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Several types of chemical messengers are employed by the nervous system for local or more diffuse signaling. Among these the peptides are the most diverse in structure and function. In nervous tissues they are typically produced by neurons or neurosecretory cells, and can therefore be specified as **neuropeptides** or **peptide hormones**, respectively. Additionally, many peptides are produced by endocrine cells or other cell types in different locations. In fact, the same peptide can be expressed by all these cell types in a given animal. Neuropeptides and peptide hormones are ubiquitous in the nervous and endocrine systems of all metazoans. Not only do these peptides exist in a large number of distinct molecular forms, they are also very diverse in their actions and signaling mechanisms. Thus, in a single animal species there may be more than a 100 different neuropeptides and peptide hormones (and their receptors), and each can have multiple functions. The peptides are encoded in the genome as parts of larger precursor proteins, referred to as **prepropeptides**. This direct coding means that when whole animal genomes have been sequenced the total inventory of neuropeptides and peptide hormones can be predicted. Such sequence data can also be used for analysis of neuropeptide evolution and show that some

peptide sequences are well conserved across a broad range of species, whereas others display considerable variability and some are even unique to certain taxa. In this chapter we show that in some cases not only peptide sequences, but also the receptor structures and mechanisms of action and physiological functions can be conserved from invertebrates to vertebrates.

Classically the peptides were investigated in neurosecretory and endocrine systems and many important roles of peptides as hormones could be established. Peptide hormones often regulate basic mechanisms in development, growth, reproduction, and metabolism and thus feature as critical players in the homeostasis of the organism. Several of these peptidergic systems are targets of therapeutics due to their importance in, for instance, diabetes, growth, pain regulation, and mental health.

The neuropeptides are produced by interneurons, sensory neurons, and efferent neurons such as motoneurons. Their actions are spatially restricted and depend on the morphology of the neurons releasing them, and distribution of cognate peptide receptors. Thus, neuropeptide function in neuronal circuits can be either as local or more global activators or modulators depending on the extent of their neuronal arborizations. In some cases assemblies of peptidergic neurons form neuropeptide systems with specific regulatory functions. In this chapter we will use the terms **neuropeptide** and **peptidergic neuron** for cases where peptides are produced by a neuron to act on adjacent neurons or other cell types. This signaling can be at synapses or nonsynaptic by means of volume transmission (see Chap. 8). In contrast, we use **peptide hormone** and **neurosecretory cell** (or when appropriate **endocrine cell**) where peptides are released into the circulation or body cavity and act on distant target cells with appropriate receptors.

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We show here that peptides have a huge spectrum of functions both in central neuronal circuits and as circulating hormones. We provide details on the organization and function of peptidergic neurons and neurosecretory cells, as well as other hormonal systems signaling with peptides, both in invertebrates and vertebrates. The hypothalamus-pituitary system and its analogs in insects are used to illustrate neuroendocrine regulatory systems utilizing peptides. Furthermore, we include some comparisons between distantly related animal groups to highlight the ancient evolutionary origins of several peptide signaling systems.

11.1 Neuropeptides, Peptide Hormones, and Their Receptors

The earliest neuropeptides to be sequenced and synthesized were purified from mammals, namely oxytocin and vasopressin from the posterior pituitary of cattle in the 1940s. For these achievements Vincent du Vigneaud (1901–1978) was awarded the Nobel Prize for Chemistry in 1955. With improved techniques further peptides were discovered in the brains of pig and sheep in the late 1960s. For instance, the hypothalamic peptides thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone (GnRH) were characterized independently in the laboratories of Andrew Schally and Roger Guillemin, who both received the Nobel Prize for Medicine in 1977. In the years that followed, numerous peptides were sequenced from a broad range of animals, including invertebrates. Gradually molecular biology techniques allowed discovery of peptides deduced from DNA sequences, thereby enabling identification of peptides in an even wider range of species.

11.1.1 Neuropeptides Are Derived from Larger Prepropeptides, and Generally Act on GPCRs

Neuropeptides and peptide hormones are chains of amino acids (AA) that vary in length from 3 to more than 80 AA, and may act as monomers or oligomers. They are derived from precursor proteins,

prepropeptides, or preprohormones, that are generated by regular protein synthesis (Fig. 11.1). Peptides are thus encoded in the genome and their biosynthesis requires gene transcription and subsequent translation by ribosomes. **Prepropeptides** typically consist of about 100–300 amino acids. After removal of the signal peptide by signal peptidase they are called **propeptides**. These are processed further by proteolytic enzymes acting at specific cleavage sites to release the biologically active **peptides** (Fig. 11.1). After further posttranslational processing steps the mature neuropeptides are stored in vesicles and ready for release (Fig. 11.2). Usually peptides are stored in **large dense core vesicles**, located away from the synaptic zone (Figs 11.2a, b), and a stronger depolarization is required for peptide release than for classical neurotransmitters (Fig. 11.2b). Large dense core vesicles have diameters of around 200 nm, as opposed to the clear vesicles of classical neurotransmitters with diameters of 35–45 nm. Prepropeptides often give rise to more than one peptide. In some cases, especially in invertebrates, multiple copies of identical peptides can be liberated; in other cases the precursor contains several copies of structurally related or unrelated peptides. After release neuropeptides and peptide hormones commonly act on G protein-coupled receptors (GPCRs) of different types. For a given peptide there may be more than one GPCR, especially in vertebrates. Some peptide hormones, like insulin-like peptides, activate receptor tyrosine kinases, others like atrial natriuretic peptide act on membrane guanylyl cyclase, and in mollusks one peptide (FMRFamide) is known to activate a ligand-gated ion channel. Activation of a GPCR triggers a cascade of events, usually involving second messengers, that via protein kinases produce various cellular responses such as alteration of ion channel and membrane properties, or long-term alterations that involve gene transcription and protein synthesis (see Chap. 6). After release the action of neuropeptides is limited by dilution over distance and by enzymatic inactivation by ubiquitous extracellular peptidases. Another way to terminate peptide action is by desensitization of the GPCRs, and upon sustained activation the receptors even internalize from their cell surface locations.

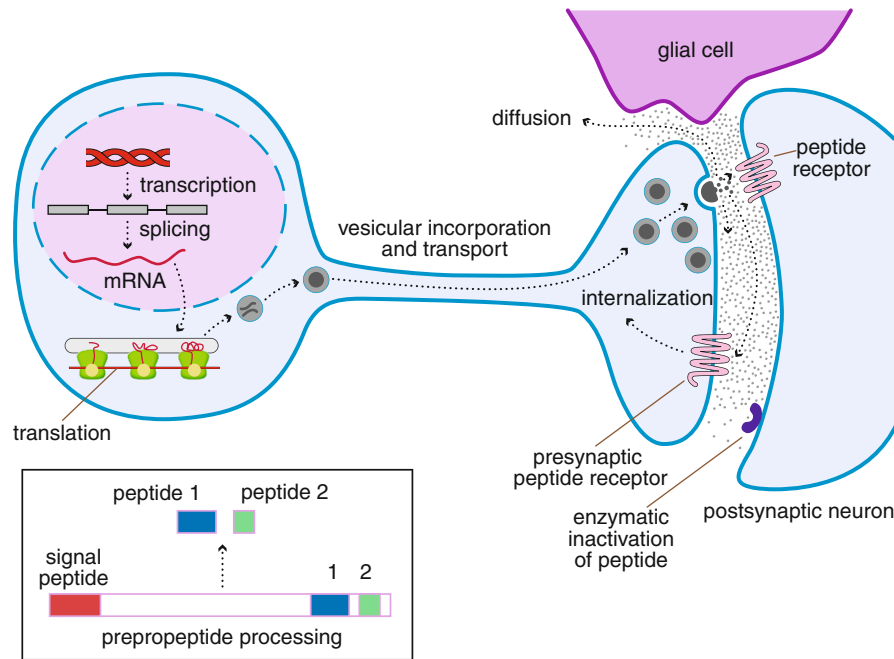


Fig. 11.1 A peptidergic neuron. Peptide biosynthesis starts with gene transcription and splicing, followed by mRNA translation at ribosomes and production of a prepropeptide (precursor). The precursor is processed into bioactive peptides (peptide 1 and 2) after incorporation into dense core vesicles. These are transported down the axon and the vesicles with mature peptides are stored in the axon termination (disproportionately enlarged in the

figure). After release, peptides act on postsynaptic receptors (GPCRs). Peptide spillover is monitored by presynaptic receptors that can regulate release of the peptide or the neuron's classical neuro-transmitter. Enzymatic inactivation of peptide occurs in the synaptic region by means of specific membrane-bound peptidases. Another factor for diminishing peptide action is diffusion away from the receptor sites (Modified from Squire 2003 [1])

11.2 Morphology and Function of Neurosecretory Cells and Peptidergic Neurons

11.2.1 Peptides Released from Neurohemal Organs Act on Distant Targets via the Circulation

Peptidergic neurosecretory cells are organized in a rather conserved fashion from arthropods to mammals. They commonly have large cell bodies (somata), where peptide biosynthesis occurs, and axons terminating in storage and release sites with access to the circulation. Often such neurosecretory axon terminals are located in specific structures called **neurohemal organs**, where several types of neurosecretory cell axons coalesce. In invertebrates it is also common that neurosecretory axon terminations are diffusely distributed on the outer surface of nerves, or even of the brain or ventral ganglia, along the intestine, or on body wall

muscle. These diffuse release sites are referred to as **neurohemal areas**. The targets of peptide hormones are reached via the circulation. Therefore, potentially, all cells in the body might respond to neurohormones, and target specificity is reached by the selective expression of hormone receptors in the target organs. At least in vertebrates the target organs of many centrally released hormones produce their own hormones that often elicit both local physiological effects and feedback on the central systems.

In many invertebrate taxa (e.g., cnidarians, flatworms, and nematodes) there is no bona fide circulatory system. Animals of these taxa have peptide-producing neurons that probably release their messengers mostly in a paracrine fashion, i.e., the peptide is released nonsynaptically to act on adjacent neighbor cells. As an example, peptide-producing neurons of the nematode *Caenorhabditis elegans* are commonly sensory-, motor-, or interneurons and part of the circuitry of the nervous system. Nevertheless, these types of neurons

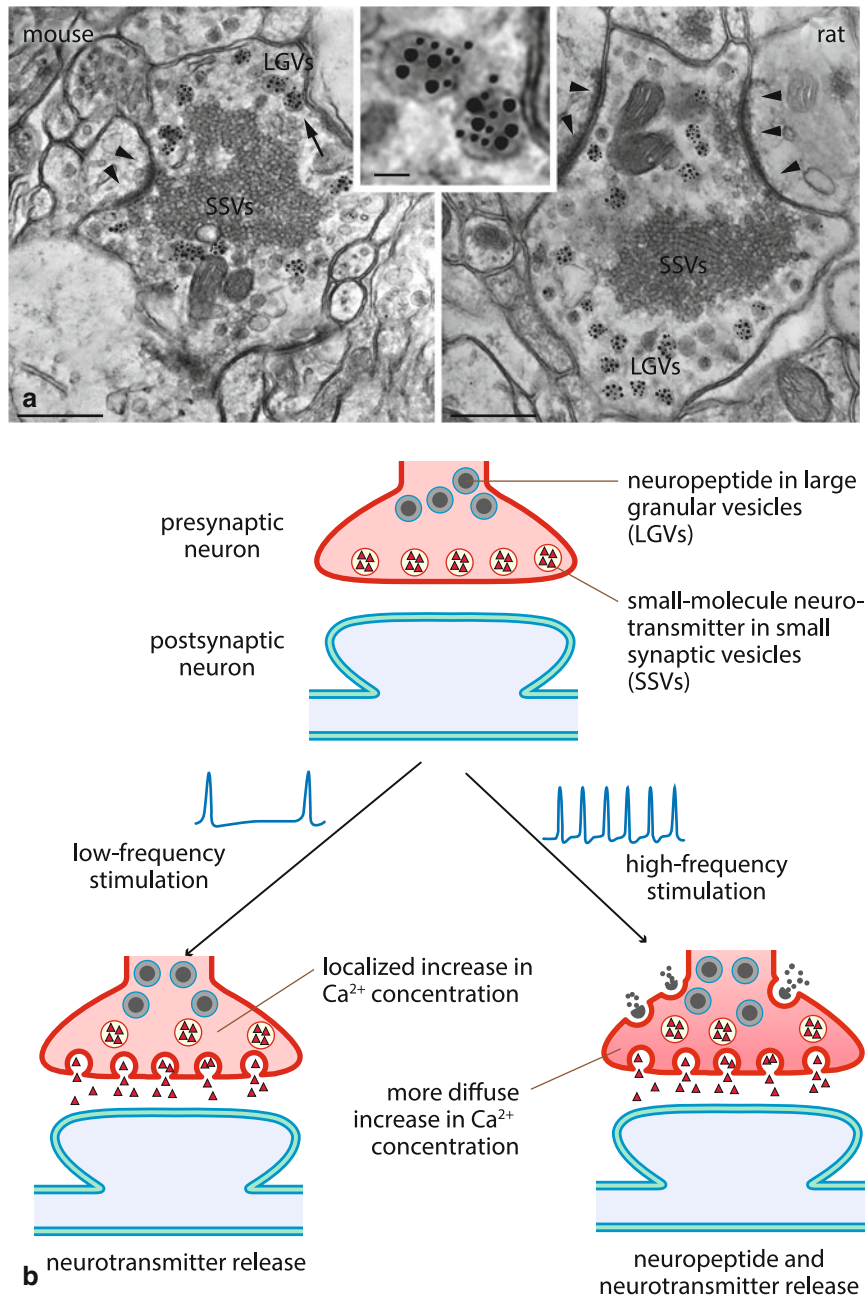


Fig. 11.2 Neuropeptides at the synapse. (a) Electron microscopic image of neuron terminations in substantia gelatinosa in spinal cord of mouse and rat with small molecule transmitter in small clear-core vesicles (small synaptic vesicles, SSVs) and two different neuropeptides in dense-core vesicles (large granular vesicles, LGVs). The peptides, calcitonin gene-related peptide (CGRP) and substance P were detected with immunogold labeling (two sizes of gold particles, shown in *inset*) in the same vesicles. The small synaptic vesicles are stored close to the active zone (*arrowheads*), whereas the dense core vesicles are located away from this, in the perisynaptic area (*arrow*) (From

Salio et al. [2] with permission from A. Merighi and Springer-Verlag). (b) Depolarization and release of small classical neurotransmitter and colocalized neuropeptide. A neuropeptide is stored in large dense vesicles that are localized perisynaptically and a small molecule (classical) transmitter in small synaptic vesicles in the active zone. These are affected differently by low- and high-frequency stimulation of the axon termination. The synaptic vesicles fuse with presynaptic membrane at low-frequency stimulus and localized calcium influx, whereas the peptidergic vesicles require more massive increase of calcium for release perisynaptically

may produce peptides that in other taxa function as peptide hormones, for instance, insulin-like peptides.

11.2.2 Peptides in the CNS Often Act Together with Other Neurotransmitters as Modulatory Cotransmitters

In the CNS there is a large variety of interneurons and sensory neurons that utilize neuropeptides for signaling or signal modulation within central circuits. These commonly have relatively small cell bodies and produce and store smaller amounts of peptides than neurosecretory cells. Target specificity is secured not only by the expression of the right GPCR in the target cell (as for neurohormones), but also by the specific branching patterns of the peptidergic neurons. The peptidergic interneurons display vast variations in morphology and their branching can be either restricted to smaller portions of the CNS or be quite extensive, suggesting more global influence. It is quite common that interneurons colocalize neuropeptides with either of a range of classical neurotransmitters, such as acetylcholine, glutamate, GABA, or monoamines. Thus, the neuropeptides may act as **cotransmitters** that modulate fast neurotransmission. Different interneurons use neuropeptides in two major forms of neuromodulation: intrinsic or extrinsic modulation. **Intrinsic modulation** is when the neuropeptide is released synaptically or nonsynaptically by a neuron within the circuit that is modulated. **Extrinsic modulation** is by neuropeptide released by neurons from outside the circuit.

11.3 Organization of Neuroendocrine and Peptidergic Systems in Invertebrates

11.3.1 From Early Surgical Experiments Demonstrating Hormones to Modern Molecular Approaches to Study Peptide Systems

The first demonstration of an invertebrate hormone produced in the nervous tissue was in experiments by Stefan Kopec (1888–1941) with the gypsy moth already in the early 1920s. In this study, removal of the brain within a certain time window in late larval

development led to a failure to pupate and timely reimplantation of a brain anywhere in the organism triggered the pupariation process (i.e., the onset of prepupal development). From these results it could be concluded that the brain secretes a pupariation-inducing hormone. This hormone, later designated **prothoracicotropic hormone (PTTH)** was identified and chemically elucidated as a large dimeric peptide produced by brain neurosecretory cells more than 60 years after Kopec's study. Interestingly, it was found that the PTTH receptor is a membrane tyrosine kinase.

Neurosecretory cells were first visualized with histochemical staining techniques that did not identify the specific hormones, but led to anatomical descriptions of some major neuroendocrine systems. Mollusks, decapod crustaceans, and larger insects were commonly used for these earlier studies. The anatomical findings made it possible to perform more precise extirpation and replacement experiments where bioactivity of neurosecretory cells could be determined. Once peptide sequences were elucidated they could be synthesized for bioassays and for production of antisera to be used for immunocytochemical identification of the cells producing them. Peptide immunocytochemistry provided a great step forward in understanding the organization of neurosecretory systems and also revealed that other types of neurons or endocrine cells produce peptides.

Today there is a huge array of molecular genetic techniques enabling the scientist to identify and specifically interfere with various components of neuropeptide and peptide hormone signaling. Over the last 10 years, the entire genomes of many species have been sequenced enabling comprehensive identification of the genes encoding neuropeptide precursors and GPCRs and many other proteins involved in peptide signaling. This has been a tremendous help for studies of the evolution of neuropeptide signaling, and has also provided directions for functional studies. In addition, tools developed for proteomics to identify and quantify proteins (e.g., MALDI-TOF mass spectrometry) have allowed identification of peptides in increasingly smaller samples down to single neurons.

It appears as if neuropeptide signaling occurs in all animal phyla alongside that conducted by small molecule neurotransmitters of different types. We focus here on a few model invertebrates, although peptide signaling has been studied also in cnidarians, flatworms, and other taxa.

11.3.2 Peptidergic Systems in Mollusks

Recently, the genome of the limpet *Lottia gigantea* was sequenced and neuropeptide-encoding genes annotated. This small genome contains 59 genes that were predicted to encode neuropeptide precursors and 8 that can give rise to insulin-like peptides or cysteine-knot protein hormones. Many of these peptides are probably ancestrally related to ones identified in insects and other invertebrates described below. Unfortunately, *L. gigantea* has not been utilized in neurobiological studies. For the two major model mollusks, the pond snail *Lymnaea stagnalis* and the marine slug *Aplysia californica*, the identification of neuropeptide precursor genes and expressed neuropeptides is mostly based on traditional cloning and biochemical techniques. More than 80 peptides have been identified this way in *Aplysia* and about 75 in *Lymnaea*, and several additional ones were predicted in connection with the *L. gigantea* genome analysis. However, only few peptide GPCRs are known so far in mollusks. Most of our knowledge on molluskan neuroendocrinology and neuropeptide function is based on research on these two snails. The snail neuroendocrine system is easily accessible and mostly composed of large individually identifiable neurons with cell bodies in constant positions. Many well defined and easily studied physiological processes were found to be under hormonal control in snails, including growth, water and ion balance, metabolism, and reproduction, as well as a number of stereotypic behaviors such as feeding, copulation, and egg laying. Here we shall look at some neurosecretory cell systems of the central ganglia in *Lymnaea* and the abdominal ganglion of *Aplysia* that have predominated in classical studies.

In *Lymnaea* there are 18 different types of neurosecretory cells within the central ganglia as distinguishable by classical staining techniques. Some cells are pigmented and named after the pigment color. Many of the snail neurosecretory cells have two sets of release sites: neurohemal areas and directly innervated peripheral targets, such as heart, genital tract, intestine, and kidney. The neurohemal areas are distributed over the surface of different nerve roots of the ganglia (Fig. 11.3). One set of cells is termed **yellow cells**. These are located in the parietal and visceral ganglia and send processes both to neurohemal areas and to direct innervation of the kidney, urether, and some other organs (Fig. 11.3). The yellow cells produce a peptide termed

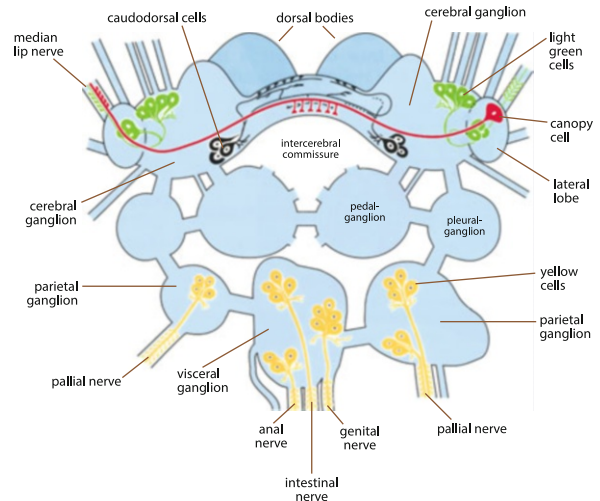


Fig. 11.3 Molluskan neuroendocrine systems. Neurosecretory cells in the CNS of the pond snail *Lymnaea stagnalis*. Four main types of neurosecretory cells are shown here: the caudodorsal cells (black), the light green cells (green), and the yellow cells (yellow), and a single so-called canopy cell (red) is drawn to the right. Note neurohemal release sites in several of the nerve roots and the neurohemal area of the intercerebral commissure. For clarity the number of cells shown here is not accurate for any of the cell types (Redrawn from Kobayashi [3] with permission)

sodium influx stimulating peptide that is important in water and ion homeostasis. The **light green cells** are located in the cerebral ganglia, and with terminations in neurohemal areas in the median lip nerves (Fig. 11.3). These cells produce insulin-like peptides and are known to be involved in growth control.

11.3.2.1 Peptides Control Metabolic Physiology and Complex Behavioral Sequences, Like Egg-Laying Behavior

One distinct neurohemal organ in *Lymnaea* is the so-called **intercerebral commissure** located in the cerebral ganglion (Fig. 11.3). This neurohemal organ is innervated by the approximately 100 peptidergic **caudodorsal cells** (CDCs) that also extend axons into cerebral ganglion neuropils. The CDCs produce a peptide precursor that contains egg-laying hormone and several smaller peptides, all of which contribute to initiation and integration of processes connected to egg-laying behavior. In *Aplysia* the hormonal cascade that initiates and orchestrates egg-laying behavior has been studied in detail [4]. Egg laying lasts more than an hour and involves specific head movements, increase of respiratory pumping, and inhibition of locomotion and feeding, and eventually release of eggs. Initiation

of egg laying is preceded by extended bursts of firing of two clusters of so-called **bag cells** located around nerve roots anteriorly in the abdominal ganglion. The bag cells produce a peptide precursor that contains egg-laying hormone (ELH) and several smaller peptides, such as α , β , and γ -bag cell peptides (BCPs), all of which are co-released. The bag cells are multipolar and supply branches to neurohemal areas in the abdominal nerves as well as to cell bodies of certain neurons inside the abdominal ganglion. When activated, the bag cells release massive amounts of ELH and BCPs in a coordinated fashion. The actions of these peptides are complex: ELH and all three BCPs act nonsynaptically on neurons in the abdominal ganglion and ELH acts as a hormone to induce ovulation. The ELH gene-derived peptides thus act at different levels: in the CNS they induce prolonged modulation of circuits controlling egg-laying behavior, leading to inhibition of feeding and locomotion and stimulation of respiratory pumping, whereas in the periphery the peptides act on muscles of the hermaphrodite gland and heart (see Chap. 23).

11.3.3 Peptidergic Systems in Crustaceans

The only report so far on a genome-wide sequencing of crustacean neuropeptide genes is from the water flea *Daphnia pulex*. According to this there are 43 neuropeptide precursor genes that encode 73 predicted peptides. Most of these are more related to neuropeptides found in insects than those found in decapod crustaceans. Expressed sequence tag data analysis combined with mass spectrometry of expressed peptides has indicated quite a large number of neuropeptides in some other crustaceans. For example, in the lobster *Homarus americanus* 84 peptides representing 15 or more precursor genes were identified. Peptide GPCRs have, however, not yet been identified in crustaceans, although four putative insulin receptors were annotated in *Daphnia*.

11.3.3.1 Peptide Hormones Produced by Neurosecretory Cells in the Eyestalk Control Many Physiological Functions

In decapod crustaceans, such as crabs, crayfishes, and lobsters, the predominant cerebral neuroendocrine system is that of the eyestalks, the X-organ-sinus gland

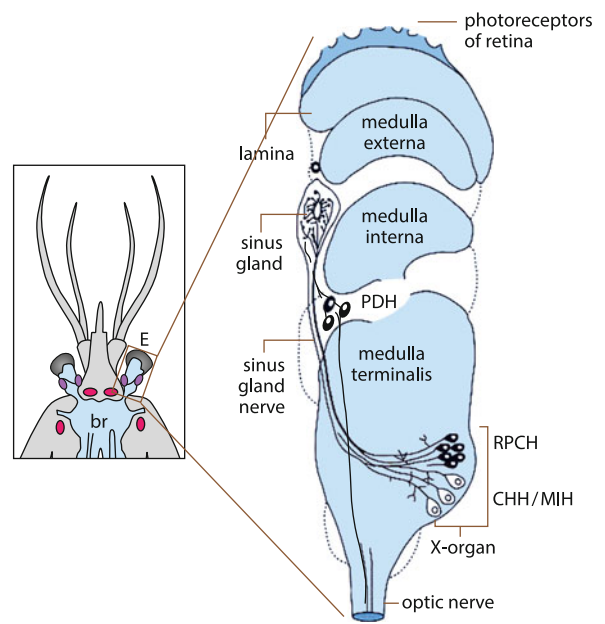


Fig. 11.4 A crustacean neuroendocrine system. Neuroendocrine system associated with the optic lobes of the shore crab *Carcinus maenas*: the X-organ–sinus gland. Neurosecretory cells producing crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH), and red pigment-concentrating hormone (RPCH) are located in the X-organ and send axons via the sinus gland nerve to the sinus gland. In the center of the sinus gland there is a hemolymph lacuna (part of the ophthalmic artery). Another set of neurosecretory cells located near the medulla interna produce pigment-dispersing hormone (PDH). Also these have axon terminations in the sinus gland. The inset shows the head with position of eyestalks (E) and brain (br) (Redrawn from Hartenstein [5] with permission)

system shown in Fig. 11.4. There are, however, also thoracic and abdominal neurohemal organs or areas (Fig. 11.5): the pericardial organ (PO), postcommisural organs, as well as the surface of ganglia and several nerves. The PO is situated within the pericardial cavity on either side of the heart (Fig. 11.5). Finally, the intestine contains large numbers of peptide-producing endocrine cells.

The neurosecretory cells of the X-organ are localized in the so-called medulla terminalis of the eyestalk neuropils (Fig. 11.4). Their axons terminate in the sinus gland, a neurohemal organ adjacent to the ophthalmic artery in the distal eyestalk. Different X-organ cells produce the peptide hormones red pigment-concentrating hormone (RPCH) and members of the crustacean hyperglycemic hormone/molt inhibiting hormone (CHH/MIH) family.

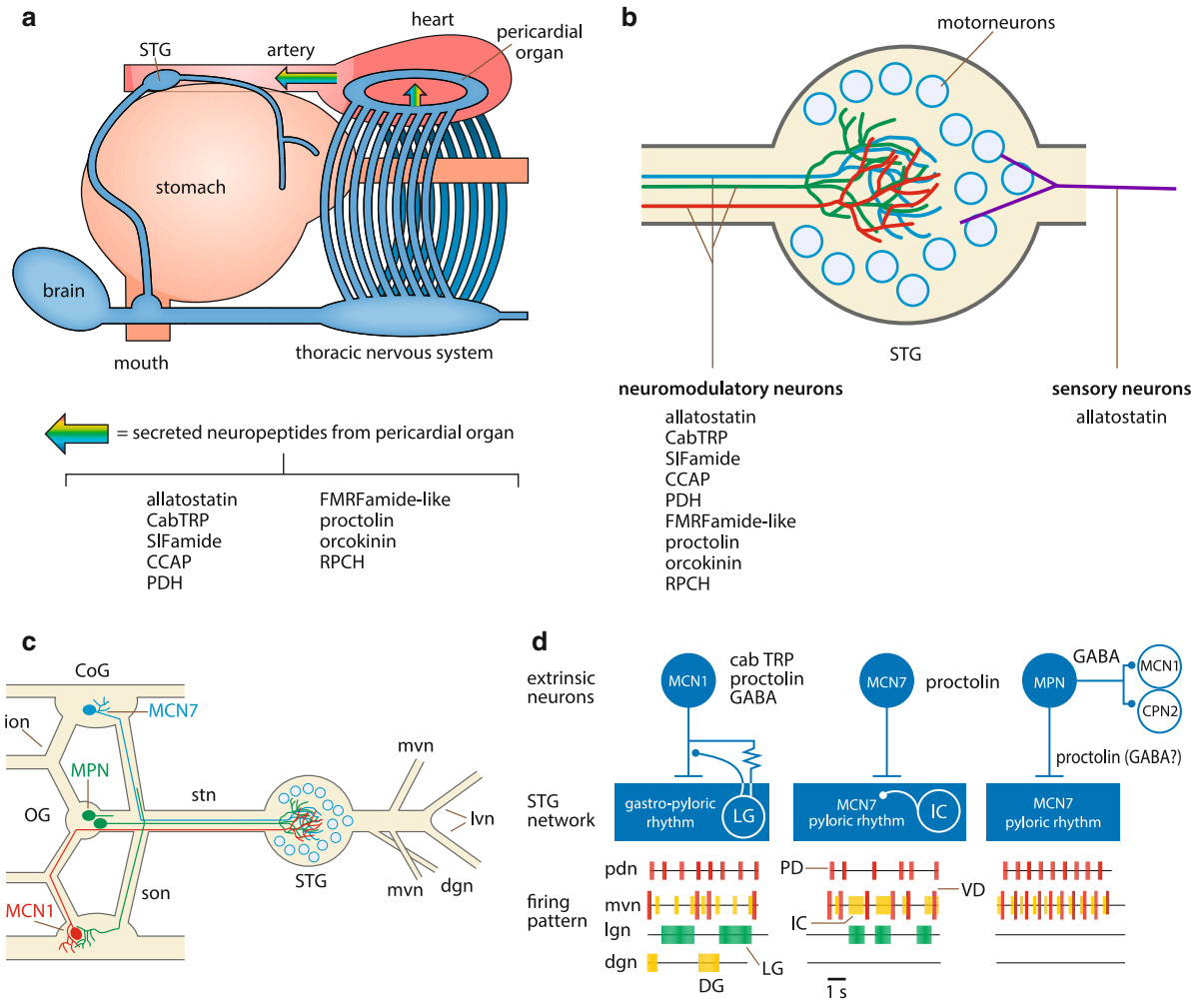


Fig. 11.5 Central actions of neuropeptides. Peptides in the stomatogastric nervous systems act both as hormones and as centrally released neuromodulators. **(a)** The stomatogastric ganglion (*STG*) is situated in the dorsal artery, directly anterior to the heart. The pericardial organs are neurosecretory structures that release many amines and neuropeptides directly into the circulatory system at the level of the heart. Some of the studied neuropeptides of the system are listed. **(b)** The *STG* is directly modulated by terminals of descending neurons and ascending sensory neurons. These direct neural inputs release many small classical neurotransmitters and neuropeptides into the neuropil of the *STG* (Redrawn from Marder and Bucher [6] and Nusbaum et al. [7] with permission). **(c)** The stomatogastric nervous system, including the soma location and axon projection patterns of the three proctolin neurons that innervate the stomatogastric ganglion (*STG*). The modulatory proctolin neuron (*MPN*) (shown in green) occurs as a functionally equivalent pair of neurons in the esophageal ganglion (*OG*). Each commissural ganglion (*CoG*) contains a single copy of modulatory commissural neuron 1 (*MCN1*) (shown in red) and modulatory commissural neuron 7 (*MCN7*) (shown in blue). Abbreviations: *dgn* dorsal gastric nerve, *ion* inferior oesophageal nerve, *lvn* lateral ventricular nerve, *mvn* medial ventricular nerve, *son* superior esophageal

nerve, *stn* stomatogastric nerve (Redrawn from Nusbaum et al. [7] with permission). **(d)** Concerted actions of the neuropeptide proctolin, tachykinin-related peptide (*CabTRP*), and the inhibitory transmitter GABA in different neurons of the crab stomatogastric system. The distinct stomatogastric ganglion motor patterns elicited by the three proctolin-containing projection neurons (*MCN1*, *MCN7*, and *MPN*) are shown. This includes a summary of their transmitter content and additional synaptic actions by which they elicit the indicated stomatogastric ganglion (*STG*) motor patterns. These additional actions include: (1) *MCN1*: presynaptic inhibition of *MCN1* by the *LG* neuron in the *STG* and electrical coupling to the *LG* neuron; (2) *MCN7*: strong excitation of the *IC* neuron; (3) *MPN*: synaptic inhibition of projection neurons in the commissural ganglia. *Lower panels*: rhythmic impulse bursts in *STG* neurons are represented by labeled boxes. Abbreviations: Nerves: *pdn* pyloric dilator nerve, *mvn* medial ventricular nerve, *lgn* lateral gastric nerve, *dgn* dorsal gastric nerve, Neurons: *PD* pyloric dilator neuron, *IC* inferior cardiac neuron, *VD* ventricular dilator neuron, *LG* lateral gastric neuron, *DG* dorsal gastric neuron. Legend: *t-bar* transmitter-mediated excitation, *filled circle*, transmitter mediated inhibition; resistor, electrical coupling (Modified from Nusbaum et al. [7] with permission)

Another group of peptidergic cells outside the X-organ also send axons to the sinus gland; these produce pigment-dispersing hormone (PDH). PDH and RPDH regulate pigment migrations or other light adaptational mechanisms in the compound eyes and they act antagonistically on pigment distribution in chromatophores in the epidermis. Both these peptides are also produced by neurons in other parts of the CNS and thus act in neuronal circuits. Circulating CHH has pleiotropic functions, including regulation of blood sugar levels, salt and water balance, molting, and reproduction, whereas MIH inhibits ecdysteroid hormone production by acting on the molting glands (Y-organs). Production of both these peptide hormones appears to be restricted to the X-organ cells.

11.3.3.2 In the Stomatogastric System, Peptides Control and Regulate the Motor Rhythms in the Foregut

One part of the nervous system where neuropeptide modulation within neuronal circuits has been extensively studied is the stomatogastric system of decapod crustaceans. This system is composed of a small set of ganglia, including the stomatogastric ganglion (STG) depicted in Fig. 11.5. The STG contains rhythm-generating networks composed of about 30 interneurons and motoneurons that control the foregut and stomach muscles during feeding. The circuitry of the STG is functionally flexible and can produce a set of dynamic output patterns, subserving a repertoire of chewing and filtering behaviors. The initiation and modulation of motor rhythms in the STG circuits are largely regulated by neuropeptides or peptide hormones. These reach the circuits either from extrinsic or intrinsic STG neurons, or via the circulation from the pericardial organ. More than a dozen different neuropeptides have been identified in neurons innervating the STG (Fig. 11.5b). Most peptides are excitatory and initiate activity in the quiescent STG or increase the frequency of existing rhythms, some produce a fusion of two rhythms to form a novel pattern. A case of peptide cotransmission can be illustrated by a set of STG neurons expressing the neuropeptide proctolin that colocalizes with another peptide, the tachykinin CabTRP, and the inhibitory classical transmitter GABA in different patterns (Fig. 11.5c, d). Experiments showed that proctolin can produce three different actions in the rhythm-generating network depending on which transmitters are coreleased

(Fig. 11.5c, d). The STG of crustaceans is the best studied invertebrate neuronal network in terms of peptide and transmitter actions in intrinsic and extrinsic neuromodulation. The stomatogastric ganglion also provides excellent insight into how the same network can generate very different activity patterns depending on its modulatory input.

11.3.4 Peptidergic Systems in Insects

Genome sequencing projects have provided good estimates of the total number of peptide precursors in several insects, and proteomics, by means of mass spectrometry, has identified many neuropeptides and peptide hormones expressed in nervous and neuroendocrine tissues. Thus, we know that there are 40 or more peptide precursor genes in insects and approximately 70–100 peptides can be produced [8]. The number of peptide-activated GPCRs is between 40 and 50. Many of the insect peptides have been investigated for functional roles. In general, insect neuropeptides and peptide hormones regulate many aspects of development, growth, metabolism, homeostasis, and reproduction, as well as specific behaviors. Neuropeptides also act as neuromodulators or cotransmitters in central neuronal circuits.

11.3.4.1 The Corpora Cardiacia and the Corpora Allata are the Main Cerebral Neurohemal Structures in Insects

The most prominent neuroendocrine system in insects is that of the protocerebral portion of the brain, referred to as the **brain-retrocerebral complex** (Fig. 11.6a). This complex is anatomically well conserved among insects and the brain portion consists of neurosecretory cells in two bilateral groups of neurons, the median and the lateral neurosecretory cells (MNCs and LNCs, Fig. 11.6b). Both cell groups send axons posteriorly to release sites in neurohemal organs, the **corpora cardiacia** (CC) and **corpora allata** (CA), that are associated with the anterior aorta (Fig. 11.6a, b). Several different peptide hormones are produced in MNCs and LNCs, as will be detailed later. In each of the CC and CA there is also a glandular portion with endocrine cells producing hormones: the peptide adipokinetic hormone (AKH) in CC and the terpenoid juvenile hormone (JH) in CA. In both CC and CA, there are also axon terminals

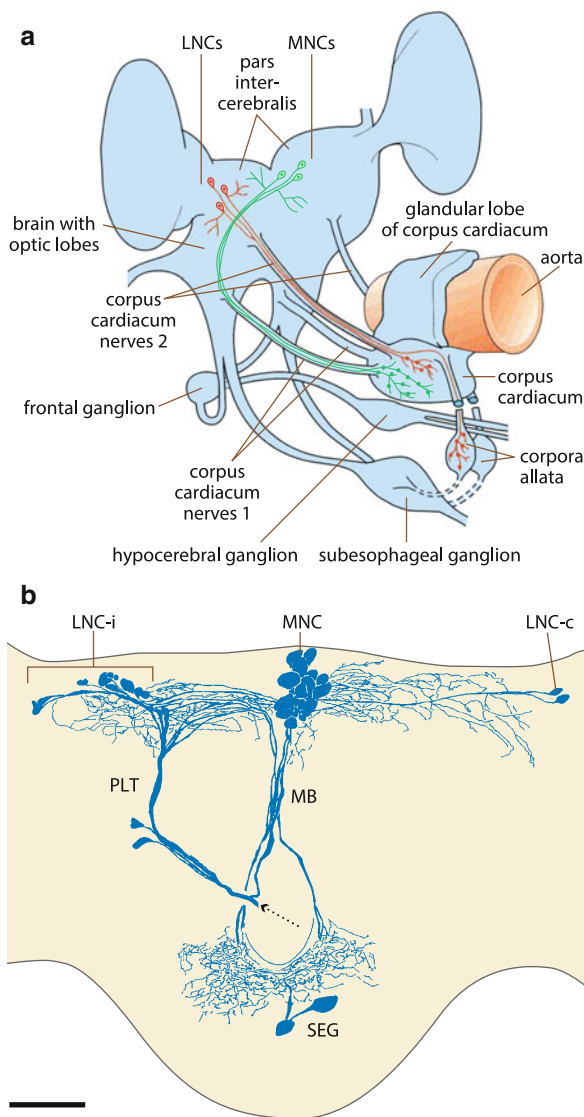


Fig. 11.6 (a) The neuroendocrine system of the brain-retrocerebral complex of the locust *Locusta migratoria*. Two groups of neurosecretory cells are shown schematically in the brain, lateral (*LNCs*) and median (*MNCs*) neurosecretory cells. These cells are shown only on one side of the brain (note that the *MNCs* send axons across to the contralateral corpus cardiacum nerve 1). There are many more neurons in each group and the different cell types produce a total of up to ten different peptide hormones. (b) Neurosecretory cells of the blowfly brain revealed by cobalt backfilling of one of the nerves to corpora cardiaca (arrow). Two main groups of neurosecretory cells are visualized in the dorsal brain, lateral (*LNCs*) and median (*MNCs*) neurosecretory cells. The majority of the *LNCs* are ipsilateral (*LNC-i*) and a few (*LNC-c*) are found on the contralateral side. Two axon tracts from the cells join the nerve to the corpora cardiaca: the posterior lateral tract (*PLT*) and the median bundle (*MB*). Two cells can also be seen in the subesophageal ganglion (*SEG*) (Redrawn from Shiga et al. [9] with permission)

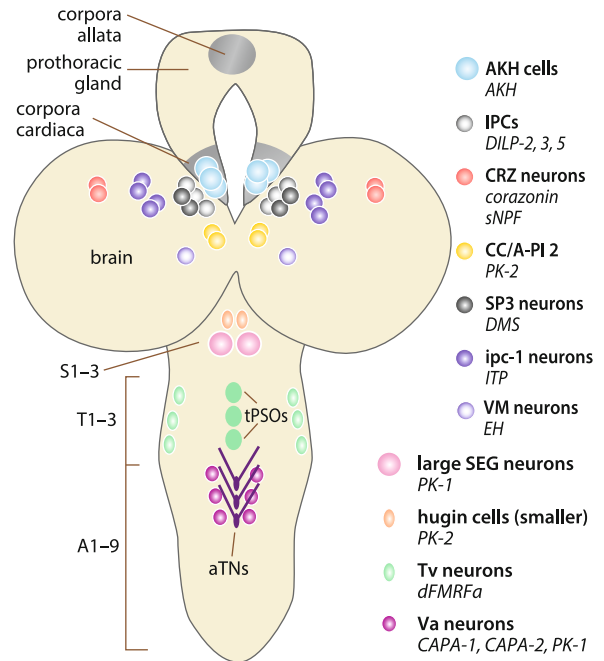


Fig. 11.7 Neurosecretory cells in a *Drosophila* larva. The brain neurosecretory cells send axons to neurohemal sites in the corpus cardiacum or corpus allatum in the ring gland and in ventral nerve cord to thoracic perisymphatic organs (*tPSOs*) or abdominal transverse nerves (*aTNs*). The color-coded cells express different neuropeptides: adipokinetic hormone (*AKH*), insulin-like peptides (*DILP-2, 3, 5*), corazonin, short neuropeptide F (*sNPF*), pyrokinins 1 and 2 (*PK-1* and 2), myosuppressin (*DMS*), ion transport peptide (*ITP*), eclosion hormone (*EH*), FMRFamides (*dFMRFa*), and capability peptides (*CAPA-1* and 2) (Redrawn and updated from Wegener et al. [10] with permission)

from peptidergic neurons in the brain that control production or release of *AKH* and *JH* by the endocrine cells. One set of brain neurosecretory cells that release peptides into the circulation via *CA* controls hormone production elsewhere. This is the system initially studied by Kopec in the 1920s: a few *LNCs* release *PTTH*, which controls production of the steroid hormone ecdysone in the prothoracic glands. Thus, the brain-retrocerebral complex of insects bears some similarities to the hypothalamus-pituitary of vertebrates that will be described later: brain neurosecretory cells with terminals in a neurohemal organ that also displays intrinsic endocrine cells and where hormone production and release is under control by systems of peptidergic brain neurons producing releasing factors.

Many MNCs and LNCs produce peptide hormones (Figs. 11.6b and 11.7). Peptide products of 11 precursors have been detected in the *Drosophila* MNCs and LNCs. In adult flies three of these are insulin-like, others are diuretic or antidiuretic hormones, or peptides regulating aspects of feeding and metabolism. The larval neurosecretory cells produce the same peptide hormones, and there are a few additional ones that have developmental roles, such as, for example, PTH and pyrokinins (Fig. 11.7).

11.3.4.2 Many Peptidergic Cells are Organized Segmentally in Thorax and Abdomen, Including Some That Control Molting Behavior

There are also peptidergic neurosecretory cells in most of the thoracic and abdominal ganglia (Fig. 11.7). These cells have their release sites in various locations in the body segments. From each ganglion an unpaired median dorsal nerve bifurcates and runs laterally to release sites in the lateral heart nerves, the heart muscles, tracheal trunks, and body wall diaphragms (Fig. 11.8). Along the median and transverse nerves there are enlarged neurohemal organs, designated perisymphatic organs, that are densely supplied by peptidergic axon terminals (Fig. 11.8). In some insects, like in adults of *Drosophila* and other flies, the dorsal neurohemal organs and areas of the body ganglia have merged with the neural sheath on the dorsal ganglion surface. Finally, there are endocrine cells in the intestine that produce a number of different peptides with local paracrine or remote hormonal functions.

More recently, a system of endocrine cells was discovered attached to the main tracheal trunks of the body. These so-called **Inka cells** produce ecdysis-triggering hormones (ETH) and are part of a complex peptidergic regulatory system which also includes various neurons and regulates ecdysis motor behavior in the moth *Manduca sexta* and in *Drosophila* (Figs. 11.9 and 11.10) [11]. Ecdysis behavior occurs when the developing insect sheds its old inflexible cuticle to enable growth. In *Manduca*, a cascade of hormonal actions, starting with release of the neuropeptide corazonin, followed by ETH released from Inka cells and eclosion hormone (EH) are formed by central neurosecretory cells in a positive feedback loop. ETH triggers responses in central interneurons and motoneurons that lead to synchronized and rhythmic muscle contractions in the body

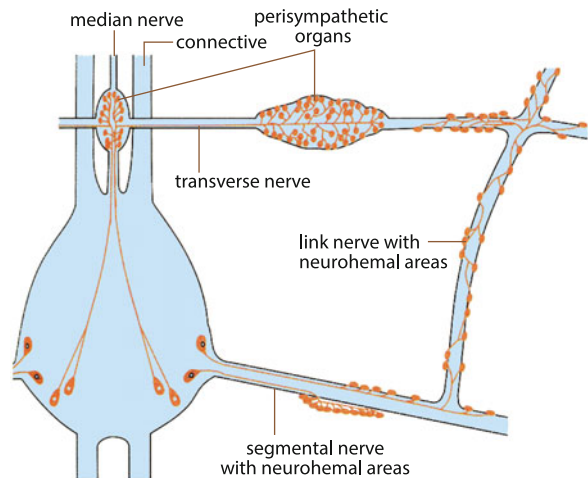


Fig. 11.8 Neuroendocrine systems in the body of insects. Details of neurohemal organs and areas in the abdominal neurosecretory system of an insect (generalized). This arrangement is typical for insects with unfused abdominal ganglia such as moths, locusts, and cockroaches. In some insects, like adults of dipteran flies (including *Drosophila*), the axons from neurosecretory cells all terminate in the dorsal neural sheath of the fused ganglia or in some segmental nerve roots

wall. These contractions break the old cuticle and enable the growing larva to escape from it. In *Drosophila* a very similar cascade has been identified and the central peptidergic neurons expressing the ETH receptor were identified which provided clues to action of further peptides in the ecdysis behavior control as shown in Figs. 11.9 and 11.10. An interesting discovery was recently made in the Oriental fruitfly, *Bactrocera dorsalis*, namely that EH acts on a **receptor guanylyl cyclase (GC)** expressed on Inka cells to increase cyclic GMP levels and thereby induce massive release of ETH. This is the first demonstration of a peptide receptor of the membrane-bound GC type in invertebrates. A GC receptor for atrial natriuretic peptide and related peptides has been known for several years in mammals.

11.3.4.3 In the CNS, Peptides act as Neuromodulators and Cotransmitters

Neuropeptides representing most of the identified precursors have also been localized to different types of interneurons in the insect CNS suggesting that they serve as neuromodulators or cotransmitters in central circuits. Examples of neuropeptides expressed in

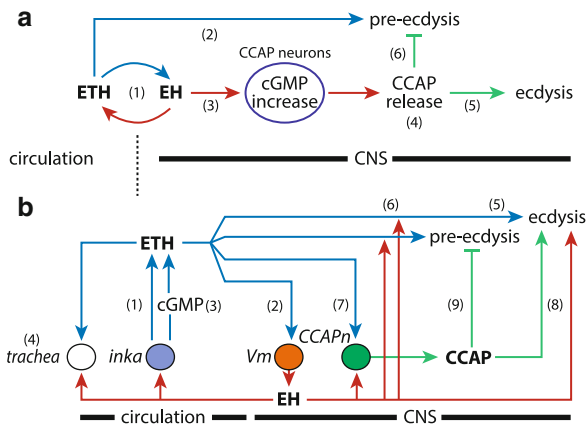


Fig. 11.9 Peptidergic cascade regulating ecdysis in insects. (a) Model for actions and relationships among ecdysis controlling neuropeptides in the moth *Manduca sexta*. Ecdysis is triggered by two neuropeptides: eclosion hormone (EH) and ecdysis triggering hormone (ETH). At ecdysis, EH is released from brain neurons (Vm) into the CNS and into the blood, and ETH is secreted from the peritracheal Inka cells into the blood. The release of these two neuropeptides is controlled by a positive feedback, in which EH and ETH stimulate release of each other (1). ETH also acts on the CNS to trigger pre-ecdysis behavior that prepares the animal for ecdysis (2). EH released within the CNS elicits an increase in cGMP levels in neurons that produce the peptide CCAP (3) which induces release of CCAP (4). CCAP then induces ecdysis (5) and inhibits pre-ecdysis (6). (b) Model for ecdysis control in *Drosophila*. The endocrine cascade is initiated by an EH-independent ETH secretion from Inka cells (1). This release induces release of EH from central Vm neurons (2), which stimulates further ETH release via increases in cGMP (3). Increased EH and ETH in circulation causes air-filling of the trachea (4). ETH peptides also act on neurons in the CNS to turn on pre-ecdysis and ecdysis behavior (5). This also requires action of EH neurons (Vm) on ETH output (6). ETH and EH regulate CCAP release from central CCAP neurons (CCAPn) (7) which inhibits pre-ecdysis (9). ETH mutants show some ecdysis-like behaviors, which could be controlled independently by EH and CCAP (8). Colored circles indicate known target cells and EH, ETH, and CCAP actions are indicated in red, blue, and green, respectively (Redrawn from Ewer [11] with permission)

central interneurons of *Drosophila* are neuropeptide F (NPF), pigment dispersing factor (PDF), and tachykinin-like peptide (DTK), which have been implicated in regulation of feeding behavior and aggression (NPF), circadian clock function (PDF), and modulation of olfactory sensory input and locomotor behavior (DTK), respectively. Some further examples of peptide functions are given in Sect. 11.4.

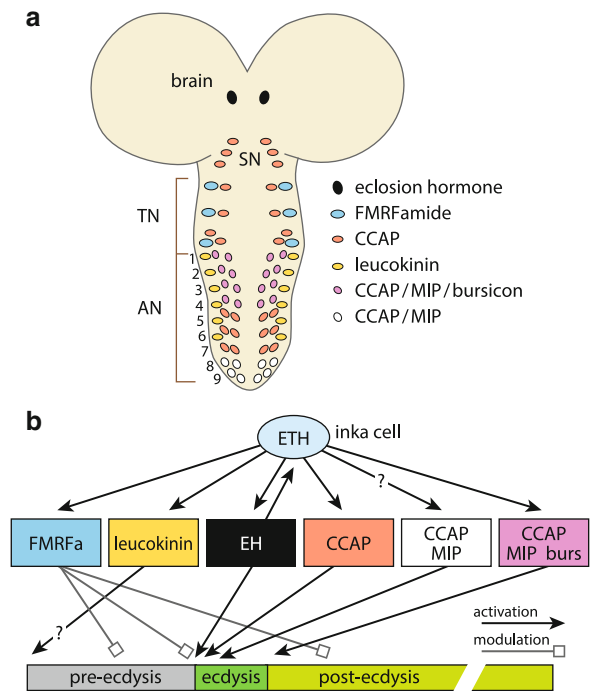


Fig. 11.10 Neurons and neuropeptides in ecdysis control in *Drosophila*. (a) Schematic depiction of peptidergic neurons in the larval CNS expressing the ecdysis triggering hormone receptor ETHR-A. These neurons respond to ETH in a sequence and trigger ecdysis behavior as depicted in (b). The color coding shows the expression of various neuropeptides in the neurons. ETH is released from peritracheal Inka cells. The Inka cells express a membrane-bound receptor guanylyl cyclase that is activated by eclosion hormone (EH). The first trigger of pre-ecdysis has not been conclusively identified. CCAP crustacean cardioactive peptide; MIP myoinhibitory peptide; burs, bursicon (tanning hormone) (Redrawn from Kim et al. [12] with permission)

11.3.5 Peptidergic Systems in the Nematode *C. elegans*

11.3.5.1 Peptides in Nematodes are Produced by Neurons, Sensory Cells, and Muscle

In *C. elegans* there is a fixed number of neurons and other cells. The nervous system of the adult hermaphrodite worm is composed of 302 neurons, classified into 118 different neuron types, connected by approximately 7,000 synapses [13]. These neurons form an anterior nerve ring, a ventral and a

dorsal nerve cord, and small ganglia anteriorly and posteriorly (Fig. 11.11). No distinct neuroendocrine system has been distinguished in nematodes in the sense that there are no neurons with axons terminating in specific neurohemal organs. However, in the pharynx there are two neurons considered to be neurosecretory cells because they have varicose axons running along the border to the body cavity, the **pseudocoel**. These cells produce serotonin and peptides generated from at least two precursors (neuropeptide-like precursors, *nlp* 13 and 18). In *C. elegans* most neuropeptides are located in sensory, motor, and interneurons of various types, but also in other cell types. We may thus have to consider peptidergic signaling in *C. elegans* and other nematodes mostly as a form of paracrine signaling. As an example, insulin-like peptides that act in hormonal signaling to regulate growth, reproduction, and metabolism in mollusks, insects, and vertebrates exist also in this nematode. Here, many insulin-like peptides are produced primarily in sensory neurons and other neurons, suggesting paracrine action as the main signaling form.

In *C. elegans* 109 peptide precursor genes and more than 50 peptide GPCRs have been identified, some of which are ancestrally related to ones in insects and vertebrates. The peptide precursors have been divided into three major groups: FMRFamide-like (*flp*), insulin-like (*ins*), and neuropeptide-like precursors (*nlp*). There are 26 *flp*, 37–39 *ins*, and 45 *nlp* genes and many of these have been localized to neurons. Out of the 302 neurons, 160 express *flp* genes; some of these neurons even express multiple *flps*. Additionally, muscles in head and pharynx as well as cells of the uterus and vulva express *flps*. The *ins* genes are seen in sensory and other neurons as well as in intestine, hypodermis, pharynx, and vulva. Finally, the *nlp*s are seen in different patterns in various types of neurons and in gonadal cells. Screens using RNA interference to diminish levels of peptides or their GPCRs have shown effects on various behaviors such as egg laying, locomotion, and sensory processing. Since many neuropeptides are expressed in sensory neurons it seems that peptide functions in the worm are important in mediating environmental inputs. We will discuss further the issue of peptide signaling in *C. elegans* in the next section.

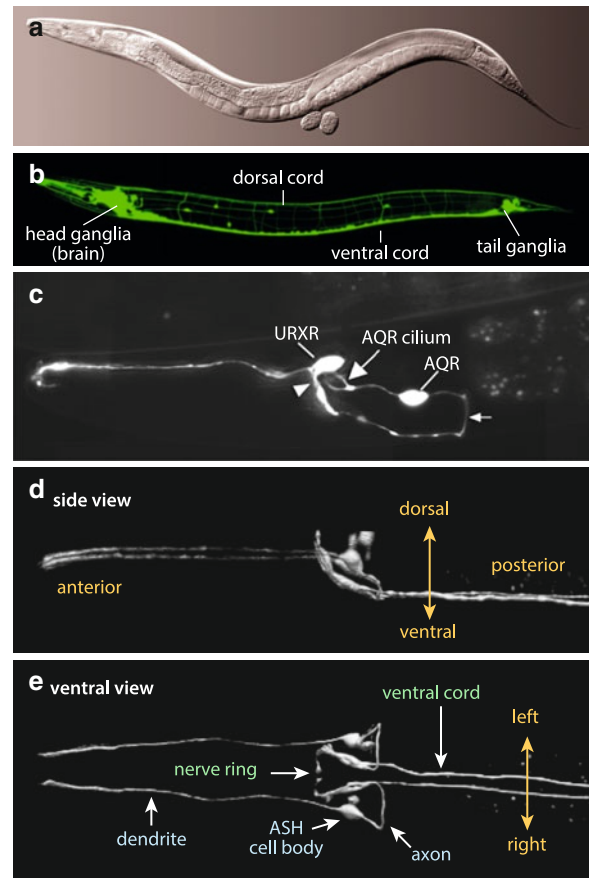


Fig. 11.11 Peptides in the nervous system of the nematode worm *C. elegans*. Anterior is to the left in all images. (a) Nomarsky contrast image of the worm (The image was kindly provided by Maria Gallegos, California State University, East Bay, CA, who owns the copyright) (b) The worm's nervous system is displayed with green fluorescent protein revealing the head and tail ganglia and cords connecting these (Image kindly provided by Dr. Harald Hutter, Simon Fraser University, Burnaby, BC, Canada, who owns the copyright) (c) A pair of neurons designated URX are oxygen-sensing neurons and express neuropeptide Y receptor-like receptor NPR-1. The ligands of this receptor are the peptides encoded by *flp-18* and *flp-21* and the neurons are important for social feeding behavior. Also the AQR neuron expresses NPR-1. Image from WormAtlas (www.wormatlas.org), with permission from Dr. Zeynep Altun-Gultekin, Albert Einstein College of Medicine, Bronx, NY, who owns the copyright. (d and e) Side view and ventral view of a pair of peptidergic sensory neurons designated ASH. These neurons express glutamate and the neuropeptides NLP-3, NLP-15, and FLP-21 (the latter FMRFamide like), as well as the receptor NPR-1 (see WormAtlas). These neurons are involved in avoidance responses to touch of the “nose” of the worm (Image from Dr. Harald Hutter, who owns the copyright). We also thank Drs Cori Bargmann and David Hall for help with these images

11.4 Comparing Functional Roles of Some Neuropeptides and Peptide Hormones Across Phyla

Gene sequencing projects have provided ample evidence for a strong evolutionary conservation of amino acid sequences of a number of peptides and peptide GPCRs. Some peptide signaling systems appear to be functionally conserved among invertebrates and even extend into the vertebrates. This section will provide a few examples of peptide signaling in invertebrates that seem more or less conserved over great evolutionary distances.

11.4.1 Insulin-Like Peptides Are Conserved Across Phylogeny

Genes encoding insulin-like peptides (ILPs) and ILP receptors have been identified in many invertebrate species, including the mollusks *Aplysia*, *Lymnaea*, and *Lottia* as well as *C. elegans* and *Drosophila*. There are 38 insulin-like peptides (ILPs) in the worm and 8 ILPs in the fly. In *Drosophila* some of the ILPs resemble mammalian insulin, others are relaxin-like, and at least one relates to insulin-like growth factors (IGFs). In both the worm, the fly, and the snails a single tyrosine kinase type ILP receptor has been identified, designated *daf-2* in *C. elegans* and *dInR* in *Drosophila*. The entire signal pathway in growth control, downstream of the ILP receptor, is conserved from *C. elegans* to mammals (see Fig. 11.12).

Experimentally impaired ILP signaling in *C. elegans* leads to pleiotropic effects, including increased fat storage, stress resistance, and constitutive entry into a diapause-like state called the **dauer**, as well as extended life span. Similarly, in *Drosophila* interference with insulin signaling affects growth, carbohydrate and lipid storage, stress resistance, female fertility and longevity [15]. The ILPs of mollusks are involved in regulation of growth and associated metabolic processes, in control of glycogen levels and gonadotropic activity. Thus, in general, ILP signaling in invertebrates combines some functions seen for vertebrate insulin, IGFs, and relaxin.

11.4.2 NPF and NPY Control Several Behaviors – Including Feeding – Across Species

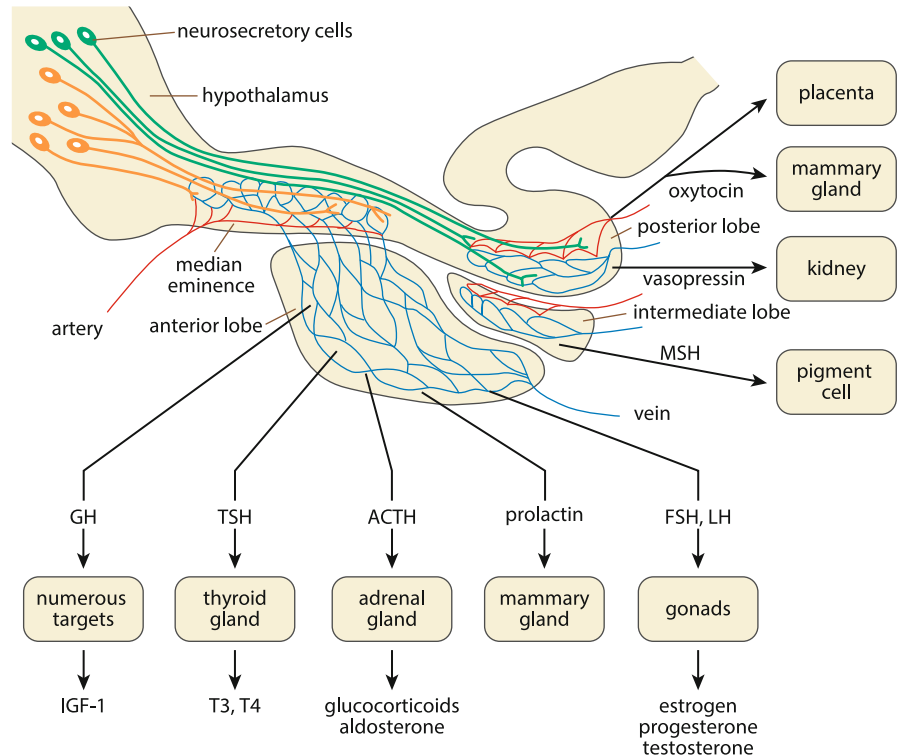
Another peptide-signaling system that appears to be partly conserved throughout evolution is that of the invertebrate neuropeptide F-like (NPF-) and vertebrate NPY-like peptides. As described in Sect. 11.6, NPY is a strong stimulator of feeding in mammals. In *Drosophila* the 36-amino-acid-long NPF is expressed in about 20 brain interneurons and activates an NPF receptor (NPFR1) distantly related to the mammalian NPY receptors and the NPF receptor of *C. elegans*. Genetic interference with the expression of NPF or NPFR1 in *Drosophila* produced phenotypes with modified feeding behavior (e.g., appetite and food choice), foraging, social behavior, ethanol sensitivity, and aggressive behavior. A male-specific role in the **circadian clock** has also been discovered. Intriguingly, a developmental change in NPF signaling in late larvae of *Drosophila* coincides with the distinct behavioral switch from continuous feeding to food aversion and wandering. All the above-mentioned roles of NPF in *Drosophila* are assumed to be mediated by release from brain interneurons and not hormonal actions.

In *C. elegans* NPF signaling also regulates feeding behavior. The worm NPF receptor (*Npr1*) displays a natural polymorphism that positively correlates with a drastic behavioral change: the two variants of *Npr1* are associated with social or solitary feeding, respectively. *Npr1* gene knockout worms display clumping, which suggests that the normal role of this receptor is to inhibit social feeding. The natural ligands for *Npr1* are peptides derived from the two genes, *flp-21* and *flp-18*. These are, however, shorter than NPF and NPY and not clearly evolutionarily related (in contrast to their receptors). A peptide resembling NPF of *Drosophila* has also been identified in *Aplysia* where NPF signaling has been implicated in circuits regulating feeding behavior.

11.4.3 AKH/GnRH Are Similar Peptides but Have Different Functions in Different Species

An example where sequences of peptide and GPCR are conserved, but functions are divergent is a *C. elegans*

Fig. 11.13 Hypothalamus and pituitary in mammals. Peptides are released from hypothalamic neurons into separate capillary networks. The anterior network transports neuropeptides to the anterior lobe of the pituitary where they regulate the release of pituitary peptide hormones. The posterior network receives peptides from large hypothalamic neurons whose axons project to the posterior lobe of the pituitary. These peptides are forwarded to the general circulation that transports them to distant target organs. Some mammals also have an intermediate pituitary lobe with a separate capillary network



posterior pituitary), and the urophysis in bony fishes and cartilaginous fishes. In addition, a large number of neuropeptides are produced in the brain and the spinal cord as well as in peripheral neurons. Some peptides are also synthesized in various non-neuronal cell types and released as hormones. In this section we will focus on the peptides involved in the communication between the nervous system and various endocrine systems, primarily the hypothalamus-pituitary connection. Often these peptides have been referred to as neuroendocrine peptides due to their role in this communication and several of these are produced both in neurons and in endocrine or neurosecretory cells. However, we will use terms neuropeptide and peptide hormone in the following. The neuropeptides that serve functions more exclusively within the brain will be discussed below in Sect. 11.7.

11.5.1 Hypothalamus, Neurohypophysis, Adenohypophysis

The hypothalamus and pituitary are usually considered to comprise the most prominent **neuroendocrine** system in vertebrates. The **pituitary gland** (also called hypophysis) has even been named the “master gland”

because it controls, via its various hormones, many functional systems throughout the body. Anatomically the pituitary consists of two clearly distinguishable parts with distinct developmental origins (Fig. 11.13). The anterior lobe is called the **adenohypophysis** and is formed by the dorsal part of the oral cavity, an embryonic structure named **Rathke’s pouch** after the nineteenth century German embryologist Martin Rathke (1793–1860). The posterior lobe is called the **neurohypophysis** because it is an extension of the hypothalamus located in the diencephalon (the posterior part of the forebrain). Between the anterior and posterior pituitary lobes an intermediate lobe arising from Rathke’s pouch is present in many groups of vertebrates. The intermediate lobe is very modest or almost nonexistent in some adult mammals but is prominent in other vertebrate classes.

11.5.1.1 Hypothalamic Neuropeptides act as Releasing Factors in the Pituitary Gland

The hypothalamus and pituitary are highly vascularized and contain 2–3 capillary networks, depending on the particular class of vertebrates. Peptides are released into these networks and transported to their target receptors. In mammals an anterior artery enters

at the base of the hypothalamus in a region called the median eminence. Peptides released here from hypothalamic neurons are transported by capillaries to the endocrine cells in the anterior lobe of the pituitary (Fig. 11.14a) where they bind to receptors that regulate the release of hormones. Therefore, these hypothalamic peptides are called releasing hormones/factors. The pituitary hormones are small proteins which are transported to target cells elsewhere in the body. Dorsally or rostradorsally to the median eminence is a group of hypothalamic neurons called the arcuate nucleus which has important functions as a sensory region where the hypothalamus can detect hormones in the blood arriving from elsewhere in the body. This sensing is possible because the arcuate nucleus, like the median eminence, lacks the blood-brain barrier (BBB). Here hormones such as insulin from the pancreas, leptin from adipose tissue, and ghrelin from the stomach can influence the activity of hypothalamic centers involved in the regulation of feeding and metabolism.

One striking difference in the anterior pituitary between vertebrate classes is that teleost fishes do not depend on a capillary network to transport the hypothalamic neuropeptides to the endocrine cells. Instead, these neurons extend their axons directly all the way to the endocrine cells and release their neuropeptides (releasing factors) onto them (Fig. 11.14b). In the posterior pituitary of all species, on the other hand, peptides are released from axon terminals of large hypothalamic neurons and are transported directly to the rest of the body via the systemic blood circulation and reach target organs such as the kidneys or, in mammals, the uterus and mammary glands.

Finally, the intermediate lobe, where present, has a distinct set of endocrine cells that are innervated by hypothalamic neurons, and there is also a separate capillary network for the intermediate lobe. This lobe releases a set of related peptide hormones called **melanocyte-stimulating hormones (MSH)** that are derived from the same precursor as ACTH. This prepropeptide is called POMC, for pro-opiomelanocortin. One of the MSH peptides, alpha-MSH, corresponds to the aminoterminal 13 amino acids of ACTH and is released after cleavage by an endopeptidase present in the intermediate pituitary but not in the ACTH-producing cells of the anterior pituitary. Some adult mammals lack an intermediate lobe, including humans, whales, and elephants and several others, most of which have few or no melanocytes.

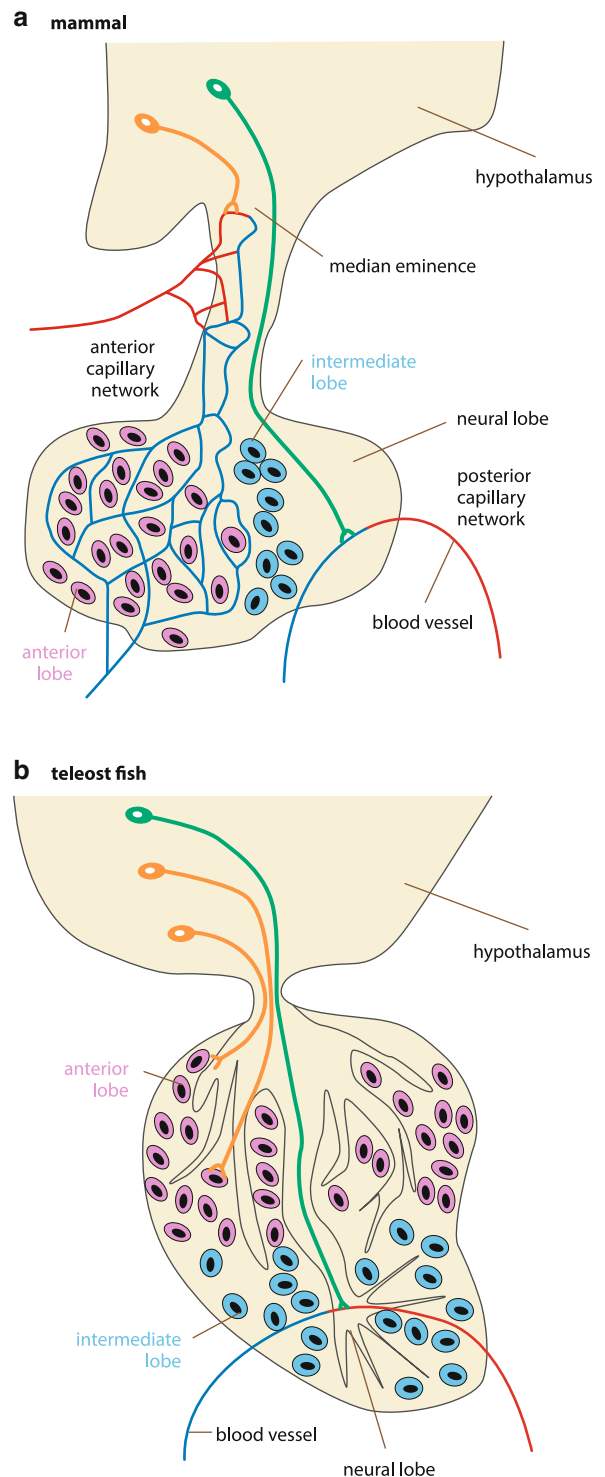


Fig. 11.14 Comparison of hypothalamus-pituitary connections in mammals (a) and bony fishes (b). The major difference is that the mammalian hypothalamus releases peptides into a capillary network that carries these signals to the anterior lobe, whereas in bony fishes all three lobes are innervated by hypothalamic neurons. The intermediate lobe is minor or absent in some mammals (Redrawn from Zohar et al. [16])

11.5.1.2 Pituitary Hormones act on Distant Targets, Including Thyroid, Adrenal Gland, and Gonads

The release of pituitary hormones in vertebrates constitutes some of the most well-characterized systems of integration of multiple regulatory inputs as well as extensive feedback mechanisms. Each pathway of pituitary hormone regulation and action is called an “axis”, such as the hypothalamus-pituitary-thyroid axis (HPT), the hypothalamus-pituitary-adrenal axis (HPA), and the hypothalamus-pituitary-gonad axis (HPG). For each of these, multiple inputs are integrated in the hypothalamus and result in modulation of the release of each of the pituitary hormones. The mechanisms have been investigated in mammals, chickens, amphibians, and bony fishes. The central features of the regulation are described below. Not all differences between vertebrate classes can be elaborated here.

11.5.2 Hypothalamus-Anterior Pituitary-Peripheral Organ Axes

In descriptions of the hypothalamic-pituitary-target networks, the pituitary hormones are often taken as starting point and the present description will use this approach. The **anterior pituitary** of mammals typically manufactures six peptide hormones belonging to three families (Fig. 11.15). One family consists of growth hormone (GH) and prolactin (PRL), the second family is comprised of the three glycoproteins called thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), and the third family has a single member named adrenocorticotrophic hormone (ACTH). A seventh hormone is present in fishes, namely somatolactin (SL) which is related to growth hormone. Some vertebrate groups, particularly among teleost fishes, have additional duplicates of some of the hormones. Functional differences between these duplicates are still incompletely known.

11.5.2.1 The HPT Axis: Thyroid Hormones Control Hormones that Control Metabolic Rates

The pituitary cells that produce TSH (a heterodimer whose two polypeptides are called alpha and beta) are primarily regulated by stimulation from the

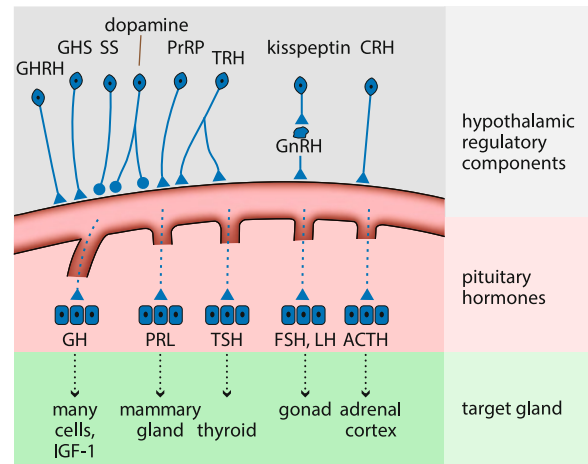


Fig. 11.15 Regulation of hormonal factors from the brain (top), offer hypothalamic regulatory components and pituitary hormones to the target glands and their hormones

hypothalamic neuropeptide TRH (TSH-releasing hormone, Fig. 11.15). Its production and release from the hypothalamic neurons is regulated by factors such as ambient temperature and metabolism. TRH is unusual in vertebrates because it is one of very few peptides that occurs in multiple identical copies in the same precursor: six in human, seven in the frog *Silurana (Xenopus) tropicalis*, and eight in goldfish. In chickens there are at least four copies with flanking consensus processing sites and a few more that may no longer be cleaved. TRH triggers the release of TSH, which binds to receptors in the thyroid gland and stimulates a variety of processes that lead to release of the thyroid hormones T3 and T4, which are iodinated derivatives of the amino acid tyrosine. These will reach almost all cells in the body and stimulate their metabolic rate by binding to nuclear receptors after uptake by transport proteins.

11.5.2.2 The HPG Axis Controls the Gonads and Sexual Drive

The two relatives of TSH, namely FSH and LH, have their primary roles in reproduction. They have the same alpha subunit as TSH but have distinct beta subunits. FSH and LH are released from a population of endocrine cells in the pituitary that are stimulated by the hypothalamic peptide GnRH (gonadotropin-releasing hormone). Recently it has been found that the GnRH-producing neurons are themselves regulated by another peptide called kisspeptin which

stimulates GnRH release [17]. (The name is derived from the famous so-called ‘kisses’ manufactured by a prominent U.S. chocolate manufacturing company located in Pennsylvania, U.S.A. where the research team was based that discovered the peptide). The hypothalamus integrates a broad range of stimuli to regulate reproduction with these neuropeptides. FSH and LH are released into the systemic circulation and bind to receptors in the gonads to regulate gametogenesis and ovulation, often in response to seasonal changes.

11.5.2.3 Growth, Metabolism, and Electrolytes are Controlled by Several Systems

GH, PRL and intermediate lobe SL are produced by separate cell types in the anterior pituitary. The two first-mentioned have been investigated in great detail, whereas SL, as the most recently discovered member of the family, is still only partially known. SL is present in bony fishes (Actinopterygii) as well as in lungfish, but has been lost in the tetrapod lineage. GH release is stimulated by the peptides GHRH (growth hormone releasing hormone) and ghrelin, also called GHS (growth hormone secretagogue), while inhibition is exerted by somatostatin (SS) and a non-peptide, namely dopamine. GH acts on many cell types and has anabolic effects. Its release occurs primarily after onset of sleep in mammals and after physical activity. Many of the peripheral effects of GH are mediated by IGF-1 (insulin-like growth factor 1) that acts locally in the target tissues.

PRL cells are mainly regulated by a tonic inhibitory signal from dopamine and in some vertebrates also stimulatory signals from TRH and the neuropeptide PrRP (PRL-releasing peptide). PRL has an extremely long list of effects in various vertebrates, the most well-known of which is lactation in mammals. However, PRL existed long before this role evolved and its most prominent effects are on reproduction, growth (particularly seasonal growth), and on electrolyte balance in fishes that alternate between freshwater and seawater.

Also several other neuroendocrine systems that utilize neuropeptides and peptide hormones have been investigated in great detail. One such system with fascinating complexity concerns the appetite-regulating peptides (Fig. 11.16). Ingestion of

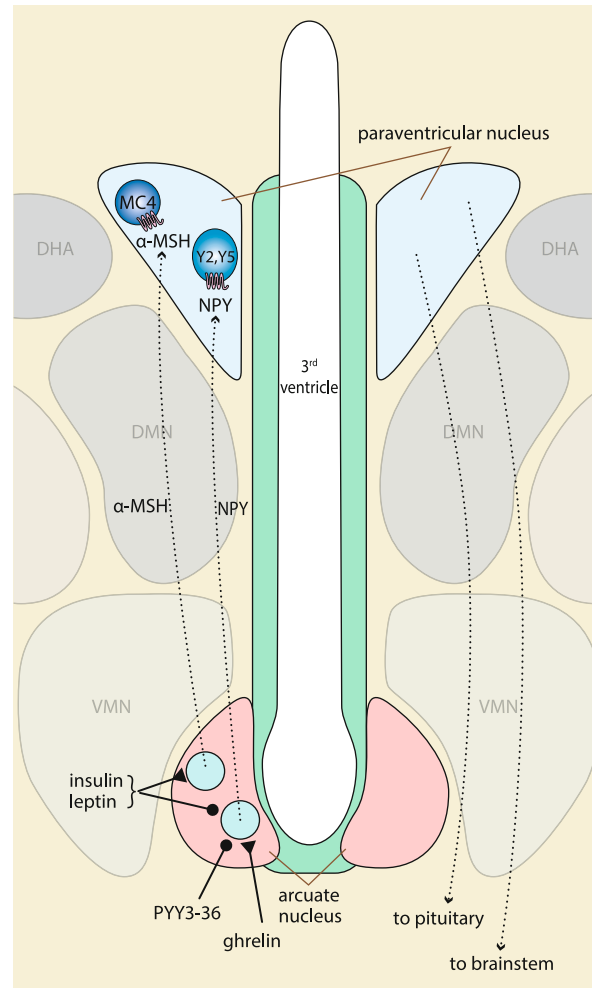


Fig. 11.16 Schematic outline of neuronal pathways that regulate hunger and satiety in a coronal section of the rat hypothalamus. The neurons in the arcuate nucleus can respond to hormones in the blood. MC4 is a receptor for α -MSH. Y1 and Y5 are NPY-receptor subtypes. Ascending pathways are shown in the *left part* of the figure and descending pathways in the *right part* for clarity only, in reality there is bilateral symmetry

food leads to the release of gastrointestinal peptides such as insulin, CCK (cholecystokinin), peptide YY (PYY), and pancreatic polypeptide that bind to receptors in the nervous system and reduce appetite. The peptide leptin, now often described as a “long-term reporter” of fat stores in adipocytes, also reduces appetite. Absence of leptin in mice or humans results in lack of appetite inhibition, resulting in overeating and obesity. Insulin, leptin, and PYY bind to receptors on neurons in the basal part of the hypothalamus called the arcuate nucleus, resulting in inhibition of

the appetite-stimulating neurons that release NPY in the paraventricular nucleus (PVN) of the hypothalamus. In addition, insulin and leptin stimulate a different population of neurons in the arcuate nucleus that produce the peptide α -MSH from the precursor POMC (the same peptide that is produced in the intermediate lobe of the pituitary in other species), and release α -MSH in the PVN where it induces satiety. Thus, food intake is reduced by the combined effects of increased release of the satiety-generating α -MSH and diminished release of the hunger-generating NPY. Output pathways from the PVN neurons to the pituitary and the brain stem result in the behavioral changes that terminate feeding. In the opposite situation, when the stomach is empty, the peptide hormone ghrelin is released from gastric endocrine cells and binds to the NPY neurons in the arcuate nucleus, resulting in increased NPY release in the PVN and onset of feeding behavior. This system is a clear example how “peripheral” peptide hormones and neuropeptide pathways interact.

Thus, it is clear that the related peptides NPY and PYY have opposing roles in appetite regulation: NPY is the most powerful endogenous stimulator of feeding, acting within the hypothalamus, whereas PYY reduces appetite by inhibiting the NPY neurons as shown in Fig. 11.16. Also a tetrapod-specific copy of PYY, the pancreatic polypeptide (PP), is released after meals and reduces appetite. In the context of appetite regulation the three peptides exert their effects through distinct receptors as shown in the figure. NPY has its receptors in the paraventricular nucleus of the hypothalamus. PYY as an endocrine signal binds to receptors on neurons in the arcuate nucleus in a more open region of the blood–brain barrier. The site of action of PP is not yet entirely clear, but its receptors may reside on the vagal nerve, bringing the signal to the brain stem for further relay to the hypothalamus.

11.5.2.4 The HPA Axis Controls Stress Responses, with a Network of Positive and Negative Feedback Loops

ACTH release is stimulated by the neuropeptide CRH, corticotropin-releasing hormone (or *CRF*, for factor). ACTH release is stimulated by stressful stimuli, both metabolic stress and in mammals more psychological types of stress (see Chap. 24). ACTH binds primarily to

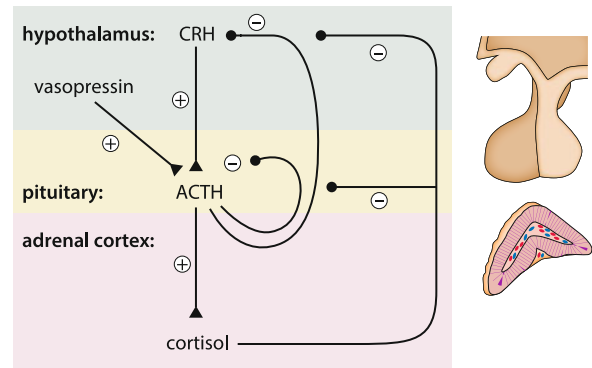


Fig. 11.17 Hypothalamus-pituitary-adrenal axis. *Plus signs* mean stimulation, *minus signs* mean inhibition

receptors in the adrenal cortex where it stimulates production of steroids such as cortisol in mammals which leads to increased blood glucose and increased blood pressure, both of which may be part of a stress reaction.

The HPA axis (Fig. 11.17) is centered around the pituitary peptide hormone ACTH excised from the POMC precursor. The biosynthesis and release of ACTH is primarily regulated by the hypothalamic neuropeptide CRH. ACTH acts on its receptor MC2 (melanocortin receptor 2) on adrenal cortex cells that produce steroid hormones. One of these, cortisol, exerts feedback regulation on multiple steps in the axis: it inhibits transcription of prepro-CRH as well as the release of CRH and it inhibits the transcription of the CRH receptor on the ACTH-producing pituitary cells as well as the transcription of POMC and the release of ACTH. In addition, ACTH has been reported to have an inhibiting effect on CRH release from the hypothalamic neurons as well as on its own release from the pituitary. One of the effects of ACTH on the adrenal gland is to stimulate the release of aldosterone, a mineralocorticoid that increases sodium retention in the kidneys and thereby increases blood pressure. The release of ACTH is stimulated by vasopressin. Thereby vasopressin stimulates retention of both salt and water because its direct effect on the kidneys is to increase water retention.

11.5.3 Posterior Pituitary Neurohormones

In mammals, the **posterior pituitary lobe** releases two peptide hormones from axons extending from

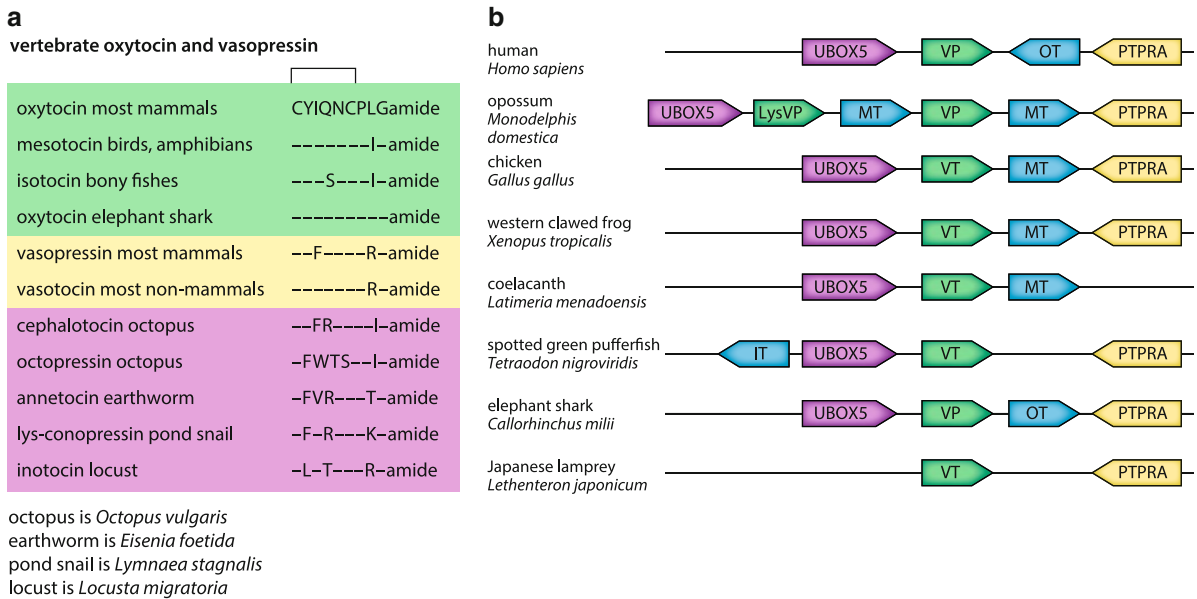


Fig. 11.18 Oxytocin and vasopressin sequence comparison.

(a) Amino acids are shown with single-letter code. The top sequence is shown as master sequence. Dashes mean identity to the master sequence. Oxytocin orthologs are shown with gray background and vasopressin orthologs with yellow background. Invertebrate sequences are shown with pink background (*Octopus vulgaris*, *Eisenia foetida*, *Lymnaea stagnalis*, *Locusta migratoria*). All peptides are cyclized by a disulfide bond between the two cysteines and they all have a carboxy-terminal amide group. (b) Schematic outline of chromosomal region harboring the oxy-

tocin (*OT*)/mesotocin (*MT*)/isotocin (*IT*) (blue) and the vasopressin (*VP*)/vasotocin (*VT*) (green) genes in various gnathostomes. LysVP is vasopressin with a lysine residue instead of arginine (see Fig. 11.18a). Adjacent genes: *Ubox5* stands for “U box domain containing 5” and *Ptpra* is “protein tyrosine phosphatase, receptor type, A”. As the lamprey has a single peptide gene, it is likely that the gene duplication that generated oxytocin and vasopressin from a common ancestral gene took place in a gnathostome ancestor after the cyclostomes had branched off

large (“magnocellular”) hypothalamic neurons – the closely related nonapeptides oxytocin and vasopressin. **Vasopressin** acts as a peptide hormone to increase blood pressure and decrease urinary volume. **Oxytocin** in its hormonal role in mammals stimulates the milk letdown reflex (milk ejection) as well as uterus contractions during parturition. The corresponding two hormones are found in all gnathostomes (jawed fishes), although they have been named differently in various animal groups as will be described below. Here, oxytocin has other roles in reproduction and water-salt balance. Notably, both oxytocin and vasopressin also have prominent roles as neuropeptides within the brain, influencing for instance, social behaviors.

These peptides were two of the earliest peptides to be characterized in multiple vertebrate species, and when different peptide sequences were reported the peptides were given new designations, which unfortunately resulted in a confusing plethora of names. Only

recently has gene characterization confirmed that oxytocin is the ortholog (species homolog) of mesotocin (birds, amphibians) and isotocin (bony fishes), whereas vasopressin is the ortholog of non-mammalian vasotocin (Fig. 11.18a). A local gene duplication gave rise to this ancestral pair before the origin of gnathostomes (Fig. 11.18b). Other peptide families have been described by using information about chromosomal location of the genes for assignment of orthology versus paralogy (sequence comparisons have been ambiguous).

11.5.4 The Urophysis

A population of large neurons was discovered in the caudal part of the spinal cord in skates by the American zoologist Ulric Dahlgren (1870–1946) in 1914. These cells were also identified in teleost fishes and were named **Dahlgren cells** (Fig. 11.19). They were found

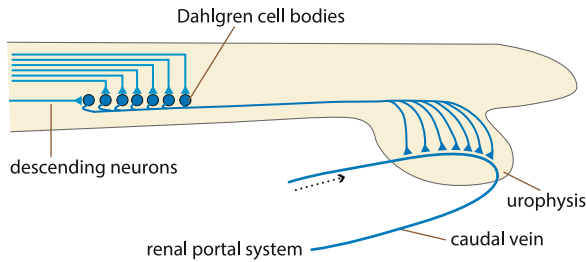


Fig. 11.19 Organization of Dahlgren cells in the teleost fish urophysis in the caudal spinal cord

to extend their axons to the posterior tip of the spinal cord where they form a neurohemal organ called the **urophysis** or the caudal neurosecretory system. The presence of the urophysis in both cartilaginous fishes, actinopterygian fishes, and the sarcopterygian lungfishes shows that it was present in the gnathostome ancestor and was subsequently lost in tetrapods. Two peptides have been discovered in the urophysis and were named urotensin I and II. Both of these are also expressed elsewhere in the nervous system. They are structurally unrelated: urotensin I also exists in mammals where it is called urocortin and belongs to the CRH family (corticotropin-releasing hormone). Urotensin II, on the other hand, is a distant relative of somatostatin. In teleost fishes both urotensin I and urotensin II are involved in osmoregulation.

11.6 Neuropeptides as (Co)Transmitters in the Brain of Vertebrates

Many of the peptides mentioned in the preceding sections also have roles as neuropeptides entirely within the brain. To name a few, these include oxytocin and vasopressin that were originally discovered in the posterior pituitary but are now known to have prominent effects on behavior, CCK that was first found in the gastrointestinal tract, and substance P found in multiple tissues. In the vertebrates the number of peptides participating in neuronal signaling is in the order of 100, accompanied by a roughly equal number of GPCRs that mediate their actions.

The nerve terminals that release neuropeptides from large dense-core vesicles also frequently release classical neurotransmitters from small synaptic vesicles (see Fig. 11.2). The first case of such coexistence was

reported in 1977 by Tomas Hökfelt and colleagues [18] at the Karolinska Institute in Stockholm and concerned somatostatin colocalization with noradrenalin (or rather the enzyme that makes noradrenaline, i.e., dopamine- β -hydroxylase). This was followed by findings of substance P coexistence with 5-hydroxytryptamine, CCK with dopamine, and numerous other examples with one or more neuropeptides coexisting with classical neurotransmitters.

The number of neuropeptides and receptors seems to have increased considerably in the early stages of vertebrate evolution due to the two genome duplications (tetraploidizations) that took place in the gnathostome ancestor. Peptide genes that were duplicated as a result of these tetraploidizations include NPY, somatostatin, tachykinins, opioid peptides, relaxins, and other insulin-like peptides. Several peptide families expanded further in the third tetraploidization that took place in the bony fish lineage before the radiation of teleost fishes. In addition, many local peptide gene duplications have occurred in various vertebrate lineages. Although many new peptides have been discovered, several more probably remain to be identified because there are many GPCRs whose ligands are still unknown (so-called orphan receptors) but are expected to be peptides. Many of the vertebrate neuropeptides have homologs in invertebrates, but some may be unique to the vertebrates (see above).

Prominent peptidergic systems in the brains of mammals include oxytocin and vasopressin, somatostatin, CRH, and others. Two of the more widespread systems in mammals are those involving CCK and somatostatin. The octapeptide CCK was initially discovered as a peptide hormone produced by endocrine cells in the duodenum, released after meals and contributing to satiety as well as gastrointestinal functions (see above). Later it was found to be widely distributed in the brain in mammals, including the cerebral cortex and particularly in interneurons where it is thought to have modulatory roles. One effect of CCK is increased anxiety. Somatostatin consists of 14 amino acids and was initially identified as a hypothalamic peptide inhibiting the release of growth hormone (somatotropin) from the pituitary. It has subsequently been found in many parts of the brain and like CCK it is present in many cortical interneurons, as well as in the gastrointestinal tract in both endocrine cells and neurons. Somatostatin in the

cerebral cortex is involved in inhibitory modulation of, for instance, somatosensory information.

NPY (neuropeptide Y) is also widespread in interneurons of the brain cortex. The NPY system consists of 3–4 related peptides all of which are 36 amino acids in length. One of these is almost exclusively neuronal in mammals, namely NPY itself. PYY (peptide YY), on the other hand, is almost exclusively produced in endocrine cells in mammals, but is expressed in the brain in teleost fishes as well as lampreys. This illustrates the close interrelationship of peptides in the neuronal and endocrine systems. The roles that NPY and PYY play in satiation control are explained above. Teleost fishes have duplicates of both NPY and PYY, but it remains to be explored what these duplications mean regarding functional diversification or specialization.

A family of vertebrate opioid peptides is expressed in the brain, including endorphin, dynorphin, enkephalins, and orphanin, arising from four large precursors encoded by four separate genes. This family, too, expanded at the dawn of vertebrate evolution along with its four receptors. The opioid peptides are important signals to reduce pain and many pharmaceuticals that relieve pain are agonists on opioid receptors (see Chap. 21). They also stimulate reward mechanisms and are thought to be involved in motivation. In humans they play key roles in the development of certain types of drug dependence and substances of abuse such as morphine and heroin.

11.6.1 Circadian Rhythms Are Controlled by Peptides

Several peptides influence the circadian rhythm in vertebrates. The two related peptides VIP (vasoactive intestinal peptide) and PACAP (pituitary adenylate cyclase-activating polypeptide) are released from different neurons in the suprachiasmatic nucleus of the hypothalamus where they act on GPCRs. PACAP binds to two related receptors whereas VIP binds to only one of them. The receptors have slightly different roles in the circadian regulation. Other peptides that affect the circadian rhythm include NPY and vasopressin.

A somewhat unusual neuropeptide system is formed by the peptides orexin A and B, also called hypocretins, that arise from the same precursor. Each peptide is

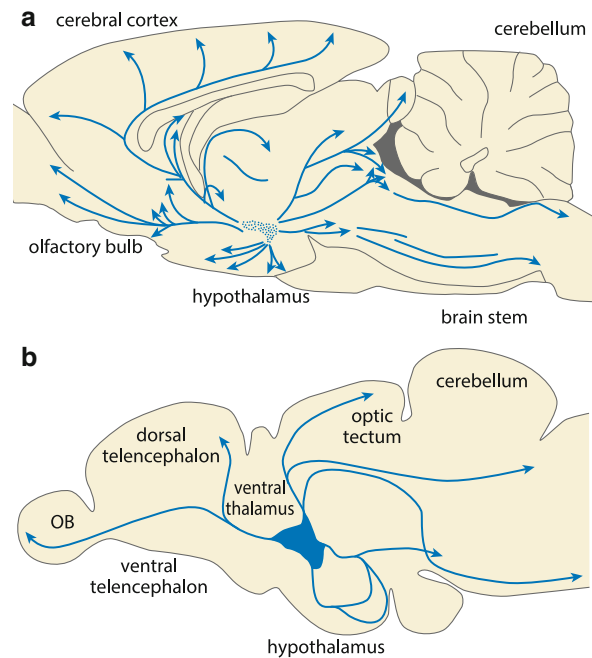


Fig. 11.20 Orexin distribution in the rat (a) and zebrafish brain (b), shown in parasagittal sections. Note that the brains are not drawn in the same scale. The rather limited population of hypothalamic cells that produce the two orexins have unusually wide axonal projections for a peptide system (a, Redrawn from Kilduff and Peyron [19] with permission. b, Redrawn from Panula [20] with permission)

approximately 30 amino acids long and they are produced by a rather small population of neurons in one nucleus of the hypothalamus. These neurons have quite widespread projections to be a neuropeptide system and reach many remote targets via long axons (Fig. 11.20) [19]. One important role of these neurons in mammals is to maintain wakefulness by acting on two GPCRs. In mammals, this alerting system can fail for a number of reasons resulting in dysregulation of sleep-wakefulness, more specifically loss of wakefulness and immediate entrance into so-called REM sleep, rapid eye movement sleep, i.e., the type of sleep associated with dreaming in mammals. The sleep disorder is called **narcolepsy** and has been described not only in humans but also several other mammalian species, particularly dogs. Human narcolepsy is usually due to loss of the neurons that produce the orexins, perhaps due to autoimmunity, but one case is known where early onset narcolepsy is due to a deleterious mutation in the gene encoding prepro-orexin. In dogs with an hereditary

type of narcolepsy, deleterious mutations are present in one of the two orexin receptors.

11.6.2 Peptides Influence Social Behavior

The closely related neuropeptides oxytocin and vasopressin described above have been found to have profound effects on social behavior in several mammals. Two species of North American voles have become famous because their reproductive and social behaviors differ greatly although they are closely related, namely the highly social and monogamous prairie vole *Microtus ochrogaster* and its asocial and promiscuous relative, the montane vole *Microtus montanus*. One species is monogamous with the male and the female forming a life-long pair, whereas the other species is described as promiscuous because male and female only meet for copulation whereupon the male leaves the female to rear the young. Many observations point to prominent roles for the oxytocin-vasopressin system in the regulation of these behaviors, such as clearly different receptor distribution in the brains of the two species. Inactivation of the oxytocin gene in mice results in specific blocking of reproductive memory. Recent studies have reported that subsets of humans with disturbed social behavior, such as autism, have genetic differences in the genes encoding these peptides or their receptors. In line with this, it is believed that oxytocin is essential for social cohesion, allowing people to form productive and meaningful relationships, in particular by mediating the development of trust.

11.7 Summary

Neuropeptides and peptide hormones play critical roles in development, growth, and reproduction and they are also involved in the regulation of most aspects of daily life of an animal. In mammals peptidergic regulatory systems are complex and often include multiple neuropeptides and peptide hormones that act at different levels in so-called neuroendocrine axes, that also involve feedback regulation from the distal cells in the pathway. Three prominent axes are distinguished in vertebrates: the hypothalamus-pituitary combined with either the thyroid gland, the adrenal glands, or the gonads. Invertebrate neuroendocrine

systems are based on similar principles. Functional overlap between components is present in many neuroendocrine systems, particularly in the vertebrates (due to gene duplicates), but defects in single genes of peptides or their receptors can nevertheless produce serious alterations in physiology and behavior. Thus, research in peptide signaling is important for future development of pharmaceutical therapies as well as pest control agents. Examples were given to illustrate how neuropeptides can be involved in the regulation of anxiety, pain, appetite, reward systems, clock functions, and sleep.

Peptide functions in invertebrates are less known than in vertebrates. One reason is that the invertebrates display tremendous diversity in organization of nervous and neuroendocrine systems, as well as considerable differences in physiology and behavior. At the molecular level some ancestral evolutionary relationships are obvious because the amino acid sequences of many peptides and peptide receptors display striking similarities between *Drosophila*, *C. elegans*, and vertebrates. Nevertheless, it has been more rare to find convincing examples of evolutionary conservation of the functional aspects of peptide signaling. One reason for this is that most peptides appear to be pleiotropic in their functions even in invertebrates. Therefore it is not clear what the original or even main function of a peptide is and more studies are required in invertebrates to resolve this before meaningful comparisons can be made. Probably the best example of peptide signaling that is conserved both at the molecular and functional levels is provided by the insulin-like peptides and their tyrosine kinase receptors. The original role of insulins may be in the regulation of growth since this is a common feature in all studied organisms.

One of the most striking conclusions emerging from studies of biology concerns the enormous diversity of paths taken in evolution, yet often using the same or similar components in a variety of different ways (a process coined exaptation by Stephen Jay Gould to refer to a feature that performs a given function but that was not produced by natural selection for its current use). Thus, we should view the diversity in organization of neuroendocrine and peptidergic systems, especially among invertebrates, as a fascinating challenge rather than a nuisance. Analysis of the complexity of neuropeptide signaling systems will certainly benefit from a broad comparative approach involving studies of many different types of animals.

References

- Squire LR (ed) (2003) *Fundamental Neuroscience*. Academic Press, Amsterdam
- Salio C, Lossi L, Ferrini F, Merighi A (2006) Neuropeptides as synaptic transmitters. *Cell Tissue Res* 326:583–598
- Kobayashi H (ed) (1987) *Atlas of endocrine organs, vertebrates and invertebrates*. Kodansha, Tokyo
- Brown RO, Pulst SM, Mayeri E (1989) Neuroendocrine bag cells of *Aplysia* are activated by bag cell peptide-containing neurons in the pleural ganglion. *J Neurophysiol* 61:1142–1152
- Hartenstein V (2006) The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. *J Endocrinol* 190:555–570
- Marder E, Bucher D (2007) Understanding circuit dynamics using the stomatogastric nervous system of lobsters and crabs. *Annu Rev Physiol* 69:291–316
- Nusbaum MP, Blitz DM, Swensen AM, Wood D, Marder E (2001) The roles of co-transmission in neural network modulation. *Trends Neurosci* 24:146–154
- Roller L, Yamanaka N, Watanabe K, Daubnerova I, Zitnan D et al. (2008) The unique evolution of neuropeptide genes in the silkworm *Bombyx mori*. *Insect Biochem Mol Biol* 38:1147–1157
- Shiga S, Toyoda I, Numata H (2000) Neurons projecting to the retrocerebral complex of the adult blow fly, *Protophormia terraenovae*. *Cell Tissue Res* 299:427–439
- Wegener C, Reint T, Jänsch L, Predel R (2006) Direct mass spectrometric peptide profiling and fragmentation of larval peptide hormone release sites in *Drosophila melanogaster* reveals tagma-specific peptide expression and differential processing. *J Neurochem* 96:1362–1374
- Ewer J (2005) Behavioral actions of neuropeptides in invertebrates: insights from *Drosophila*. *Horm Behav* 48:418–429
- Kim YJ, Zitnan D, Galizia CG, Cho KH, Adams ME (2006) A command chemical triggers an innate behavior by sequential activation of multiple peptidergic ensembles. *Curr Biol* 16:1395–1407
- Hall DH, Altun ZF (2008) *C. elegans Atlas*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, p 348
- Puig O, Marr MT, Ruhf ML, Tjian R (2003) Control of cell number by *Drosophila* FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev* 17:2006–2020
- Géminard G, Arquier N, Layalle S, Bourouis M, Slaidina M et al. (2006) Control of metabolism and growth through insulin-like peptides in *Drosophila*. *Diabetes* 55:S5–S8
- Zohar Y, Muñoz-Cueto JA, Elizur A, Kah O (2009) Neuroendocrinology of reproduction in teleost fish. *Gen Comp Endocrinol* 165:438–455.
- Roseweir AK, Millar RP (2009) The role of kisspeptin in the control of gonadotrophin secretion. *Hum Reprod Update* 15: 203–212
- Hokfelt T, Elfvin LG, Elde R, Schultzberg M, Goldstein M et al. (1977) Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. *Proc Natl Acad Sci USA* 74:3587–3591
- Kilduff TS, Peyron C (2000) The hypocretin/orexin ligand-receptor system: implications for sleep and sleep disorders. *Trends Neurosci* 23:359–365
- Panula P (2010) Hypocretin/orexin in fish physiology with emphasis on zebrafish. *Acta Physiol (Oxf)* 198:381–386
- Ancil M (2009) Chemical transmission in the sea anemone *Nematostella vectensis*: A genomic perspective. *Comp Biochem Physiol Part D Genomics Proteomics* 4:268–289
- Cerdá-Reverter JM, Larhammar D (2000) Neuropeptide Y family of peptides: structure, anatomical expression, function, and molecular evolution. *Biochem Cell Biol* 78: 371–392
- Dirksen H, Neupert S, Predel R, Verleyen P, Huybrechts J, Strauss J, Hauser F, Stafflinger E, Schneider M, Pauwels K, Schoofs L, Grimmelikhuijzen CJ (2011) Genomics, transcriptomics, and peptidomics of *Daphnia pulex* neuropeptides and protein hormones. *J Proteome Res* 10: 4478–4504
- Grönke S, Clarke DF, Broughton S, Andrews TD, Partridge L (2010) Molecular evolution and functional characterization of *Drosophila* insulin-like peptides. *PLoS Genet* 6(2): e1000857
- Gwee PC, Tay BH, Brenner S, Venkatesh B (2009) Characterization of the neurohypophysial hormone gene loci in elephant shark and the Japanese lamprey: origin of the vertebrate neurohypophysial hormone genes. *BMC Evol Biol* 9:47
- Hartenstein V (2006) The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. *J Endocrinol* 190:555–570
- Husson SJ, Mertens I, Janssen T, Lindemans M, Schoofs L (2007) Neuropeptidergic signaling in the nematode *Caenorhabditis elegans*. *Prog Neurobiol* 82:33–55
- Kastin AJ (ed) (2006) *The handbook of biologically active peptides*. Elsevier, Amsterdam
- Kits KS, Boer HH, Joose J (1991) *Molluscan neurobiology*. North-Holland, Amsterdam
- Kobayashi H (ed) (1987) *Atlas of endocrine organs, vertebrates and invertebrates*. Kodansha, Tokyo
- Lopez-Bermejo A, Buckway CK, Rosenfeld RG (2000) Genetic defects of the growth hormone-insulin-like growth factor axis. *Trends Endocrinol Metab* 11:39–49
- Nässel DR, Winther ÅM (2010) *Drosophila* neuropeptides in regulation of physiology and behavior. *Prog Neurobiol* 92: 42–104
- Papadimitriou A, Priftis KN (2009) Regulation of the hypothalamic-pituitary-adrenal axis. *Neuroimmunomodulation* 16:265–271
- Strand FL (1999) *Neuropeptides: regulators of physiological processes*. MIT Press, Cambridge
- Taghert PH, Nitabach MN (2012) Peptide neuromodulation in invertebrate model systems. *Neuron* 76:82–97
- Veenstra JA (2010) Neurohormones and neuropeptides encoded by the genome of *Lottia gigantea*, with reference to other mollusks and insects. *Gen Comp Endocrinol* 167:86–103
- Winter MJ, Ashworth A, Bond H, Brierley MJ, McCrohan CR, Balment RJ (2000) The caudal neurosecretory system: control and function of a novel neuroendocrine system in fish. *Biochem Cell Biol* 78:193–203
- Wynne K, Stanley S, McGowan B, Bloom S (2005) Appetite control. *J Endocrinol* 184:291–318

Further Readings