Oliver von Bohlen und Halbach and Rolf Dermietzel

Neurotransmitters and Neuromodulators

Handbook of Receptors and Biological Effects

2nd completely revised and enlarged edition

WILEY-VCH Verlag GmbH & Co. KGaA

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Contents

Preface for the Second Edition

The overwhelming success of our handbook prompted us to embark on a second edition which keeps in line with the original concept of the book, namely to provide a comprehensive source of information on the extraordinary complex field of neurotransmitter and neuromodulator chemistry and function. Apparently the "handyness" of the format was one of the key features that made the book so popular. In this new edition we have updated the *Neurotransmitters* and *Neuromodulators* parts which now include, among others, some additional paragraphs such as D-amino acids and their relationship to the NMDA receptor, and the endocannabinoids. In addition, each chapter has been extended by a paragraph on *Neurological Disorders and Neurodegenerative Diseases*, and the list of references for further reading is renewed. Extensive progress has also been made concerning the uncovering and functional identification of growth factors and neuropeptides with neuromodulatory functions. Here we have concentrated on some factors which gained increasing attention during recent years, such as the family of fibroblast growth factors, the neurotrophins and new neuropeptides of the G-protein coupled receptors (GPCRs) like the ghrelin and the hypocretin/orexin family.

The make up of the book has also been improved by redrawing all sketches which depict the distribution of transmitters and neuropeptides. Coloring of most of the diagrammatic representations made the outfit more appealing.

We thus hope that the new edition keeps track with the speed of progress in this field and that the reader will find the requested information in a straightforward and comfortable way.

Finally, we would like to express our gratitude to Dipl. Biol. Helga Schulze who spent extraordinary efforts on the drawing of almost all the new graphical art work and the redrawing of the old sketches of neurotransmitter and neuromodulator distribution. We also would like to thank Dr. Georg Zoidl who contributed a section on proteomics in the *Methods* part.

January 2006

PD Oliver von Bohlen und Halbach
Prof. Dr. Rolf Dermietzel

Preface for the First Edition

A handbook on neurotransmitters and neuromodulators is necessarily a work in progress. For example, although our knowledge about neuromodulators – their numbers, molecular composition and some of their functions – has increased considerably over the past ten years, the tempo of progress in this field is likely to increase in the near future as the results of genomic cloning and proteomic research become available. Quite apart from these developments, there is now a need for a comprehensive source of information in a format, which is convenient and accessible. For this reason this handbook was conceived as a "handy" book, allowing the reader rapid access to essential information on the main classes of neurotransmitters and neuromodulators. In order to draw informative profiles, we decided to concentrate on certain basic features which characterize each class of substance, namely: *General Aspects and History, Localization, Biosynthesis and Degradation, Receptors and Signal Transduction* and *Biological Effects*. This concept is kept throughout the book. Each chapter is followed by a brief list of *Further Reading*. Although such a strict guideline places some restrictions on the flow of information and results in some overlap, we nevertheless considered it helpful in providing both a broad outline of each substance and also points of comparison between them. Necessarily, this concept is encylopaedic, but nothing more was intended. The absence of detailed discussion and justification for many statements has inevitably given the book a somewhat dogmatic tone, but the bibliographies provide further sources of information for readers wishing to pursue particular issues.

We expect this book to be a useful companion on the laboratory shelf rather than a heavyweight in a librarian's cupboard. We would appreciate comments about the work from colleagues, and from junior and senior students.

This book is dedicated to all of those who suffered from our absence during the process of data collection and writing, especially our families, our friends and coworkers.

June 2001

O. von Bohlen und Halbach
R. Dermietzel
D. Ballantyne

1
Introduction

The paramount functional property of nerve cells is their ability to receive, conduct and store information. Although diverse cell types can perform one or other of these functions most effectively, neurons are unique insofar as they integrate the ability of information transmission with network behavior, which accounts for experience-dependent mechanisms such as memory storage, learning and consciousness. In order to perform these tasks, neurons are structurally and functionally polarized. This is apparent in their tripartite structural differentiation into a cell soma, an axon and dendrites. While the soma harbors the biosynthetic machinery – the nucleus, ribosomes, the endoplasmatic reticulum and the Golgi apparatus including mitochondria for energy supply – the axon is furnished with molecular and subcellular components for the propagation of action potentials away from the cell body to distant targets. Dendrites constitute sets of branched cytoplasmic processes that extend from the cell body and result in an enlargement of the soma for signal reception.

The crucial structural links for signal transmission between neurons are specialized junctions, which are referred to as synapses. Signal transmission between chemical synapses involves the release from presynaptically located sites of molecules (neurotransmitter or neuromodulator) which bind to receptors in the membrane of the target cell, the postsynaptic membrane. Direct transmission of action potentials has also evolved in the form of electrical synapses, which are constituted by gap junctions.

With the exception of the sites of electrical synapses, nerve cells are electrically isolated from one another by an intersynaptic cleft.

A change in the electric potential of the presynaptic neuron triggers the release of a chemical substance, which diffuses across the synaptic cleft and elicits an electrical change at the postsynaptic neuron.

The release of neuroactive substances is linked to the arrival of an action potential at the presynaptic terminal, which elicits the opening of voltage-dependent Ca^{2+} channels (so-called L-type channels). The increase of Ca^{2+} in the presynaptic terminals is the key event that activates the molecular machinery for excocytosis of synaptic vesicles and ultimately triggers the release of the neuroactive molecules. Following diffusion through the intersynaptic cleft, the neurotransmitter binds to the postsynaptic receptor complex. This leads by confor-

mational changes or allosteric mechanisms to the opening of ion channels followed by voltage change at the postsynaptic site.

Depending on the nature of the neuroactive substance and the type of receptor to which they bind, neuroactive substances produce effects that have either a rapid onset and a brief duration or a slow onset and a more prolonged duration.

Neurons are specialized to synthesize a variety of neurotransmitters, and in turn, their activity may be modulated be neurotransmitters released from other neurons. For decades, neurons were believed to constitute monofunctional units with respect to neurotransmitter production and secretion (Dale's principle). However, a large body of evidence now indicates that individual neurons are able to synthesize different neuroactive substances and process them for secretion. This evidence does not, in principle, violate Dale's idea that the neuron is a monofunctional entity, but it does lead to a modification of this paradigm, i.e. the functional phenotype of a differentiated neuron is monospecific in respcet of its neurotransmitter efficacy. The synthesis and release of more than one neuroactive substance from a single neuron substantially augments the range of variability of chemically coded signals. The full significance of this increase is far from being understood. The neuroactive messengers synthesized in an individual neuron belong to two different classes: the neurotransmitters and the neuromodulators.

The first class, the neurotransmitters, includes substances which are responsible for intersynaptic signal transmission, whereas the second, the neuromodulators, exerts a modulatory function on postsynaptic events. Thus, neurons can synthesize and release individual neurotransmitters and are able to produce and release co-transmitter in the form of the neuromodulators.

Brain tissue is composed not only of neurons, but also of supporting cells, the so-called glia. Glial cells are classified into four categories: astrocytes, ependymal cells, microglia cells and oligodendrocytes.

Astrocytes provide mechanical and metabolic support for neurons since they can synthesize and degrade neuroactive substances. They are essential for balancing ion homeostasis and may be involved in neurotransmitter-triggered signaling, thereby constituting a non-neuronal link of spatial signal transmission (Cornell-Bell and Finkbeiner 1991; Cooper 1995). Astrocytes are equipped with neurotransmitter transporters capable of taking up released neuroactive substances and so terminating signals involved by these substances. Glial neurotransmitter transporters also contribute to the synthesis of new neuroactive substances by recycling the captured and/or degraded neurotransmitter metabolites to neurons for reuse.

Ependymal cells line the internal cavities of the central nervous system and seem to play a role in stem cell generation in the central nervous system. They are also involved in controlling volume-transmitted exchanges (see below) of neuroactive substances at the cerebrospinal/interestitial fluid interphase. The primary function of oligodendrocytes is to insulate axons via myelin sheets and thereby provide the cellular substrate for saltatory action potential propagation

in the central nervous system. Finally, microglia represent the brain-specific mononuclear macrophage system essential for immune response of brain tissue and repair mechanisms.

Further Reading

Bruzzone, R., Giaume, C. (1999): Connexins and information transfer in glia. *Adv. Exp. Med. Biol.* **468**: 321–337.

Byrne, J. H., Roberts, J. L. (eds) (2004): *From Molecules to Networks*. Elsevier Science, Amsterdam, and Academic Press, London.

Cooper, M. S. (1995): Intracellular signaling in neuronal–glial networks. *Biosystems* **34**: 65–85.

Cornell-Bell, A. H., Finkbeiner, S. M. (1991): Ca^{2+} waves in astrocytes. *Cell Calcium* **12**: 185–204.

Kandel, E. C., Schwartz, H. S., Jessell, T. M. (eds) (2000): *Principles of Neural Sciences*. MacGraw–Hill, New York.

Kettenmann, H., Ransom, B. R. (eds) (2003): *Neuroglia*. Oxford University Press, Oxford.

Rouach, N., Glowinski, J., Giaume, C. (2000): Activity-dependent neuronal control of gap-junction communication in astrocytes. *J. Cell Biol.* **149**: 1513–1526.

Zigmond, M. J., Bloom, F. E., Landis, S. C., Roberts, J. L., Squire, L. R. (eds) (2002) *Fundamental Neuroscience*. Elsevier Science, Amsterdam, and Academic Press, London.

1.1 Neuroactive Substances

A variety of biologically active substances, as well as metabolic intermediates, are capable of inducing neurotransmitter or neuromodulator effects. A large diversity of neuroactive substances regarding their metabolic origin exists. The molecular spectrum of neuroactive substances ranges from ordinary intermediates of amino acid metabolism, like glutamate and GABA, to highly effective peptides, proteohormones and corticoids.

Recent evidence indicates that neuronal messengers convey information in a complex sense entailing a variety of processes. These include:

- reciprocal influence on the synthesis of functionally linked neuronal messengers;
- induction of different temporal patterns in terms of short-term and long-term effects;
- shaping of network topology including synaptic plasticity during long-term potentiation.

Chemical neurotransmission is not restricted to central nervous synapses but occurs in peripheral tissues as well, including neuromuscular and neuroglandular junctions.

Neuroactive molecules target receptors with pharmacologically different profiles. The existence of multiple sets of neuronal receptors for a single neurotransmitter seems to be the rule rather than the exception. This receptor multiplicity seems to mirror at molecular level the functional diversity of neuronal networks.

Although functional overlap between neurotransmitters and neuromodulators is quite common, this classification has proven useful for practical purposes.

1.1.1 Neurotransmitters

Neurotransmitters are the most common class of chemical messengers in the nervous system. A neuroactive substance has to fulfill certain criteria before it can be classified as a neurotransmitter (R. Werman 1966).

- It must be of neuronal origin and accumulate in presynaptic terminals, from where it is released upon depolarization.
- The released neurotransmitter must induce postsynaptic effects upon its target cell, which are mediated by neurotransmitter-specific receptors.
- The substance must be metabolically inactivated or cleared from the synaptic cleft by re-uptake mechanisms.
- Experimental application of the substance to nervous tissue must produce effects comparable to those induced by the naturally occurring neurotransmitter.

A neuroactive substance has to meet all of the above criteria to justify its classification as a neurotransmitter.

Based on their chemical nature, neurotransmitters can be subdivided into two major groups: biogenic amines and small amino acids (Fig. 1.1).

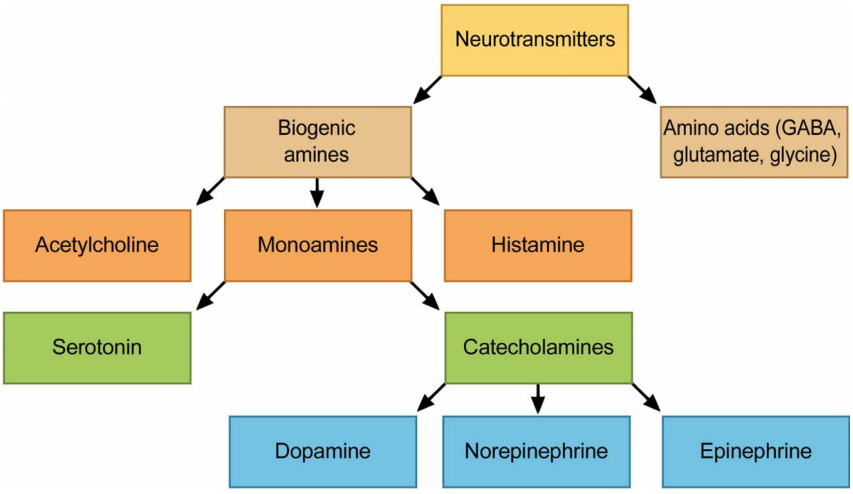

Fig. 1.1 Differentiation of neurotransmitters based on their chemical structures.

1.1.2 Neuromodulators

In contrast to neurotransmitters, neuromodulators can be divided into several subclasses. The largest subclass is composed of neuropeptides. Additional neuromodulators are provided by some neurobiologically active gaseous substances and some derivatives of fatty acid metabolism (for details see Sections 4.2, 4.10, 4.23). The history of the discovery of neuropeptides goes back to the 1960s and 1970s, when it became evident that the regulatory factors of the pituitary gland are peptidergic substances and that some enteric peptides are also synthesized in the central nervous system.

Neuropeptides are synthesized by neurons and released from their presynaptic terminals, as is the case for neurotransmitters. Like neurotransmitters, some of the neuropeptides act at postsynaptic sites, but since they do not meet all of the above criteria they are not classified as such. These neuropeptides are frequently labeled "putative neurotransmitters" (for example: endorphins).

Other neuropeptides are released by neurons, but show no effects on neuronal activity (e.g. follicle-stimulating hormone (FSH) produced in gonadotrophs of the anterior pituitary). These neuropeptides target to tissues in the periphery of the body and can therefore be classified as neurohormones. Consequently, not all neuropeptides function as neuromodulators.

The fact that peptides are synthesized in neurons and are able to induce specific effects via neuronal receptors led de Wied (1987) to formulate the neuropeptide postulate; in essence, this states that peptides which are of neuronal origin and exert effects on neuronal activities are classified as neuropeptides.

The term neuropeptide is no longer used in this restricted sense, because most neuropeptides not only influence neurons, but also non-neuronal tissues. In addition, neuropeptides seem also to link brain activities with other body functions, the most prominent of which is the neuro-immune axis. Thus, neuropeptides are involved in diverse physiological processes, including development, growth, body homeostasis, behavior and immune responses.

Neuropeptides are generated from large precursor molecules. Maturation of the precursor can lead to the formation of one or more peptides (Table 1.1). The biosynthesis requires a cascade of cellular processes to translate the genetic information into the generation of the biological active substance.

The synthesized neuropeptides are stored in vesicles and released upon an adequate stimulus. Different neuropeptides have been found to coexist in a single neuron. Equally, colocalization of neuropeptides with neurotransmitters is quite common.

In the case of colocalization with neurotransmitters, neuropeptides are capable of modulating the effects of the co-released neurotransmitter(s).

Since the formulation of the neuropeptide postulate, a large number of neuropeptides and receptors have been discovered. To date, more than 50 different neuropeptides have been described which are biologically active. We consider the list of neuromodulators and neuropeptides (shown in Table 1.1) to be far

Table 1.1 The most common neuropeptide precursor molecules which lead to one or more biologically active neuropeptides by proteolytic cleavage.

Neuropeptide family	Precursor	Active peptide
Angiotensin	Angiotensinogen	Angiotensin I
		Angiotensin II
		Angiotensin (1–7)
		Angiotensin IV
Calcitonin I gene (CALC I) products	Pro-CALC I	Calcitonin
	Pro-CGRP I	Calcitonin gene related peptide I (α-CGRP)
Calcitonin II gene (CALC II) products	Pro-CGRP II	Calcitonin gene related peptide II (β-CGRP)
Cholecystokinin (CCK)	Pro-CCK	CCK-8
		CCK-33
		CCK-58
Dynorphin gene products	Prodynorphin	Dynorphin A
		Dynorphin B
		α-Neoendorphin
		β-Neoendorphin
Enkephalin gene products	Proenkephalin	Met-enkephalin
Melanin-concentrating hormone gene products	Pro-MCH	MCH
		Neuropeptide Glu-Ile (NEI)
		Neuropeptide Gly-Glu (NGE)
Neurotensin gene products	Proneurotensin	Neurotensin (NT)
		Neuromedin N
Preprotachykinin A (PPTA) gene products	PPTA	Substance P
		Neuropeptide K
		Neurokinin A
		Neuropeptide γ
Preprotachykinin B (PPTB) gene products	PPTB	Neuromedin K
Proopiomelanocortin (POMC)	POMC	α-Melanocyte-stimulating hormone (α-MSH)
		β-Melanocyte-stimulating hormone (β-MSH)
		ACTH
		β-Endorphin
		α-Endorphin
		γ-endorphin
		β-Lipoprotein (β-LPH)
		Corticotropin-like intermediate peptide (CLIP)

Table 1.1 (continued)

Neuropeptide family	Precursor	Active peptide
Somastostatin	Prosomatostatin	Somatostatin-12 Somatostatin-14 Somatostatin-28
Vasoactive intestinal peptide gene products	Pro-VIP	Vasoactive intestinal peptide (VIP) PHM-27/PHI-27
Vasopressin gene products	Provasopressin	Vasopressin (VP) Neurophysin II (NP II)

from being complete and expect an increase in number in the near future, including the precursor molecules. Some derivatives of fatty acids are also known to elicit biological effects in neurons. These lipid neuromodulators have effects which are functionally similar to that of neuropeptides. Like neuropeptides, the neuroactive fatty acids are generated from precursors by a sequence of diverse enzymatic steps.

Similar to neurotransmitters and neuropeptides, the neuroactive derivatives of fatty acids bind to membranous receptors, which leads to downstream signal transduction. Some diffusable gases, like nitric oxide and carbon monoxide, can also act as chemical messengers. The gaseous messengers are generated intracellularly by the activity of specific sets of enzymes. Because of their short half-life and diffusion-dependent radius of activity, they operate in restricted areas, mostly in the proximity of their synthesizing neurons.

In contrast to neurotransmitters and most of the common neuromodulators, nitric oxide does not bind to its own high-specific membraneous receptors because its lipophilic character allows it to pass membranes freely (however, it could bind to guanylyl-cyclase).

Further Reading

Byrne, J. H., Roberts, J. L. (eds) (2004): *From Molecules to Networks*. Elsevier Science, Amsterdam, and Academic Press, London.

Brogden, K. A., Guthmiller, J. M., Salzer, M., Zasloff, M. (2005): The nervous system and innate immunity: the neuropeptide connection. *Nat. Immunol.* **6**: 558–564.

de Wied, D. (1987): The neuropeptide concept. *Prog. Brain Res.* **72**: 93–108.

de Wied, D., Diamant, M., Fodor, M. (1993): Central nervous system effects of the neurohypophyseal hormones and related peptides. *Neuroendocrinology* **14**: 251–302.

Hökfelt, T. (1991): Neuropeptides in perspective: the last ten years. *Neuron* **7**: 867–879.

Hökfelt, T. (2003): Neuropeptides: opportunities for drug discovery. *Lancet Neurol.* **2**: 463–472.

Sossin, W. S., Sweet, C. A., Scheller, R. H. (1990): Dale's hypothesis revisited: different neuropeptides derived from a common prohormone are targetted to different processes. *Proc. Natl Acad. Sci. USA* **87**: 4845–4848.

Werman, R. (1966): Criteria for identification of a central nervous transmitter. *Comp. Biochem. Physiol.* **18**: 745–766.

1.2 Receptors and Transporters

Signal transduction at the cellular level is defined as the transmission of a signal from the outside of a cell into the cell interior. The initial step in signal transduction is performed by the "first messengers", which overcome the barrier of the plasma membrane by interacting with a receptor or a ligand-gated channel. In nervous tissue, "first messengers" are neurotransmitters and neuromodulators, which signal the postsynaptic cell to modify its electrical behavior.

Most common receptors are membrane-bound oligomeric proteins which:

- recognize a ligand with high affinity and selectivity;
- convert the process of recognition into a signal that results in secondary cellular events.

The molecular make-up of a membrane-bound receptor consists of three distinct structural and functional regions: the extracellular domain, the transmembrane-spanning domain and the intracellular domain.

Receptors are characterized by their affinity to the ligand, their selectivity, their number, their saturability and their binding reversibility. So-called isoreceptors form families of structurally and functionally related receptors which interact with the same neuroactive substance. They can be distinguished by their response to pharmacological agonists or antagonists.

Isoforms of receptors can occur in a tissue-restricted manner, but expression of different isoreceptors in the same tissue is also found.

The binding of a ligand to a receptor induces a modification in the topology of the receptor by changing its conformation; and this allows either an ion current to flow, so-called ionotropic receptors, or elicits a cascade of intracellular biochemical events, the metabotropic receptor. Mediators of the intracellular events often consist of "second messengers", like cAMP or cGMP (see below).

The design of intramembraneous receptors is quite variable. Some receptors consist of single polypeptides exhibiting three domains: an intracellular and an extracellular domain linked by a transmembrane segment. Other receptors are also monomeric, but folded in the cell membrane and thus form variable intra- and extracellular as well as transmembrane segments. A large group of receptors consists of polymeric structures with complex tertiary topology.

Receptor categories are completed by cytosolic and nuclear receptors, though these are functionally less relevant so far as neuronal signal transmission is concerned. After the binding of a ligand to a cytosolic receptor, a complex is formed which consists of the receptor and the ligand. This complex is translocated to the cell nucleus and can influence gene transcription. The most common cytosolic receptors are corticoid receptors, which are the targets of the membrane-permeable steroids. Neurotransmitter receptors are commonly located on both the presynaptic and the postsynaptic site and can be ionotropic or metabotropic.

A special class of receptors is referred to as autoreceptors. These are located presynaptically and they bind neuroactive substances released by the presynaptic

cell. Through the activation of autoreceptors, the turnover of released neuroactive substances can be modulated by feedback mechanisms. Thus, autoreceptors seem to be involved in limiting transmitter release and thereby balancing the amount of neurotransmitter concentration in the synaptic cleft.

Another class of receptors comprises the so-called heteroreceptors. Heteroreceptors are also located at presynaptic sites. In contrast to autoreceptors, they can bind neuroactive substances different from the neurotransmitter released by the "host" neuron. Heteroreceptors are therefore able to influence the release of neuroactive substances from their own "host" cell after stimulation by a neurotransmitter from a different source.

The primary signal, which is elicited by the binding of the ligand to the receptor, must be amplified to generate the transmembrane signal. Two distinct amplification mechanisms are of major importance:

- A change in membrane potential, resulting from an agonist-induced change in ion flux. The change in ion flux can arise either directly via ligand-gated ion channel receptors or, indirectly, via an effector-mediated change (for example via G proteins) in channel conductance.
- Activation of a phosphorylation–dephosphorylation cascade. Such a cascade can be initiated either directly, via a receptor that possesses intrinsic ligand-modulated tyrosine kinase activity or indirectly via effector-generated signal mediators, such as cAMP, diacylglycerol (DAG), or elevated intracellular calcium, that on their own can activate protein kinases.

On the basis of their signal transduction pathways, receptors can be classified into two major groups. One group consists of receptors belonging to the gated ion channels, the ionotropic receptors.

The other consists of receptors which act through activation of several enzymes and thus need a cascade of enzymatic events for their signal transduction: this is the group of the metabotropic receptors.

1.2.1 Ionotropic Receptors

Signal transduction of membrane-bound receptors can entail a single step consisting of the activation of gated ion channels. In this case, the receptor forms an ion channel. Binding of the ligand to the receptor opens the ion channel and ions can cross the membrane, driven by an electrical gradient. This current produces a net inward flow of positive or negative charge with the consequence of a change in conductance followed by a postsynaptic potential response. The receptor allows the signal to flow (in the form of ions) from outside the cell into the cell or vice versa.

The activation of ionotropic receptors results in fast signal propagation, but the induced electrical effects are mainly short-lasting without metabolic consequences.

1.2.2 Metabotropic Receptors

More complex forms of signal transduction of membrane-bound receptors involve a coupling of ligand and receptor followed by the activation of different intracellular signal transduction pathways.

After binding of the ligand, the signal is transferred into the cell and leads to the activation of "second messengers", like cyclic nucleotides (cAMP, cGMP), calcium (Ca^{2+}), inositol-trisphosphate (IP3), diacylglycerol (DAG), as well as to the phosphorylation of proteins. Phosphorylation involves in many cases the activity of cAMP-dependent protein kinase (PKA) and DAG-activated protein kinases.

The downstream response modifies intracellular processes, for example the release of neuroactive substances, an altered activity of ion channels or changes in enzymatic activity, particularly kinase cascades. The modifications are induced slowly and the resulting effects can be long-lasting.

G protein-coupled receptors

The most prominent group of metabotropic receptors consists of G protein-coupled receptors (GPCRs). These reveal a uniform molecular composition. The extracellular domain is formed by the N-terminal sequence of the receptor, which has potential glycosylation sites. Since this domain is exposed to the extracellular space, it constitutes the ligand-binding site. The tertiary structure of G protein-coupled receptors exhibits a conserved motif of seven membrane-spanning segments, which are connected by alternating extracytoplasmic and cytoplasmic loops. The C-terminal segment is in the cytoplasm (see Fig. 1.2).

The signal arising form the ligand–receptor interaction is forwarded to membrane-bound GDP/GTP-binding proteins inside the cell. These proteins are termed G proteins.

Each G protein is a heterotrimer consisting of a GTP-binding subunit (α-subunit) and two further subunits (β- and γ-subunits). Subclasses of the α-subunit form the different G protein subtypes G_s, $G_{i/o}$ and G_q. In response to receptor activation, the G_s proteins stimulate adenylate cyclase. This stimulation leads to catalytic formation of cAMP from cytosolic ATP.

The most profound effect of cAMP is the activation of cAMP-dependent, calcium-independent protein kinases through binding to the regulatory subunits of the latter. By allosteric mechanisms the kinases alter their conformation into the active enzymatic form. In this activated form they are able to catalyze the transfer of the γ phosphate from ATP to specific amino acid residues, such as serine and threonine for PKA, and tyrosine for PKC, resulting in phosphorylation of the protein and so, as a consequence, resulting in changes in energy equilibrium.

G_i proteins inhibit adenylate cyclase in response to receptor activation. In turn, they can activate K^+ channels and, additionally, they decrease the influx of calcium through voltage-gated Ca^{2+} channels. G_q proteins activate phospholipase C in response to receptor activation.

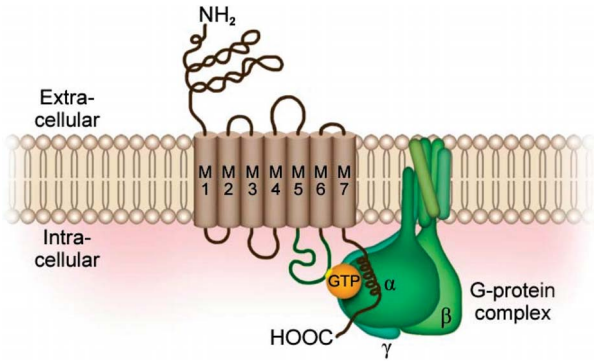

Fig. 1.2 Prototype of a G protein-coupled receptor (GPCR). The domain topology of the single subunits consists of seven transmembrane domains (TM1–TM7) with the amino terminus on the extracellular site and the carboxyl terminus on the cytoplasmic site. GPCRs are homologous to rhodopsin for which detailed structural data are available. The receptor can actively isomerize between the inactive and active state. A receptor domain of the third intracytoplasmic loop significantly affects the specificity of the G protein coupling and contains the main site for receptor coupling to G proteins. Each G protein is a heterotrimer consisting of a GTP-binding subunit (α subunit) and two further subunits (β and γ subunits). For further details see text and Fig. 3.15.

Phospholipase C hydrolyzes the membrane-bound lipid phosphatidyl-4,5-bisphosphate (PIP2) to form diacylglycerol (DAG) and inositol-trisphosphate (IP3). Subsequently, the cytosolic IP3 binds to specific receptors on the endoplasmic reticulum (ER) where it induces the opening of ER-bound Ca^{2+} channels and the release of Ca^{2+} from this store into the cytoplasm. Intracellular elevation of calcium elicits multiple effects on cellular metabolism. These include the activation of Ca^{2+}-dependent enzymes, e.g. calmodulin and its related kinases, activation of motor ATPases for vesicle trafficking, a mechanism that underlies exocytosis of neurotransmitter and receptor dynamics at postsynaptic sites.

Cytosolic DAG binds to cAMP-independent protein kinase C (PKC) and leads to its activation. In addition, DAG induces the opening of plasmalemmal Ca^{2+} channels and thus augments the effects of IP3.

Not all metabotropic receptors target to the G protein system for signal transduction. Other signal transduction systems take advantage of pathways involving guanylate cyclase or tyrosine kinase.

Deorphanizing GPCRs

A total of about 800 GPCRs are found in the human genome, which seems to represent the largest of all gene families. GPCRs can bind a variety of ligands which can be classified into chemosensory receptors, which bind olfactory-gustatory ligands, chemokines and chemoattractants, and the neuromodulator receptors. Some 367 receptors have been found in the human genome on the basis of their sequence homology. Most of them were “orphans” because the ligands

were unknown. "Deorphanizing" the GPCRs by exogenous expression in adaequate expression systems has been used to identify a large scale of new potential neuromodulators and neurotransmitters. The first to be found were the serotonin receptor 5-HT_{1A} and the dopamine D2 receptors. By the mid-1990s, about 150 GPCRs had been paired to 75 known transmitters. Since the number of potential transmitters was about 15 whereas ~200 GPCRs remained orphans, there was a need to identify further new ligands. The use of tissue extracts as a source of new transmitters instead of potential transmitters was rendered successful with the discovery of a new neuropeptide, nociceptin/orphanin FQ (see Section 4.24), as the neuroactive ligand of the GPCR ORL-1.

Deorphanization of GPCRs is still an expanding field of pharmacological reseach and the estimated number of deorphanized GPCRs are ~7–8 per year. Some of the most prominent ligands paired by this approach are: hypocretins/orexins, prolactin-releasing peptide, apelin, ghrelin, metastin, neuropeptide B, neuropeptide W and neuropeptide S.

Guanylate cyclase-coupled receptors

Cyclic guanosine monophosphate (cGMP) resembles cAMP in its chemical composition, with a substitution of guanosine for adenosine as nucleotide.

In contrast to the membrane-bound adenylate cyclase, guanylate cyclase occurs in both membrane-bound and cytosolic forms.

The two forms of guanosine cyclases have different functions and follow different routes of activation. The soluble form is activated by nitric oxide (NO) and by free radicals, whereas the membrane-bound form is a part of a transmembranous receptor. Cyclic GMP activates a specific cGMP-dependent protein kinase (PKG) and leads to a downstream activation of a 23-kDa protein (known as G substrate).

It is thought that the cGMP-induced signaling pathway involves the inhibition of phosphatases through the G substrate, so prolonging the effects of phosphorylation, catalyzed by other signal transduction cascades.

Tyrosine kinase-coupled receptors

The molecular backbone of tyrosine kinase-coupled receptors is a single membrane-spanning polypeptide which separates the N-terminal segment from the cytoplasmatic C-terminal domain.

The binding of a ligand to the extracellular domain is followed by receptor activation. Tyrosine kinases initiate a cascade of intracellular phosphorylation steps.

Prominent members of the tyrosine kinase-coupled receptor family are the insulin receptor and receptors for diverse growth factors, e.g. the group of neurotrophic growth factors.

Cytokine receptors

Cytokine receptors are classified into four families (type I, II, III and IV) but only type I receptors seem to be expressed in the central nervous system. The prolactin receptor, some interleukin receptors and growth hormone (GH) recep-