps of berberine biosynthesis are localized in the parenchyma cells of the root cortex (see ◘ Fig. 9.1).

Two different routes for the papaverine pathway have been suggested. One pathway is initiated by the methylation of (*S*)-reticuline to generate (*S*)-laudanine (Han et al. 2010). A second methylation at the 3′ position of laudanine leads to laudanosine (*N*-methyltetrahydropapaverine); both are known alkaloids from the opium poppy. Laudanosine is found in small amounts in opium poppy and toxic to humans by inducing epileptic seizures, bradycardia and hypotonia. In the human body, it can arise as a degradation product of medically used drugs for muscle relaxation, including atracurium and cisatracurium, *N*-demethylation, aromatization and dehydrogenation of laudanosine yields papaverine.

The other pathway starts with (*S*)-coclaurine via (*S*)-norreticuline leading to (*S*)-norlaudanine, (*S*)-tetrahydropapaverine and finally papaverine (Desgagne-Penix and Facchini 2012). The identification of a norreticuline 7-*O*-methyltransferase (N7OMT), which uses norreticuline to produce norlaudanine provides evidence for the latter pathway (Pienkny et al. 2009). Papaverine accumulates to high levels in the latex of some poppy species and can act as a phosphodiesterase inhibitor (Han et al. 2010).

Whereas all pathways downstream of reticuline begin with the (*S*)-epimer, conversion to the (*R*)-epimer of reticuline is a required entry step into the morphinan alkaloid biosynthetic pathway. The change in configuration is known to be achieved by an oxidation-reduction process and the intermediate 1,2-dehydroreticulinium ion. Via salutaridine and salutaridinol, thebaine is formed with the help of Cyt P450 enzymes, which catalyse C-C bond formation, reduction and acetylation. Demethylation of thebaine leads mainly to codeinone and further to codeine, another demethylation step yields morphine. Codeine and morphine are the best known narcotic analgesics. The characteristics that allow these opiates to bind strongly to the opioid receptor are defined by the "morphine rule": (1) tertiary nitrogen (a nitrogen atom connected by single bonds to three other atoms) with a small alkyl group attached, (2) a quaternary carbon (a carbon attached by single bonds to four other atoms), (3) a benzene ring or its equivalent attached to the quaternary carbon and (4) a two-carbon chain between the quaternary carbon and the tertiary nitrogen.

In *Papaver*, the pathway from dopamine to thebaine takes place in the sieve element of the phloem, and the necessary enzymes are imported from the companion cells (Ziegler et al. 2009). The synthesis of codeine and morphine can take place either in the sieve element or in the adjoining laticifers (Weid et al. 2004). The cytoplasm of laticifers contains many vesicles, which sequester the alkaloids. The latex in the laticifers, a milky liquid containing resins, proteins and secondary metabolites, is harvested by cuttings the capsule.

However, latex-containing laticifers are also found in the stem. A derivative of thebaine is oripavine, which is also an opiate.

In Ranunculaceae, as analysed in *Thalictrum flavum* (meadow-rue), the benzylisoquinoline alkaloid metabolism takes place in endodermis, pericycle, protoderm, cortex or pith tissues and does not involve vascular cell types as in Papaveraceae (Bird et al. 2003; Samanani et al. 2005; Samanani et al. 2006).

Apparently, morphine can also be synthesized in mammalian tissues. The presence of common intermediates of morphine biogenesis between plants and mammals suggests analogous pathways. Several enzymes for morphine synthesis have been identified in mammals, for instance, an enzyme catalyzing the conversion of thebaine into morphine. Dopamine seems to be the precursor for morphine in mammals. This suggests that trace amounts of morphine and related alkaloids can be produced in animals and are not always taken up with dietary plants (Laux-Biehlmann et al. 2013; Kramlinger et al. 2015).

Bicuculline, another isoquinoline alkaloid the biosynthesis pathway of which is not yet understood, has been isolated from Fumariaceae such as *Corydalis*. It acts as an antagonist of $GABA_A$ receptors, thereby mimicking epilepsy (Johnston 2013).

12.4 Phenethylamine Alkaloids (Derived from Tyrosine, e.g. Mescaline)

Tyramine (derived from tyrosine) is also the starting point for phenethylamine alkaloids or monoamine alkaloids. Tyramine itself is unable to cross the blood-brain barrier, and therefore does not have any psychoactive effects after ingestion. Hordenine is synthesized by the stepwise *N*-methylation of tyramine, which is first converted to *N*-methyltyramine, and which, in turn is methylated to hordenine. This compound was first isolated from the cactus *Ariocarpus fissuratus*, but later also from barley (*Hordeum vulgare*) seedlings. It can act as an allelochemical repressing growth and germination of plants in the surrounding. Hordenine is sold as a nutritional supplement with claims that it would stimulate the central nervous system and promote weight loss by enhancing metabolism. However, there is no scientific evidence for such effects.

When tyramine is hydroxylated it yields 2-DOPA (L-dihydroxyphenylalanine) and carboxylation then creates dopamine. Dopamine can be further methylated and hydroxylated (◘ Fig. 12.4). This results in the production of mescaline, a psychedelic alkaloid, which can be isolated from several cacti, such as the South American peyote cactus (*Lophophora williamsii*). In humans dopamine gives rise to the catecholamines, noradrenaline and adrenaline (epinephrine). These animal hormones and neurotransmitters are also produced in some plants, for instance, dopamine is found in in the pulp of banana (*Musa acuminata*), plantain (green or cooking bananas, *Plantago major*) and the fruit of avocado (*Persea americana*) (Kulma and Szopa 2007; Soares et al. 2014).

12.5 Phenylethylamine (Derived from Phenylalanine, e.g. Ephedrine)

When only the carbon skeleton of phenylalanine is utilized and transformed into 1-phenyl-1,2-propanedione and then transaminated, cathinone is formed. This component can be found in Khat (*Catha edulis,* Celastraceae) and acts as an amphetamine-like

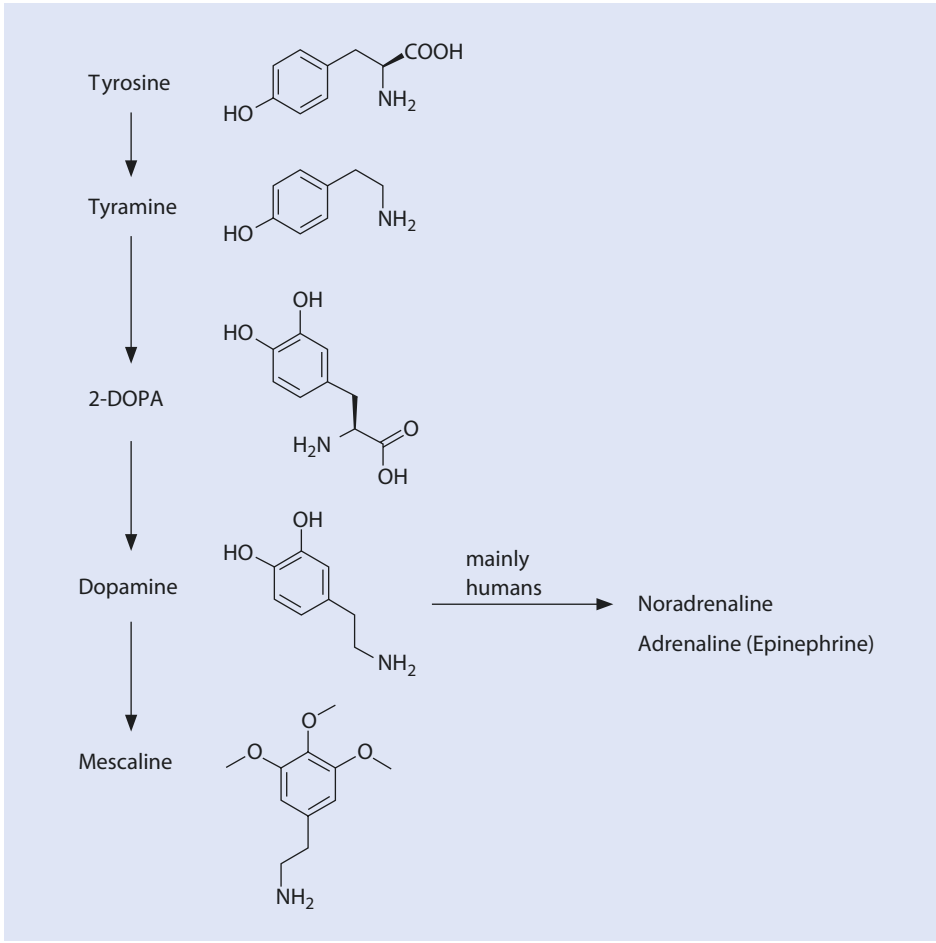

Fig. 12.4 Schematic overview of the biosynthetic pathway of phenethylamine alkaloids and representative members

stimulant. A carbonyl reduction of cathinone leads to norephedrine and norpseudoephedrine. From Khat also cathine (norpseudoephedrine) can be isolated with similar functions. A similar compound, ephedrine, is found in *Ephedra* (Ephedraceae), and can be used as a nasal decongestant and bronchial dilator against asthma. Ephedrine has already been described in Chinese medicine. Some dietary supplements also include ephedra, with the most popular uses being for improvement of weight loss and athletic performance (see ▶ Sect. 7.2).

12.6 Alkaloids from Condensation of Tyrosine and Phenylalanine

12.6.1 Amaryllidaceae (Norbelladine)

Tyramine (from tyrosine) and 3,4-dihydroxybenzaldehyde (from phenylalanine) are condensed to norbelladine, and after different phenol coupling reactions, either galantamine

(inhibitor of the acetylcholine esterase, approved as symptomatic treatment for Alzheimer's disease), haemanthamine (an alkylating agent, inducing DNA-damage), or lycorine (poison found, for instance, in daffodil bulbs causing nausea) is synthetized (Jin 2016). Some of these compounds, for instance, galantamine, have allelopathic functions for the plants.

12.6.2 Phenylethylisoquinoline Alkaloids

Dopamine (from tyrosine) and 4-hydroxydihydrocinnamaldehyde (from phenylalanine) are condensed in a Mannich-like reaction (see ▶ Fig. 9.2d) to a phenylethylisoquinoline derivative, which gives rise to autumnaline and further to colchicine, which contains an unusual tropolone ring (Bentley 2001; Larsson and Ronsted 2014). Colchicine is obtained from the autumn crocus, *Colchicum autumnale*, and extracts of this plant are used to alleviate the symptoms of rheumatism and gout. They also have antimitotic activity due the interaction of colchicine with tubulin, which prevents spindle formation. However, of natural compounds interacting with microtubule dynamics (like vinblastine and vincristine, see ▶ Sect. 12.2) colchicine is not used in chemotherapy because it is too toxic at the required concentrations.

12.7 Pyridine Alkaloids (e.g. Nicotine), Pyrrolidine Alkaloids (e.g. Hygrine) and Tropane Alkaloids (e.g. Atropine, Scopolamine and Cocaine) All Derived from Ornithine and Arginine

Ornithine, a non-protein amino acid, is derived from the urea cycle, by removing urea from arginine by arginase. Ornithine is as a precursor for tropane alkaloids and one part of the pyridine alkaloids. It is transformed into putrescine by a decarboxylase. Putrescine is associated with foul odour. In *Nicotiana* (tobacco) it was shown that this decarboxylase cannot only act on ornithine but also on lysine, thereby producing cadaverine, another compound associated with the odour of decay of dead organisms (▶ Sect. 12.10) (Lee and Cho 2001; Bunsupa et al. 2016). This bifunctional enzyme is localized inside the chloroplast. In contrast, more specific ornithine decarboxylases are localized in the cytosol, allowing compartmentalization of putrescine and cadaverine in plant cells (Fuell et al. 2010). Putrescine is methylated to *N*-methylputrescine. This is catalysed by the putrescine *N*-methyltransferase (PMT) and is the rate-limiting step in this pathway. Oxidative deamination of *N*-methylputrescine by the action of a diamine oxidase leads to the 4-methylaminobutanal aldehyde, followed by a spontaneous cyclization that eventually produces the central intermediate, *N*-methyl-Δ^1-pyrrolinium cation.

To form nicotine, the most abundant pyridine alkaloid in tobacco, *N*-methyl-Δ^1-pyrrolium is condensed with 3,6-dihydronicotinic acid (◘ Fig. 12.5). Therefore, the structure of nicotine contains a pyridine ring together with a pyrrolidine ring. In plants 3,6-dihydronicotinic acid is derived from aspartatic acid and glycerine-3-phosphate via quinolinic acid to form nicotinic acid, the pyridine unit (Dewey and Xie 2013; Kajikawa et al. 2017). Nicotinic acid is an essential component of metabolic coenzymes such as NAD^+ and $NADP^+$ and has to be taken up with nutrition by animals as vitamin B_3. Nicotine acts on animal nicotinic acetylcholine receptors (Green et al. 2013).

Nicotine can be *N*-demethylated to form nornicotine, which is an undesirable derivative because it constitutes the precursor of the carcinogen *N*′-nitrosonornicotine. Nicotine

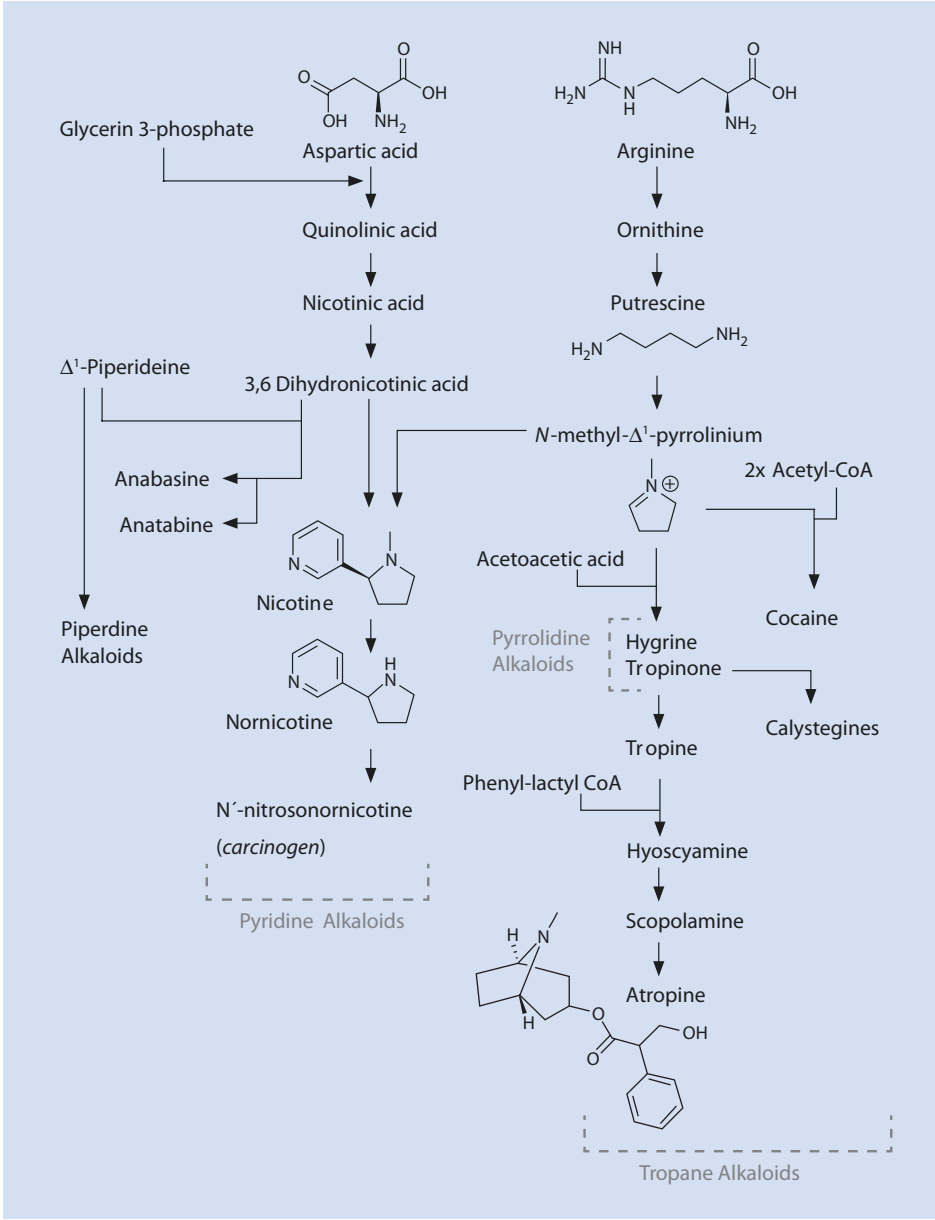

Fig. 12.5 Schematic overview of the biosynthetic pathway of pyridine alkaloids, pyrrolidine alkaloids, tropane alkaloids and piperidine alkaloids and representative members of various subfamilies

is synthesized in the roots of *Nicotiana* (Solanaceae) and is then transported to the aerial parts of the plant where it can also be found in trichomes (Cai et al. 2013). Nicotine levels in plants rise upon attacks from herbivores, and the pathway is induced by jasmonate and wounding. Leaves contain higher levels of nornicotine than of nicotine. Furthermore, in *Nicotiana repanda* it was shown that in trichomes nornicotine is acylated with fatty acids

to produce *N*-acyl nornicotine, which is more toxic than nicotine. This compound can be secreted to coat the leaf surface (Laue et al. 2000).

Arecoline is found in the fruit of the *Areca catechu* palm (Palmae/Arecaceae), used for betel quids and is a tetrahydronicotinic acid derivative. The nuts are chewed for their stimulating effect on alertness; continued usage is a major risk for cancer of the mouth and oesophagus (Horenstein et al. 2017). Ricinine (from *Ricinus communis*, Euphorbiaceae, castor bean) contains a nitrile group as a side group to the pyridine ring and is probably formed by dehydration of a nicotinamide derivative. The castor oil plant is toxic due to the presence of ricin (a protein) and the ricinine alkaloid.

Condensation of the *N*-methyl-Δ^1-pyrrolium cation with acetoacetic acid is proposed to yield hygrine (found mainly in coca leaves), a pyrrolidine alkaloid. Hygrine cyclization or alternative routes lead to tropinone, a branch point for two pathways, which both lead to tropane alkaloids (Drager 2006). They depend on the stereochemistry of the reduction. In the first pathway tropinone reductase I produces tropine, which is then fused with phenylalanine-derived (*R*)-phenyllactyl-CoA to the bicyclic littorine and further to hyoscyamine. Hyoscyamine 6β-hydroxylase, an α-ketoglutarate-dependent dioxygenase, converts hyoscyamine to its epoxy derivative, scopolamine, in two sequential steps (Hashimoto et al. 1993). Scopolamine forms atropine upon racemization (Fig. 12.5). Atropine, hyoscyamine and scopolamine can be found in many members of the Solanaceae family, such as *Atropa belladonna* and several *Datura* species. Some enzymes needed to catalyse the steps to yield scopolamine are localized at the pericycle in the roots of *Atropa belladonna* and *Hyoscyamus muticus*, whereas others are localized in the endodermis or cortical cells, which means that intermediates of this pathway have to traffic between different cell types (Pramod et al. 2010). Hyoscyamine and scopolamine can then be translocated to the aerial parts of the plant, but the hyoscyamine 6β-hydroxylase is also found in leaves of *Duboisia myoporoides* and *Hyoscyamus senecionis*, suggesting that scopolamine biosynthesis could be taking place mainly in leaves at least in some plant species (Dehghan et al. 2013; Kohnen et al. 2018). The synthesis of *N*-demethylated tropane alkaloids such as norlittorine and norhyoscyamine is induced under stress conditions, which could be a detoxification mechanism for cells (Al Balkhi et al. 2012). Atropine, hyoscyamine and scopolamine are strong antagonists of the human muscarinic acetylcholine receptor (see ▶ Sect. 5.1).

The second pathway from tropinone is via tropinone reductase II, leading to pseudotropines from which calystegines (selective glucosidase inhibitors) are synthesized (Scholl et al. 2001). Calystegines are nortropane alkaloids bearing between three and five hydroxyl groups at various positions and in various orientations. They function as selective glucosidase inhibitors due to their structural similarity with monosaccharides and occur mainly in the Solanaceae and Convolvulaceae. This pathway has been shown to localize to the companion cells of sieve elements in the phloem of potato (Petersson et al. 2013).

Epibatidine alkaloids are also compounds with a nortropane ring system, but their biosynthesis and biological source are not yet clearly understood. They have been first isolated from poison frogs, *Epipedobates*. Epibatidine has an analgesic effect 200 times that of morphine, yet it targets a specific subset of nicotinic acetylcholine receptors (NAchRs) rather than opioid receptors. Frogs eat this poison with their diet (mainly insects, like ants and beetles) before depositing it in their skin. One amino acid replacement, which evolved three times in poison frogs, decreases epibatidine sensitivity of the frog nicotinic acetylcholine receptor. This comes at the cost of acetylcholine sensitivity of this receptor, suggesting a mechanism how the frog protects itself from the poison (Tarvin et al. 2017).

N-Methyl-Δ^1-pyrrolium is also the precursor for the biosynthesis of cocaine (Fig. 12.5). The additional carbon atoms required for the synthesis of cocaine are derived from acetyl-CoA, by addition of two acetyl-CoA units to the *N*-methyl-Δ^1-pyrrolinium cation (Drager 2006). The (*S*)-enantiomer can cyclize to form the tropane ring system of cocaine. The tropane ring system undergoes hydrolysis and SAM-dependent methylation. Methylecgonone is reduced via NADPH for the formation of methylecgonine with the help of the methylecgonone reductase. The benzoyl-CoA required for esterification of methylecgonine to form the cocaine diester is synthesized from phenylalanine via cinnamic acid, and the enzyme needed is an acyltransferase (Schmidt et al. 2015). Cocaine is a rare alkaloid restricted to some species of *Erythroxylum* (Erythroxylaceae). The South American *Erythroxylum coca* shrub can have up to 1% dry weight of cocaine in its leaves and has been cultivated for religious and medicinal purposes for more than 8000 years (Bieri et al. 2006).

Tropane alkaloid biosynthesis is distributed among different families of the angiosperms, but it seems that it has evolved independently in different lineages. In species of the Solanaceae, which produce compounds such as hyoscyamine, atropine and scopolamine, the enzyme that is important for the reduction of the keto group in the tropane ring, the tropitone reductase I, belongs to the short-chain dehydrogenase/reductase family. In *Erythroxylum coca*, which accumulates mainly cocaine, a protein of the aldo-keto reductase family carries out this reaction (methylecgonone reductase), which has higher homologies to the chalcone reductase, an enzyme of flavonoid biosynthesis (Jirschitzka et al. 2012). Cocaine is also mainly accumulating in young developing leaves and, in contrast to Solanaceae, is not found in roots.

12.8 Pyrrolizidine Alkaloids (Derived from Spermidine and Putrescine)

Pyrrolizidine alkaloids are formed by the condensation of spermidine and putrescine (both derived from ornithine) or two putrescines to form homospermidine, leading to necine (Ober and Kaltenegger 2009). Pyrrolizidine alkaloids are composed of a necine base present as esters with one or more necic acids. They are produced especially in the families Asteraceae, Boraginaceae, Heliotropiaceae, Apocynaceae and the Orchidaceae.

Senecionine is produced from retronecine by the addition of two molecules of L-Ile. Pyrrolizidine alkaloids are found in many species, as a defence mechanism against herbivores, but have been studied mostly in Fabaceae (e.g. *Crotalaria*), Asteraceae (e.g. *Senecio*) and Boraginaceae (e.g. *Heliotropium*, *Symphytum* and *Cynoglossum*). Examples are the hepatotoxic compounds seneciphylline (from *Senecio*) and echimidine (from *Echium*).

Pyrrolizidine alkaloids accumulate in the plant as polar *N*-oxides, facilitating their transport within the phloem from the roots (where they are usually produced) to the above-ground organs and maintaining them in a non-toxic form. *N*-oxides are then modified by species-specific enzymes. The *N*-oxides are changed back to the tertiary amines in the gut of an herbivore, where they then exhibit their hepatotoxic, genotoxic and carcinogenic potential (Lindigkeit et al. 1997; Ruan et al. 2014).

12.9 Purine Alkaloids (Derived from Xanthosine, e.g. Caffeine)

Purine nucleotides provide the basis for purine alkaloids (Ashihara et al. 2008). Xanthosine (a xanthine conjugated with a ribose), derived from adenine, leads to the synthesis of theobromine and further to caffeine by splitting off the ribose moiety (Fig. 12.6). At least three *N*-methyltransferases are needed in this pathway to add the respective side chains. Caffeine is produced mainly in young leaves and immature fruits of coffee plants and continues to accumulate gradually during the maturation of these organs. Caffeine catabolism involves removing of the methyl groups and leads to theophylline and finally xanthine. Caffeine is not only present in *Coffea arabica* (coffee, Rubiaceae) but also in tea (*Camellia sinensis,* Theaceae), mate (*Ilex paraguariensis,* Aquifoliaceae) and cacao (*Theobroma cacao,* Byttnerioideae). Citrus flowers accumulate purine alkaloids including caffeine mainly in the androecium, and the floral nectar can have trace quantities, which attract honey bees (Weckerle et al. 2003). Caffeine biosynthesis seems to have independently evolved in several species and not always the canonical pathway is used (Huang et al. 2016).

Theacrine is found in cupuaçu (*Theobroma grandiflorum,* Byttnerioideae) and in Chinese tea species (*Camellia assamica var. kucha,* Theaceae). It is synthesized from caffeine and shows anti-inflammatory, sedative, hypnotic and analgesic effects along with effects similar to those caused by caffeine. Theobromine and theophylline can be produced

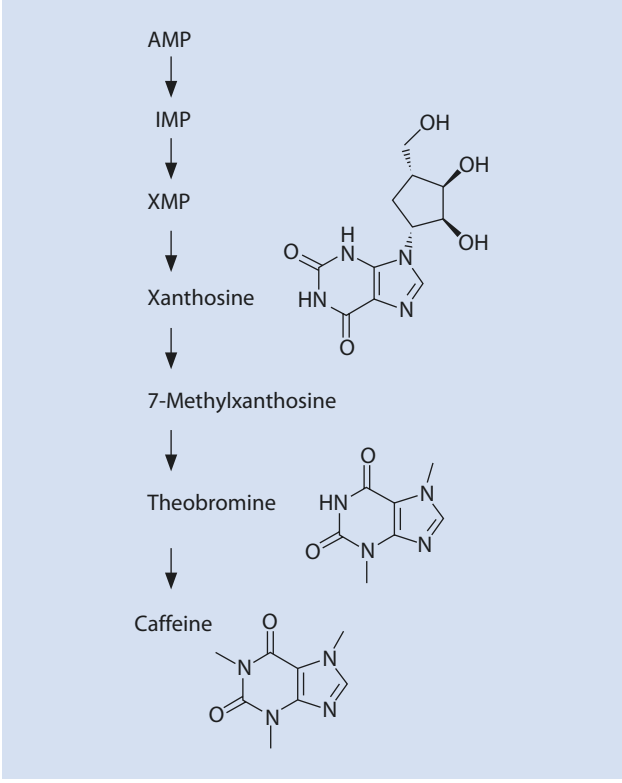

Fig. 12.6 Schematic overview of the biosynthetic pathway of purine alkaloids and representative members. Abbreviations: AMP adenosine monophosphate, IMP inosine monophosphate, XMP xanthine monophosphate

from caffeine in the human liver by removal of a methyl group, but they are also naturally occurring in the cacao plant, the leaves of the tea plant and the cola nut and can therefore be found in tea and chocolate.

12.10 Piperidine Alkaloids (e.g. Lobeline), Quinolizidine Alkaloids (e.g. Cytisine) and Indolizidine Alkaloids (All Derived from Lysine)

Mainly three different alkaloid groups can be derived from lysine: The piperidine alkaloids, the quinolizidine alkaloids and the indolizidine alkaloids (Bunsupa et al. 2012; Bunsupa et al. 2016). While ornithine forms 5-membered pyrrolizidine rings, the extra methylene group in lysine allows the formation of a 6-membered piperidine ring. The lysine amino acid is first processed into cadaverine (see above) by a lysine decarboxylase (which can also act on ornithine, see ▶ Sect. 12.7 in ▶ Chap. 12).

Piperidine alkaloids are lobeline (present in Asterales), pelletierine (present in Myrtales, e.g. pomegranate, *Punica granatum*) and piperine (present in Piperales). To form these, cadaverine is oxidized by a copper-dependent amine oxidase to yield 5-aminopentanal, which is spontaneously cyclized to a Δ^1-piperideine Schiff base. Side chains are either formed by attaching acetic acids or derivatives from cinnamoyl-CoA (from the shikimate pathway) via a Mannich reaction (see ◘ Fig. 9.2d). These alkaloids are produced in the chloroplast and protect the plants against herbivorous insects (Bunsupa et al. 2012). Coniines are similar in structure but seem to have a fatty acid precursor rather than lysine (see ▶ Sect. 13.3.4).

Lobeline can be extracted from the plant and seeds of *Lobelia* (Campanulaceae). *Lobelia inflata* is also called the Indian tobacco or asthma weed. Mainly due to the presence of lobeline, it acts as a respiratory stimulant and has similar effects as nicotine but weaker. It is used in the treatment of central nervous system disorders and drug abuse (Dwoskin and Crooks 2002; Kaniakova et al. 2014). *Piperine* is known for its pungent taste, especially derived from the dried unripe fruit of *Piper nigrum* (black pepper, Piperaceae) and other *Piper* species. The stereoisomer of piperine, chavicine, is slowly transformed to piperine on storage, leading to a loss in pungency. Piperine has been attributed many medicinal properties, among them that it can influence the activity of metabolic enzymes and transporters, thereby changing the pharmacokinetics of therapeutically drugs and/or increasing their bioavailability (Meghwal and Goswami 2013; Lee et al. 2018).

In *Nicotiana,* Δ^1-piperideine can condense with 3,6-dihydronicotinic acid (which is otherwise a precursor for nicotine, see ▶ Sect. 12.7 in ▶ Chap. 12, ◘ Fig. 12.5). This leads to anabasine and anatabine, which have a similar function as nicotine in humans and act as agonist on nicotinic acetylcholine receptors (Dewey and Xie 2013).

The quinolizidine alkaloids have a bi-heterocyclic nucleus and are formed by fusion of two lysine derivatives, either cadaverine or Δ^1–piperideine, via Mannich reactions (see ◘ Fig. 9.2d). The bicyclic ring system is closely related to the spermidine-/putrescine-derived pyrrolizidine system (see ▶ Sect. 12.8 in ▶ Chap. 12). Tiglyl-CoA, *p*-coumaroyl-CoA or feruloyl-CoA can be used to add moieties to the quinolizidine alkaloids (Panter and Keeler 1993; Bunsupa et al. 2012). They are found mainly in the Fabales, many of them in *Lupinus,* which accumulates primarily derivatives of lupinine and lupanine. Due to their presence in the crop sweet lupins, the fruits must be soaked in water to prevent toxicity following ingestion. Another quinolizidine alkaloid is sparteine from broom (*Cytisus scoparius*, Fabaceae).

It contains a tetracyclic bis-quinolizidine ring system and is formed by incorporation of a third lysine or cadaverine molecule. *Cytisine*, a tricyclic quinolizidine alkaloid found in *Baptisia*, *Cytisus*, *Laburnum* and *Sophora* species, has nicotine-like effects on the gastrointestinal tract and on the central nervous system. These plants are sometimes smoked recreationally for their stimulant effects and mild hallucinogenic properties. Cytisine is probably formed from a tetracyclic system by loss of carbon atoms. *N*-methylcytisine is often found in herbal preparations of blue cohosh, an abortifacient (from *Caulophyllum thalictroides,* Berberidaceae) (Rao and Hoffman 2002; Dugoua et al. 2008). *Anagyrine* is a partial agonist at NAchR and is nematicidal (Green et al. 2013). It can cause deformities and cleft palate in cattle ("crooked calf disease") fed on *Lupinus*, but not in sheep (Lee et al. 2007).

The majority of quinolizidine alkaloids are synthesized in the aerial parts of plants. They can accumulate in seeds, sometimes being synthetized in the seeds, but often translocated to the seeds by phloem (Lee et al. 2007). During germination, their concentration is reduced.

Lycopodium alkaloids, which are only found in plants of the families Lycopodiaceae and Huperziaceae are probably derived from quinolizidine alkaloids and are characterized by four six-membered rings. Huperzine A (from *Huperzia serrata*) acts as an inhibitor of acetylcholine esterase and is used in the treatment of Alzheimer's disease to increase the levels of acetylcholine (Xing et al. 2014; Zheng et al. 2016).

Indolizidine alkaloids are characterized by fused six- and five-membered rings, with a nitrogen atom at the ring fusion, and are formed from L-Lys via L-pipecolic acid. Typical indolizidine alkaloids are castanospermine (from chestnut, *Castanospermum austral,* Fabaceae), an inhibitor of some glucosidase enzymes, and swainsonine (from *Swainsona canescens, Astragalus sp.,* Fabaceae), which is actually not produced by the plant but by fungal endophytes (Ren et al. 2017). Swainsonine can cause poisoning in livestock. It is an inhibitor of the Golgi alpha-mannosidase II, thus inducing accumulation of mannose oligosaccharides. The resulting lysosomal storage disease affects many reproductive functions in cattle.

12.11 Steroidal Alkaloids (From Steroids Derived from the MVA Pathway)

Steroidal alkaloids are based on a C_{27} cholestane skeleton and include, for instance, solasodine and tomatidine, two nitrogen-containing analogues of steroids. Cholesterol, produced through the cytosolic MVA pathway, is the precursor in their biosynthesis (see ▶ Sect. 10.6 in ▶ Chap. 10). Similar to saponins they are also present in plants as glycosides. They often occur in Solanaceae, solasonine in several *Solanum* species and tomatine in tomato (*Solanum lycopersicum*). The toxic and bitter α-solanine, the glycoside of solanidine, can also be found in potatoes (*Solanum tuberosum*) and is mainly accumulating in green tissues (but also in green parts of the potato tuber). These compounds inhibit acetylcholine esterase (AChE) that is required to hydrolyse and inactivate the neurotransmitter acetylcholine. Therefore, some of these substances from the Solanaceae are also discussed as bioinsecticides (Chowanski et al. 2016).

Cyclopamine isolated from corn lily (*Veratrum californicum,* Melanthiaceae) antagonizes a signalling pathway that is overactive in basal cell carcinoma and other cancers. Veratridine from Liliaceae prevents the inactivation of voltage-gated sodium ion channels and can be used as an insecticide.

12.12 Terpenoid Alkaloids (Derived from the MEP Pathway, e.g. Aconitine)

Terpenoid alkaloids, as the name indicates, originate from the plastidial MEP pathway, leading to the synthesis of geranyl diphosphate (GPP, see ▶ Sect. 10.1 in ▶ Chap. 10). During biosynthesis, a nitrogen atom is added often after cyclization in the form of a β-aminoethanol, ethylamine, or methylamine. Species of *Aconitum* (Ranunculaceae, monkshood, blue rocket) and *Delphinium* (larkspurs, Ranunculaceae) contain complex diterpene-derived esters, especially in the roots. Aconitine is a diester of aconine with acetic and benzoic acids. These alkaloids are potent neurotoxins and antiarrhythmic agents because they block voltage-gated sodium channels (see ▶ Chap. 8.1). All species of *Aconitum* are potentially toxic to man and animals and must be treated with caution. Another diterpene alkaloid, methyllycaconitine, is also found in many species of *Delphinium* and has insecticidal properties. In mammals it has been used to study the nicotinic acetylcholine receptor (see ▶ Sect. 6.2 in ▶ Chap. 6).

12.13 Tropane-Related Alkaloids (Derived from Proline, e.g. Anatoxin A)

Anatoxin A is found in several freshwater cyanobacteria such as *Anabaena flos-aquae,* which can occur as an algal bloom. This component acts as a neurotoxin and is an agonist for the nicotinic acetylcholine receptor. The biosynthesis pathway seems to start from an activated proline, which is attached to an acyl carrier protein (Mejean et al. 2014). Prolyl-ACP is oxidized to 1-pyrroline-5-carboxyl-ACP (P5C-ACP) by an oxidase, and three acetate units are added. Methylation forms the homoanatoxin A precursor.

12.14 Isoxazole Alkaloids (e.g. Muscimol)

Isoxazoles (1,2-oxazole) such as ibotenic acid, muscimol and muscazone are structural analogues of γ-aminobutyric acid (GABA). They occur in some species of the mushroom *Amanita*, especially fly agaric (*Amanita muscaria*). Muscimol is derived from ibotenic acid by decarboxylation.

12.15 Muscarine

Muscarine is an agonist for the muscarinic acetylcholine receptor due to its similarity with acetylcholine and is thought to contribute to the overall psychoactivity of *Amanita muscaria*. Its biosynthesis is not yet understood, it is probably derived from pyruvic acid to yield the tetrahydrofuran-containing quaternary ammonium alkaloid.

12.16 Guanidinium Toxins (e.g. Saxitoxin)

Guanidinium toxins, such as saxitoxin (STX), tetrodotoxin (TTX) and their analogues, are alkaloids with divergent evolutionary origins but which share the common chemical feature of guanidinium moieties (functional group on the side chain of arginine).

Saxitoxin and its analogues neosaxitoxin and gonyautoxin are known as paralytic shellfish toxins, which are strong and selective voltage-gated sodium channel (Na_vCh) blockers due to the guanidinium groups. These molecules are found in many species of marine dinoflagellates and several freshwater species of cyanobacteria, and they can accumulate in mussels, leading to intoxication of humans. Propionyl-ACP is fused with arginine, and after several steps and cyclizations, saxitoxin is synthesized. This leads to a reduced purine ring system, which is not biosynthetically related to the purine alkaloids. Arginine is also a precursor for tetrodotoxin, another neurotoxin, which selectively inhibits voltage-gated Na^+-channels and thus blocks the propagation of action potentials. It contains a polar guanidino group, probably coupled to an isopentenyl diphosphate. Tetrodotoxins occur mainly in macrozoa, particularly among puffer fish, several species of marine invertebrates and a few terrestrial amphibians. This toxin makes also the Japanese dish "fugu" sometimes lethal. It is discussed that the primary source of tetrodotoxin in these animals are marine bacteria taken up with food or via symbiosis (Lago et al. 2015).

12.17 Kainoids

Several types of kainoids exist that vary within their side groups, kainic acid (kainate), domoic acid and acromelic acid are the most prominent natural occurring forms. Domoic acid is produced by diatoms (esp. *Pseudo-nitzschia sp*.) or the alga *Chondria armata* and accumulates in mollusks and other marine animals causing amnesic shellfish poisoning in humans. It is formed from the condensation of GPP (see above, ▶ Sect. 10.1) with glutamate to generate a pyrrolidine ring skeleton by cyclization that is subsequently converted to domoic acid (Savage et al. 2012). The toxin acts as an agonist of the ionotropic kainite glutamate receptor and is excitotoxic in the vertebrate central nervous system and other glutamate receptor-rich organs. Kainic acid, which is even more potent, differs from domoic acid by one side chain and is isolated from the red alga *Digenea simplex*. Acromelic acid is the toxic component of the mushroom *Clitocybe acromelalga*.

Take Home Messages

- Alkaloids always contain a nitrogen atom, usually within a heterocyclic ring.
- They are mainly synthetized from aromatic amino acids (Phe, Tyr, Trp) but also some other amino acids, ornithine, spermidine, xanthosine and the terpenoids.
- Many toxic components are present within this group and many have shown to interact with mammalian receptors or channels such as ibogaine, psilocybin, morphine, codeine, bicuculline, mescaline, acreoline, atropine, cocaine, caffeine, cytisine, aconitine, anatoxin A, muscimol, guanidinium toxins and kainoids.
- Not only many plants synthetize alkaloids, but also some algae, diatoms and mushrooms are able to produce them. Alkaloids found in animals are often taken up by their diet.

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Minor Groups of Secondary Metabolites

13.1 Quinone – 206

13.2 Polyketide (e.g. Cannabinoids) – 206

13.3 Fatty Acid-Derived Metabolites – 208
13.3.1 Polyacetylenes (e.g. Cicutoxin) – 209
13.3.2 N-Acylethanolamines – 210
13.3.3 Alkylamides, N-acylamides (e.g. Capsaicin) – 210
13.3.4 Conium Alkaloids (Pseudoalkaloids, e.g. Coniine) – 211

13.4 Non-protein Amino Acids – 211

13.5 Acetylcholine – 212

References – 212

A. Böttger et al., *Lessons on Caffeine, Cannabis & Co*, Learning Materials in Biosciences,
https://doi.org/10.1007/978-3-319-99546-5_13

What You Will Learn in This Chapter

Besides the classical groups of alkaloids, phenylpropanoids and isoprenoids, several other biosynthesis pathways produce important classes of secondary metabolites. These include especially cannabinoids and fatty acid-derived compounds.

13.1 Quinone

Naphthoquinone biosynthesis can use chorismate, a phenylpropanoid, as a precursor (see above, ▶ Chap. 11). The addition of 2-oxoglutarate leads to the intermediate 2-succinylbenzoate. In addition, the shikimate/mevalonate or polyketide pathway can be utilized for modifications. 2-carboxy-1,4-naphthoquinol is a branch point leading to the biosynthesis of phylloquinone (vitamin K1), two-ring naphthoquinones such as lawsone (red-orange pigment present in the leaves of the henna plant, *Lawsonia inermis*), juglone (a phytotoxic and therefore allelopathic compound mainly found in walnut, *Juglans regia*) and the three-ring anthraquinones such as alizarin (a red dye isolated from Rubiaceae).

13.2 Polyketide (e.g. Cannabinoids)

Polyketides are structurally and functionally diverse secondary metabolites produced in bacteria, fungi, and plants. They are synthesized from a starter unit, usually an acyl-CoA, fused sequentially with malonyl-CoA and catalysed by polyketide synthases (PKSs), a reaction similar to fatty acid biosynthesis. PKSs are large, multifunctional enzymes with several enzymatic domains able to generate complex structures. Intramolecular cyclation can occur by Claisen condensation, aldol condensation, and lactonization. Additionally, units can be added, such as acetate, malonate, propionate, butyrate, and glycolate. Polyketide synthesis in fungi or bacteria can be combined with non-ribosomal peptide synthesis, where carboxylic and amino acid extender units are sequentially added to a growing acyl or peptidyl chain (similar to ergot biosynthesis, see above, ▶ Sect. 12.2). Polyketides from bacteria are often used as antibiotics or immunosuppressants, e.g. lovastatin (p resent in fermented tea or rice, used for the treatment of hypercholesterolemia due to an inhibition of the HMG-CoA-Reductase) and actinorhodin (an antibiotic).

Phytocannabinoids are terpeno-phenolic compounds predominantly produced in *Cannabis sativa* (Cannabaceae). The precursors of cannabinoids are derived from the polyketide pathway starting with the short-chain fatty acid hexanoate via hexanoyl-CoA (Flores-Sanchez and Verpoorte 2008; Stout et al. 2012). Sequential aldol condensation with three molecules of malonyl-CoA leads to *olivetolic acid* with the help of the polyketide synthase olivetolic acid cyclase (OAC)(Gagne et al. 2012). A prenyltransferase adds GPP derived from the MEP pathway leading to the formation of cannabigerolic acid (CBGA) (◘ Fig. 13.1). Oxidocyclases are responsible for the diversity. The main compounds are Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabichromenic acid (CBCA). The decarboxylated derivatives are Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabichromene (CBC) (Raharjo et al. 2004). The decarboxylation occurs non-enzymatically by harvesting or heating. The *Cannabis* variety used for textiles contains the cannabinoids CBDA and CBCA at high concentrations in contrast to THCA (Kim and Mahlberg 1997; Kim and Mahlberg 2003). CBCA accumulates mainly

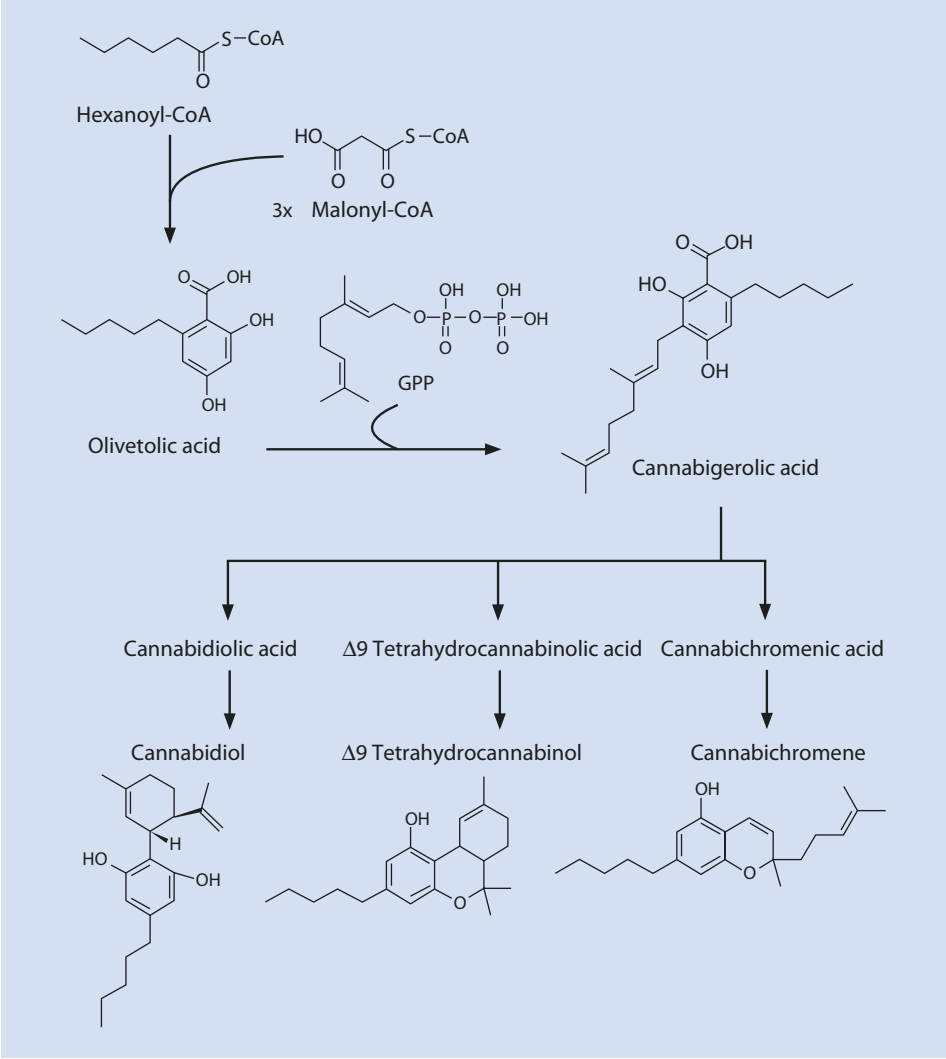

Fig. 13.1 Schematic overview of the biosynthetic pathway of cannabinoids. Abbreviations: *GPP* geranyl pyrophosphate

in young plants. Propyl cannabinoids (cannabinoids with a C3 side chain, instead of a C5 side chain), such as tetrahydrocannabivarinic acid (THCVA), are synthetized from a divarinolic acid precursor instead of olivetolic acid. These have also been reported to be present in *Cannabis* (Hillig and Mahlberg 2004; Flores-Sanchez and Verpoorte 2008). Thus, THCA is the major cannabinoid in the drug-type *Cannabis*, while CBDA predominates in fibre-type hemps. These compounds are produced and accumulate in the trichomes of *Cannabis sativa,* especially in glandular capitate-stalked and capitate sessile trichomes (Kim and Mahlberg 2003). THCA is synthesized in the storage cavity, and the enzyme THCAS is also transported to this compartment. This seems to be a protective measure for the plant since accumulation of cannabinoids in cells induces apoptosis (Sirikantaramas et al. 2005).

Olivetolic acid is also the starting point for biosynthesis of other compounds, for instance, primin, which accumulates in the trichomes of *Primula obconica* (Primulaceae) and causes dermatitis.

In hops (*Humulus lupulus*, Cannabaceae) specialized trichomes, the so-called peltate glandular trichomes, can be found especially at the base of hop cones. They contain, in addition to terpenes, many C5-prenylated polyketides, with the major compound being xanthohumol, a prenylflavonoid. Further oxidized prenylated polyketides are the so-called bitter acids such as humulone. These polyketides provide flavour for beer, especially through the bitter taste of humulene. The non-ketone part of xanthohumol originates in phenylpropanoid metabolism (see ▶ Chap. 11), namely, chalconaringenin. Transfer of DMAPP to chalconaringenin allows prenylation and yields desmethylxanthohumol, which is methylated to form xanthohumol. Xanthohumol was found in significant amounts in male hop plants, presumably due to the presence of lupulin glands on anthers (Nagel et al. 2008). Humulone is derived from leucine to form isovaleryl CoA and furthermore, after the addition of three malonyl CoA, to phlorisovalerophenone. Two additional aromatic prenylation steps, where the prenyl group is added via DMAPP, and final oxidation lead to humulone.

Dinoflagellates seem to have specialized in the production of secondary metabolites of polyketide origin (Rein and Snyder 2006; Van Wagoner et al. 2014). There is a frequent occurrence of five-, six-, seven-, eight- and even nine-membered all *trans*-fused ether rings as a backbone. These rings can be found in isolation (e.g. amphidinolide), in spiroketal formation (e.g. okadaic acid) or in as many as fourteen ether rings continuously fused (e.g. brevetoxin). For dinoflagellates it was observed that the C1 carbons of acetate were frequently missing, and pendant methyl groups were derived from acetate methyl, in addition to *S*-adenosyl methionine (SAM). Brevetoxins, especially known from *Karenia brevis,* have 10–11 fused ether rings and an unusual terminal aldehyde group derived from an acetate methyl carbon. Brevetoxins are neurotoxins that bind to voltage-gated sodium channels in nerve cells, causing neurotoxic shellfish poisoning. Brevenal has four fused ether rings and appears not to share the sodium channel activity of the brevetoxins but seems to act as a brevetoxin antagonist (Bourdelais et al. 2004; Bourdelais et al. 2005; Gold et al. 2013). Gambiertoxin is produced by dinoflagellates from the genus *Gambierdiscus*. They can be transformed into ciguatoxin in predatory fish guts, with molluscs being the vector. It is a neurotoxin that is not destroyed by the cooking process and constitutes a polyether with ring structures. Okadaic acid found in *Dinophysis* and *Prorocentrum* is known to cause diarrheic shellfish poisoning by inhibiting protein phosphatases 1 and 2A (Nielsen et al. 2016).

13.3 Fatty Acid-Derived Metabolites

Fatty acids, polyacetylenes and polyketides share the biosynthetic scheme of condensing multiple acetate units and forming poly-β-keto-chains but differ in the chain extension mechanism. Fatty acid composition varies between plant families. Very long-chain fatty acids, which include the polyunsaturated fatty acid arachidonic acid, are characteristic of algae, mosses and ferns. Flowering plants produce long-chain fatty acids with a chain length of C16 and C18 and zero to three *cis* double bounds, for instance, linoleic acid. Respectively, the derivatives can also vary between different plants (◘ Fig. 13.2). Many of the fatty-acid derived metabolites accumulate in trichomes.

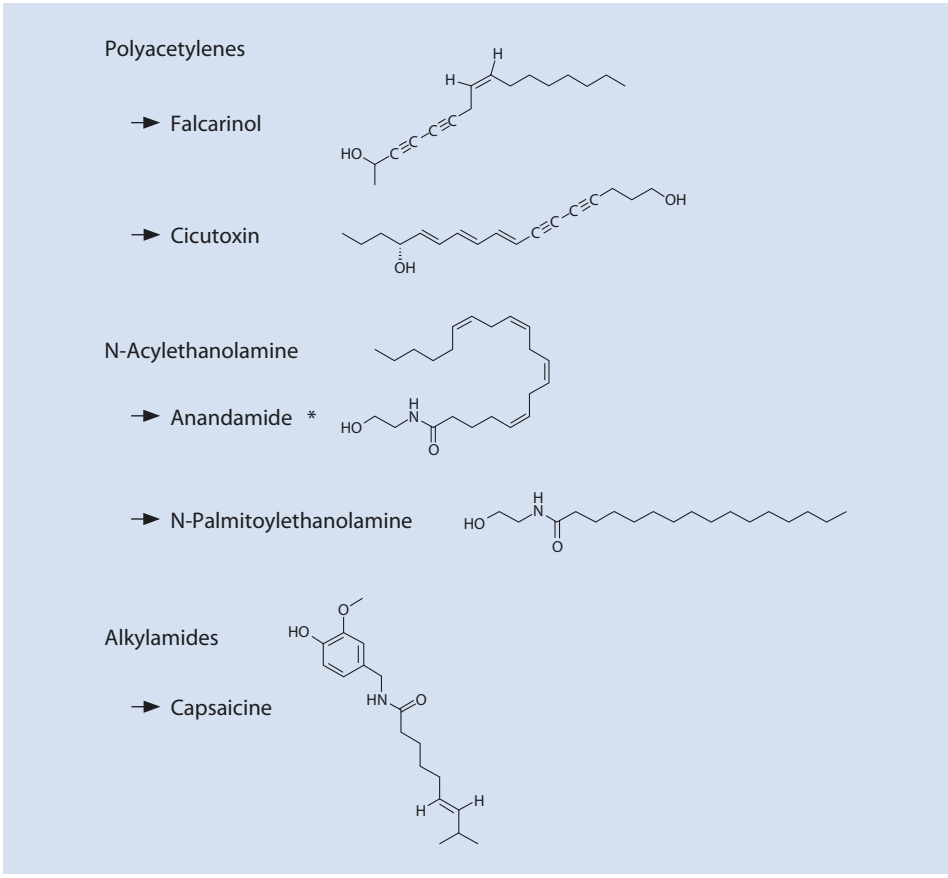

Fig. 13.2 Representative members of fatty acid-derived secondary metabolites. *, compound only in animals not in plants

13.3.1 Polyacetylenes (e.g. Cicutoxin)

Polyacetylenes are hydrocarbons with more than one triple bond (Fig. 13.2). They are also sometimes called polyyne, which are organic compounds with alternating single and triple bonds. Polyacetylenes are mainly produced by plants of the families Apiaceae, Araliaceae, Asteraceae, Campanulaceae, Olacaceae, Pittosporaceae and Santalaceae. Three fatty acids are usually the starting point for most acetylated products: crepenynic acid, stearolic acid and tariric acid (Minto and Blacklock 2008; Negri 2015). The crepenynate pathway starts with acetate-derived acyl lipids provided from primary metabolism and leads to the conversion of linoleic acid to crepenynic acid. Further insaturation reactions by desaturases, acetylenase and hydrolases lead to the triple bonds. Additionally, alkyne moieties can be added, oxidations shorten the chain, or (and) hydroxyl, epoxide and ketone groups are introduced for specificity. Polyacetylenes can also be glycosylated with different saccharides. Decarboxylation leads to C_{17} polyynes and other polyacetylenes characterized by a skeleton with an unpaired number of carbon atoms. Falcarinol found in carrot (*Daucus carota,* Apiaceae), other vegetables from this family (such as parsnip,

fennel, celery and parsley) and ivy derives from the crepenynate pathway (Dawid et al. 2015) (Fig. 13.2). Falcarinol has been made responsible for the bitter taste and alleged allergenic, antibacterial, antimycobacterial and antifungal activities of these herbs and vegetables. Furthermore, falcarinol shows significant binding interactions with both cannabinoid receptors (Leonti et al. 2010). Also panaxydol and panaxynol, found in ginger (*Panax ginseng,* Araliaceae), are derived from the crepenynate pathway. They have been attributed cytotoxic activity against several human tumour cells. Cicutoxin is a C_{17}-polyacetylene from water hemlocks (*Cicuta sp*., Apiaceae), and a related compound is oenanthotoxin from *Oenanthe crocata* (Apiaceae). Cicutoxin acts as noncompetitive antagonists of the $GABA_A$ receptor by binding to the picrotoxin binding site within the chloride channel to block ion flow through the channel (Berger et al. 2018).

13.3.2 *N*-Acylethanolamines

N-acylethanolamines are fatty acid derivatives found in many eukaryotes. Several variations can be distinguished, *N*-arachidonoylethanolamine (AEA, anandamide), *N*-palmitoylethanolamine and *N*-oleoylethanolamine. In animals, they are generated from membrane phospholipids through hydrolysis or by sequential deacylation of N-arachidonoyl phosphatidylethanolamine or similar structures. Anandamide is one of the endogenous ligands of the cannabinoid receptor (therefore called endocannabinoid), whereas the other *N*-acylethanolamines are receptor-inactive (Felder et al. 1993). Only recently, the presence of *N*-acylethanolamines in plants has been demonstrated, but it is not clear if the biosynthesis is similar to synthesis of these compounds in animals. In contrast to animals, plants probably do not generally produce arachidonic acid, therefore plants do not produce anandamides and only *N*-acylethanolamines that do not interact with CB receptors (Fig. 13.2). Nevertheless, *N*-acylethanolamines (e.g. *N*-linoleoylethanolamide, *N*-oleoylethanolamide and *N*-palmitoylethanolamide) have been shown to inhibit the enzyme fatty acid amid hydrolase (FAAH), which inhibits anandamide breakdown and thus leads to an increase in endocannabinoid levels (Coulon et al. 2012a, b); see Fig. 5.7b. *N*-Acylethanolamines are highest in seeds of various plant species, such as *Medicago truncatula* (Kilaru et al. 2007) and the cacao tree (*Theobroma cacao*, Malvaceae) and decrease upon germination. They also seem to interact in plants with abscisic acid (ABA) signalling pathways (Teaster et al. 2007; Keereetaweep et al. 2013; Blancaflor et al. 2014).

13.3.3 Alkylamides, *N*-acyl amides (e.g. Capsaicin)

Alkylamides comprise about 200 chemically related compounds and are amines acylated with a variety of different unsaturated fatty acid. Usually, they have an aliphatic, cyclic or aromatic amine residue and a C8 to C18 saturated or unsaturated chain (including double or triple bonds or both), which can also be aromatic. As the nitrogen atom of alkamides is not part of a heterocyclic ring, these compounds are classified as pseudo alkaloids. There structure resembles animal endocannabinoids such as anandamide; therefore, alkamides are highly active in the central nervous system. Plants belonging to the Asteraceae, Convolvulaceae, Euphorbiaceae, Menispermaceae and Rutaceae families specialize in the biosynthesis of alkamides with both amine and acid aliphatic residues.

The over 20 different alkylamides of *Echinacea* have attracted attention due to their pharmacological activities such as immunostimulatory activity, and they have been used for the treatment of cold, flu, respiratory infections and inflammations, but they also act as cannabinomimetics (Raduner et al. 2006; Woelkart et al. 2008). Their amine group is usually an isobutylamine or 2-methylbutylamine, and the acyl moiety has between 11- and 16-carbon atoms with a *trans*-double bond situated at the 2-position and additional double bonds or acetylenic bonds (Raduner et al. 2006; Rizhsky et al. 2016). Alkamides have been reported to be present in other plants, for instance, affinin in *Heliopsis longipes* (Compositae). Affinin stimulates GABA release from brain cells and can be used as analgesic for toothache (Rios et al. 2007).

The amide capsaicin is the hot component in chilli peppers (*Capsicum annuum*, Solanaceae) (Fig. 13.2). The initial burning effect of capsaicin is found to affect the pain receptors (TRPV1), making them less sensitive. The aromatic portion of capsaicin is derived from phenylalanine through ferulic acid and vanillin, this aldehyde being the substrate for transamination to yield vanillylamine. The acid portion of the amide structure is a branched-chain fatty acyl-CoA being produced by chain extension with three malonyl residues from isobutyryl-CoA (derived from valine)(Aza-Gonzalez et al. 2011). Capsaicin starts accumulating in fruits of *Capsicum* upon ripening. Its biosynthesis occurs in the epidermis cells of the placenta in the fruit and they accumulate on the interlobular septum (Stewart et al. 2007).

13.3.4 Conium Alkaloids (Pseudoalkaloids, e.g. Coniine)

The hemlock poisons are toxic to animals and can be found in seeds, vegetative or flowering parts of some plants. The precursor for coniine is a fatty acid, capric (octanoic) acid, which is transformed into the ketoaldehyde by successive oxidation and reduction steps. L-alanine is used for the transamination reaction, leading to 7-amino-octanone. Cyclization leads to the N-containing heterocyclic ring of γ-coniceine and then reduction to coniine. γ-coniceine is even more toxic than coniine. Other biosynthesis pathways (malonyl and butyryl CoA, which are cyclized or a lysine precursor) have been discussed but seem unlikely (Panter and Keeler 1989). Coniine is found in poison hemlock (*Conium maculatum*, Apiaceae), in seeds of *Cicuta maculata* (Apiaceae, therefore also called cicutine) and in *Sarracenia* (Sarraceniaceae) (Reynolds 2005; Green et al. 2013). Other derivatives are methylconiine, *N*,*N*-dimethylconiine, conhydrinone, pseudoconhydrine and *N*-methyl pseudoconhydrine.

13.4 Non-protein Amino Acids

Besides the 20 amino acids used for protein biosynthesis additionally many non-proteinogenic amino acids exist. One of them is willardiine derived from uracil, naturally found often in Fabaceae such as *Acacia willardiana, Mimosa asperata,* and pea (*Pisum sativum*). Willardiine acts as an AMPA-type glutamate receptor agonist, while 5-iodowillardiine is a selective kainite-type glutamate receptor agonist.

Also the γ-aminobutyric acid (GABA) neurotransmitter is a non-protein amino acid. Others mimic the function of neurotransmitters. Quisqualic acid, for instance, mimics L-glutamic acid. It can be isolated from seeds of *Quisqualis* species (Combretaceae) and

flowers of zonal geranium (*Pelargonium,* Geraniaceae). It is responsible for causing paralysis in some beetles.

L-canavanine is a structural analogue of L-arginine and a plant-derived insecticide, detected by the gustatory receptor (GR), a G-protein-coupled receptor of insects, to avoid intoxication. L-canavanine is occurring in several Fabaceae and is considered as a strong allelochemical, which is often stored in seeds. Its biosynthesis involves the conversion of L-canavaninosuccinic acid to L-canavanine and fumaric acid

Caramboxin is similar to phenylalanine with additions at the phenol ring. It acts as a neurotoxin, especially the caramboxin from star fruit (Syn. carambola, *Averrhoa carambola,* Oxalidaceae). Patients with kidney disease can accumulate caramboxin after digestion of bananas, oranges, tomatoes, nuts, broccoli, beans and star fruit, and the neurotoxin can enter the brain, where it overactivates glutamate receptors in the brain leading to hiccups, vomiting, weakness, mental confusion and psychomotor agitation and to unusually long-lasting epileptic seizures, coma and death (Garcia-Cairasco et al. 2013).

13.5 Acetylcholine

The neurotransmitter acetylcholine is mainly known from humans and animals, but it is also present in many plant species as well as in bacteria and fungi (Tretyn and Kendrick 1991). The biosynthesis in plants seems similar to the production pathway in animals, as choline acetyltransferase, which participates in acetylcholine synthesis from its precursors, choline and acetyl-CoA, and other corresponding enzymes (choline acetyltransferase, and the acetylcholine degrading enzyme, choline esterase) have been found in plants. The synthesis seems to take place mainly in young leaves. High concentrations of acetylcholine have been found in *Urtica dioica* (stinging nettle, Urticaceae) and related species but also in *Viscum album* (mistletoe, Santalaceae) and *Digitalis species* (foxglove, Plantaginaceae). In the stinging hair of nettles, acetylcholine can be found besides formic acid and histamine (Otles and Yalcin 2012); all are probably contributing to the stinging sensation.

Take-Home Messages

- The best-known polyketide is Δ9-tetrahydrocannabinol (THC) from *Cannabis sativa*.
- Fatty acid-derived metabolites are polyacetylenes (such as falcarinol and cicutoxin), *N*-acylethanolamines, alkylamides (such as capsaicin) and coniine.
- Non-protein amino acids can interfere with human receptors.

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Correction to: Plant-Derived Drugs Affecting GPRCs

Correction to

Chapter 7 in: A. Böttger et al., *Lessons on Caffeine, Cannabis & Co*, Learning Materials in Biosciences, https://doi.org/10.1007/978-3-319-99546-5_7

In the original publication of Figure 7.5, the formulas for Caffeine and Theophylline had been inadvertently exchanged. The correct formula for the two compounds is shown below (Fig. 7.5).

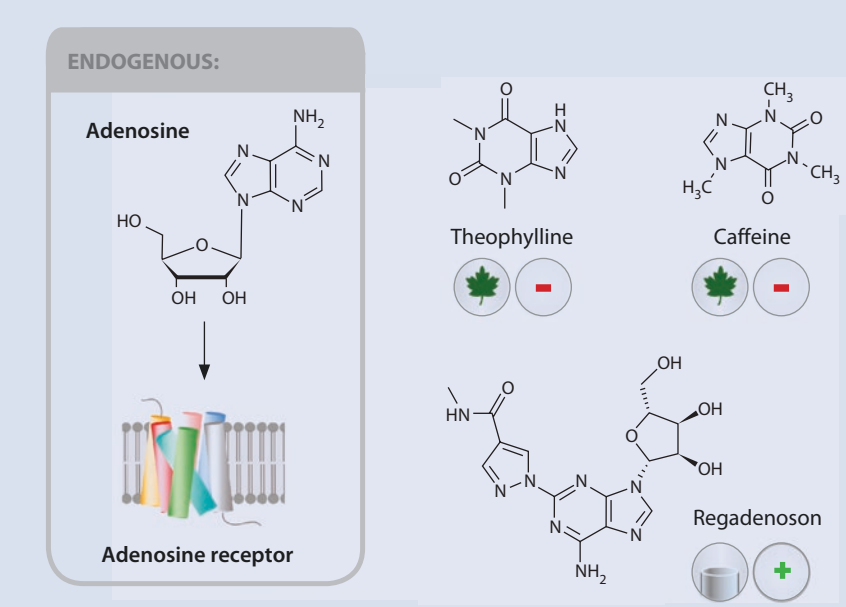

Fig. 7.5 Examples of natural and synthetic compounds that act as agonists or antagonists of the adenosine receptor

The updated online version of this chapter can be found at https://doi.org/10.1007/978-3-319-99546-5_7

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Supplementary Information

Glossary – 216

A. Böttger et al., *Lessons on Caffeine, Cannabis & Co*, Learning Materials in Biosciences,
https://doi.org/10.1007/978-3-319-99546-5

Glossary

Compounds

Acid Acidic, can donate a hydrogen. Carboxylic acids: acidity is associated with carboxyl group (–COOH) (ends in *-oic acid* or *-ic acid*)

Alcohol Derivatives of hydrocarbons, in which an –OH group has replaced a hydrogen atom (end in *–ol*)

Aldehyde Carbonyl group, where the C atom is linked to an H and to a carbon atom or two hydrogen atoms (H-(C=O)-R/H)

Alkane Consists entirely of single-bonded carbon and hydrogen atoms and lacks any other functional groups (ends in *–ane*)

Alkene A hydrocarbon, which is unsaturated (C=C), which means that it contains at least one carbon-to-carbon double bond. Also called olefins (ends in *–ene).* Alkenes that contain two double bonds are called diene

Alkyne Contains one or more carbon-carbon triple bonds (ends in *–yne*)

Amide Derived from carboxylic acids where the -OH group of the –COOH functional group has been replaced by an $-NH_2$ group to form a $-CONH_2$ group (ends in *–amide*); secondary amides are marked with "*N*" to designate that the alkyl group is on the nitrogen atom, tertiary amides with two "*N*" before the name

Amine Derivatives of ammonia (NH_3) in which one or more of the hydrogens have been replaced by an organic group (alkyl or aryl group); amines are formed if amino acids are decarboxylated; primary amines have one alkyl group bound to the N; a nitrogen bound to four alkyl groups will necessarily be positively charged and is called a 4°-ammonium cation

Cation Positively charged ion

Enol Alcohol groups substituted directly onto alkenes (R-(C-OH) = C-R´); good nucleophiles; called "alkene-ols" or enols

Ester Combination of alcohols with acids (R-(C=O)-O-R´)

Ether Organic compound that contains an oxygen between two alkyl groups (R-O-R)

Glycoside Acetal derivatives formed when a monosaccharide reacts with an alcohol in the presence of an acid catalyst (ends in *-oside*)

Hydrocarbon Organic compound consisting only of carbons and hydrogens

Imine Or Schiff base, is a compound with a C=N double bond

Ketone Carbonyl group, where the C atom is linked to two carbon atoms (R-(C=O)-R'); removal of the amino group from an amino acid leads to a keto acid (OH-(C=O)-R)

Lactones Cyclic esters with a ring of two or more carbon atoms and a single oxygen atom with a ketone group at one of the carbons adjacent to the other oxygen; they are formed by intramolecular esterification (end in *–one*)

Ternary compound Ionic compound composed of three or more elements and compounds that contain polyatomic ions

Side Groups

Acetyl group Carbonyl group, where the C atom is linked to a methyl group (CH_3-(C=O)-R)

Alkyl group Contains a point for an attachment that forms if a hydrogen atom is removed from an alkane

Allyl group Consists of a methylene bridge ($-CH_2-$) attached to $-CH=CH_2$ ($H_2C=CH-CH_2-$)

Carbonyl group C=O

CoA Coenzyme A consists of a β-mercaptoethylamine group linked with an amid linkage to the vitamin pantothenic acid; this generates a reactive high energy bond, which delivers the acetyl groups to other compounds; the simplest form is acetyl-CoA

Methyl group Alkyl derived from methane, $-CH_3$

PP Pyrophosphate, an allylic diphosphate ester bond that energizes the compound when removed

Reactions

Alkylation Addition of a saturated hydrocarbon to an alkene to yield a saturated hydrocarbon

Aminotransferase Transfers the amino group from a primary amine (NH_2) to a ketone or aldehyde. The enzyme requires pyridoxal-5′-phosphate (PLP) as a cofactor.

Claisen reaction Carbon-carbon bond formation between two compounds; one of them a enolate anion and then nucleophilic addition of the enolate anion onto carbonyl and loss of the leaving group (see Fig. 9.2c)

Decarboxylation Removal of a CO_2

Dehydrogenases Removal of two hydrogen atoms from the substrate, passing them to a suitable acceptor (such as NAD)

Mannich reaction Carbon-carbon aliphatic bond formation, which involves the combination of an amine, an aldehyde or ketone, and a nucleophilic carbon (see Fig. 9.2d)

Monooxygenases Direct addition of oxygen from molecular oxygen to the substrate (see Fig. 9.3)

Nomenclature

(*R*) or (*S*) Prefix; the chiral centre in a molecule is assigned a prefix (R or S), according to whether its configuration is right- or left-handed

***N*-** Prefix; "*N*" is used to indicate the group that is attached to the nitrogen; used for secondary amides and amines; tertiary amides and amines have two "*N*" as prefix

Nor- Prefix to name a structural analogue that is derived from a parent compound by the removal of one carbon atom along with the accompanying hydrogen atoms