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-

Neurotransmitters and Neuromodulators. Handbook of Receptors and Biological Effects. 2nd Ed.
Oliver von Bohlen und Halbach and Rolf Dermietzel

ISBN: 3-527-31307-9

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).

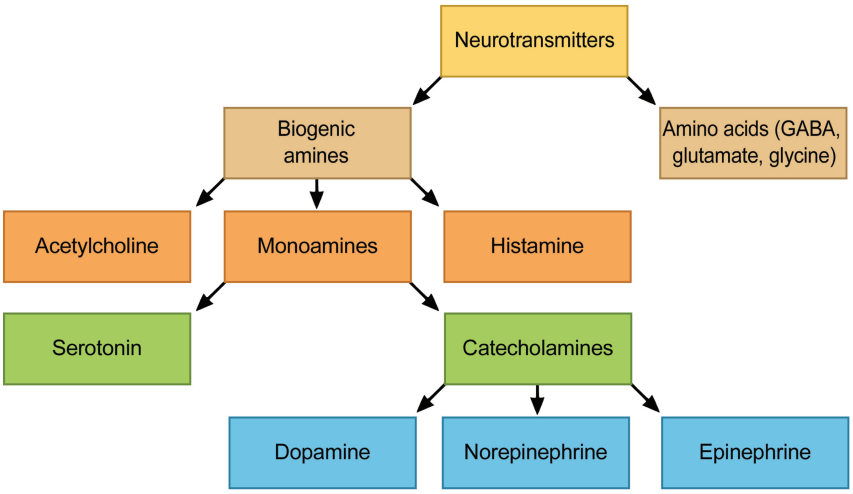

Fig. 1.1 Differentiation of neurotransmitters based on their chemical structures.

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.

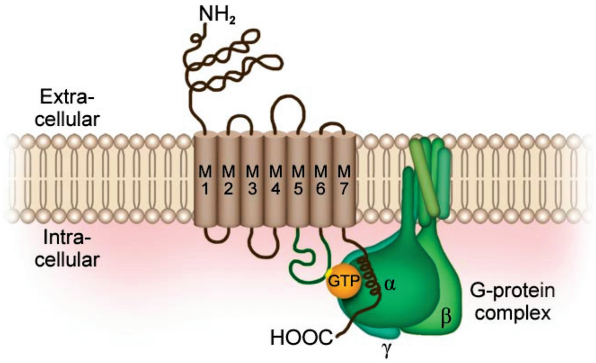

Fig. 1.2 Prototype of a G protein-coupled receptor (GPCR). The domain topology of the single subunits consists of seven transmembrane domains (TM1–TM7) with the amino terminus on the extracellular site and the carboxyl terminus on the cytoplasmic site. GPCRs are homologous to rhodopsin for which detailed 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-

tors belong to the type I cytokine receptors. The exact signal transduction pathways of cytokine receptors are largely unknown and are the subject of intensive investigation.

1.2.3 Receptor Regulation

The affinity of receptors to their appropriate ligands can be decreased by various mechanisms, one of which involves allosteric changes in the receptor molecule, which can result in decreased affinity. Binding of the ligand to its receptor is reversible. The dissociation of the ligand from the receptor results in a decay of the evoked effect after its removal or inactivation.

Receptors comprise highly dynamic moieties, in terms of lateral mobility, membrane insertion and turnover. The concept of a receptor as a "mobile" or "floating'' intramembranous constituent has evolved along with the understanding of the general properties of cell surface proteins. Receptors, like other cell surface constituents, can perform complex protein–protein interactions, resulting in the internalization and intracellular redistribution of both, the receptor and the ligand.

Neuromodulators as well as neurotransmitters can influence the number and sensitivity of receptors. Under normal physiological conditions, the number of receptors is finite and the receptors are saturable. Depending on the concentration of neuroactive substances, the number can increase (up-regulation) or decrease (down-regulation).

For instance, the chronic presence of receptor antagonist can lead to an increase in receptor number, which is often accompanied by an increase in sensitivity to the specific agonist.

In contrast, receptor down-regulation occurs in response to continuous stimulation (for example due to the chronic administration of an agonist) and is frequently accompanied by desensitization.

The regulation of receptor sensitivity is orchestrated by several feedback mechanisms, which may involve the receptor itself (i.e. by allosteric mechanisms at the level of the receptor itself), or subsequent steps in the signal transduction pathways, which result in a reduction in receptor number, a loss of coupling to G proteins or phosphorylation of the receptor by protein kinases. The latter mechanism induces a conformational change in the receptor, which modifies its affinity to the ligand.

Desensitization can also occur after binding of the ligand to the receptor and subsequent internalization of the ligand–receptor complex by endocytosis. This internalization rapidly limits the duration of the signal of the "first messenger".

Once the endosome carrying the ligand–receptor complex is transported intracellularly, it becomes acidified by an ATP-dependent mechanism. This results in dissociation of the ligand from the receptor. The receptors are further targeted to the Golgi apparatus, where sorting takes place, with either partial degradation or recycling of the receptors to the cell membrane.

1.2.4 Transporters

In order to fuel cells with essential hydrophilic metabolites, transporter systems are required which overcome the phospholipid barrier of the plasma membrane. At nerve terminals, transporters have evolved for high-affinity uptake of neurotransmitters and some neuromodulators. These transporters control the temporal and spatial concentrations of extracellular transmitters via a rapid re-uptake into the nerve terminals. Equally, astrocytes participate in transporter-mediated uptake of neurotransmitters, serving a kind of servo-function in extracellular neurotransmitter cleansing. It is assumed that as much as 80% of released GABA is recaptured by GABA-transporters, indicating that transporter systems play an essential role in controlling the concentration of released neurotransmitters.

Termination of the activity of neuroactive substances is an essential prerequisite for controlled neuronal excitation. For instance, overexcitation of neurons induced by some excitatory neurotransmitter, which are not recaptured adequately, can exert severe damage to neurons (so-called "excitotoxity"). Glutamate, for example, is the most widespread excitatory neurotransmitter in the central nervous system and influences numerous neuronal networks. To limit receptor activation during signaling and to prevent overstimulation of glutamate receptors that would trigger excitotoxic mechanisms and cell death, extracellular concentration of glutamate is strictly controlled by transport systems from both neuronal and glial sites.

Further Reading

Barry, P.H., Lynch, J.W. (2005): Ligand-gated channels. *IEEE Trans Nanobiosci*. **4**: 70–80.

Bolander, F.F. (1989): *Molecular Endocrinology*. Academic Press, San Diego.

Civelli, O. (2005): GPCR deorpanization: the novel, the known and the unexpected neurotransmitter. *Trends Pharmacol. Sci*. **26**: 15–19.

Kennedy, M.B. (1992): Second messengers and neuronal function. In: *Effects and Mechanisms of Action, vol. 3*, ed. Negro-Vilar, A., Conn, P.M., CRC Press, Boca Raton, pp 120–142.

Keramidas, A., Moorehouse, A.J., Schofield, P.R., Barry, P.H. (2004): Ligand-gated ion channels: mechanisms underlying ion selectivity. *Prog. Biophys. Mol. Biol*. **86**: 161–204.

Kubo, Y., Tateyama, M. (2005): Towards a view of functioning dimeric metabotropic receptors. *Curr. Opin. Neurobiol*. **15**: 289–295.

LeBeau, F.F., El Manira, A., Griller, S. (2005): Tuning the network: modulation of neuronal microcircuits in the spinal cord and the hippocampus. *Trends Neurosci*. **28**: 552–561.

Mons, N., Cooper, D.M.F. (1995): Adenylate cyclases: critical foci in neuronal signaling. *Trends Neurosci*. **18**: 536–542.

Simon, M.I., Strathmann, M.P., Gautam, N. (1991): Diversity of G proteins in signal transduction. *Science* **252**: 802–808.

Sonders, M.S., Quick, M., Javitch, J.A. (2005): How did the neurotransmitter cross the bilayer? A closer view. *Curr. Opin. Neurobiol*. **15**: 296–304.

Vizi, E.S. (2000): Role of high-affinity receptors and membrane transporters in nonsynaptic communication and drug action in the central nervous system. *Pharmacol. Rev.* **52**: 63–89.

1.3 Distribution and Localization of Neurotransmitters and Neuromodulators

Neurotransmitters and neuromodulators reveal a spatially organized distribution in the central nervous system. For instance, glycine is the most abundant inhibitory neurotransmitter in the spinal cord, norepinephrine is focally synthesized in the locus coeruleus of the brain stem and neuropeptides are most abundant in hypothalamic nuclei. When describing neurotransmitter distribution, one has to take into account that axonal transport can carry neuroactive substances anterogradually to reach distant targets. Consequently, high concentrations of neuroactive substances can be found in nerve terminals, well separated from the source of their biosynthesis. Thus, in constructing a descriptive map of neurotransmitter distribution, it is important to differentiate between soma-bound and terminal localization.

Additional techniques, e.g. *in situ* hybridization, provide further insights into a descriptive neurotransmitter-based map of the brain. This technique has the limitation that it provides signals mainly from the cell soma where the highest concentration of cRNA occurs and gives no information about terminal localizations.

The coexistence of neurotransmitter and neuropeptides as originally described by Hökfelt adds an additional level of complexity to a descriptive approach of local maps of neuroactive substances.

The fact that neurotransmitter and neuropeptide coexist in single neurons necessitates the presence of pre- and postsynaptic receptors for both classes of neuroactive substances to induce their effects. In describing the functional relationships of both neuroactive substances, Lundberg and Hökfelt (1985) summarized possible effects which arise from their coexistence:

- A neurotransmitter acts on one type of postsynaptic receptor.
- A neurotransmitter acts on multiple types of postsynaptic receptors.
- A neurotransmitter also acts on presynaptic receptors to affect its own release.

Nerve terminals carry different neuroactive substances, which are stored in small vesicles (neurotransmitters) and in large dense-cored vesicles (neurotransmitter and neuromodulator). If these vesicles are co-released, they can interact in the following ways:

- inhibition of the release of the neuromodulator by the neurotransmitter via presynaptic action;
- inhibition or enhancement of the release of the neurotransmitter by the neuromodulator via presynaptic action;
- modulation of the activity of the postsynaptic neuron via postsynaptic receptors by the neuromodulator.

In some cases, coexpressing neurons exhibit specific projection patterns: this is the case in the basal ganglia.

Gibbins (1989) summarized some principles in neurotransmitter architecture which result from the coexistence of variable neuroactive substances:

- A target cell can be innervated by different types of neurons, which express the same neuroactive substances.
- Functionally closely related neurons can express different neuropeptides.
- Neurons can express biochemically related neuroactive substances, since different neuromodulators can be synthesized from a common precursor.
- Although some different members of a neuromodulator family are generated by differential processing of a common precursor, not all active forms coexist in a single neuron.

Further Reading

Fuxe, K., Grillner, S., Hökfelt, T., Ölson, L., Agnati, L.F. (eds) (1998): *Towards an Understanding of Integrative Brain Functions*. Elsevier, Amsterdam.

Gibbins, I.L. (1989): Co-existence and co-function. In: *The Comparative Physiology of Regulatory Peptides*, ed. Holgren, S., Chapman & Hall, New York, pp 308–343.

Lundberg, J.M., Höckfelt, T. (1985): Coexistence of peptides and classical neurotransmitters. In: *Neurotransmitters in Action*, ed. Bousfield, D., Elsevier, New York, pp 104–118.

1.4 The Blood–Brain Barrier

Neuroactive substances are not exclusively expressed in the central nervous system, but are also common in peripheral tissues. One prominent example is represented by the gastro-intestinal peptides which possess a chimeric function as enteric hormones and neuromodulators. This dual function requires mechanisms that restrict or facilitate the entry of neuroactive substances from the periphery into the central nervous system. The highly regulated system for controlled exchange of neurobiologically relevant substances resides at the border between the general blood circulation and the brain parenchyma in the form of the blood–brain barrier.

The existence of a functional barrier between the blood and the brain was first demonstrated by Paul Ehrlich at the beginning of the 20th century. He observed that, when injected into the circulation, dyes like methylene blue stained the parenchyma of most organs of the body but not the brain. Injection of dyes into the cerebrospinal fluid, however, led to a staining of the brain, but not the body. These experiments were the first demonstration that a barrier between the blood and the brain exists and that this barrier blocks all free transport, regardless of the direction from which the barrier is approached by the substance.

While the capillaries of most non-neural tissues are permeable to molecules smaller than 30 kDa, capillaries in the mammalian central nervous system show a high degree of selectivity concerning permeation coefficients. The permeability of substances into brain is governed by specific chemical properties of the molecules. Although the term blood–brain barrier implies a general impermeability, it is best considered as selectively permeable. The blood–brain barrier

does not provide a passive barrier between the brain and the body, but constitutes a kind of active filter that regulates the flow of substances between both compartments by structural and metabolic elements. The most effective structure that restricts the free access of dissolved hyrophilic molecules into the brain is the high-resistance tight junction that seals the interendothelial cleft. The active components, which regulate exchange in both directions, are specific transporters and enzymes which, for example, include amino acid transporters, glucose transporters and transporters for essential elements like the transferrin transporter for iron.

Among the neuroactive substance that are transported through the barrier are some neuromodulators and their analogs. Lipid-soluble substances like alcohol and steroids can penetrate the endothelial barrier freely.

A second barrier exists at the circumventricular organs (CVOs). The CVOs are located close to the ventricles of the brain and they include the *chorioid plexuses,* the median eminence, the *organum vasculosum* of the *lamina terminalis,* the subfornical organ, the subcommissural organ, the area postrema, the neurohypophysis and the pineal gland.

In contrast to the common brain parenchyma, these structures are equipped with leaky fenestrated capillaries, which allow the transfer of substances through the endothelium, and a tight junction between the covering ependymal cells blocks free passage into the cerebro-spinal fluid.

In general, substances can cross the blood–brain barrier by four different pathways:

- penetration via pores and trancytosis;
- transmembrane diffusion;
- carrier-mediated mechanisms and transporters;
- retrograde neuronal transport, so by-passing the blood–brain barrier.

Some chemical properties enable substances to cross the blood–brain barrier. The properties which affect permeability include lipid solubility, molecular weight and the ability to form electro-neutral complexes. Some neuroactive substances, like α-MSH, are known to cross the blood–brain barrier by this mechanism.

The most convenient route for molecules to cross the blood–brain barrier is by making use of specific receptor-mediated mechanisms or by transporters. In order to do this, the substance has first to bind to a receptor on the endothelium; and second, the formed ligand–receptor complex has to be internalized and transferred via endosomes into the endothelial cytoplasm. Finally, the ligand–receptor complex has to be degraded and the ligand can then be released by exocytosis on the opposite side of the barrier.

Some peptides, like MSH, can cross the barrier primarily by transmembrane diffusion, a non-saturable mechanism largely dependent on the lipid solubility of the peptide. Other neuroactive substances utilize highly specific transporters for crossing the blood–brain barrier, which can operate unidirectionally or bidirectionally. The unidirectional transport can be from the blood to brain parenchy-

ma, as is the case for Leu-enkephalin, or in opposite direction, as is the case for the neuropeptides Tyr-MIF-1 and Met-enkephalin. This latter seems to depend on the peptide transport system-1 (PTS-1), which carries small peptides with an N-terminal tyrosine from the brain to the blood. This system preferentially transports two peptides, namely Tyr-MIF-1 and Met-enkephalin.

Bi-directional transport has been described, for example, for the gonadotropin-releasing hormone GnRH.

The transport systems are highly specific and each system carries its own complement of substrates. Another route of entry to the brain parenchyma utilized by some neurotoxins and viruses is to by-pass the barrier by retrograde transport from peripheral nerve endings.

Specific transport systems, which carry peptides through the blood–brain barrier, have important neuro-regulatory functions. Pharmacological manipulation of the blood–brain barrier can therefore provide therapeutic strategies for the efficient delivery of drugs which are impermeable under normal conditions.

Further Reading

Banks, W.A., Kastin, A.J. (1985): Permeability of the blood–brain barrier to neuropeptides: the case for penetration. *Psychoneuroendocrinology* **10**: 385–399.

Banks, W.A., Kastin, A.J., Fischman, A.J., Coy, D.H., Strauss, S.L. (1986): Carrier-mediated transport of enkephalins and *N*-Tyr-MIF-1 across blood–brain barrier. *Am. J. Physiol.* **251**: E477–E482.

Begley, D.J. (1994): Peptides and the blood–brain barrier: the status of understanding. *Ann. N.Y. Acad. Sci.* **739**: 89–100.

Dermietzel, R., Krause, D. (1992): Molecular anatomy of the blood–brain barrier as defined by immunocytochemistry. *Int. Rev. Cytol.* **127**: 57–109.

Dermietzel, R., Spray, D.C., Nedergaard, M. (eds) (2006): *Blood–Brain Barrier: From Ontogeny to Artificial Membranes*. Wiley–VHC, Weinheim.

Greenwood, J., Begley, D.J., Segal, M.B. (1995): *New Concepts of a Blood–Brain Barrier*. Plenum Press, New York.

Jaspan, J.B., Bank, W.A., Kastin, A.J. (1994): Study of passage of peptides across the blood–brain barrier: biological effects of cyclo (His-Pro) after intravenous and oral administration. *Ann. N.Y. Acad. Sci.* **739**: 101–107.

Nag, S. (ed) (2003): *The Blood–Brain Barrier*. Humana Press, Totowa, N.J.

Pardridge, W.M. (2001): *Brain Drug Targetting. The Future of Brain Blood Development*. Cambridge University Press, Cambridge.

Pardridge, W.M. (2000): Receptor-mediated peptide transport through the blood–brain barrier. *Endocrinol. Rev.* **7**: 314–330.

1.5
Volume Transmission and Wiring Transmission

Intercellular communication in the brain can be grouped in two broad classes, as proposed by Agnati and coworkers (1986). Based on some general features of signal transmission, the authors differentiate between wiring transmission (WT) and volume transmission (VT). Transmission by WT is defined as a mode for intercellular communication, which is mediated via a relatively constrained

cellular chain (wire), while transmission by VT consists of a three-dimensional diffusion of signals in the extracellular fluid for distances larger than the synaptic cleft. Transmission by WT thus exhibits (like classic synaptic transmission) a one-to-one ratio with respect to the number of signal source structures and the number of signal targets, whereas transmission by VT shows a one-to-many ratio. With respect to the neuroactive substances and their function in brain tissues, as described above, neurotransmitters as well as neuromodulators convey both modes of function. In fact, it is now generally believed that there exists in the brain some kind of non-synaptic, hormone-like, modulatory transmission besides synaptic transmission. This concept is supported by recent findings of the neurotrophic effects of some neuropeptides and neurotrophins. Furthermore, data on gaseous transmitters like NO have given strong support to this view. For instance NO, once released, can affect the electro-metabolic state of numerous neurons not in synaptic contact with the neuron source of the signal. In addition to the classic mode of wiring transmission in the form of synaptic communication as described above, we will give here a brief account of the basic elements of transmission by VT, as summarized recently by Zoti et al. (1998).

The general features of VT are:

- a cell source of the VT signal, neuronal or non-neuronal, from which a signal molecule can be released into the extracellular cerebral fluid (ECF);
- a VT signal diffusing in the ECF for a distance larger than the synaptic cleft;
- communication trails in the extracellular space in form of preferred diffusion pathways;
- a cell target of the VT signal, that is, a cell possessing molecules capable of detecting and decoding the message.

Model systems for VT are the highly divergent monoaminergic pathways of the brain, e.g. the dopaminergic mesostriatal system. Both morphological and functional evidence indicates that dopamine acts as a VT signal in the striatum.

The existence of a functionally coupled syncytium provided by astrocytes and postnatal neurons has led to an extension of the concept of VT transmission (Dermietzel 1998). If the route of VT is generally regarded as through extracellular cerebral fluid, primarily via diffusion, then gap junctions, which represents the structural correlate of electrical synapses, may provide a second highly regulated route of VT which can be defined as intercellular as opposed to the extracellular VT. This intercellular VT could serve as a route parallel to the extracellular VT, allowing coordinated responses of functionally coupled neurons or glial compartments. In fact, recent evidence indicates that neurotransmitter coupling via gap junctions exists. For instance, the inhibitory neurotransmitter glycine can be provided by glycinergic amacrine cells to cone bipolar cells in the retina (Vaney et al. 1998). Although evidence for intercellular volume transmission of neurotransmitters is still circumstantial, one has to expect new concepts in transmitter trafficking and function in the near future.

Further Reading

Agnati, L. F., Fuxe, K., Zoli, M., Zini, I., Toffano, G., Ferraguti, A. (1986): A correlation analysis of the regional distribution of central enkephalin and beta-endorphin immunoreactive terminals and of opiate receptors in adult and old mice rats. Evidence for the existence of two main types of communication in the central nervous system; the volume transmission and the wiring transmission. *Acta Physiol. Scand*. **128**: 201–207.

Alexander, D. B., Goldberg, G. S. (2003): Transfer of biologically important molecules between cells through gap junction channels. *Curr. Med. Chem*. **10**: 2045–2058.

Dermietzel, R. (1998): Gap junction wiring: a new principle in cell-to-cell communication in the nervous system. *Brain Res. Rev.* **26**: 176–183.

Vaney, D. I., Nelson, J. C., Pow, D. V. (1998): Neurotransmitter coupling through gap junctions in the retina. *J. Neurosci*. **18**: 10594–10602.

Zoti, M., Torri, C., Ferrari, R., Jansson, A., Zini, I., Fuxe, K., Agnati, L. F. (1998): The emergence of the volume transmission concept. *Brain Res. Rev.* **26**: 136–147.