Vertebrate Protein and Peptide Hormones 4

Contents

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Readers familiar with how proteins are made may skip the following section. For those who are not familiar, we provide a short introduction to this process from which all life has arisen. The mechanism of forming structures from the genetic blueprint is obviously as old as life itself because it is common to all forms of life on Earth.

4.1 Translation

4.1.1 Reading Genetic Information: Transcription

Genetic information if encoded in the chromosome by means of the sequence of four bases—adenine, cytosine, guanine, and thymine—in the double strand of deoxyribonucleic acid genetic information is coded for in the chromosomes. This information is transcribed into a single-stranded ribonucleic acid when a gene is activated. In the case of bacterial, viral, or many yeast genes, the RNA is directly coupled to ribosomes with whose help single amino acids are added to a protein sequence according to the code in the DNA.

4.1.2 Coding and Other Sequences

In eukaryotic cells the coding information on the DNA double strand is interspersed with noncoding chromosomal regions, which will never be used for protein synthesis. The coding sequences are called exons, those without coding information, introns.

4.1.3 Splicing

The primary RNA transcript still contains exons and introns. By a process called splicing the introns are removed. Splicing is performed using enzymatically active RNAs and proteins. These proteins are called splicing factors.

Many RNAs can be spliced to different products, alternative splicing; for obtaining differentially spliced RNA the just-mentioned splicing factors are responsible that are found in a cell-type-specific manner. Thirty different splicing factors have been found; their regulation is not yet well understood.

4.1.4 RNA Cap

Eukaryotic RNA has on its 5′ end an additional structure, the so-called RNA cap that reduces the RNA degradation in the cytoplasm.

4.1.5 Nuclear Export of Messenger RNA

RNA when spliced and capped is called messenger RNA (mRNA). This mRNA is exported through the nuclear membrane with the help of transfer proteins and thus reaches the cytosol.

4.1.6 Docking to Ribosomes

In the cytosol two ribosomal subunits aggregate with the mRNA. Transfer RNA will load the amino acids into the ribosomes that will then be added to the protein sequence according to the genetic code. This process is called translation.

4.1.7 Translational Termination

A termination signal within the RNA sequence lets the ribosomal subunits fall off the mRNA. mRNA and ribosomes can be reused.

4.1.8 Membrane and Secretory Proteins

In the case of secreted proteins or membrane proteins this general translation pathway is extended. During translation membrane proteins are integrated into membranes although secretory proteins are not translated into the cytosol, but into special, membrane-sealed, cellular compartments, from where the secreted proteins, for example, hormones, are finally secreted.

These compartment are vesicles of the ER where synthesis of membrane proteins and secretory cell products takes place. These vesicles themselves are enclosed with a double membrane like the cell membrane. Other cellular compartments with separate double membranes are the eukaryotic nucleus, prokaryotes (i.e., bacteria or blue algae do not possess a nucleus), mitochondria, where energy is gained from sugars, and the Golgi apparatus, where protein maturation occurs. Secretory granules that contain the mature hormones ready for secretion are also separated from the cytosol by a double membrane.

Later, we demonstrate that some proteins of the steroid synthetic pathway are localized to mitochondria, some are found in the ER, and others stay in the cytosol. There exists a topological separation of different enzymatic functions of steroid-forming cascades.

4.2 Posttranslational Modification: Hormone Maturation

Precursors of protein/peptide hormones are formed at the membrane of the ER and they are translocated through this membrane into the ER vesicles. Therein and in other matured vesicles hormone maturation will occur.

4.2.1 Removal of the Signal Peptide

The first 22–30 amino acids of a precursor protein that is formed at the ER membrane are called signal peptides. Once the growing polypeptide chain has reached the interior of the ER the enzyme signal peptidase cleaves off this signal peptide, a process that is performed for membrane and secretory proteins.

4.2.2 Folding and Disulfide Bridges

The growing polypeptide chain moves through the ER pore as a linear strand. Within the ER this strand is folded into the three-dimensional structure characteristic for any protein. Folding is achieved with the help of chaperones, for example, heat shock proteins.

The three-dimensional protein structure resulting from folding contains mainly helices and β sheets. Other areas exist in an unordered form. Hydrogen bonds are essential structural elements for the maintenance of a given three-dimensional structure, as well as ionic and nonionic interactions between the amino acids of an individual protein. The use of supercomputers has not yet made it possible to create a general algorithm for protein folding: folding prediction has only been possible with varying success.

Coupled with folding is the generation of intramolecular disulfide bonds thereby covalently linking two cysteine residues. These disulfide bonds together with the interactions just mentioned determine the three-dimensional protein structure. The glycoprotein hormones such as thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), choriogonadotropin (CG) or nerve growth factor, as well as the insect hormone bursicon, form a special cysteine knot (Fig. 4.1); two pairs of disulfide bonds with short amino acid sequences between adjacent cysteines form a belt. The third disulfide bond is directed through this belt (Fig. 4.1). Modifications of this knot structure render the protein nonfunctional. The proper formation of the cysteine knot is indispensable for these hormones. It appears almost self-evident that this structure had been conserved during evolution whereas other amino acids were exchanged. The distances between two cysteine residues and thus the chain length in between remained constant during vertebrate evolution, which gives a clue for the conservation of the functional properties, too.

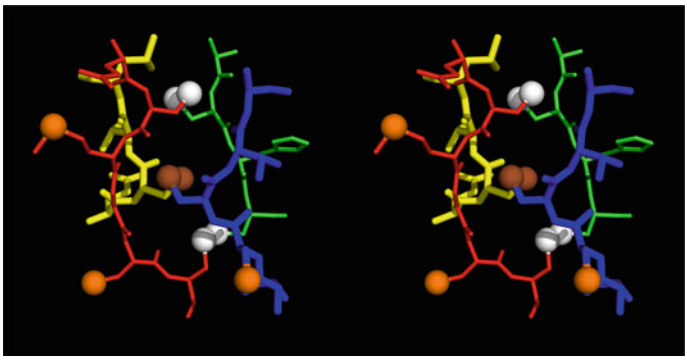


Fig. 4.1 Stereo view of the cysteine knot of the gonadotropin α-chain: two disulfide bonds (*white*: sulfur atoms) between Cys28 (*red* chain, amino acids 27–32) and cys82 (*green* chain, amino acids 81–84), and between Cys32 (*red*) to Cys84 (*green*) form a ring through which reaches the third disulfide bond between Cys10 (*yellow* chain, amino acids 9–12) and Cys60 (*blue* chain, amino acids 58–62) (*brown*: sulfur atoms) (Source: GenBank 1HRP and PyMOL)

4.2.3 Protein Complexes

The next step during hormone formation is the aggregation of identical or different polypeptides to larger complexes. This is a general feature not only of hormones, but also of many other proteins.

Within hormones the glycoprotein hormones are complexes of two different polypeptides. The first chain, the α chain is the common chain of four different glycoprotein hormones, and the β chain is characteristic for the four hormones: LH, FSH, TSH, and CG. Singular α or β chains are nonfunctional. The complex of both chains is necessary to give rise to the proper structure that triggers the hormone receptor on the target cell.

Oxytocin has equally been found in a complex with other peptides, the neurophysins. Whereas α-glycoprotein and β-glycoprotein hormone chains are transcribed from different genes, the oxytocin and the neurophysins are coded for in the same gene and transcribed into a single protein which is then processed during hormone maturation, however, the separated peptides stay together in a complex. At the final stage in the secretory granule, the mature oxytocin is no longer complexed to neurophysins.

4.2.4 Glycosylation

This step again primarily concerns the glycoprotein hormones. Several asparagine residues are substituted with oligosaccharides. In the Golgi apparatus these contain mannose-rich oligosaccharides. These mannoses form a sorting signal that leads the way to the secretory granules. In later vesicles the mannoses are partially

replaced with other sugars and acquire fucoses and terminal *N*-acetylneuraminic acids, the latter the characteristics of mature glycoproteins. Addition of sugars and replacement of mannoses are common processes of glycoprotein synthesis and not restricted to hormones.

4.2.5 Prohormone Convertases

4.2.5.1 Introduction

We now discuss the special pathways of hormone maturation. As shown above, the first processing of the newly formed polypeptide chain is performed by the signal peptidase which removes the signal peptide. Whenever a signal peptide reaches the interior of the ER it is quickly and reliably removed but the chain itself is not yet finished.

By cleaving off the signal peptide, one end of many protein and peptide hormones is exposed. This end is called the amino terminus or N-terminal end. Here we find the name giving α-amino group at carbon atom 1 of the terminal amino acid. Because all the other α-amino groups are involved in the peptide bonds (boxes in Fig. 4.2), there is only this single α-amino residue in any polypeptide chain. The opposite end of the polypeptide chain is called the carboxy-terminal or C-terminus due to the free carboxy group there, a characteristic feature of organic acids. There is only one free C-terminal carboxy group in any polypeptide inasmuch as all the others are also part of the peptide bonds.

The C-terminus of almost any vertebrate protein/peptide hormone is exposed by enzymes that recognize dipeptide motifs formed by lysine (**K**) and arginine (**R**) and cleave the polypeptide chain behind these dipeptides. These enzymes were labeled prohormone convertases (PC) because they convert the precursor chains into functional hormones (at least sometimes).

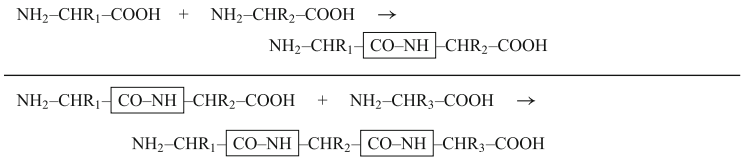


Fig. 4.2 Forming peptide bonds. R_1, R_2, and R_3 represent different amino acid side-chains (Table 16.2)—for example, R_1 is CH_3; this amino acid is called alanine; two alanines (R_1 and R_2 are CH_3) give rise to alanylalanine; and by adding a third alanine (R_3 is CH_3), we obtain alanylalanylalanine (Ala–Ala–Ala or **AAA**)

4.2.5.2 Sequences and Genes

PC1

The human *PC1* gene (other names: neuroendocrine convertase 1 (NEC1); prohormone convertase 3 (PC3)) is found on chromosome 5 at locus 5q15–21 and holds 14 exons. Its promoter is preferentially stimulated by cAMP, and also by, for example, CRH. This suggests coordinated activation of the hormone precursor proopiomelanocortin (POMC) and of its processing enzyme PC1.

The protein PC1 is a serine protease of the subtilisin/kexin type.[1]

PC2

Twelve exons of the human *PC2* gene are distributed on chromosome 20 (20p11.2). The protein precursor is formed in an inactive form and requires for its activation the coexpression of the protein 7B2 (SGNE1). Defects in either of these two genes result in hypoglycemia, hyperinsulinemia, and hypoglucagonemia, indicating that these enzymes participate in insulin precursor processing. The pathological effects are more pronounced when 7B2 is defective compared with PC2 defects.

4.2.5.3 Properties and Physiology

Prohormone convertases cleave an inner peptide bond of polypeptides. Thus, they belong to the large group of endopeptidases. Some endopeptidases cleave the bond between any two amino acids, for example, proteinase K. Others such as trypsin or chymotrypsin recognize single amino acids and cleave the polypeptide chain after these monoamino acid motifs. Prohormone convertases 1 and 2, however, recognize diamino acid motifs with lysine (**K**) and arginine (**K**).[2] The dibasic amino acid motifs are **KK**, **KR**, **RK** and **RR**.

While PC1 preferentially cleaves behind the motif **KR**(= lysyl-arginyl)[3] all four motifs are recognized and cleaved by PC2. During processing of the POMC precursor this is most important. POMC gives rise to different peptides depending on the PC active in a cell. In addition to adrenocorticotropic hormone (ACTH) β-lipotropin (β-LPH), γ-LPH, β-endorphin, and three distinct melanocyte-stimulating hormones (MSH) are formed by alternative splicing. A cell with only PC1 derives only ACTH and β-LPH from POMC-like corticotropic cells of the pituitary. Other cells in the brain express PC2. These cells form γ-LPH, β-endorphin and MSHs (Figs. 4.17 and 4.19).

The POMC example shows that PC1 or PC2 may cut a precursor chain several-fold. The neuropeptide TRH, for example, from the hypothalamus which induces TSH release in the pituitary exists in six copies in the TRH precursor. Each copy of the peptide sequence **QHPG** is preceded by a **KR** motif and followed by a **KR** or **RR**

[1] Subtilisin is a protease of *Bacillus subtilis*, kexin the prohormone convertase from *Saccharomyces*.

[2] Amino acids names using the single-letter code are always printed in bold letters of equal spacing.

[3] Amino acids within a polypeptide chain are called, for example, lysyl or arginyl.

mature TRH: **pEHP-NH$_2$**

pre-pro-TRH: (human TRH precursor)

```
MPGPWLLLAL ALTLNLTGVP GGRAQPEAAQ QEAVTAAEHP GLDDFLRQVE
RLLFLRENIQ RLQGDQGEHS ASQIFQSDWL S
KR:QHPGKR: EEEEEEGVEEEEEEEGGAVGPH
KR:QHPGRR: EDEASWSVDVTQH
KR:QHPGRR: SPWLAYAVP
KR:QHPGRR: LADPKAQRSWEEEEEEEEREEDLMPE
KR:QHPGKR: ALGGPCGPQGAYGQAGLLLGLLDDLSRSQGAEE
KR:QHPGRR: AAWVREPLEE
```

Fig. 4.3 Thyrotropin-releasing–hormone (TRH). The precursor sequence starts at **MPG** in the *upper line*, and ends in *line 8*. The motifs **KR** and **RR** are emphasized by *colons* which also indicates cleavage sites where Prohormone convertase 1 or Prohormone convertase 2 process the precursor. Amino acids are depicted by letters (See Table 16.2)

motif (Fig. 4.3). PC1 and PC2 produce six oligopeptides from the TRH precursor. TRH is essential for metabolic regulation. Multiplication of its sequence ensures that a single point mutation induces only a gradual loss thus protecting against a dominant TRH defect. Production of multiple copies of a peptide from the same precursor is economical and reduces the energy required for formation of ribosomal complexes and translational start because they are only to be complexed once.

4.2.5.4 Phylogeny

The mechanisms of hormone formation have not changed much from the very first days of primordial neuropeptides. Thus prohormone convertases are among the primordial enzymes of hormone formation, already found in invertebrates.

4.2.6 Monobasic and Dibasic Sequence Motifs in Invertebrates and Vertebrates

Viewing the many neuropeptide precursors of vertebrate and invertebrate species the common **KR** sequences are striking. These constitute by far the most frequent peptide motif recognized by prohormone convertase. **KR** is the PC1 motif. Much less frequent are the other three dibasic motifs **KK**, **RK**, or **RR** recognized by PC2. Sometimes (more in invertebrates, less in vertebrates) we find motifs for furin-like peptidases **RxxxxR** with two to four variable (= **x**) amino acids. Very rarely there are monobasic **K** or **R** cleavage sites where in mammals trypsin or chymotrypsin would cleave the chain.

Veenstra (2000) and Southey et al. (2008, 2006) have reviewed that **KR** sites are always used whereas**RR**, **KK**, or **RK** sites are less frequently used. The utilization of monobasic recognition sites is not yet understood because the identity and even more the specificity of enzymes in different taxonomical orders are far from being fully understood. Sometimes furin-like and dibasic sites in the same precursor are

used: for example, the short neuropeptide F (sNPF) precursor from the mosquito Anopheles gambiae (Fig. 5.31) is cleaved into five, from the same extract chemically identified oligopeptides. Three of these are cleaved after a dibasic, however, two of them in a furin-like motif.

4.2.7 Chopping the C-Terminus

By cleaving the TRH precursor chain at the **KR** motif, the maturation process is not yet finished. In many cases this peptide is still nonfunctional. Comparing the different TRH-cleavage products with mature TRH, we observe that they are still extended at the C-terminus.

Other peptidases than those described thus far will now chop off all amino acids from the C-terminus until they encounter a glycine residue. Glycine cannot be removed by these enzymes. Thus **QHPGKR** will be left from **QHPG**, however, **QHPG** will also remain from **QHPGRR:EDEASWSVDVTQH** because there is no glycine but that in position 4; all the other amino acids will be sequentially removed from the C-terminus. After similar processing of the other four oligopeptides six **QHPG** peptides are present.

4.2.8 Oxidation of the Terminal Glycine

The peptidyl glycine-α-amidating monooxygenase (PAM) oxidizes the terminal glycine into an amide residue (Fig. 4.4). At first the α-C atom of glycine will be oxidized. This reaction is only possible in glycine with its two hydrogen atoms at the Cα atom. The second step involves removal of glyoxal and leaves the NH_2 function. Because this is coupled to a carbonyl double bond the structural name is amide. Amides are less prone to chemical attack than amino groups. Amidation of the C-terminus increases the overall survival of a peptide in the body where many enzymes are ready to digest a lonely peptide.

Remember that precursor sequences such as peptide-**GxxKR** peptide-**GxxRR**, peptide-**GxxRK**, or peptide-**GxxKK** will result in a peptide-amide at the C-terminal end of the hormone (xx indicates small or larger peptide sequences and may also be missing).

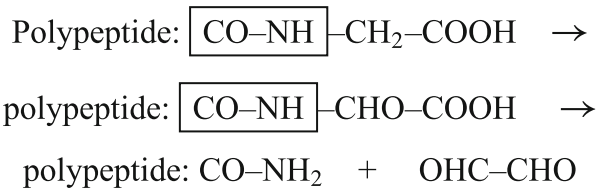


Fig. 4.4 Amidation of the C-terminal glycine

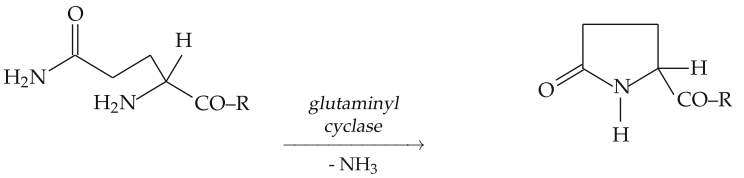


Fig. 4.5 Cyclization of the N-terminal glutamine

4.2.9 Cyclization of the N-Terminal Glutamine

The perseverance of a peptide hormone in the circulation will be further enhanced by one additional step of hormone maturation: the N-terminal glutamine (**Q**) will undergo intramolecular cyclization giving rise to a N-terminal pyroglutamic acid group (p**E**; Fig. 4.5).

The neuropeptide gonadotropin-releasing hormone thus loses its last free amino group. Such a peptide, especially if no lysyl residue is present that has an additional ϵ-amino group, is better armed against enzymatic degradation. Given that GnRH need only survive for a little more than 2 cm of bloodstream its half-life of 5 min in blood is sufficiently long enough to ensure that the receptors on the pituitary cells get triggered.

4.2.10 Esterification of Ghrelin

Without precedent among secreted peptides is esterification of ghrelin by octanoic acid. *O*-Acyltransferase—that is, the enzyme that transfers octanoic acid to the hydroxyl group of serine at position 3 of ghrelin—has been identified (Yang et al. 2008; Gutierrez et al. 2008).

Other peptides with long-chain fatty acid substitutions have these at their N-terminus or at the free lysyl amino groups. The reversible transfer of palmitic acid to the cysteine of guanosine nucleotide-binding proteins (G proteins) while forming a thioester bond suggests a special function for this modification. The acylated G-protein complexes associate with membranes and may thus facilitate hormone receptor interactions (see also Sect. 8.2.1). Gene activation by acetylation of histones also belongs to these mechanisms. β-Endorphins and α-melanocortins are also N-terminally acetylated.

Apart from octanoic acid other fatty acids including decanoic acid and its unsaturated decenoic acid have also been found as substituents of ghrelin. We would assume that further chain elongation might result in strong unspecific interactions of ghrelin with any membranes. A hormone with such long-chain fatty acids will never reach its receptor because it previously got stuck somewhere.

4.3 Peptide Hormones of the Hypothalamus and the Brain

4.3.1 Hypothalamic-Releasing Hormones

GnRH, TRH, CRH, GHRH (Table 4.1): These four neuropeptides stimulate release of hormones in the pituitary: GnRH induces the release of the gonadotropins, LH and FSH, TRH of thyrotropin (thyroid-stimulating hormones, TSH), CRH boosts corticotropin (ACTH; adrenocorticotropic hormone) release, and GHRH stimulates growth hormone (GH; older term somatotropin) secretion. After being formed in neurosecretory cells of the hypothalamus (see Sect. 10.2.1), the four neuropeptides are transferred via axonal transport into the median eminence where they will be released by appropriate stimuli. The blood capillaries will be reached by diffusion through little windows in the capillary wall. By direct transport through a portal system the four releasing hormones reach the anterior pituitary and their cellular targets leaving the capillaries again in fenestrated areas.

Such fenestrated passages between brain cells and blood vessels are called neurohemal organs. Usually the vessels in the brain are covered with a thickened layer of cells, the blood–brain barrier (BBB); in neurohemal organs the BBB is missing and a direct transport of hormones into and from the blood is permitted.

Released in the median eminence the four neuropeptides reach the pituitary straight via a portal system. The distance is not much larger than 2 cm. During this short passage the peptides are stable. In the pituitary there are again fenestrated capillaries allowing the hormones to reach the receptors on the target cells.

4.3.1.1 TRH

Introduction

TRH was the first hypothalamic neuropeptide whose structure could be determined in 1969 (Boler et al. 1969; Burgus et al. 1969). About 500 tons of sheep brain were used to extract the peptide and identify the structure pyrGlu-His-Pro-NH_2. Compared to usual peptides TRH shows three distinct characteristics:

1. It is very short, only a tripeptide.
2. The C-terminus is amidated.
3. The N-terminus is a pyroglutamic acid.

Table 4.1 The hypothalamic-releasing hormones (RH)

Name	Abbreviation	Sequence
Corticotropin RH	**CRH**	**SEEPPISLDL TFHLLREVLE MARAEQLAQQ AHSNRKLMEI I**-NH_2
Thyrotropin RH	**TRH**	p**EHP**-NH_2
Gonadotropin RH	**GnRH**	p**EHWSY GLRPG**-NH_2
Growth-hormone RH	**GHRH**	**YADAIFTNSY RKVLGQLSAR KLLQDIMSRQ QGESNQERGA RARL**-NH_2

Fact sheet 4.1: Thyrotropin-Releasing Hormone

Gene:	Chromosome 3; locus 3q13.3-q21; three exons.
Sequence:	**pEHP-NH_2**.
Synthesis and target:	TRH is preferentially synthesized in the paraventricular nucleus and acts via the median eminence on thyrotropic and lactotropic cells of the pituitary. TRH is also active as a neurotransmitter in many neurons.
Function:	Releasing hormone for thyrotropin and prolactin; major stimulator of metabolism; controls thyroid gland functions; equally active as neurotransmitter.
Receptor:	Heptahelical GPC membrane receptor.

Inasmuch as TRH was the first neuropeptide whose structure was determined, these features were very new; TRH appears to be the proverbial needle in the haystack to be looked for. The problems the protagonists in the race for the first neuropeptide structure, Schally and Guillemin, encountered can be studied in the book by Crapo (1985)

Biochemistry and Genes

On chromosome 3 (3q13.3-a21) the singular gene for the TRH precursor was found to contain three exons. After splicing and translation, the precursor contains several copies of the **QHPG** sequence; the **KR** prohormone convertase 1 recognition site is present several-fold, too. By PC1 the precursor is cleaved and the several precursor peptides then undergo maturation to the final TRH: p**EHP**-NH_2 (see Sect. 4.2). There are six copies of the **QHPG** sequence in the TRH precursor in humans, five in rats, and seven in frogs.

Physiology

TRH is the major regulator of the thyroid hormone and thus of energy homeostasis. In "lower" vertebrates TRH functions as a neurotransmitter because these animals do not synthesize thyrotropin. This neurotransmitter function is also retained in mammals, independently of the hypothalamic–pituitary–thyroidal axis.

Apart from the hypothalamus pro-TRH is synthesized in many brain regions: in the reticular nucleus of the thalamus, in the cerebral cortex, in pyramidal cells of the hippocampus, in external "plexiformal" layers of the olfactory bulb, in the sexually dimorphic nucleus, in the preoptic area, in the supraoptic nucleus, and in the substantia nigra, as well as in the pineal gland and the spinal cord.

Nonneural tissues where TRH is expressed are the mammalian pancreas and normal thyroid tissue. Frogs express TRH in their skin.

Human TRH regulates a circadian TSH rhythm with maximal release at midnight and minimal concentrations in the late afternoon. There are additional ultradian TSH peaks in 2- to 4-h intervals (see also Chap. 12). These rhythms are controlled by the suprachiasmatic nucleus and other cerebral pacemakers (Chap. 12). The limbic

system, the pineal gland, and CNS regions involved in stress responses (Sect. 11.2.1) co-influence the pulsatile TRH/TSH release.

Catecholamines are further important regulators of hypothalamic TRH neurons: α_1-adrenergic neurons from the brainstem activate hypothalamic TRH neurons. Noradrenaline induces in vitro TRH secretion and dopamine inhibits TSH release. Application of the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine diminishes the TSH release triggered by chilling (compare catecholamine biosynthesis, Fig. 7.1).

Endogenous opioids as well as somatostatin block TRH release; the latter blocks TSH release as well.

By glucocorticoids TRH mRNA transcription is directly blocked and by stimulating somotastatin indirectly. Dexamethason, a synthetic glucocorticoid, however, stimulates TRH transcription. In vivo such directly stimulating effects are counteracted by inhibitory neural influences from, for example, the hippocampus.

In its role as neurotransmitter TRH is involved in thermoregulation and in the amplification of noradrenergic and dopaminergic effects. By stimulating the preoptic area a direct influence on the regulation of body temperature is exerted. While activating the thyroid gland and thus metabolic activity, TRH indirectly enhances the body temperature and the activity of *sympathetic neurons* in the brainstem and the spinal cord.

Phylogeny

TRH is a characteristic vertebrate hormone. In agnathans (hagfish and lampreys) TRH positivity has been observed by immunocytology. Related peptides have been observed in lancelets and echinoderms: p**ESP**-amide in lancelets and p**EWP**-amide and p**EYP** together in a common precursor protein. Multiple copies of the sequences are found as in the human TRH precursor. Teleosts, frogs, birds, and mammals all express one homologous gene in the brain with the translated sequence **QHPG**. Maturation to the active p**EHP**-NH_2 is found in all these vertebrates. In *Xenopus laevis*, a second gene was identified that also shows seven peptide copies. This gene, however, has a different promoter and is predominantly expressed in the frog's skin. At least one of the TRH receptors is expressed in the *Xenopus laevis* skin, which suggests that color adaption to the environment might be regulated by TRH.

4.3.1.2 CRH

Introduction

An adequate response to stress in mammals depends on a functional hypothalamic–pituitary–adrenal axis (HPA). CRH, its receptors on corticotropic cells in the pituitary, ACTH released by these cells and its receptors, together with cortisol synthesis and release in the adrenal constitute this HPA. The indispensable role of CRH was demonstrated in the analysis of children suffering from congenital isolated adrenocorticotropic hormone deficiency where an abnormal CRH gene structure or expression was observed.

Fact sheet 4.2: Corticotropin-releasing hormone

Gene:	Chromosome 8; locus 8q13; two exons
Sequence:	**SEEPPISLDL TFHLLREVLE MARAEQLAQQ AHSNRKLMEI I**-NH_2.
Synthesis and target:	CRH is predominantly synthesized in the paraventricular nucleus, released in the median eminence and targets corticotropic cells of the pituitary; CRH is released from the placenta.
Function:	Releasing hormone for ACTH; central regulator of neuroendocrine reactions and behavior in response to stress; during gestation a potential indicator of preterm delivery.
Receptor:	Two heptahelical GPC membrane receptors, CRHR1 and CRHR2, with alternatively spliced products.

Biochemistry and Genes

CRH is derived from a preprohormone in a classical way processed as shown in the earlier chapters. The amidated C-terminus is a prerequisite for CRH receptor binding whereas the N-terminus is not required. Thus N-terminally shortened CRH peptides such as the CRH-9–41 peptide are fully biologically active. Oxidation of the methionine residue in position 38 destroys any biological activity which is a way as to inactivate CRH. The human CRH gene is found on chromosome 8 (locus 8q13) (Kellogg et al. 1989).

Physiology

CRH and vasopressin are the primary hormonal regulators of the human stress response. The observation of CRH and its receptors in the brain region apart from the hypothalamus, for example, as in the limbic system, in the central, stimulating sympathetic system of the brainstem and the spinal cord suggest this role. Intracerebral injection of CRH in animals leads to a coordinated sequence of physiological and behavioral reactions. These comprise:

- Activation of the hypothalamic–pituitary–adrenal axis
- Activation of the system of the nervus sympathicus
- Enhanced alertness
- Suppression of feeding and sexual activity
- Hypothalamic hypogonadism
- Changes in locomotor activity

These items characterize the usual behavior when stressed.

There are additional allies of this response which function as important regulators of corticotropic cells. A mutual positive interaction exists between CRH and vasopressin (AVP) release in the hypothalamopituitary unit: AVP induces CRH release and CRH stimulates AVP release. Without stress the pulses of these two hormones are more than 80 % overlapping. During stress the amplitude enlarges and if magnocellular AVP neurons are involved a continuous increase of the AVP level in plasma is observed.

CRH as well as AVP are released after stimulation by catecholamines (dopamine, noradrenaline, and adrenaline). AVP/CRH neurons on the one hand and the locus coeruleus plus noradrenergic neurons of the central stress response system are intimately mutually innervated and are regulated by the same factors in parallel. There are some ultrashort feedback loops by CRH on CRH neurons and by noradrenalin on noradrenergic neurons (Strakis and Chrousos 1997).

CRH and noradrenergic neurons as well are triggered by serotonin (5-HT) and acetylcholine and inhibited by corticosteroids and by the neurotransmitter γ-aminobutyric acid (GABA). The peptides derived from POMC and released after CRH stimulation in the pituitary such as ACTH, α-MSH, β-endorphin and further opioids such as dynorphin, exert a feedback inhibition on CRH and on noradrenergic neurons. Intracerebral injection of noradrenaline upregulates CRH, AVP, and ACTH release in the CNS, but not the ACTH secretion in the pituitary. Catecholamines, therefore, influence brain regions that are upstream of the pituitary functions and thus enhance AVP and CRH release.

AVP and CRH neurons additionally release products of the dynorphin gene together with AVP or CRH. These products including β-endorphin are potent endogenous opioids and suppress AVP and CRH effects on target cells.

CRH-Binding Protein

In addition to the CRH receptor there is a plasma CRH-binding protein (CRH-BP) with high affinity for CRH. Binding to this binding protein blocks activation of the CRH receptor by CRH. The binding protein is not related to the receptor. It has been found in the CNS, in placenta, in the amnion liquid, and in human plasma. In mice and cows, however, the binding protein has not been identified in plasma. Expression of the CRH-BP in the brain modulates reactions to stress. Deletion of the CRH-BP gene in mice increases anxiety in these mice but not in control animals. As mice do not have CRH-BP in their plasma or in the adrenal glands, there is no effect of the deletion in modified mice (Karolyi et al. 1999).

Phylogeny

Vertebrates possess three further CRH-related genes involved in the response to stress: CRH/CRF, urocortin/urotensin I, stresscopin (SCP)/urotensin III, and stresscopin-related peptide (SRP)/urotensin II. Between mammals and teleosts sequence homology is above 96 % for CRH/CRF, and above 55 % for SCP. There are similar precursor proteins and derived peptides in insects. This suggests that fight-or-flight responses and the handling of stress might have been present early in chordate evolution and that the two CRH receptors have mediated these responses.

Human urocortin (40 amino acids long and coded for on chromosome 2) preferentially binds to CRH receptor 2. Its effect is diminishing appetite and not to mediate stress. Stresscopin (40 amino acids) and stresscopin-related peptide (coded for on chromosome 3 (3q21.3; 43 amino acids)) display similar reactions. This might indicate that CRH mediates immediate reaction to stress by inducing cortisol synthesis and release and managing the metabolic changes depends on CRH, but equally on urocortin, stresscopin, or SCP.

The homology between vertebrates and insects extends to the CRH binding protein (Huising and Flik 2005). This extends the discussion of whether CRH might have already been present in common ancestors of insects and vertebrates.

4.3.1.3 GnRH

Fact sheet 4.3: Gonadotropin-Releasing Hormone

Genes:	GnRH-I: Chromosome 8; locus 8p21-p11.2; four exons GnRH-II: Chromosome 20 ; locus: 20p13; four exons
Sequences:	GnRH I: p**EHWSY GLRPG**-NH_2 GnRH II: p**EHWSH GWYPG**-NH_2.
Synthesis and target:	GnRH-I is expressed in several nuclei of the hypothalamus, released in the median eminence and triggers pituitary gonadotropic cells. Additionally, GnRH is expressed in trophoblastic cells of the placenta, to a lesser degree by T-lymphocytes. GnRH-II is preferentially expressed in kidney, bone marrow, and prostate; in the brain in the caudate nucleus, the hippocampus, and the amygdala.
Function:	GnRH-I: releasing hormone of LH and FSH; central regulator of reproduction; during gestation stimulator of choriogonadotropin . GnRH-II: found to enhance ovarian cancer invasiveness.
Receptor:	One GPCR: GnRHR1 (the human *GnRHR2* gene is a pseudogene).

Introduction

GnRH-I is the major regulator of vertebrate reproduction. Its sequence is identical for almost all mammalian species (with the known exception of guinea pigs). Other vertebrates possess this mammalian GnRH-I such as certain teleost fish or frogs. Another peptide, GnRH-II, very similar to GnRH I, is labeled according the species where it was identified: chicken-II. In fish the first GnRH is seabream GnRH (sbGnRH), the second GnRH-II, also known as chII-GnRH, and a third GnRH-III, also known as salmon-GnRH (smGnRH). Although GnRH-II is predominantly expressed in the forebrain of fish, the other two GnRH are found in midbrain. sbGnRH is the hypothalamic hormone.

Biochemistry and Genes

The human genes on chromosomes 8 and 20 are similarly organized, the introns being larger in the GnRH-I gene on chromosome 8. Alternative splicing of the GnRH-II mRNA enlarges the polypeptide by several amino acids; this alternative splicing appears tissue specific (White et al. 1998).

Using the GnRH precursor the decapeptide GnRH is formed by the sequential action of the following enzymes:

1. the signal peptidase
2. the prohormone convertase PC1

3. the exopeptidase E
4. the peptidylglycyl α-amidating monooxygenase (PAM)
5. the glutaminyl cyclase

A large collection of synthetic structural analogues was instrumental in identifying structure–function relationships:

- N- and C-terminus are required for receptor binding.
- Amino acids (AA) 1–4 are necessary to release LH or FSH.
- The side-chains of His^2–yr^5–Arg^8 are essential for full biological activity.
- Replacement of Arg^8 decreases LH and FSH secretion.
- Changing Gly^6 for Leu^6 influences the capacity for LH secretion in a more profound way than the activity to release FSH.[4]
- The secondary structure of all GnRH peptides is conserved, because the β-turn, formed by amino acids 5–8, induces a hairpin loop required for receptor binding.

Physiology

Mammalian reproduction depends on secretion of hypothalamic GnRH in all species analyzed thus far. Its regulation is controlled by multiple set points, hormones, neurotransmitters, and regulator circuits (see also Sect. 11.3).

Most critical for functional GnRH activity is pulsatile release of GnRH. Without this periodic and (only in adults) fully adjusted release pattern, any LH and FSH secretion does not take place; on the contrary continuously elevated GnRH levels in the blood lead to LH and FSH suppression. This constitutes one mechanism of contraception (see also Sect. 11.3).

During fetal development, GnRH-positive neurons migrate from the olfactory bulb to the hypothalamus. Any disturbance of this migration results due to missing hypothalamic GnRH neurons in infertility frequently combined with olfactory defects (Kallmann syndrome).

The physiology of GnRH-II[5] is far from being understood. Due to its conserved structure for 500 million years a critical role is suggested. On the other hand, in cow and sheep, the gene is present as well as the gene for the GnRH2 receptor. However, there is a mutation in the cow sequence that prohibits receptor binding and the sheep gene harbors a premature stop codon that abolishes any GnRH-II synthesis. The human GnRH2 receptor is afunctional owing to a frameshift mutation. GnRH-II binds the GnRH1 receptor, however, with a signal induction different from GnRH-I induction. An important functional role is thus not evident at all.

The teleost GnRH-III is reported to be expressed in the forebrain whereas GnRH-I and GnRH-II have been found in the diencephalon. The GnRH-III neurosecretory cells are located close to the nervus terminalis, not far from the olfactory bulb. Their axons reach the retina. It has been suggested that GnRH-III may control

[4] Genazzani et al. (1996).

[5] GnRH-I is either mGnRH, chGnRH-I, or sbGnRG; GnRH-II is always chGnRH; GnRH-III is smGnRH.

pattern recognition in animals ready to mate. It is worth noting that GnRH-I neurons originally were observed in the very same brain region. These latter, however, migrate to the hypothalamus whereas the GnRH-III neurons stay in the forebrain. In vertebrates other than teleosts, a GnRH-III gene has not been found. There are further GnRH-like genes in agnathans; these are, however, not related to the GnRH-II gene.

Phylogeny

For a long time, it was assumed that GnRH peptides were characteristic vertebrate hormones. This assumption has been discarded. There are three GnRH peptides in lampetra, presumably evolutionarily older than chondrychthyes and osteichthyes or newer vertebrates; molluscs such as *Aplysia californica* and *Octopus vulgaris* were shown to form a GnRH-like peptide of 12 amino acids, with the insertion at the same place. *Ciona intestinalis* expresses nine GnRH peptides and a 16 amino acid long GnRH-like peptide that differs from all other peptides by an elongation at the C-terminus (Table 4.2). Reports in corals about GnRH activity that could release LH from teleost cells have not been corroborated by peptide sequencing or cDNA cloning (Twan et al. 2006).

Table 4.2 Sequences of GnRH variants with mammalian GnRH as a reference

Species	Abbreviation	Sequence	Reference
Mammalian GnRH	(mGnRH)	p**EHWSYGLRPG**-NH_2	Matsuo et al. (1971)
Chicken I	(chGnRH-I)	p-------**Q**---NH_2	Miyamoto et al. (1983)
Frog	(frGnRH)	p-------**W**---NH_2	Yoo et al. (2000)
Seabream (Sparidae)	(sbGnRH)	p-------**S**---NH_2	Powell et al. (1994)
Salmon	(smGnRH)	p------**WL**---NH_2	Sherwood et al. (1983)
White fish (*Coregonus*)	(whGnRH)	p------**MN**---NH_2	Adams et al. (2002)
Guinea pig	(gpGnRH)	p-**Y**----**V**----NH_2	Jimenez-Liñan et al. (1997)
Medaka (Japanese killifish)	(mdGnRH)	p----**F**--**S**---NH_2	Okubo et al. (2000)
Chicken II	(chGnRH-II)	p----**H**-**WY**---NH_2	Miyamoto et al. (1984)
Seawolf (Anarhichadidae)	(cfGnRH)	p----**H**--**N**---NH_2	Ngamvongchon et al. (1992)
Herring	(hgGnRH)	p----**H**--**S**---NH_2	Carolsfeld et al. (2000)
Dogfish	(dfGnRH)	p----**H**-**WL**---NH_2	Lovejoy et al. (1992)
Lamprey I	(lGnRH-I)	p--**Y**-**LEWK**---NH_2	Sherwood et al. (1986)
Lamprey II	(lGnRH-II)	p----**H**-**WF**---NH_2	GenBank ABE66462
Lamprey III	(lGnRH-III)	p----**HDWK**---NH_2	Sower et al. (1993)
Tunicate I	(tGnRH-I)	p----**DYFK**---NH_2	Powell et al. (1996)
Tunicate II		p----**LCHA**---NH_2	Powell et al. (1996)
Tunicate III		p-----**EFM**---NH_2	Adams et al. (2003)

(continued)

Table 4.2 (continued)

Species	Abbreviation	Sequence	Reference
Mammalian GnRH	(mGnRH)	p**EHWSYGLRPG**-NH_2	Matsuo et al. (1971)
Tunicate IV		p**----NQ-T--**-NH_2	Adams et al. (2003)
Tunicate V		p**-----EYM--**-NH_2	Adams et al. (2003)
Tunicate VI		p**----K--Y--**-NH_2	Adams et al. (2003)
Tunicate VII		p**-----A-S--**-NH_2	Adams et al. (2003)
Tunicate VIII		p**----LA-S--**-NH_2	Adams et al. (2003)
Tunicate IX		p**----NK-A--**-NH_2	Adams et al. (2003)
Tunicate X		p**----NWWI--APGYNG**-NH_2	GenBank AB219239.1: Ikemoto and Park (2006)
Octopus		p**ENY-F-N-WH--**-NH_2	Iwakoshi et al. (2002)
Aplysia		p**ENY-F-N-WYA-**-NH_2	Zhang et al. (2008a)

Tunicate GnRHs are from *Ciona productum* (I/II), *C. intestinalis*, or *C. savignyi* III–IX

The figure from Guilgur et al. (2006) was used as a template to generate a phylogenetic tree that includes nonvertebrate GnRH sequences.

Figure 4.6 shows the characteristic three GnRH types of fish. Some species have two chII-GnRH precursors, goldfish and carp probably indicating the duplication of the entire genome.

Figure 4.6 Phylogenetic tree of GnRH variants (Sect. 4.6). Precursor sequences from GenBank (query: "gonadotropin releasing hormone" minus "receptor") were imported into ClustalW clustered with the neighbor joining method and 1,000 bootstrappings. The resulting tree was sketched using `treedyn` and illustrated using `inkscape`.
Colorsg: *red lines*: Mammals; *orange lines*: reptiles and amphibians; *blue lines*: fishes; *green lines*: cyclostomata; *dotted black lines*: invertebrates

Abbreviations: ac: Aplysia GnRH; chI, chII: chicken GnRH I/II; ci: Ciona GnRH; cf: catfish GnRH; cp: guinea pig GnRH; fr: frog GnRH; hr: herring GnRH; lp: lamprey GnRH; m: mammalina GnRH; md: medaka GnRH; ov: octopus GnRH; sm: salmon GnRH; sb: sea bream GnRH; wf: white fish GnRH.
A. anser: greylag goose; *A. burtoni*: *Astatotilapia burtoni* (cichlidae); *A. californica*: California sea hare; *A. japonica*: Japanese eel; *A. sapidissima*: American shad; *A. schlegelii*: Japanese black sea bream; *C. auratus*: goldfish; *C. carpio*: carp; *C. clupeaformis*: lake whitefish; *C. gariepinus*: African sharptooth catfish; *C. intestinalis*: vase tunicate; *C. nebulosus*: *Cynoscion nebulosus*; *C. porcellus*: guinea pig; *D. labrax*: European seabass; *D. rerio*: zebrafish; *E. macularis*: leopard gecko; *G. australis*: pouched lamprey; *G. gallus*: chicken; *H. huso*: Beluga sturgeon; *H. sapiens*: Homo sapiens sapiens; *I. fossor*: northern brook lamprey; *I. unicuspis*: silver lamprey; *L. richardsoni*: western brook lamprey; *L. tridentatus*: pacific lamprey; *M. albus*: Asian swamp eel; *M. appendix*: American brook lamprey; *M. auratus*: Golden hamster; *M. cephalus*: flathead mullet; *M. gallopavo*: turkey; *M. mordax*: Australian lamprey; *M. mulatta*: Rhesus macaque; *M. musculus*: house mouse; *M. saxatilis*: Atlantic striped bass; *M. undulatus*: Atlantic croaker; *O. aries*: sheep; *O. bonariensis*: *Odonthesthes bonariensis*; *O. latipes*: medaka, Japanese killifish; *O. masou*: cherry salmon; *O. mossambicus*: Mozambique tilapia; *O. mykiss*: rainbow trout; *O. nerka*: sockeye salmon; *O. niloticus*: Nile tilapia; *O. tshawytscha*: Chinook salmon; *O. vulgaris*: common octopus; *P. major*: Japanese madai; *P. marinus*: sea lamprey; *P. notatus*: bluntnose minnow; *R. catesbeiana*: American bullfrog; *R. dybowskii*: Dybowski's frog; *R. norvegicus*: rat; *R. rutilus*: common roach; *R. sarba*: tarwhine; *S. aurata*: gilt-head (sea) bream; *S. fontinalis*: brook trout ; *S. jardinii*: gulf saratoga; *S. ocellatus*: red drum *S. murinus*: Asian musk shrew; *S. salar*: Atlantic salmon; *S. scrofa*: wild boar; *S. trutta*: brown trout; *T. natans*: rubber eel; *T. belangeri*: northern treeshrew; *T. rubripes*: Fugu; *T. nigroviridis*: green spotted puffer; *T. vulpecula*: common brushtail possum; *T. thynnus*: northern bluefin tuna; *V. moseri*: barfin flounder; *X. laevis*: African clawed frog

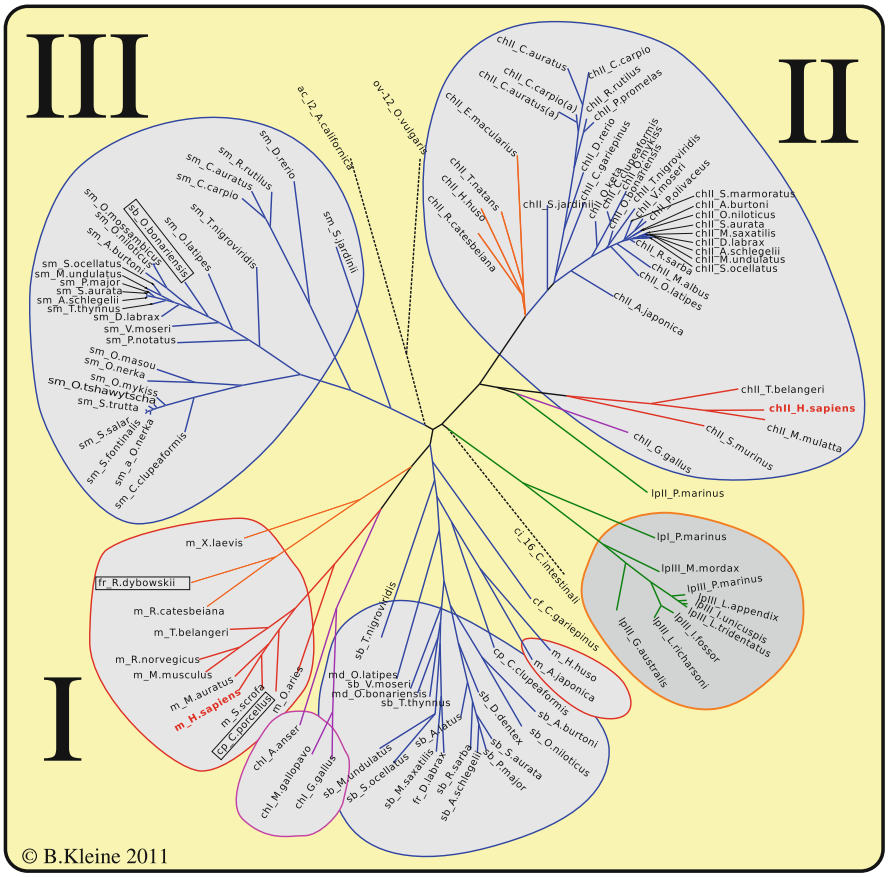


Fig. 4.6 Phylogenetic tree of GnRH variants

The ClustalW algorithm sorts the sequences first of all due to the sequence of the mature peptides (whereas the input comprised the whole precursor proteins). Further differentiation occurs due to differences in the remaining sequences, signal peptide, or associated peptide. There are additional differences separating chII-GnRH of mammals and birds. Although any variations of chII-GnRH have not been observed, smGnRH-II and GnRH (m-, chI- or sb-GnRH) show single amino acid exchanges (boxed in Fig. 4.6).

The pattern gains further complexity if we include the GnRH receptors. Up to five distinct GnRH receptor genes have been found (e.g., in the *Takifugu rupripes* genome (http://genome.jgi-psf.org/Takru4/Takru4.home.html) and in seabream (Moncaut et al. 2005). Some degree of tissue-specific differential expression of different receptor genes does not allow a definite association of receptor type and GnRH variant with any function. For this reason, the situation in mammals with the hypothalamic GnRH secretion and the derived pituitary gonadotropin release appears functionally clear. We discuss it again in Sect. 11.3.

4.3.1.4 GHRH

Fact sheet 4.4: Growth hormone releasing hormone

Gene:	Chromosome 20; locus 20q11.2; five exons.
Sequence:	**YADAIFTNSY RKVLGQLSAR KLLQDIMSRQ QGESNQERGA RARL**-NH_2.
Synthesis and target	GHRH neurons exist in the ventromedial nucleus and the arcuate nucleus; they secrete in the median eminence.
Function:	Releasing hormone of the pituitary growth hormone.
Receptor:	GPC heptahelical membrane receptor.

Introduction

Release of the growth hormone in the pituitary is regulated by stimulating (GH releasing hormone; GHRH) and inhibiting (somatostatin) neuropeptides. Both are secreted in the median eminence. Recently, ghrelin was identified to stimulate GH release, too.

Structure and Genes

The GHRH gene maps to chromosome 20q11.2. The translated RNA gives rise to a prepropolypeptide that contains a 30 amino acid long signal peptide, the GHRH sequence (1–44), the amidation signal, and the 30 or 31 amino acid long stretch of the C-terminal peptide. GHRH is posttranslationally modified like the other neuropeptides: cleaved by the signal peptidase and then by the prohormone convertase-1, shortened by endopeptidases, and finally amidated by PAM. Endopeptidase treatment at the C-terminal region forms 40 or 37 amino acid long, biologically active peptides. Further digestion to a 29 amino acid long peptide abolishes any biological activity.

Physiology

GHRH release is controlled by product feedback; it is growth hormone regulated. In the majority of brain regions the GH receptor and GHRH RNA were co-localized: in the hypothalamus, thalamus, septal region, hippocampus, dentate gyrus, or amygdala.

GHRH expression is higher in the hypothalami of male rats than in female hypothalami. This sexually dimorphic behavior is controlled by steroid hormones: Dihydrotestosterone (DHT) injection into ovariectomized rats masculinized their GH secretion. Injection of estradiol, however, diminished the GHRH secretion in male rats. In addition, the GH feedback control of GHRH release appears gender specific.

Those neurosecretory cells that release GHRH in the median eminence are found in the ventromedial nucleus and in the arcuate nucleus of the hypothalamus. They are interconnected with different CNS areas: signals from the “sleep center(s)” are stimulating and coupled to the sleep rhythm. Signals from the amygdala and from

ascending noradrenergic neurons of the brain stem are related to the activation of the stress reaction. These mediate stress-induced GH release. The ventromedial nucleus processes the secretion of hormones involved in blood glucose regulation and thus influences the GHRH release in reaction to hypoglycemia (see also Sect. 11.4).

GH release is regulated by GRHR stimulation and somatostatin (SST) inhibition. Functional and anatomically reciprocal interactions exist between the ventromedial nucleus, arcuate nucleus, and paraventricular nucleus: endogenous SST inhibits GHRH secretion from median eminence, whereas intracerebral SST injection stimulates GHRH release. GHRH neurons of the arcuate nucleus express high-affinity SST receptors. In addition to SST regulation, circadian GHRH pulses are controlled by zeitgeber of the suprachiasmatic nucleus. This circadian GHRH rhythm is synchronized to the sleep rhythm: elevated GH secretion during sleep, reduced GH release while awake.

GHRH neurons are further influenced by other neurons and their neurotransmitters: sleep-induced GH release is modulated by serotonergic and cholinergic neurons. Circadian GH pulses mediated by GHRH may be inhibited by α-antagonists *i.e.* inhibitors of catecholamine α-receptors, or substances directly blocking catecholamine biosynthesis. β_2-Agonists, stimuli of the β_2-catecholamine receptor, induce GH release presumably by stopping SST secretion. Anticholinergic drugs inhibit all GH stimulating effects but hypoglycemia. L-DOPA[6] as well as dopamine increase GH release most probably due to their local conversion into noradrenaline.

Apart from SST other CNS neuropeptides interact with GHRH neurons and contribute to GH release:

- Endogenous endorphins, in particular β-endorphin, increases GHRH and GH release.
- TRH, injected in to rat brain enhances GH release by a Ca^{2+}-dependent, cAMP-independent mechanism. In humans, TRH injection increases GH levels only in patients with acromegaly.
- Galanin, motilin and NPY[7] stimulated GH secretion from isolated rat pituitary cells. A subgroup of GHRH neurons themselves expresses neuropeptide Y which *in vitro* appears to upregulate GH release. Applied into a cerebral ventricle NPY quenches GH secretion suggesting additional regulators of GHRH and SST neurons by the inhibiting ascending noradrenergic neurons from the brainstem usually stimulating GH secretion via GHRH.

Phylogeny

Phylogenetically GHRH is closely related to another neuropeptide, PACAP.[8] By gene duplication at the beginning of mammalian evolution two different genes for

[6] 3,4-Dihydroxyphenylalanine.

[7] Neuropeptide Y.

[8] Pituitary adenylate cyclase activating peptide.

GHRH and PACAP were formed. Nonmammalian vertebrates form GHRH and PACAP by alternatively splicing the same RNA precursor. It is worth noting that the PACAP sequence is more strongly conserved in mammals than is the GHRH sequence (Montero et al. 2000).

After the event of gene duplication the GHRH exon in the PACAP gene gave rise to the PACAP-related peptide (PRP) whereas the PACAP exon of the GHRH gene mutated to the C-peptide of GHRH. PACAP is, like GHRH, amidated.

PACAP has been reported as an additional inducer of noradrenaline secretion in the adrenal glands and as a mediator of the metabolic response to elevated blood glucose levels. PACAP knockout mice and flies with PACAP defects show behavioral disorders, in $PACAP^{-/-}$ mice the metabolite 5-hydroxyindoleacetate was reduced.

PACAP and GHRH belong to the family of secretin-like peptides.

4.3.2 Gonadotropin-Inhibiting Hormone

Fact sheet 4.5: Neuropeptide VF; RF-related peptide; aka gonadotropin-inhibiting hormone (GnIH)

Gene:	Chromosome 7; locus 7p15; three exons.
Structure:	See Fig. 4.7; three peptides with a similar C-terminus, LPLRFamide, LPQRFamide, and LPLRSamide.
Synthesis and target:	Neuropeptide VF is expressed in dorsomedial neurons and in gonads and acts as a neurotransmitter preferentially on GnRH neurons in the median eminence.
Function:	Inhibitor of GnRH release and of gonadal activity.
Receptor:	Neuropeptide FF receptor 1; GPCR147 from the rhodopsin receptor family.

4.3.2.1 Introduction

Any preproproteins of human peptide hormones cleaved to release peptide hormones possess basic dipeptide motifs where either PC1 or PC2 may act. Later in this book we show that such motifs do exist in arthropods and thus most presumably already in common ancestors of vertebrates and invertebrates some 600 million years ago. There are, however, in flies, snails, or shellfish additional neuropeptides endocrine-active where monobasic recognition sites are used, however, close inspection showed that most of the peptides can be cleaved at recognition sites composed of the two basic amino acids **R/K** spaced 0, 2, 4, or 6 amino acids apart: $\mathbf{R/Kx}_N\mathbf{R/K}$ ($n = 0, 2, 4, 6$). The lack of usual or unusual cleavage sites directly raises the question of whether peptides where an N-terminal cleavage site is missing are active as neuropeptides if at all.

In Chap. 12 we deal with circannual rhythms. In all but a few species reproduction is coupled to the annual seasons. Any activity has its time, copulation, breeding,

upbringing, hibernation, or bird migration. Whereas in humans female reproductive activity cycles with a period of 28 days and humans can be sexually active on any day, in birds, for example, such behavior inhibits successful raising of the litter and would commit to much energy required, for example, for migration or survival in the cold season. In most animals reproductive activity is related to the annual cycle of seasons in males and females, at least in nondomesticated ones. A gonadotropin-inhibiting hormone in men appears dispensable, but it might definitely have a role in wild animals.

GnRH release has been previously shown to be inhibited by some neurotransmitters, however, a negative regulator had not been shown in vertebrates. Tsutsui et al. (2000) decided to look for hypothalamic peptides blocking GnRH release and identified in quail a dodecapeptide doing just that. They called this peptide gonadotropin inhibitory hormone (GnIH). Because this peptide has never been shown released into the circulation we would call the naming premature. GnIH belongs to the RFamide family of peptides present in vertebrates and invertebrates. The human GnIH homologue is cleaved from the neuropeptide-VF precursor protein.

4.3.2.2 Structure and Gene

The gene for neuropeptide VF is located on the short arm of chromosome 7. GnIH and their mammalian homologues RF-related peptides (RFRP) are characterized by the C-terminal **LPxRF** (x = **L** or **Q**). The human neuropeptide VF precursor contains in contrast to the chicken one only two GnIH homologues, the RFRP-2 (on *blue* background) has a C-terminal serine (**S**) instead of phenylalanine (**F**) (Fig. 4.7).

RFRP have unusual prohormone convertase motifs in contrast to other vertebrate hormones in this book: C-termini possess a rarely used **Rx**$_N$**R** (n = 0, 2, 4, or 6) motif where the PC1 can act. Some singular arginines (**R**) might be recognized by other endopeptidases. However, inspecting the precursor sequences in this

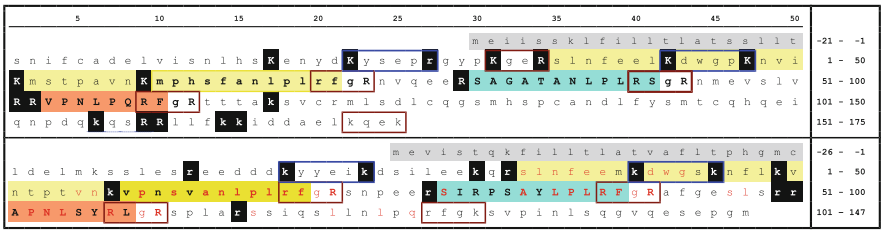


Fig. 4.7 Chicken gonatotropin inhibiting hormone (GnIH) and the human homologue, RFamide-related peptide (RFRP). The *upper* sequence displays the human preproprotein, the *lower* that of chicken. Signal peptides are on *gray* background, RFRP-1 on *yellow*, RFRP-2 on *blue*, and RFRP-3 on *orange* background. Monobasic and dibasic peptid motifs. are *inverted*. *Red* and *blue* frames indicated unusual PC1 motifs. Conserved amino acids are shown *red* in the chicken sequence. RFRP-1 is labeled by Tsutsui as a presumable **LPLRF**amide peptide due to the unknown cleavage mechanisms for the N-terminus (Tsutsui 2009) (Source: Swiss-Prot Q9HCQ7 (human) and GenBank BAE17049 (chicken))

book, hormonal activity does not rely on enzymes cutting after singular arginines. Whenever these are present in a precursor, there are multiple copies of peptide sequences and there are other peptides cleaved at dibasic sites of the **R/Kx$_N$K/R** with n = 0 the most obvious. Whether RFRP-1 is an active neurotransmitter is not clear and is obviously questioned by the most active group which called it only a presumable neuropeptide (Tsutsui 2009).

The GnIH/RFRP receptor is called in mammals the neuropeptide FF receptor (OT7T0222 or GPCR147) which is triggered by other RFamides, too.

In a very recent review, Tsutsui and Ubuka (2014) report an overview of GnIH in birds and mammals. They demonstrate that GnIH receptors on GnRH neurons influence the release of this hormone. What is actually blocked is the interaction of GnRHR with the $Gs_{\alpha\ s}$ subunit, which is responsible for activating adenylate cyclase and cAMP.

4.3.2.3 Physiology

The original observation in quail that GnIH directly inhibits pituitary LH/FSH release (Tsutsui et al. 2000) could not be confirmed in mammals. Isolated quail pituitaries where incubated with GnIH and showed reduction of LH/FSH secretion. Whereas quail GnIH is expressed in the paraventricular nucleus (Tsutsui et al. 2000) RFRP *e.g.* in rats it is expressed in the dorsomedial hypothalamus (Rizwan et al. 2009. RFRP axons originating in dorsomedial hypothalamus cell bodies reach in rats to the median eminence, however, not to its external border from where neuropeptides would be released into the hypothalamic–pituitary portal system (Rizwan et al. 2009).

Inhibition of gonadotropin secretion in mammals is achieved by inhibiting not the gonadotropin releasing cells in the pituitary but by blocking the secretion of GnRH in the pituitary. RFRP influences the membrane firing of GnRH neurons, mostly inhibitory: 40 % of the investigated neurons reduced ion channel openings when treated with RFRP-3, in 10 % of these firing was enhanced and about half of neurons remained unchanged (Ducret et al. 2009).

Apart from brain GnIH/RFRP expression has also been observed in gonads. In birds (quail, chicken, starling) GnIH and its receptor GPCR147 were detected in theca and granulosa cells, in interstitial testes cells, and in the epididymis. In hamster RFRP-3 was identified in spermatocytes and spermatids together with GPCR147. There was a circannual rhythm of RFRP expression (Bentley et al. 2008).

Tsutsui and Ubuka (2014) report that by RNA interference in white-crowned sparrow, "Birds reduced resting time, spontaneous production of complex vocalizations, and stimulated brief agonistic vocalizations. GnIH RNAi further enhanced song production of short duration in male birds when they were challenged by playbacks of novel male songs. These behaviors resembled those of breeding birds during territorial defense. The overall results suggested that GnIH gene silencing induces arousal." They have other facts about blocking influence on arousal.

GnIH and its mammalian analogues might thus fulfill a role in the circannual regulation of reproductive activity which, however, has not been sufficiently analyzed in order to give a general picture. Such regulation as mentioned above might be

necessary for the survival of wildlife species. The fact that GnIH expression is induced by melatonin would fit (Ubuka et al. 2005) into the scheme, melatonin being the hormone used to estimate in molecular terms the duration of nighttime in mammals.

4.3.2.4 Phylogenesis

Thus far LPxRF amides have been observed only in vertebrates (mammals, amphibians, birds, and fish) and in hagfish, but not in lamprey, where a similar precursor was found, but with slightly modified peptides (Tsutsui and Osugi 2009; Tsutsui and Ubuka 2014).

4.3.3 Neuropeptide Y

Neuropeptide Y (NPY) neurons are broadly distributed a protein. The structure and functions are discussed in Sects. 4.10 and 11.5. In the hypothalamus NPY neurons localize predominantly to the arcuate nucleus. NPY release from there controls feeding and CRH release. In the periphery NPY is often formed in noradrenergic neurons.

4.3.4 Agouti-Related Protein

Fact sheet 4.6: Agouti-related

Gene:	Chromosome 16 ; locus 16q22; four exons
Sequence:	See Fig. 4.8
Synthesis and target:	AgRP is predominantly formed in the arcuate nucleus and controls feeding by binding to the melanocortin receptor 4 (MC-R4). This receptor is found on cells of the paraventricular nucleus, the dorsal motor nucleus of the vagus, and in the raphe nucleus, areas involved in energy homeostasis.
Function:	AgRP inhibits activation through MC4-R and thus permits enhanced feeding.
Receptor:	GPC heptahelical receptor: melanocortin 4 receptor.

4.3.4.1 Introduction

By discovering the *Agouti* gene regulation of skin pigmentation could be better understood. In humans the agouti protein is—in contrast to rodents—not restricted to the skin, but equally expressed in adipose tissue, in the testes and ovaries, in heart, and in kidney and liver (Dinulescu and Cone 2000). The agouti protein functions as an MSH antagonist at the melanocortin receptor I (MC-R1) in rodent melanocytes.

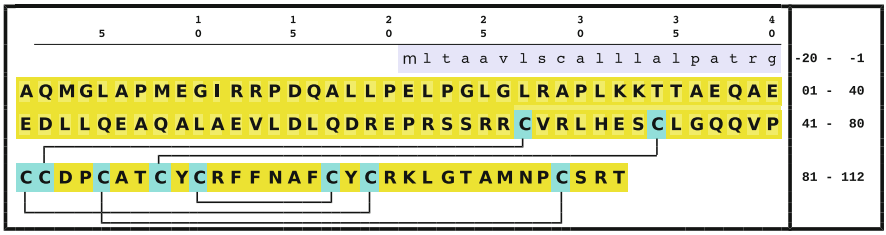


Fig. 4.8 Sequence and disulfide bridges of agouti-related protein. The signal peptide is shown in *lowercase*. The intramolecular disulfide bonds are depicted by *lines* between cysteine residues (Source: NP_001129; disulfide bonds were identified by Bures et al. 1998)

Agouti-related protein (AgRP; Fig. 4.8) has a similar antagonistic role as agouti, but at the hypothalamic MC-R4. It is involved in the regulation of feeding.

4.3.4.2 Structure and Gene

The AgRP gene was mapped to chromosome 16 (16q22). AgRP is 131 amino acids long, and its C-terminal region (82–131) is antagonistically active as is AgRP. The intramolecular cysteine bonds are functionally indispensable.

4.3.4.3 Physiology

Neurons in the arcuate nucleus produce agouti-related protein (AgRP). This protein is a specific antagonist of the melanocortin 4 receptor MC4-R. By inhibiting MSH association with the MC4-R and thus the suppression of feeding AgRP stimulates feeding. Mice with defects of MC4-R develop gluttony and adiposity.

4.3.4.4 Phylogeny

Until now, AgRP sequences are limited to vertebrates (Klovins et al. 2004). The proteins are characterized by 10 cysteine residues. It has not been possible thus far to delineate the divergence of agouti and AgRP.

4.3.5 Somatostatin

4.3.5.1 Introduction

The releasing hormones thus far mentioned mediate hormone secretion in the pituitary. In contrast to these hypothalamic somatostatin (SST or SRIF as *somatotropin release inhibitory* factor) blocks the pituitary release of the growth hormone. GH secretion is thus controlled by the balance of activating GHRH and inhibiting somatostatin. Other hypothalamic inhibitory peptides for any of the other hormones in the pituitary have not (yet?) been found, however, prolactin release is tonically suppressed by the catecholamine dopamine.

Fact sheet 4.7: Somatostatin

Gene:	Chromosome 3; locus 3q28; two exons
Sequence:	SS14: **AGCKNFFWKTFTSC** SS28: **SANSNPAMAPRERKAGCKNFFWKTFTSC** (see Fig. 4.9); an intramolecular disulfide bond forms a cyclic peptide; amino acids **FWK** are essential for receptor binding.
Synthesis and target:	Hypothalamic somatostatin is formed mainly in the paraventricular nucleus, to a lesser degree in the arcuate nucleus or ventromedial nucleus. Its targets are somatotropic cells in the pituitary and GHRH neurons in the arcuate nucleus and ventromedial nucleus. Somatostatin synthesis in the gastrointestinal tract serves regulation of endocrine cells therein, often in a paracrine way.
Function:	Somatostatin is an inhibitor of multiple endocrine functions.
Receptor:	Five human somatostatin receptors have been identified. Their cell type specific expression may explain the divergent somatostatin effects on target cells.

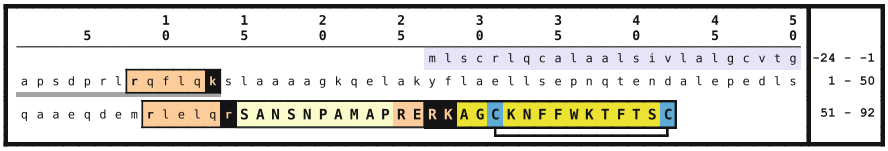


Fig. 4.9 The somatostatin precursor and its derived peptides. From the precursor prosomatostatin (PSS) furin cleaves off somatostain-28 (SST-28) and SST-14 apart from a short N-terminal peptide PSS(1–10) whereas by Prohormone convertase1 only SST-14 can be released. The two cysteines by generating an intramolecular disulfide bond form the ring structure of SST (Source: GenBank NP_001039)

In the search for GnRH, Burgus, Ling, Butcher, and Guillemin (1973) isolated from about 500,000 ovine hypothalami a cyclic tetradecapeptide inhibiting GH release from the pituitary. At the same time they were able to report the isolation of human somatostatin.

4.3.5.2 Structure and Gene

Somatostatin is derived from a precursor by proteolytic cleavages by either PC1 or furin (Fig. 4.9). PC1 can only cleave off the short SST-14 variant, whereas furin liberates the longer SST-28, SST-14 and an additional N-terminal peptide.

Expression of the somatostatin gene on chromosome 3 is controlled by stimulating signals increasing intracellular cAMP and by repressive influences of thus far unknown character.

4.3.5.3 Physiology

Somatostatin generation is not restricted to the hypothalamus. SST is an inhibiting agent of different endocrine and neuronal processes. In the GI tract SST attenuates multiple hormones (see Sect. 4.10); in the mammary glands milk ejection is suppressed. Apart from the hypothalamus SST neurons are located to other brain areas. Different SST functions do not originate from SST variants themselves

(no functional differences found between SST-28 and SST-14). These different functions arise by differentially expressed somatostatin receptors that mediate type-specific signal transduction pathways and are specifically expressed by cell type on various target cells (Sect. 8.2.4).

The independence of the multiple SST functions is due to the short SST half-life in blood (below 3 min) and due its rapid inactivation. For therapeutic reason an SST agonist was developed with similar SST receptor binding but an enhanced life span in blood: octreotide (Fig. 14.1).

Pituitary GH release is controlled twice by SST. SST secretion into the median eminence will inhibit GH release by SST receptor-mediated suppression of GH release in somatotropic cells. The second inhibitory signal is through direct SST action on GHRH secreting neurons still in the hypothalamus (see Müller et al. 1999).[9]

4.3.5.4 Phylogeny

SST is present in a variety of invertebrates. The paracrine gastrointestinal regulation from and within pancreatic islets is, however, a vertebrate achievement.

- *SST gene duplication:* although there is a singular SST gene in the human chromosome as in other mammalian genes, fish have two different SST genes; compared to mammalian SST the product of this second SST gene has one to four amino acid exchanges (Sheridan et al. 2000).
- *Cortistatin:* In 1996 de Lecea et al. reported another peptide with strong homology to somatostatin: cortistatin (CST), which appears to play an important role for sleep regulation. The peptide homology is 10 of 14 amino acids; all residues involved in receptor coupling are conserved (Fig. 4.10). The cysteine forming the intracellular disulfide bond and thus the cyclic peptide are conserved as well. The cortistatin gene maps to chromosome 1 (1p36.22) and comprises two exons. Three different CST peptides have been isolated: CST-14, CST-17, and CST-29.

  CST is expressed in several tissues: in the cerebral cortex and in the hippocampus, furthermore in pancreas, gut, kidneys, testis, and leukocytes. The final proof, however, is lacking for some of these inasmuch as sometimes only the presence of RNA, but not of the CST peptide has been confirmed.

  Unlike SST CST binds not only to SST receptors but the growth hormone secretagogue receptor, too (GHS-R): this receptor was first observed more

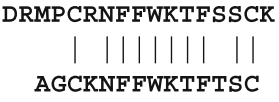


Fig. 4.10 Cortistatin-17 and somatostatin-14: sequence comparison

[9] SST secretion is inhibited by the pancreatic polypeptide (PP) (section 4.10.6; Kim W, et al., FEBS Letters, 588:3233–3239)

than 20 years ago and has recently been identified as a ghrelin receptor. The MrgX2 (mas-related gene), first shown to mediate pain and nociception, binds CST as well (Robas et al. 2003). Proadrenomedullar peptides after binding to MrgX2 generate elevated blood pressure by inhibiting catecholamine release from sympathetic neurons or chromaffine medullary adrenal cells. In contrast to SST CST is expressed by several types of immune cells (Gonzalez-Rey et al. 2006) inhibiting endotoxin-induced cytokine release and thus protecting against lethal outcome of endotoxic shock.

In spite of these differences the endocrine functions of SST and CST are very similar with respect to central GH regulation, prolactin control, and GI-driven insulin release. The two peptides appear to be mutually restorable.

- *Somatostatin in invertebrates:* By immunological means somatostatin or somatostatin-like peptides (already with the disulfide bond) have been found in neurons of protostomes; in deuterostomes SST was equally found in neurons, and in the gut mucosa, too: in singular neuroendocrine cells in invertebrates, however, in vertebrates in the known Langerhans islets combined with insulin (and glucagon and PNP[10]; Conlon et al. (1988); Falkmer et al. (1985)).
- *SST family in vertebrates* Tostivint et al. have recently demonstrated that the genes for SST, CST, and urotensin II/urotensin-related peptide (UII/URP) arose by two gene duplications. The original precursor gave rise to a tandem of SST/CST or UII/URP genes. Such a tandem exists in very early vertebrates suggesting duplication early in development. The tandem may then be duplicated with the entire genome, an event which is timed to early fish evolution (Tostivint et al. 2006).

4.3.6 Substance P

Substance P (SP) is used in the brain to adapt to stress (Sect. 4.11.2).

4.3.7 Proopiomelanocortin

POMC neurons synthesize the β-endorphine contributing to the reaction to stress as well as α-MSH which is involved in control of food uptake. Alternative processing of POMC is described in Sect. 4.4.1.

4.3.8 Ghrelin

Ghrelin is also a mediator of food intake. It is discussed in Sect. 4.8.2.

[10]Pancreatic polypeptide.

4.3.9 Kisspeptin

Fact sheet 4.8: Kisspeptin

Gene:	Chromosome 1 (1q32); three exons
Sequence:	See Fig. 4.11
Synthesis and target:	Kisspeptin is formed by neurons in the arcuate nucleus and the paraventricular nucleus; it is released via synapses in the preoptic region and controls GnRH secretion.
Receptor	heptahelical GPC receptor: GPR54

4.3.9.1 Introduction

While studying tumor metastasis Lee et al. (1996) identified a protein fully blocking metastasis without inhibiting melanoma cell proliferation. GPR54 was identified as the receptor for this kisspeptin protein (Kotani et al. 2001). GPR54 knockout mice were viable but did not demonstrate sexual maturation which in turn led to kisspeptin's role in GnRH secretion.

4.3.9.2 Structure and Genes

The *KISS1* gene mapping to chromosome 1 has three exons, the first one noncoding.

Kisspeptins (Fig. 4.11) are derived from the primary KISS-1 protein by post-translational modifications. Aside from kisspeptin 54 the literature reports smaller kisspeptins with chain length of 10 to 14 amino acids. The peptidase for tissue-specific processing is not yet identified.

4.3.9.3 Physiology

Kisspeptins supposedly have two roles: they block metastases of tumor as well as of placenta cells and, centrally, they control GnRH release in the median eminence:

1. *Metastasis inhibiting function:* From the first description on, the number of reports on suppression of invasive tumor migration has been ever increasing. In

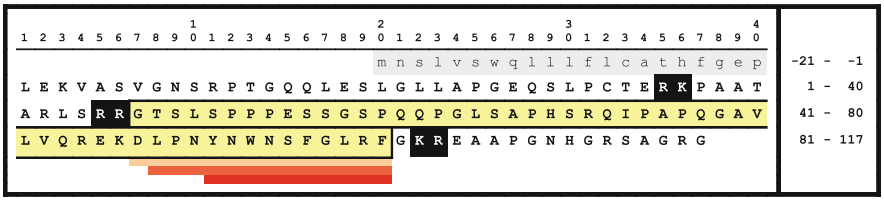


Fig. 4.11 Primary sequences of the KISS-1 protein and of derived kisspeptins. By Prohormone convertases the Kiss-1 gene product (*upper case*) is processed giving rise to the kisspeptin-54 *yellow*; after further C-terminal amidation, smaller kisspeptins 14, 13, and 10 (*orange to red*) are cleaved off by proteolytic digestion (Source: GenBank: NP_002247)

some tumors suppression of NF-κB translocation into the nucleus by kisspeptin was shown. In addition different signal pathways via protein kinase A or protein kinase C were shown. Blocking metastasis may be related to CXCR4 signal transduction; CXCR4 is seen as an important player in metastasis and in the interactions of cells with the environment (Navenot et al. 2005). In mice Bilban et al. (2004) have shown that kisspeptin and its receptor regulating trophoblast invasion into the maternal endometrium are predominantly expressed in early gestation: at term only the measurable Kiss RNA was 30 times less than in the third month of gestation.

2. *Regulation of GnRH release:* Kisspeptin is expressed in neurons of the arcuate nucleus and released in the paraventricular nucleus and the preoptic nuclei. Estradiol is known to control kisspeptin release; whether GABA or further neurotransmitters modulate kisspeptin secretion is thus far unknown. The control of the pulsatile secretion is further unknown. Neurons in the paraventricular nucleus are at least involved in the preovulatory LH peak; the regulation of this is not known either in women nor in any animal.

4.3.9.4 Phylogeny

Thus far kisspeptins and the kisspeptin receptor are found in vertebrates: primates, rodents, fish. **RF**amides, however, are among the primordial neuropeptides and found in those species with the earliest existence of neurons and neurosecretion.

Similarly kisspeptin during maturation of gonads was observed in fish as in man: male *D. rerio* express kisspeptin maximally during the first formation of sperm.

4.3.10 Galanin

Fact sheet 4.9: Galanin

Gene:	Chromosome 11; locus 11q13.2; six exons
Sequence:	see Fig. 4.12
Synthesis and target:	Galanin is released from CNS neurons, from neurons of the enteric nerve system, as well as by cytotrophoblastic cells of the placenta, and acts in endocrine and paracrine fashions on cells regulating feeding, insulin release, memory, and reproduction.
Function:	Galanin is a regulator of different aspects of homeostasis and of reproduction. Its presence is essential for maturation of mammary glands and of milk synthesis.
Receptor:	The three human galanin receptors are heptahelical GPC membrane receptors.

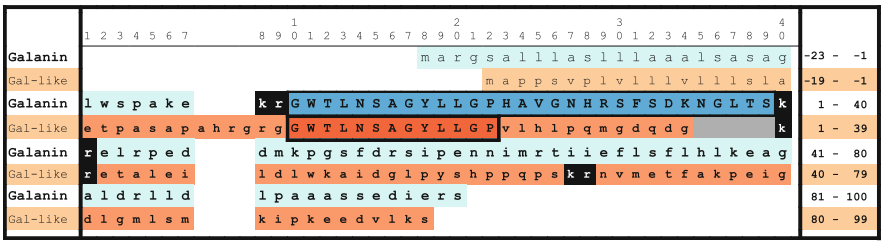


Fig. 4.12 The galanin preproproteins: after removal of the signal peptide(*upper line*) the galanin peptide (*in uppercase* and *blue*) is liberated from the proprotein by Prohormone convertase1. Dibasic peptide motifs are shown *inversely*. An alternative galanin-related peptide lacks the first Prohormone convertase1 motif; additional spaces are added to superimpose the homologous partial galanin sequence and the second **KR** motif (Source: GenBank CAA01907 and NP_149097)

4.3.10.1 Introduction

Galanin was first identified as a gut peptide (Tatemoto et al. 1983). Later on it was shown to be expressed in multiple types of neurons. Its expression in the human placenta could also be shown (Kleine et al. 2001). Recent literature demonstrates divergent roles of galanin.[11] Experimentally proven is a direct relationship to uptake of a fatty enriched diet. The indispensable role of galanin for mammary gland maturation and function has already been shown before.

4.3.10.2 Structure and Genes

The preprotein is transcribed from the gene on chromosome 11 (close to a metalloproteinase gene). After removal of the signal peptide the PC1 cuts galanin from the proprotein. In animals the terminal glycine is amidated. Due to an amino acid change from glycine to serine, nonamidated human galanin remains and its sequence is prolonged by one amino acid.

First in swine and then in other mammals an alternative galanin-related peptide (GalrP) was found. The human GalrP lacks the first PC1 motif. The sequence identical to galanin is thus N-terminally elongated (Fig. 4.12).

Three galanin receptors belong to GPC heptahelical membrane receptor families. Gal-R1 and Gal-R3 induce adenylate cyclase and thus cAMP elevation, and Gal-R2 signals via phospholipase C.

4.3.10.3 Physiology

Experiments in mice have demonstrated galanin's functions in at least two pathways: galanin-defective ($Gal^{-/-}$) mice were viable and fertile which precludes any essential role in reproduction. However, once these $Gal^{-/-}$ females had given birth they were unable to feed their offspring because the mammary glands were afunctional. $Gal^{-/-}$ mice utilized a fat-enriched diet to a lesser extent than

[11] Issue 12 of Cellular and Molecular Life Sciences in the year 2008 (Vol. 65) was dedicated to modern galanin research and comprises a couple of reviews on the subject.

comparable wildtype mice. Whether the phenomena are important in humans is not yet proven.

In addition, *Gal*$^{-/-}$ mice do not show estradiol-induced prolactin stimulation. Thus, galanin appears to be an estrogen-dependent stimulator of pituitary prolactin synthesis and release. Galanin receptor mutations may be factors of prolactinoma development.

Neurons exclusively forming galanin may not exist: galanin has been co-localized to neurons and cells expressing multiple other hormones or neurotransmitters: GnRH, GHRH, prolactin, vasopressin, CRH, oxytocin, substance P, CGRP, noradrenaline, or acetylcholine. Using patch clamp techniques, galanin was shown to inhibit neurons by elevating potassium currents (triggered by Gal-R1 and Gal-R3) and by downregulating calcium currents. Galanin also blocks synaptic plasticity, for example, when memory develops (*long-term potentiation*). Especially in the arcuate nucleus galanin presynaptically reduces GABA release as well as postsynaptically via galanin receptors. Singular effects by galanin stimulation have been observed in the dorsal–vagal complex where calcium currents were decreased.

Apart from the CNS is galanin expressed in the anterior pituitary, in the adrenal medulla, in the pancreas, the urogenital tract, and in skin (Wynick and Bacon 2002; Tortorella et al. 2007; Bauer et al. 2008). Neurons innervating the heart, kidneys, or gut have also been found to be galanin positive by immunocytochemistry.

When axons are cut, galanin and its receptors are upregulated. Galanin also seems relevant for nociception and neuronal development in spinal ganglia in mice (Hobson et al. 2008). Animal models suggest effects of galanin on learning and memory (Miller 1998; Rustay et al. 2005).

Galanin might also play a role during reproduction: it is hypothalamically co-expressed together with GnRH; the count of galanin plus GnRH double positive neurons was fivefold elevated in female rats compared to male. This difference may be due to a testis-dependent epigenetic regulation (Merchenthaler 1998).

Most recent articles suggest that galanin characterizes metastatic breast cell tumors. Further such markers are vascular endothelial growth factor (VEGF) and related drugs induced during tissue hypoxia by hypoxia-induced factor 1 (HIF1). Given that galanin is required for mammary gland maturation such a role is feasible (Bertucci and Birnbaum 2009).

4.3.10.4 Phylogeny

Galanin has been identified thus far in vertebrates.[12] However, proteins with homology have been found in placozoans, that is, before protostomes and deuterostomes developed separately. Allatostatin receptors in insects share up to 50 % homology with vertebrate galanin receptors.

[12]In the protein library there are some sequences labeled "potentially galanin" from bacteria; these sequences, according to our own ClustalW analysis, do not show any homology with vertebrate galanins.

4.3.11 Melanin-Concentrating Hormone

Fact sheet 4.10: Melanin concentrating hormone (MCH)

Gene:	Chromosome: 12 ; locus: 12q23.2 ; three exons
Sequence:	**DFDMLRCMLGRVYRPCWQV** (Fig. 4.13).
Synthesis and target:	MCH is released from the lateral hypothalamic area and acts on MCH receptors in brain and in the periphery.
Function:	Mammalian MCH is a neuropeptide regulator of feeding behavior and of energy expenditure; in fish and amphibia it is active as a melanophore-concentrating hormone and triggers lightening of the skin.
Receptor:	Two GPCR MCH-R1 (aka orphan receptor SLC-1) and MCH-R2 with different functional and topological characteristics.

4.3.11.1 Introduction

The adaption of the fish skin to the environment expressed as darkening or lightening has been suggested to be controlled by mutually antagonistic peptides since the year 1931 (Hogben and Slome 1931). The hormone for darkening turned out to be the MSH (from POMC), however, a melanin-concentrating hormone could only be identified in 1983 by Kawauchi et al. (1983) in salmon. A homologous hormone was later isolated and cloned from different mammals including humans. Several mammalian MCH share the same structure; in mice there are two amino acid exchanges and in sheep the sequence appears truncated (Pissios et al. 2006).

4.3.11.2 Structure and Gene

The original MCH from salmon is a 17 amino acid long cyclic peptide with an intramolecular disulfide bridge; human (and common mammalian) MCH has two additional N-terminal amino acids and few exchanges compared to the salmon sequence. The peptide is released from a precursor which in addition to the signal peptide and the MCH bears two other peptides called neuropeptide GE and neuropeptide EI (due to the termini), the latter being amidated (Fig. 4.13). The human *MCH* gene on chromosome 12 has three exons; the next neighbors are a nucleoporin and IGF1.

The MCH receptor was identified to be the orphan GPCR SLC-1 which was relabeled to MCH-R1. The receptor couples to different G- proteins and activates different signaling pathways: increase in intracellular free calcium, suppression of forskolin stimulated cAMP, stimulation of phophoinositol pathways, and triggering of extracellular-signal-regulated kinases (ERK). The receptor is preferentially expressed in the brain, highly in the piriform cortex and the olfactory tubercle, and with lower density in the nucleus accumbens and the amygdala. Further expression has been found in the arcuate nucleus and the ventromedial nucleus.

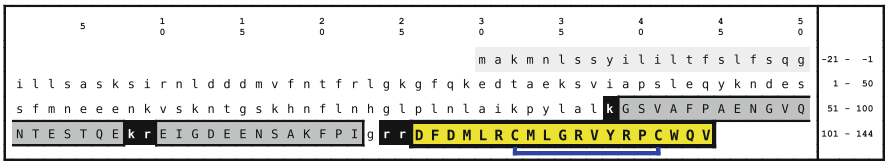


Fig. 4.13 Primary sequence of human melanin concentrating hormone (MCH). The signal peptide is highlighted *light gray*, the MCH peptide *yellow* and two other peptides *dark gray*. Endopeptidase motifs are *inverted*. The disulfide bridge of MCH is indicated by *blue lines* (Source: GenBank NM_002665.2)

By Northern blot and in situ hybridization analysis of human and monkey tissue, Sailer et al. (2001) showed that expression of MCH-R2 mRNA is restricted to several regions of the brain, including the arcuate nucleus and the ventral medial hypothalamus, areas implicated in regulation of body weight.

4.3.11.3 Physiology

The original function in fish (lightening of skin) has been lost during further vertebrate development. Today human MCH is considered an important regulator of food intake and energy expenditure. This has been shown by different authors, either in model animals or in humans (for reviews, see Nahon 2006; Flier 2004; Pissios et al. 2006). The first hint for this event stems from direct intracerebral injections of MCH into rat brains (Qu et al. 1996) which induced food intake. Careful analysis by a couple of laboratories identified the lateral hypothalamic area (LHA) as the place where most MCH perikarya were located with projections into many other brain areas. This is conclusive for a neurotransmitter role of MCH in induction of feeding and energy expenditure. The LHA, in addition, comprises another type of cell directly related to appetite and feeding behavior: orexin neurons and orexins likewise active as feeding control neurotransmitters.

Whereas POMC and other neurons almost always show a membrane potential, MCH neurons are mostly quiet and become active upon stimulation, either by synaptic contacts or, as shown recently, by elevated glucose levels. Upon stimulation MCH neurons secrete MCH over synapses and, to some lower degree, into the circulation. The role of MCH in periphery, however, has not attracted attention as do the neurotransmitter actions, and it thus largely speculative. Skin cells and some other cell types have been shown to express MCH-R1.

Most instructive have been MCH knockout mice: These mice were hypophagic (reduced food intake) and lean compared to wildtype littermates. This leanness was attributed to an enhanced energy consumption in these MCH-deficient mice.

Long-term exposure to intracerebroventricular MCH led to an increased body weight in mice, which increased even further with a parallel 33 % fat diet (one fifth compared with 1/20 (with normal diet) within 14 days of MCH exposure). Rats kept in the cold appear to depend on MHC to adapt to the low temperature by activating brown adipose tissue's fat consumption: blocking MHC expression by antisense RNA resulted in a dramatic weight loss compared to controls. Using an MCH

receptor antagonist it could be shown in obese mice that this treatment reduced food uptake, stopped body weight gain, instead reduced body weight, lowered the overall fat content, and reduced hypercholesterolemia, hyperinsulinemia, hyperglycemia, and hyperleptinemia associated with obesity in these animals.

These facts demonstrate clearly a neuropeptide control of MCH on feeding, energy mobilization, and accumulation of energy stores.

The interaction of MCH neurons with other central centers of feeding are dealt with in the chapter about feeding (Sect. 11.5.8).

4.3.11.4 Phylogeny

Any MCH-like protein has only been described in vertebrates.

4.3.12 Orexins

Fact sheet 4.11: Orexins/Hypocretin (HCRT)

Gene:	Chromosome 17; locus 17q21; two exons.
Synthesis and target:	Two orexins, A and B, cleaved from the same precursor; orexins are formed in neurons (neurosecretory cells?) of the lateral hypothalamus and the enteric nervous system (ENS) and almost exclusively released as neurotransmitters.
Structure:	The peptide structures have been determined by NMR analysis (Kim et al. 2004a).
Function:	Orexins are involved in regulation of feeding and the maintenance of a regular sleep cycle.
Receptor:	Two heptahelical GPCR, HCRT-R1 and HCRT-R2; HCRT-R1 is recognized by orexin-A, whereas HCRT-R2 is bound by both orexins.

4.3.12.1 Introduction

Orexins, also known as hypocretins (i.e., incretins of the hypothalamus, belonging to the secretin protein family), were identified in 1998 as peptides contributing to the regulation of feeding (de Lecea et al. 1998). In the meantime it has been additionally found that they play a role in the regulation of sleep because a defect in the orexin gen triggers familiar narcolepsy, and acquired narcolepsy can be induced by destruction of orexin neurons in the lateral hypothalamus. The original assumption of an exclusive presence of orexin neurons in the brain has been discarded when orexin formation could also be observed in the enteric nerve system (ENS) (Kirchgessner 2002).

4.3.12.2 Structure and Gene

The gene for the orexin precursor has been found on the long arm of chromosome 17. After removal of the 33 amino acids of the signal peptide orexin A is released

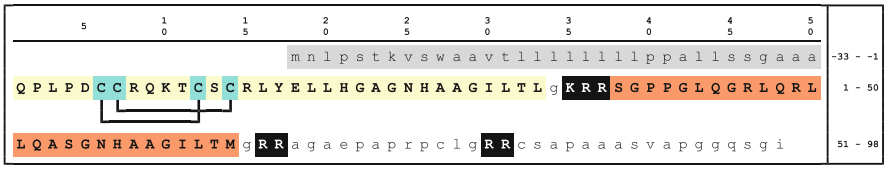


Fig. 4.14 Orexin precursor and its primary sequence. Following the 33 amino acids of the signal peptide (highlighted *gray*) there are two orexin peptides: orexin A (on a *yellow background*) harboring two disulfide bridges (*lines* connecting *blue* cysteines) and the cysteine lacking orexin B (on *orange*) (Source Swiss-Prot: O43612.1.; PyMOL)

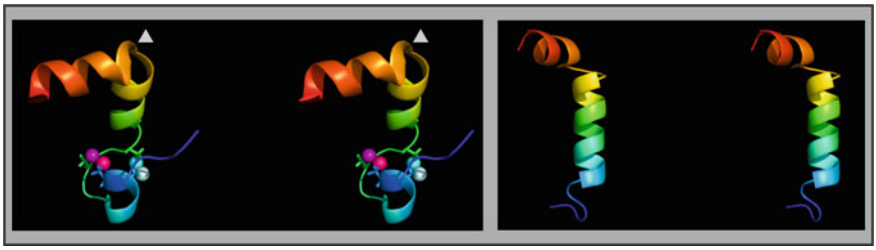


Fig. 4.15 Structures of the human orexins. The stereo models of orexin A (*left*) and orexin B (*right*) show a *blue* colored N-terminus (in the lower part of the image) and a *red* colored C-terminus. The *arrowheads* in the orexin A image indicate a β-turn

by PC1 and orexin 2 by PC2. Therefore the release of orexin B is coupled to the expression of the PC2. The extent of expression of this enzyme in the lateral hypothalamus and its regulation in the brain region has not yet been conclusively analyzed. Other prohormone convertase—for example, furin—do not play any role due to the lack of peptide motifs in the orexin preprotein.

Orexin-A contains two N-terminal disulfide bridges missing in orexin-B. This difference is already present in fish *e.g.* ABF29871.1 from codfish. The two peptides are C-terminally amidated; orexin-A has an N-terminal pyroglutamate (p**E**; Fig. 4.15). These peptides have been analyzed by NMR (Fig. 4.16). The C-terminal helices share structural homology; the sequence homology is enhanced in the C-terminal region compared to the N-terminus.

Two heptahelical GPCR have been shown to be orexin receptors. Their sequence homology is 64 %. The HCRT-R1 activates upon ligand binding hypoxia-inducible factor (HIF-1a). This HIF-1a activation leads to increased glucose uptake and enhanced glycolysis. In contrast to the hypoxia-induced activation ATP is not formed by anaerobic glycolysis but by stimulating the citrate pathway and oxidative phosphorylation (Sikder and Kodadek 2007). HRCT-2 is linked in dogs to inherited narcolepsy (Lin et al. 1999): an insertion of 226 base pairs in intron 3 of the *HCRT-2* gene causes aberrant splicing and a shortened and afunctional receptor protein.

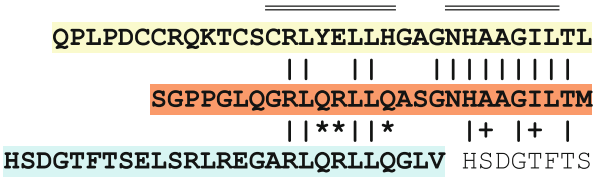


Fig. 4.16 Homology of orexins and secretin. The homology of both orexins is highest in those helices Kim et al. (2004a) determined. Also the homology to the eponymous secretin is obvious (compare the fact sheet 4.36). Orexins are indeed the secretine of the hypothalamus. This homology is mostly due to the helices *Doppelmarkierung* above the orexin-A sequence. The first eight amino acids of secretin are homologous to the C-teminal sequence of both orexins

4.3.12.3 Physiology

In the brain orexin neurons are almost exclusively found in the lateral hypothalamus which is among others a center for the regulation of feeding. The orexin neurons of rat project their axon in different brain regions (Hagan et al. 1999): the lateral hypothalamus itself, the perifornical nucleus, the dorsal raphe nucleus, the periaqueductal gray, as well as into the paraventricular and centromedial nuclei of the thalamus. Most intensively orexin neuron axon were found in the *locus coeruleus*. Here mostly probably noradrenergic neurons are synaptically connected to orexin-A. The neurons in the LC[13] are especially involved in the regulation of stress (see Sect. 11.2.1) and in the control of alertness and sleep. Orexin intracerebroventricularly injected prolonged states of alertness, enhanced locomotor activity, and reduced sleep periods. There are related findings that lack of orexin or a defective orexin receptor are at the origin of narcolepsy (Lin et al. 1999; Chemelli et al. 1999; Ebrahim et al. 2003, 2005). Orexin is thus applied when alertness should be enhanced.

Orexin positive cells are additionally found in the enteric nervous system in the mucosa of humans and animals: in the *plexus myentericus* and the *plexus submucosus* about one of four cells was orexin and leptin positive (Kirchgessner and Liu 1999). The authors suggest, therefore, that orexins have an important role in energy homeostasis.

4.3.12.4 Phylogeny

Thus far orexin sequences have been found in vertebrates and lampreys. In the latter case (XP_002598524) an orexin is not formed when a PC2 is active, only fragments thereof. In skate (*Leucoraja ocellata*) two orexins are coded for, disulfated bridges are present, and intramolecular PC2 motifs are absent. In the codfish Xu and Volkoff (2007) there are two peptides, 50 and 29 amino acids long, released by PC1. In humans, only the orexin-A is released by the PC1, not orexin-B, which raises doubts about its role as a neuropeptide, and both peptides can be targeted by the PC2 (see Fig. 4.14).

[13] Locus coeruleus.

4.4 Anterior Pituitary Hormones

4.4.1 POMC

Fact sheet 4.12: Proopiomelanocortin (POMC)

Gene:	Chromosome 2 ; locus: 2p23; three or four exons.
Sequence:	See Fig. 4.17.
Synthesis and target:	The prohormone is formed in corticotropic cells of the anterior pituitary, within melanotropic cells of the pars intermedia, in the hypothalamus and in skin. Due to the expression of PC1 and/or PC2 ACTH, β-endorphins and/or MSH peptides are synthesized.
Receptor:	Heptahelical G protein-coupled receptors: melanocortin receptors, opioid receptors.

Fig. 4.17 (Page 59). Primary sequences of POMC from vertebrates.
Colours code: peptide motifs *white, bold and uppercase*: on *red* **KR**, on *blue* **RR**, on *orange* **KK**, on *dark gray* **RK**; signal peptide: *gray* background; peptides: MSHs: *bold red*; ACTH: α-MSH plus *green*; lipocortin: *pink*; β-endorphin: on *yellow*; amino acids on *black* : changes compared to the human sequence. The POMC analogue from *P. marinus* (AAC59724.1) is not shown due to missing space. This peptide contains a much elongated α MSH-like peptide, a β endorphin-like peptide, but no ACTH. The remaining part of that peptide cannot be aligned, the cysteines in the N-terminal peptide are out of place and additional pro-hormone convertase motives are missing .

Introduction

Proopiomelanocortin (POMC) constitutes the precursor of seven different peptide hormones. It is expressed predominantly in corticotropic cells of the adenohypophysis as well as in melanotropic cells of the pars intermedia which in adult life is regressed. After processing POMC in the anterior pituitary adrenocorticotropic hormone (ACTH), β-lipotropin (LPH) as well as β-endorphin are stored in vesicles. In contrast, in the intermediate lobe melanocortins (MSH) and acetyl-β-endorphin are formed. POMC is further expressed in the hypothalamus, in the testis, in the ovary, in the adrenal medulla, in placenta, in the lungs, in skin, and especially in circulating monocytes and in tissue macrophages.

Structure and Genes

The *POMC* gene on chromosome 2 gives rise to two variants differing by an additional exon of 50 bp in the 5′ untranslated region. The POMC mRNA in pituitary and hypothalamus is about 1.1 kilobases (kB) long, whereas extracranial RNA has only 800–900 bp. In tumors there is a further RNA of about 1.4 kB size.

From the different POMC RNAs the same precursor protein is translated: POMC with 267 amino acids in humans. From this precursor, different fragments are cleaved by PC1/PC2. Three different types of peptides are known: melanocortins, adrenocorticotropic hormone (corticotropin, ACTH), and endorphins (see Fig. 4.17). These peptide hormones have different action. Which neuroendocrine cells which of these hormones releases is determined by the expression of the prohormone convertases. In addition, from bovine POMC it is known that

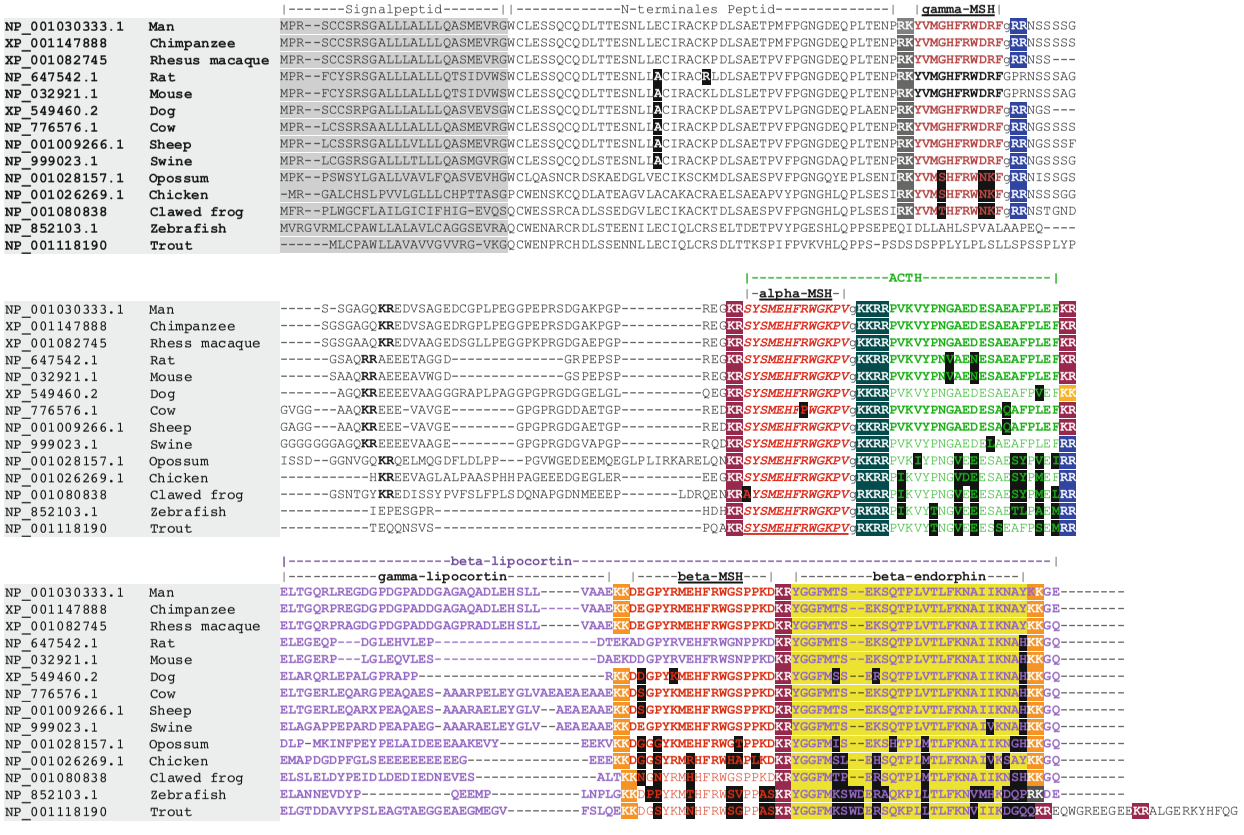


Fig. 4.17 Primary sequences of POMC from vertebrates

different glycosylations influence the use of the peptide motifs by the prohormone convertases (Birch et al. 1991).

The *POMC* gene is predominantly expressed in corticotropic cells of the pituitary, in neurons of the arcuate nucleus and the nucleus of the solitary tract, and in keratinocytes and melanocytes of the skin.

The dog sequence shown in Fig. 4.17 has been retracted from GenBank due to low quality. Another sequence (XP_849463) still shows the **KK** motif (at the end of the *middle block*) which indicates that ACTH may not be a product from the anterior pituitary in dog, swine, opossum, chicken, and frog because the PC1 motif is lacking. In fish, however, there are two *POMC* genes and only the one shown of zebrafish has mutation translated into the **KR** to **KK** change. The gene product of the second gene can be processed to give rise to ACTH (Alsop and Vijayan 2009).

The corticotropic cells of the pituitary express predominantly PC1 forming thus ACTH, β-Lipotropin and β-Endorphin from POMC. In contrast, melanocytes of the intermediate lobe express additional PC2 which in turn forces α-MSH synthesis. POMC neurons of the ventral hypothalamus equally synthesize α-MSH.

Phylogeny

POMC and the peptides derived thereof have thus far been found but in vertebrates and agnathans. The genome of the urochordate *S. purpurata*[14] contains neither a POMC precursor nor any melanocortin receptor (Burke et al. 2006). There are, however, reports that parasitic *Schistosoma mansoni* expresses POMC as a defense against vertebrate immune attack and releases MSH, ACTH, and β-endorphin peptides. Since these reports in 1992 (Duvaux-Miret et al. 1992), a POMC gene from *S. mansoni* has not been published. Furthermore, an isolation of γ-MSH from leech has been reported. In the blue mussel POMC derived peptides were reported as well. Again any genomic sequence has yet to be found.

In most vertebrates a single POMC exists; a second POMC gene exists in fish after an additional genome duplication (de Souza et al. 2005). Fish POMC*α* is expressed in the hypothalamic nucleus lateralis tuberis, in the anterior pituitary, and in the intermediate lobe, whereas POMCβ is found in the preoptic region and faintly in the pituitary stalk. The endorphin sequence is retained only in POMCα.

Comparing selected mammalian and other vertebrate POMC sequences (Fig. 4.17), you may notice that:

- The α-MSH sequence is conserved from teleosts on; there are two amino acid exchanges in clawed frog and in cows. The dibasic sequence motifs are conserved, too. Apart from *X. laevis* the glycines for C-terminal amidation and the N-terminal serine that is acetylated are always present.
- In rats and mice β-MSH is lacking because the **KK**-cleavage site is gone.
- Teleosts do not have a γ-MSH.

[14] Strongylocentrotus purpuratus.

- Within mammals ACTH has two characteristic exchanges in rodents. Nonmammalian ACTH have some constant differences compared to mammals. The lack of a PC1 motif in dog, swine, opossum, or chicken raises the question whether and how these animals make ACTH. There is no report in the literature on the problem. In *D. rerio* a second POMC rescues this defect, however, in tetrapods and birds only a single gene has ever been reported.
- γ-Lipocortin is the most variant part of the different POMC regions.
- Most interesting is the high conservation of the N-terminal peptide: the four cysteines are at similar positions and equally spaced. With the exception of opossum there are but two positions with alternative amino acids although any function of this sequence has not yet been described.

The melanocortin receptors reflect the different genome duplications (Fig. 4.18). In lamprey i.e. before the second genome duplication two MCR were found. After the next genome duplication vertebrates have five MCR genes which in humans are on chromosomes 16 and 18 with synteny tom, for example, fugu (Klovins et al. 2004). After the third genome duplication restricted to fish in the thus far analyzed species several genes got lost including both the MCR3 genes in *Takifugu rubripes* as well as the γ-MSH (as shown in fugu, zebrafish, and trout). MCR3 is the specific receptor for γ-MSH in humans and other species analyzed.

The topological distribution of the hormone targets merits attendance.

In humans melanocortin receptor 1 (MCR1) is expressed in skin: in melanocytes, keratinocytes, fibroblasts, endothelial cells, and antigen presenting cells. This receptor binds α-MSH and ACTH with equal specificity. In leukocytes MCR1 mediates the inflammation inhibiting effects of α-MSH. MCR2 is the receptor in the adrenal cortex mediating the steroidogenic activity of ACTH. This receptor is not activated by MSHs. MCR3 is expressed in CNS, gastrointestinal tract, and in placenta. γ-MSH exhibits the highest activity for this receptor. MCR4 is specially expressed in CNS. α-MSH and ACTH activate this receptor more strongly than β-MSH or γ-MSH. Finally, MCR5 is the α-MSH receptor of sebaceous glands but found in other tissues, too. Obviously the receptor has its role in the regulation of exocrine glands.

Compared with humans, the distribution and functional specificity in *T. rubripes* is different: truMcR1 is faintly expressed but in CNS; truMcR2 in brain and in the adrenal, (truMcR3 is lacking); truMcR4 and truMcR5 are found in brain, in the adrenal, and in gut (truMcR4), respectively; and in the eye (truMcR5). As in humans in *T. rubipes* truMcR2 is stimulated exclusively by ACTH, not by any MSH. truMcR1 and truMcR4 show much stronger activation by ACTH than by α-MSH (Klovins et al. 2004): this is different from humans and interpreted by the authors as a hint to an ancestral function of ACTH.

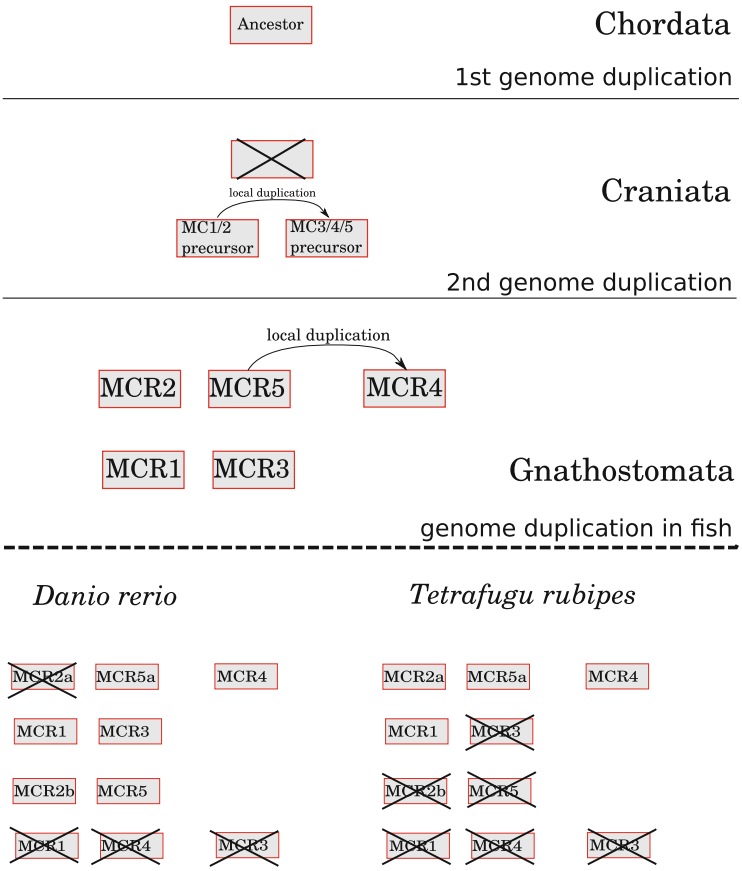


Fig. 4.18 Hypothesis of melanocortin receptor gene (MCR) development. After an initial genome duplication of an unknown ancestor gene one gene is lost and the other locally duplicated again. A second genome duplication was followed by doubling of one of the resulting genes. The pattern of five MCR is found in the majority of vertebrates. In fish a third genome duplication took place. Not all of the genes were maintained which gave rise to characteristic patterns like those of *D. rerio* or *T. rubripes* (Source: redrawn due to Klovins et al. 2004)

4.4.1.1 ACTH

Fact sheet 4.13: Adrenocorticotropic hormone (ACTH)

Sequence:	**SYSMEHFRWG KPVGKKRRPV KVYPNGAEDE SAEAFPLEI**
Synthesis and target:	In POMC forming cells by activity of prohormone convertase 1
Function:	Stimulation of steroidogenesis in the adrenal
Receptor:	Heptahelical G-protein-coupled receptor: melanocortin 2 receptor

Introduction

ACTH is the effector hormone of the HPA axis and stimulates hormone release in the adrenals: glucocorticoids as well as mineralocorticoids are released after ACTH stimulation, as well as adrenaline whose synthesis is stimulated by ACTH which induces the last enzyme of adrenaline synthesis: PNMT (see Fig. 7.1).

Physiology

ACTH is released from adrenocorticotropic cells in the pituitary by hourly pulses. This secretion thus results from hypothalamic CRH release into the median eminence and into the hypophyseal portal system. During night, the quantity of ACTH is twice to thrice as high as during daytime (see Fig. 12.2). ACTH stimulates by its effect on the adrenal MCR2 receptors intracellular cAMP resulting in steroid hormone synthesis and (because these are not stored in granules) release. Of paramount importance is the cortisol formation.

Regulation of POMC→ACTH release in the pituitary results from stimulation of intracellular cAMP by CRH and binding of transcription factor Nur to Nur-responsive elements in the POMC promoter. Feedback inhibition by glucocorticoids is due to direct interaction of ligand-bound glucocorticoid receptors with Nur, thus blocking its interaction with DNA (Murakami et al. 2007).

ACTH's half-life in human blood was determined to be 19 min (Keenan et al. 2004).

4.4.1.2 Endorphins

Fact sheet 4.14: Endorphins

Sequence:	`YGGFMTSEKS QTPLVTLIKN AIIKNAYKKG`
Synthesis and target:	In POMC expressing cells by the action of PC1
Function:	Endogenous opioid
Receptor:	Heptahelical G-protein-coupled receptor: μ-opioid receptor (MOR)

Introduction

Endorphins are endogenous opiates . These are released, for example, as a reaction to pain. Inasmuch as β-endorphin release is coupled to ACTH release, reactions to stress are metabolic changes, circulatory reactions mediated by glucocorticoids or adrenaline, and on the other hand analgesic effects by endorphins. Triggering the feeling of happiness by endurance sports appears to be an adaption to stress.

Physiology

As shown in Fig. 4.19 β-endorphin and ACTH are formed in equal amounts by the POMC *processing* by PC1. Endorphin synthesis takes place in all cells where MSH is formed. Endorphins thus can be released from all those cells/tissues where ACTH is formed and from those MSH forming cells in hypothalamus, pituitary, skin, additional tissues, and leukocytes.

β-Endorphin has a role in analgesia: β-endorphin is one endogenous ligand of μ-opioid and δ-opioid receptors (MOR and DOR). Both receptors are found in neurons that trigger pain, so-called nocireceptors communicating injury, chemical attack, heat, or coldness to the nocicenters of the brain. MOR neurons are densely found in the periaqueductal gray of the midbrain. As do other analgesics β-endorphin blocks the activity of nocireceptors.

It is not yet clear whether endorphins act directly or indirectly via other analgesic mediators. Endorphins 1 and 2 have, for example, a greater affinity to MOR than endorphins. However, the proteins where they should be cleaved from have not yet been found. Apart from endorphins and endomorphins, enkephalins and dynorphins are analgesically active as well.

Information about a phylogeny of endomorphins and nocireception has not yet been published.

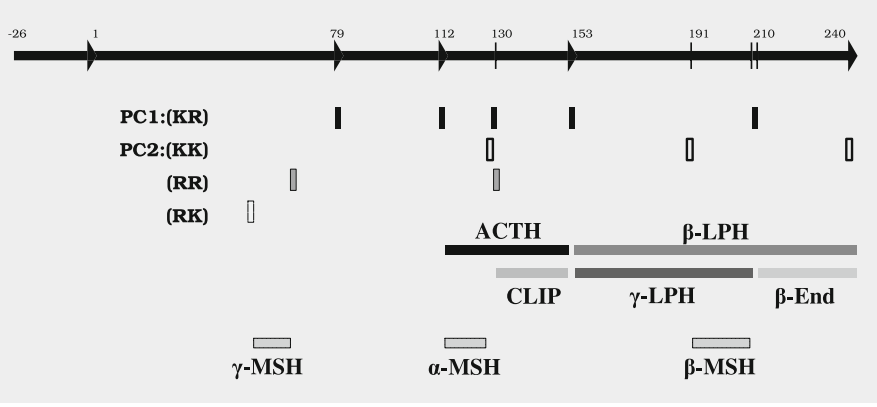


Fig. 4.19 POMC: prohormone convertases and alternative peptides. By the prohormone convertases PC1 and/or PC2, proopiomelanocortin (POMC) peptides are cleaved from the precursor: adrenocorticotropin (ACTH), lipocortin (LPH), melanocortin (MSH), endorphin (End), and corticotropin-like intermediate peptide (CLIP). PC1 cleaves but after Lys-Arg (**KR**), whereas PC2 cleaves after Lys-Lys (**KK**), Arg-Arg (**RR**), or Arg-Lys (**RK**). Which peptides are formed depends on the type of endocrine cell and of its convertase expression

4.4.1.3 Melanocortins

Fact sheet 4.15: α-, β-, and γ-Melanocyte stimulating hormone (MSH)

Sequence:	α-MSH	Acetyl-**SYSMEHFRWGKPV**-NH_2
	β-MSH	**DEGPYRMEHFRWGSPPKD**
	γ-MSH	**YVMGHFRWDRF**-NH_2
Synthesis and target:	MSH are made within POMC neurons in the CNS, in skin, in the cells of the pars intermedia, and in other peripheral tissues by the action of PC1 and PC2, PAM, and other enzymes. They act on melanocytes and centrally on centers of satiation.	
Function:	Centrally MSH acts as antagonist of NPY and agouti-like peptides and induces satiety. In melanophorous cells it triggers formation and aggregation of melanin. MSH further inhibits leukocyte activation.	
Receptor:	Heptahelical G-protein-coupled receptors: melanocortin receptors (MCR) 1,2,3,4,5.	

Introduction

Not long ago, it was observed that in humans melanocortin and its receptors are important regulators of the balance of appetite and satiety. In animals adaptations of coat color to the environment are triggered by melanocortin stimulation of melanocytes (Penzlin and Ramm 2008).

MSH, in addition, is regarded as link between the neuroendocrine system and the immune system because MSH receptors are functionally active on different leukocyte and lymphocyte cell types. Stress-induced skin reactions are thought to be of ectopic ACTH and melanocortin origins.

Structure

The N-terminus of α-MSH is acetylated by the peptide acetyl transferase. Both α-MSH and γ-MSH are C-terminally amidated, but β-MSH is not. The MSH consensus sequence is **YxMxHFRWxxx**.

Physiology

Functions of MSH peptides depend on the target organ: regulation of food uptake occurs in the CNS, induction of melanin synthesis in the skin is dependent on sunlight, and inhibition of leukocyte activation in the circulation.

The latter effect is due to protein kinase A activation by enhanced cAMP after MSH binding to the MCR. This PKA blocks phosphorylation of IκB which in the cytosol is complexed by NFκB. Without this IκB phosphorylation dissociation of the complex after receptor-triggered leukocyte stimulation is blocked and thus the signal transduction by NFκB transport into the nuclease does not take place, blocking several activation patterns (Catania 2007).

The central regulation of food uptake is normally blocked by MSH released in the hypothalamus and by its binding to MCR on NPY neurons in the arcuate

nucleus which is called a tonical suppression. By the release of AgRP—triggered by ghrelin, for example—this suppression is removed and NPY neurons become active, triggering appetite.

During control of melanin synthesis in human skin, UV light activates POMC expression in melanocytes and keratinocytes. Due to PC2 expression in these cells MSH is formed which, in turn, stimulates pigmentation.

4.4.2 TSH

Fact sheet 4.16: Thyroid stimulating hormone (TSH)

Gene α chain:	Chromosome 6 (6q12-q21); four exons.
Gene β chain:	Chromosome 1 (1p13); three exons.
Sequence:	Figs. 4.20 and 4.21.
Synthesis and target:	TSH is formed in thyrotropic cells of the pituitary and released by TRH trigger. TSH acts on TSH receptors in the thyroid.
Receptor:	Heptahelical G-protein-coupled receptor: The TSH-R stimulates adenylate cyclase.

4.4.2.1 Introduction

Thyroid-stimulating hormone and the group of gonadotropins described thereafter constitute the group of glycoprotein hormones all built from heterodimers. The larger α-chain has an identical amino acid sequence in the different glycoprotein hormones (LH, FSH, CG, TSH) and is coded for by the TSH-α gene (Fig. 4.20). The glycoprotein hormones differ by their β-chains (Fig. 4.21). In addition they differ in glycosylation and furthermore in sulfation, the latter also rendering α-chains different.

Investigating potential ligands for some leucine-rich G protein-coupled receptors (GPCR), which are evolutionarily ancient among the TSH-R and other glycoprotein hormone receptors, an additional α2 and β5 chain were found in humans whose

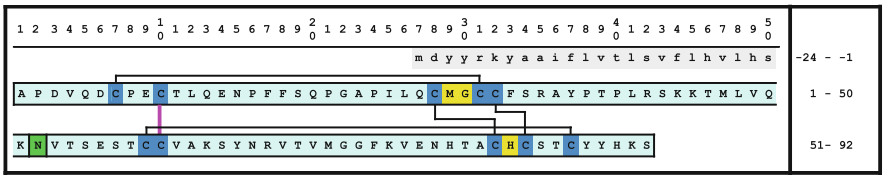


Fig. 4.20 α-chain of thyroid-stimulating hormone (TSH). The signal peptide with 24 amino acids (*gray background*) is followed by 92 amino acids of the mature TSH-α chain; disulfide bridges are indicated by black lines between the different cysteine residues **C**. The boxed asparagine (**N**) is glycosylated (Source: GenBank P01215)

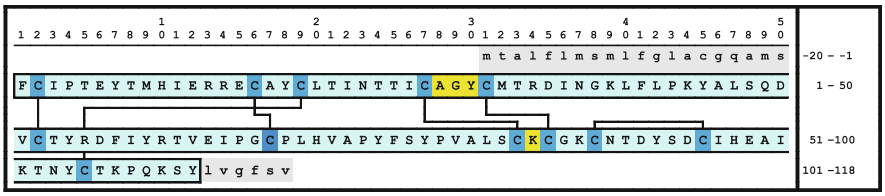


Fig. 4.21 thyroid stimulating hormone (TSH) β-chain. In front of the 112 amino acids of the mature TSH-β-chain there is on the precursor protein a signal peptide of 20 amino acids and at the end an additional six amino acid long peptide (on *gray background*). Disulfide bridges are drawn as lines between *blue* cysteine residues (**C**). The boxed asparagine (**N**) is glycosylated (Source: GenBank P01222)

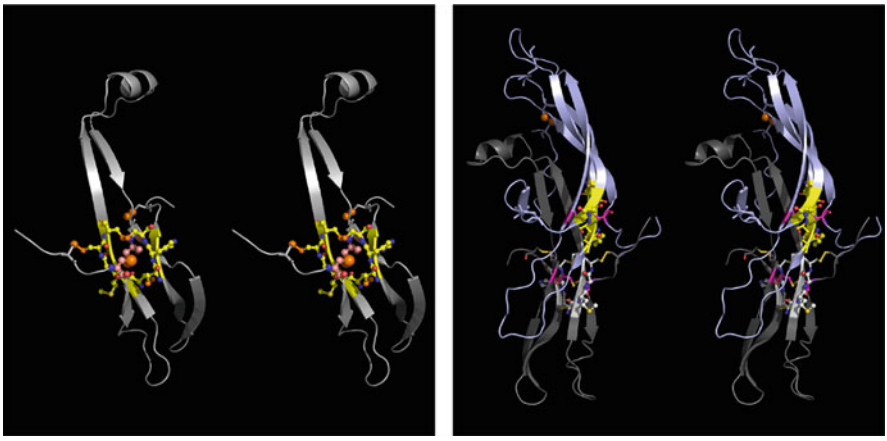


Fig. 4.22 Structure of choriongonadotropin (hCG). ***Left:*** the α chain backbone contains three loops partially twisted held together by beta sheets and five disulfide bridges. Beta sheets are indicated by *arrows*. Cysteines are shown as *spheres/wire frame*, sulfur atoms as *orange spheres*. The cysteine residues marked by *yellow carbon atoms* construct a plain which is pierced by a third disulfide bridge (*ping carbon atoms, large orange sulfur*). The other two disulfide bridges keep the N-terminus and the C-terminus close to this cysteine knot. ***Right:*** in the entire hCG both chains are complexed. The β chain itself has another cysteine knot (*arrow*) formed by three disulfide bridges. The other β chain disulfide bridges maintain a structure into which the α-chain can glide. ***Stereo:*** in order to get the three-dimensional view focus behind the images; both will eventually superimpose and be fused into a single image (Source: 1HRP, und PyMoL. Scripts for the construction are included in Appendix 16.3)

presence was confirmed in rodents as well as in drosophila. The heterodimer α2/β5 stimulates the TSH-R as TSH does (Nakabayashi et al. 2002; Sudo et al. 2005).

4.4.2.2 Structure and Genes

The TSH heterodimer contains the 92 amino acid long α-chain (Fig. 4.20) and the 112 amino acid long β-chain (Fig. 4.21). Structurally characteristic and indispensable is the cysteine knot. This element characterizes the family of nerve growth factors (NGF), too.

The human TSH genes are located on chromosome 6 (α) and chromosome 1 (β) with four, respectively, three exons. The three-dimensional glycoprotein hormone structure was first determined from FSH (Fig. 4.22).

Intact cysteine knots of both chains are required for synthesis and posttranslational sorting into the secretory granules; for receptor binding, not all disulfide bridges are necessary.

4.4.2.3 Physiology

TSH synthesis is stimulated in thyrotropic pituitary cells by the action of thyrotropin-releasing hormone (TRH) and (by TRH triggering again) is released into the portal system. TRH stimulates phosphorylation of cAMP reactive elements binding proteins CREB which recruits CREB-binding protein (CBP) which then, in cooperation with P-LIM,activates the TSH-α promoter. The β-TSH promoter is activated by CBP plus the pituitary transcription factor Pit1.

4.4.2.4 Phylogeny

(is discussed together with all other glycoprotein hormones (Sect. 4.4.3.4).

4.4.3 LH, FSH, CG

Two of the human three gonadotropins are made and released from the pituitary, luteinizing hormone (LH) and follicle stimulating hormone (FSH); the third, choriogonadotropin (CG) is of placental origin and *the* sign of pregnancy. When the possibility of pregnancy can be excluded the presence of CG in blood is indicative of a chorion carcinoma.

4.4.3.1 LH/CG

Fact sheet 4.17: Luteinizing hormone (LH)

Gene α chain:	Chromosome 6 (6q12-q21); four exons.
Gene β chain LH:	Chromosome 19 (19p13.32); three exons.
Sequence:	Fig. 4.23.
Synthesis and target:	LH is synthesized in gonadotropic cells of the pituitary; its release into the portal system is triggered by GnRH. Target of LH/CG is the common LH-receptor on cells in the gonads: for men the Leydig cells and for women theca cells.
Receptor:	Heptahelical GPCR: the LH/CG receptor activates the adenylate cyclase.

Introduction

Inasmuch as the structure of LH and CG is very similar and because they bind to the same receptor, they are treated together here.

Fact sheet 4.18: Choriogonadotropin (CG)

Gene α chain:	Chromosome 6 (6q12-q21); four exons.
Gene β chains CG:	Chromosome 19 (19p13.32); *CGB*; *CGB1*; *CGB2*; *CGB5 CGB7*; *CGB8*.
Sequence:	Fig. 4.23.
Synthesis and target:	CG is synthesized in placental cytotrophoblastic cells and in the syncytium (in the presence of GnRH). Targets of CG are CG receptors in the corpus luteum and the endometrium.
Receptor:	Heptahelical GPCR: The LH/CG receptor activates the adenylate cyclase.

Structure and Genes

With the crystal structure of human CG determined (Lapthorn et al. 1994; other CG have only been found in primates, the CG of horses is a LH) it became evident that gonadotropins as well as NGF,[15] PDGF,[16] TGF-β display a so-called cysteine knot (see Fig. 4.1). Two disulfide bridges together with some beta sheets form a ring; across the ring the third disulfide bridge is formed (Fig. 4.1). Cysteine residues of gonadotropins are conserved in vertebrates, not only the β-chains, but the α-chains as well. Equally conserved is the asparagine residue at position 30 of LH/CG which is N-glycosylated; other N-glycosylation sites are not fully conserved: compare the sequence of LH (Fig. 4.23) and of TSH (Fig. 4.21).

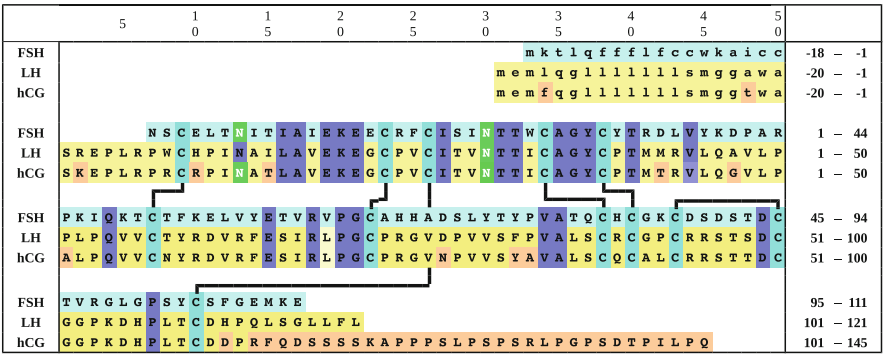


Fig. 4.23 Sequence comparison of three human glycoprotein hormone beta chains: follicle-stimulating hormone (FSH), luteinizing hormone (LH), and choriogonadotropin (hCG). With an N-terminal FSH shift by six Amino acid all cysteine residues are placed at identical positions. Cysteines are on *blue background*. The cysteines in the mature peptide are conserved. Conserved amino acids or groups of amino acids are on *violett background*. The 25 Amino acid that distinguish CG and LH are on *light orange background*. N-glycosylation sites are *white on green*. Disulfide bridges are according to the Swiss-Prot annotations (Source: FSH: NM_001018090, LH: P01229, hCG: P01233)

[15]Nerve growth factor.

[16]Platelet-derived growth factor.

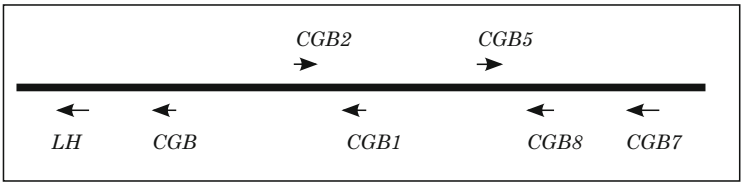


Fig. 4.24 Organization and orientation of the human luteinizing hormone (LH) and choriongonadotropin (CG) genes on chromosome 19 (Source: http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?TAXID=9606&CHR=19&MAPS=genes-r&QSTR=chorionic+gonadotropin&QUERY=uid%28-2038742931%29&BEG=48%2C940K&END=49%2C160K&oview=default)

Those N-glycosylated asparagines are conserved, too. (Primary structures from http://www.chem.gla.ac.uk).

Sequences of CG and LH are more than 90 % identical, whereas FSH is only about 30 % homologues. Within these 30 % are the fully conserved cysteines and the distances in between which helps to retain the conserved structure (Fig. 4.23).

The LH and CG genes are located on chromosome 19, separated only by the pseudogene of neutrophin. The order of the genes is shown in Fig. 4.24.

Physiology of LH

LH release is triggered (like FSH) by pulsatile GnRH. In addition, LH synthesis is regulated by aktivins and inhibins. Increased concentrations of progesterone which are made in the corpus luteum block LH formation.

LH acts on male and female gonads. In the testes testosterone synthesis and release are stimulated in Leydig cells. (The release is immediate by diffusion: steroids are not (except in rare instances) collected in granules and not released from these latter by receptor-mediated vesicle–membrane fusion; they diffuse from the cells and are (often) bound by serum transport proteins.) In the menstrual cycle LH from pituitary stimulates testosterone formation in follicular theca cells. This testosterone diffusing to the granulosa cells is aromatized into estradiol. Due to sufficient elevation of circulating estradiol on cycle day 14 a sharp increase of LH release (LH surge) leads to ovulation. In mice kisspeptin neurons of the anteroventral periventricular nucleus increase, upon elevated estradiol, their GnRH release and thus the LH surge whereas GnRH release from neurons of the arcuate nucleus seems to be inhibited (Han et al. 2005). The increase of serum progesterone once the corpus luteum menstrualis is formed reduces LH and FSH release.

Physiology of CG

When ovulation is not followed by pregnancy, the corpus luteum is spent after 14 days and degenerates. The progesterone release finishes and inhibition of LH and FSH release is abrogated. In the case of pregnancy, however, the trophoblast developing from the fertilized egg releases CG which again stimulates progesterone

synthesis from the corpus luteum. This CG-supported progesterone synthesis is accomplished in later pregnancy by the fetus itself.

Being one of the very first proteins secreted from the trophoblast raised the question about CG's role during nidation. During these studies the effects of progesterone and estradiol were confirmed that facilitate nidation. There was, however, a direct CG effect on the synthesis of glycodelin (Cameo et al. 2004). Simultaneous activation of Notch-1 by CG and progesterone should have an antiapoptotic effect on the endometrium. A special role of CG on the endometrium was observed because the LHCGR in endometrial cells does not activate adenylate cyclase and thus increase cAMP but induces via a rapid phosphorylation of ERK 1/2 (*extracellular-signal-regulated kinase*) cyclooxygenase synthesis and thus prostaglandin E_2-synthesis. It could be possible that this signal transduction is due to a splice variant of LHCGR (Cameo et al. 2004).

Phylogeny

This is presented in Sect. 4.4.3.4.

4.4.3.2 FSH

Fact sheet 4.19: Follicle stimulating hormone (FSH)

Gene α chain:	Chromosome 6 (6q12-q21); four exons.
Gene β chain:	Chromosome 11 (11p13); three exons.
Sequence:	Fig. 4.23.
Synthesis and target:	FSH is formed and released by gonadotropic cells of the pituitary upon GnRH stimulation. Target of FSH are FSH receptors (FSHR) in the gonads: in men on Sertoli cells and in women on follicular granulosa cells.
Receptor:	Heptahelical GPCR: FSHR activates the adenylate cyclase.

Introduction

In the ovary FSH (aka follitropin) induces follicle maturation. In testis FSH stimulates sperm-forming Sertoli cells.

Structure and Genes

The FSH β chain is coded for on chromosome 11 in three exons. Its gene product associates, as do other glycoprotein hormones, with the α chain. Dias (2001) and others have shown that the mode of N-glycosylation of the arginines N^{7}_{β} and N^{31}_{β} influences the potency of FSH: different patterns of glycosylation are differentially active. Some patterns in fact are endogenous antagonists and block FSHR activity.

Physiology

In the pituitary LH and FSH are synthesized by the same gonadotropic cells. No hint exists that these two hormones are sorted into selected vesicles which then might secrete either LH or FSH. On the contrary, tracer experiments suggest that all

intracellular vesicles contain both LH and FSH. With reference to Sect. 11.3 there are, however, different LH and FSH levels at different stages of the menstrual cycle. Elevated estradiol suppresses serum FSH concentration although LH remains high. In sheep and rats an estrogen-responsive element (ERE) in the FSHR gene promoter is used to block FSH transcription. Such an ERE is lacking in the LH receptor gene. In contrast to FSH regulation is the LH synthesis in pituitary gonadotropic cells not inhibited but stimulated. A blocking effect of estradiol on LH synthesis thus must take place in the hypothalamus where GnRH formation is inhibited by estradiol.

FSHR are present on granulosa cells in the wall of ovarian follicles and on Sertoli cells in testis.

4.4.3.3 GPHA2B5

Fact sheet 4.20: Thyrostimulin (GPHA2B5)

Gen α-chain-2:	Chromosome 11 (11q13); three exons.
Gen β-chain-5:	Chromosome 14; two exons.
Sequence:	α-chain **MPMASPQTLV LYLLVLAVTE AWGQEAVIPG CHLHPFNVTV RSDRQGTCQG SHVAQACVGH CESSAFPSRY SVLVASGYRH NITSVSQCCT ISGLKKVKVQ LQCVGSRREE LEIFTARACQ CDMCRLSRY** β-chain **MKLAFLFLGP MALLLLAGYG CVLGASSGNL RTFVGCAVRE FTFLAKKPGC RGFGSPRMPA GVAVRPGRNP FWNPPILKPI IESVPTTRPN R**
Synthesis and target:	GPHA2B5 is expressed in some corticotropic pituitary cells, in the retina, in skin, and in testis. It activates the TSHR.
Receptor:	Heptahelical GPCR: TSHR activates the adenylate cyclase.

Introduction

In 2002 Hsu et al. described an additional glycoprotein hormone that they had identified at first by sequence analysis of the published human genome and known mRNA sequences and whose expression they investigated later on. Most surprisingly, this GPHA2B5 was also found and expressed in invertebrates.

Structure and Genes

The second human GPH α-chain gene was localized to chromosome 11 and contains three exons, and the fifth GPH β-chain to chromosome 14 with two exons. The GPHA2 protein is up to 35 % homologous to the common α-chain of LH/FSH/CG/TSH, however, 9 of 10 cysteine residues are conserved with respect to position and spacing. In GPHB5 10 of 12 cysteines are conserved including those of cysteine knot. Nakabayashi et al. (2002) could show that the two chains associate. The presence of a functional dimer was, however, challenged by Alvarez et al. (2009) showing that the structural requirements for dimerization are most probably not met and if at all only at very high protein concentrations. To date any functional

dimer has not been isolated from any species. In invertebrates, the genes for GPHA2 and GPHB5 are linked, although this linkage is lost in vertebrates.

Physiology

GPHA2B5 was shown to activate the TSH receptor (Nakabayashi et al. 2002) and induce thyroid hormone. This led to its name, thyrostimulin.[17] Compared to the expression of GPHA2 in the anterior pituitary the GPHB5 expression in 2000-fold reduced (Nagasaki et al. 2006). Double positive for both chains were only a few corticotropic pituitary cells (Okada et al. 2006). A simultaneous expression of both chains was also found in humans and rat in the eye, skin, ovaries, and in testis with unknown impact. In contrast to TSH release, thyrostimulin release is not stimulated by TRH.

Regulation of both genes seems to be differentially regulated, *e.g.* in lancelets, where the distribution of both genes is shown to differ (Dos Santos et al. 2009) The expression of GPHA2 in human pancreas is regulated by the organ-specific transcription factor isl-1 (Suzuki et al. 2007) and not dependent on triiodothyronine or TRβ1. GPHB5 regulation might probably be modified by inflammatory cytokines that induce NKκB to translocate to the nucleus and bind to a responsive element on the GPHB5 promoter (Suzuki et al. 2009).

Whether homodimers of GPHA2 are by themselves active is an open, but testable, question. In lancelets *i.e.* at the beginning of vertebrate evolution, both GPHA2 and GPHB5 are expressed, GPHA2, however, in a much more restrictive way.

Exploring the role of these novel gonadotropin chains, van Zeijl et al. (2011) recently generated GPHB5 knockout mice. These knockout mice displayed a remarkable reduction of serum thyroxine. The authors concluded that the lack of GPHB5 influences the hypothalamic pituitary thyroid axis and that GPHB5 might have a role during development (Trudeau 2009, see also). Tando and Kubokawa (2009) analyzed expression of GPHA2B5 in the prevertebrate lancelets where the genes were expressed in an analogue of vertebrate pituitary. The finding gives rise to the hypothesis that GPHA2B5 is the precursor of all gonadotropins.

In *D. melanogaster*[18] fly gpha2b5 activated the orphan receptor DLGR1, but not the related DLGR2. Fly gpha2b5 did not activate the human TSHR, but a chimeric fly gpha2/human GPHB5 did which is interpreted as the conservation of the functional structure over millions of years (Sudo et al. 2005).

Sellami et al. (2011) reported expression of Gpha2 and Gphb5 in the same few abdominal neurons in drosophila, in larvae as well as in adults. They did not isolate the protein.

[17]The name thyrostimulin, however, has been used since 1950 for an at that time unknown compound with thyroid-activating properties (Roche et al. 1950).

[18]Drosophila melanogaster.

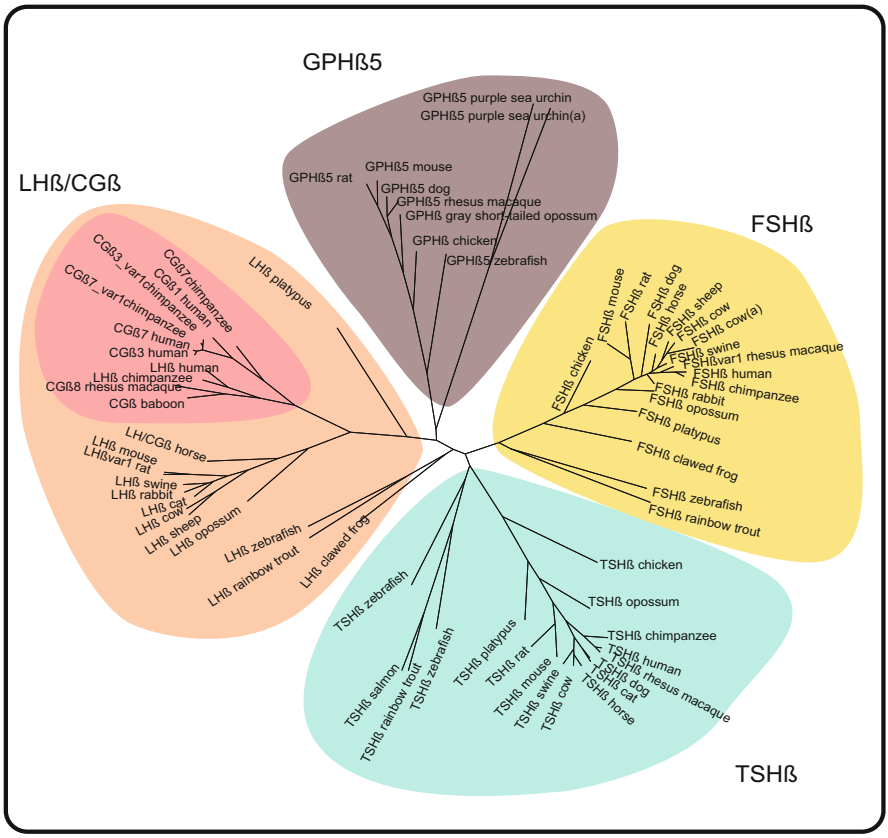


Fig. 4.25 Evolutionary tree of glycoprotein hormone β chains. Using 65 reference sequences from GenBank and ClustalW (neighbor joining method/1,000 bootstrapping steps) phylogenetic dependencies were identified and drawn using the program TreeDyn. Four trees matching the four β chains of follicle-stimulating hormone (FSH), choriogonadotropin/luteinizing hormone (CG/LSH), thyroid stimulating hormone (TSH), and glycoprotein hormone B5 (GPHB5) can be separated. The primate CG subtree is part of the LH tree. Please note that horse CG is a LH. CG from *Oryctolagus cuniculus* (AF362079; rabbit) is obviously a database error. Of closely related variants, only one variant is shown. Some CG-β chains were omitted for clarity reasons

4.4.3.4 Phylogeny of Glycoprotein Hormones

Figure 4.25 demonstrated that separation of LH/FSH/TSH predates vertebrate development or is at its very earliest stage. Dos Santos et al. (2009) have shown that separation of GPHA2 and the common GPHA1 genes coincides with vertebrate origin. They have also shown that the linkage to one chromosome got lost during these stages. The more ancient GPHA2 and GPHB5 genes and proteins are of bilaterial origin lacking in cnidarians and can be found in most of the species analyzed with a few exceptions such as the honeybee although other insects have these genes and GPHA2 is not found in chicken. The uncertain role of thyrostimulin for deiodothyronine/thyroxine release has been discussed in the last chapter.

Note that horse choriogonadotropin does not belong to the CG tree, but belongs to the LH tree. Different CG/LH genes are only present in primates.

4.4.4 Growth Hormone

Fact sheet 4.21: Growth hormone, GH

Gene:	Chromosome: 17; locus: 17q24; four exons.
Sequence:	see Fig. 4.26.
Synthesis and target:	GH release from the pituitary is regulated by GHRH and somatostain. It acts on GH receptors in liver and stimulates IGF-1 synthesis.
Function:	GH acts via IGF1 on growth. During fetal development it has a specific role on nerve system development.
Receptor:	GH receptor is a transmembrane protein that dimerizes upon ligand binding and thus activates STAT proteins.

4.4.4.1 Introduction

The human growth hormone (GH, aka somatotropin, Fig. 4.27) is made by somatotropic cells in the anterior pituitary.

4.4.4.2 Structure and Genes

Within the human *GH* gene locus on chromosome 17 (17q22-q24) for pituitary GH there are also genes for the placental GH variant and three other prolactin-like hormones. (chorionic somatomammotropins (CSH); Chen et al. 1989[19]). Variants arise by alternative splicing, the regulation of which is not yet known. Pituitary GH has 191 amino acids; with alternative splicing a 20 amino acid variant arises instead of the 22 kDa[20] proteins.

In Fig. 4.26 exon-intron boundaries are shown by vertical bars. Variants of GH (lines 2–3) arise by a splicing that removes exon three and/or four (items 01 and 02). The lack of exon three and four modifies the tetrahelical structure (see Fig. 4.27). There some cases of growth hormone deficiency where such an afunctional variant blocks the GH receptor inhibiting the function of GH. GH_var2 (item 03) results from the use of alternative splice acceptor site in exon3.

The GH crystal structure has been determined. In Fig. 4.27 you may notice the high degree of helices characteristic for this hormone. The different helices are

[19] http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?TAXID=9606&CHR=17&MAPS=genec%2Cgenes-r&QSTR=somatotropin&QUERY=uid%28-2146581366%29&BEG=63%2C800K&END=63%2C950K&oview=default

[20] Kilodalton.

[21] Protein Data Bank.

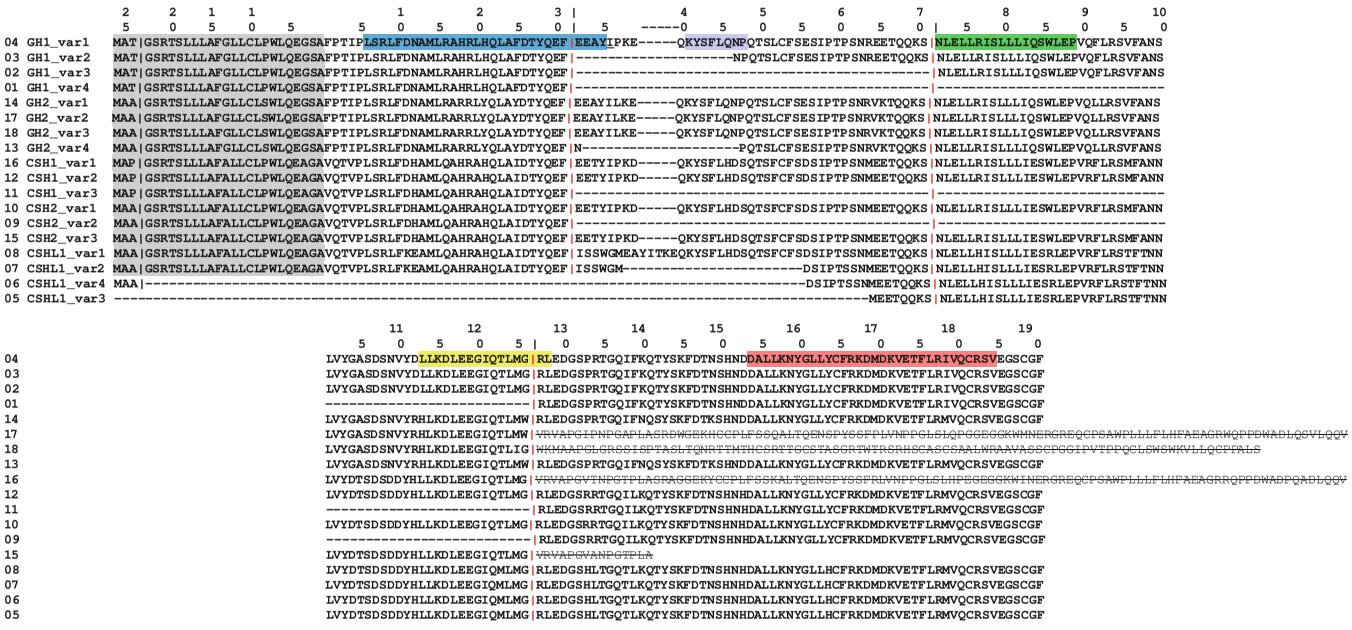


Fig. 4.26 Comparison of human somatotropin splice variants. Exon boundaries are marked by *vertical lines*, lacking amino acids by - - -. With the exception of GH1_var2 gaps coincide with exons. Numbering is according to GH_var1. In variants 15, 16, 17, and 18, intron 4 is not removed which results in new unrelated sequences (*crossed out*). The helices of GH!_var1 are *color coded*. (sources: GH1_var1 NP_000506.2 (04); GH1_var2 NP_072053.1 (03); GH1_var3 NP_072054.1 (02); GH1_var4 NP_072055.1 (01); GH2_var1 NP_002050.1 (14); GH2_var2 NP_072051.1 (17); GH2_var3 NP_072052.1 (18); GH2_var4 NP_072050.1 (13); CSH1_var1 NP_001308.1 (16); CSH1_var2 NP_072166.1 (12); CSH1_var3 NP_072167.1 (11); CSH2_var1 NP_066271.1 (10); CSH2_var2 NP_072170.1 (09); CSH2_var3 NP_072171.1 (15); CSHL1_var1 NP_072101.1 (08); CSHL1_var2 NP_072103.1 (07); CSHL1_var3 NP_001309.3 (05); CSHL1_var4 NP_072102.1 (06))

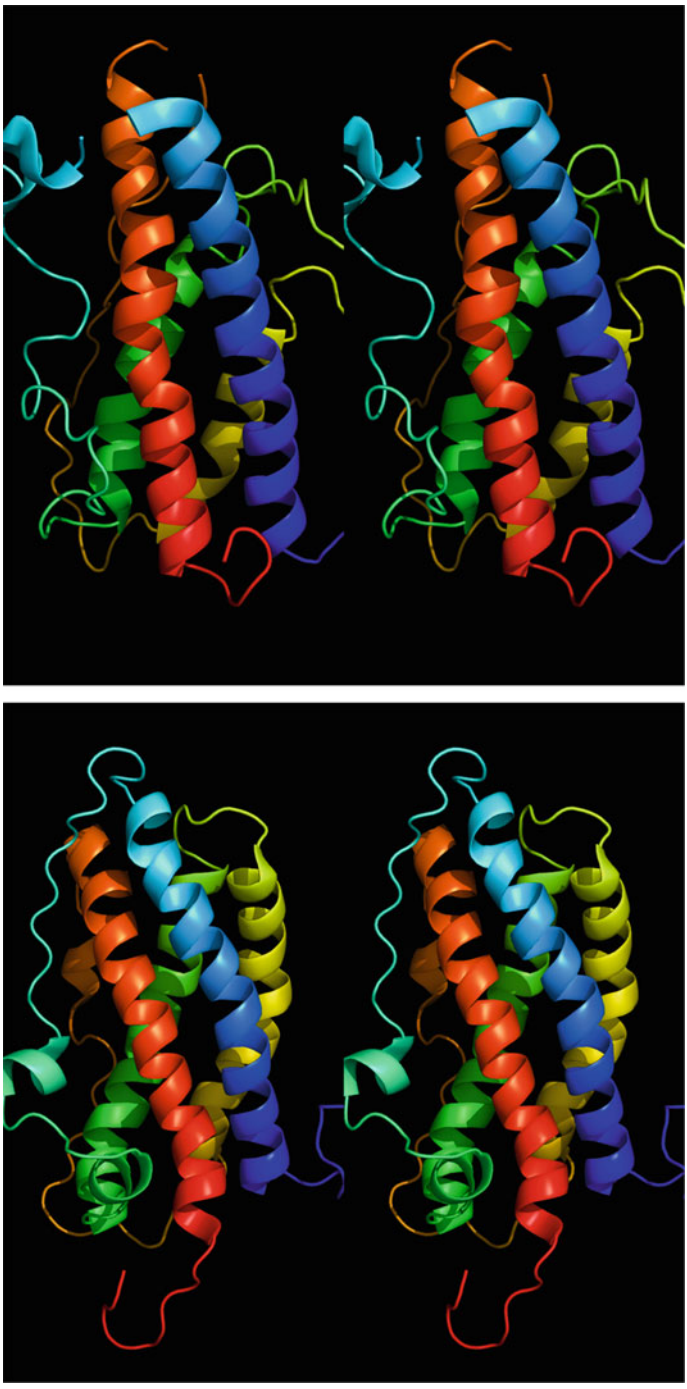


Fig. 4.27 Stereo views of the growth hormone (*above*) and the prolactin molecule (*below*) (Source: Chantalat et al. 1995; PDB: 1HGU (GH); Keeler et al. 2003; PDB: 1N9D, Model 1 (prolactin))

underlined in Fig. 4.26. Activation of the receptor leads to dimerization upon ligand binding: one GH molecule binds to one receptor. The dimer recruits the second receptor molecule.

4.4.4.3 Physiology

GH acts directly using the GH receptor or mediated by insulin-like growth factor 1 (IGF1). IGF1 is formed in hepatocytes stimulated by GH. GH release is pulsatile and reaches (in children) maximal rates about 1 h after the onset of deep sleep. Adult men have six to eight pulses in 24 h whereas women have a less regular pulse rate; in addition women release, per pulse, higher GH rates than men, most probably due to a stronger estrogen influence.

Pertinently elevated GH concentrations *e.g.* due to pituitary tumors may lead to acromegaly, whereas a GH deficit is at the origin of dwarfism. This condition may also be due to nonfunctional GH receptors.

4.4.4.4 Phylogeny

As are prolactin and leptin, GH is a member of the so-called class I cytokines such as interleukin-6, IL11, IL12, erythropoietin, and G-CSG (Huising et al. 2006). The phylogenic context of these proteins suggests a prevertebrate origin (Huising et al. 2006). An IL-6 receptor homologue has been found in flies, but in the literature and in GenBank no class-I cytokine from an invertebrate species has been described although a couple of invertebrate species genomes have been completely sequenced.

The phylogenetic tree of GH has some peculiarities according to Huising et al. (2006): GH genes from sturgeon seem to be more closely related to mammalian GH than to those from amphibians. In order to explain this discrepancy the authors discuss different mutational velocities.

In fish there is another hormone, somatolactin that does not have a homologue in other vertebrate families. This is presented in Sect. 4.5.1.

4.4.5 Prolactin

4.4.5.1 Introduction

Prolactin is produced by lactotropic cells of the pituitary. These develop from common GH- and prolactin-producing cells.

4.4.5.2 Structure and Genes

In the GH gene cluster on chromosome 17 several somatomammotropic, placental, and prolactin-related proteins are coded for (see the previous section), the prolactin gene, however, is found on chromosome 6. Homology between GH and prolactin with respect to amino acids is mainly due to the conservation of disulfide bridges. The crystal structure of prolactin, however, is similar to that of GH: both have the same four long helices (Keeler et al. 2003) (Fig. 4.27). Complexed with zinc GH binds to the prolactin receptor, confirming strong structural similarity between prolactin and GH. Whether prolactin binds to the GH receptor is not described.

Fact sheet 4.22: prolactin (PRL)

Gene:	Chromosome: 6; locus: 6p22.2-p21.3; five exons.
Sequence:	see Fig. 4.28.
Synthesis and target:	Prolactin is formed by lactotropic pituitary cells and acts on prolactin receptors: these are located in the woman's breast, in the ovary, in testis, sexually independent in the CNS, and in adipose tissue and several other organs.
Function:	Prolactin regulates milk production and supports the functions of the corpus luteum. Prolactin plays a role in the establishment of mother/child relations.
Receptor:	Prolactin receptor is a transmembrane receptor which on ligand-induced dimerization stimulates the JAK/STAT signal transduction pathway.

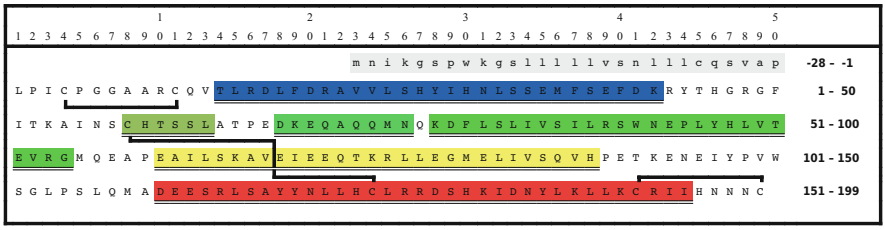


Fig. 4.28 Primary structure of prolactin: disulfide bridges are indicated by *black lines*, helices are *colored* according to Fig. 4.27, arginine 31 (N; *green on black*) is potentially glycosylated (Source: GenBank NP_000939, Swiss-Prot P01236)

Prolactin can be differentially glycosylated (Lewis et al. 1989); N-glycosylated molecules appear to be more active.

4.4.5.3 Physiology

Despite many efforts, a hypothalamic-releasing peptide for prolactin has not been found to date. Prolactin secretion is negatively controlled by the catecholamine dopamine (see Chap. 7). Prolactin is released, like GH, in pulses during sleep. TRH (Sect. 4.3) is one stimulus of prolactin release. Recently, a potent prolactin-releasing peptide (PRLRP) from the pars intermedia has been cloned and functionally tested in humans and rodents (see Sect. 4.5.2). The PRLRP receptor could also be identified. In contrast to the other hypothalamic-releasing hormones, no PRLRP axons could be found in the median eminence, furthermore PRLRP is made in the pars intermedia. Whether PRLRP is thus a true hypothalamic prolactin-releasing hormone is doubtful. In addition, the pars intermedia is degenerated in adults. Whether PRLRP has a different role in nonprimates remains to be answered.

During pregnancy maternal prolactin promotes—in concerted action with estrogens, progesterone, and placental lactogens—functional maturation of mammary glands. After birth prolactin promotes milk production.

The tumor of prolactin-producing lactotropic cells, the prolactinoma, is the most frequent tumor in the pituitary. In a subgroup of cases prolactin is secreted together with GH. Because normal lactotropic cells are controlled by dopamine, in many cases the prolactinoma can be controlled by the dopamine agonist bromocriptin.

Inasmuch as strongly elevated prolactin serum levels caused by prolactinoma cells block pulsatile GnRH release from the hypothalamus and thus pituitary gonadotropin release, prolactin has been regarded as a contraceptive drug. The prolactin levels during lactation, however, are not sufficient to inhibit follicle maturation and ovulation.

In male animals prolactin controls the yearly rhythms of testis function and thus sexual activity: prolactin stimulates testis growth. In men prolactin has been found elevated after orgasm. Prolactin receptors are present on Leydig cells, spermatids, spermatocytes, and widely distributed in testis epithelium (Bartke 2004). For these reasons prolactin is regarded as important for reproductive activity. In addition, prolactin controls maternal functions such as nest building and brood care (Freeman et al. 2000).

4.4.5.4 Phylogeny

Like GH, prolactin is a class-I cytokine which has been found only in vertebrates.

4.5 Hormones of the Pars Intermedia

4.5.1 Somatolactin

Fact sheet 4.23: Somatolactin

Sequence:	Fig. 4.29.
Synthesis and target:	Somatolactin is made in the fish's pars intermedia.
Receptor:	A cloned somatolactin receptor from salmon is a class-1 cytokine receptor.

4.5.1.1 Introduction

As mentioned earlier, in fish an additional pituitary homologue of GH and prolactin has been found, somatolactin. Somatolactin is produced in the intermediate lobe. There are two histologically distinct cell types, one expressing POMC and secreting MSH, whereas the other cells were found in 1990 to produce somatolactin (Ono et al. 1990). As in lactotropic and thyrotropic cells, SL expression is dependent on the pituitary transcription factor Pit1.

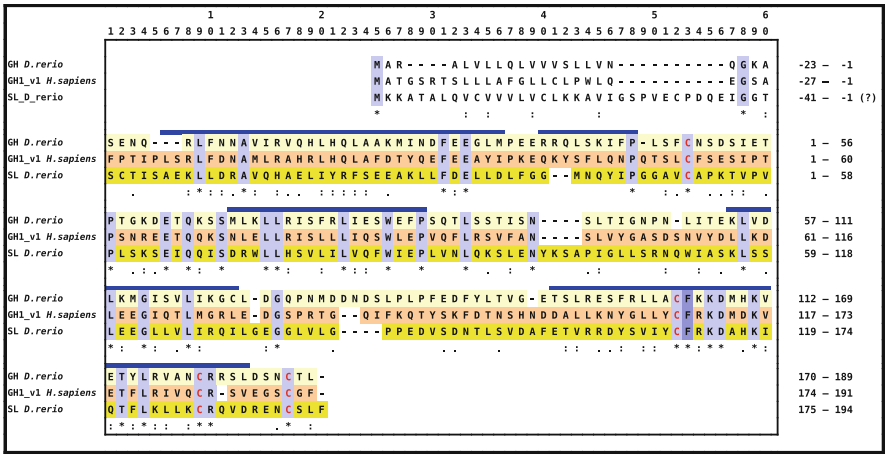


Fig. 4.29 Primary structure of somatolactin (*yellow*) from zebrafish compared to growth hormones (GH) from zebrafish (*light yellow*) and humans (*light orange*). Four cysteine residues (*red on light blue*) are conserved. As does prolactin, somatolactin has an additional N-terminal disulfide bridge. Identical amino acids are in *light blue*. The program ClustalW indicates highly conserved (:) and partially conserved (.) amino acids. Overall homology between GH and SL from zebrafish is 24 % in relation to somatolactin and between human GH and zebrafish somatolactin 22 % respectively. The helices of human GH are indicated by *blue* bars (Sources: NP_001032763, NP_001018328, NP_000506; ClustalW version 2.0.8)

4.5.1.2 Biochemistry and Genes

Somatolactin is a class-I cytokine such as GH and prolactin. The sequence homologies are only marginal; the tetra-helical structure, however, as well as the position and spacing of the cysteine residue are conserved. The somatolactin receptor appears to be related to GH and prolactin receptors.

4.5.1.3 Physiology

The functional role of somatolactin is far from being well understood. It has been observed that it may influence HCO_3^- exchange (Kakizawa et al. 1997); during sexual maturation somatolactin was found to increase (Taniyama et al. 1999). The *ci* mutant from medaka (*Oryzias latipes*) bears a somatolactin deletion that results in inappropriate adaptation of pigment cells to environmental changes. Other changes compared to wildtypes have not been detected. This led the authors to suggest that somatolactin preferentially inhibits proliferation and morphogenesis of leucophores.

Very recently Wan and Chan reported ectopic expression of somatolactin genes in zebrafish that lead to an increased transcription of IGFs, insulin, leptin, sterol regulatory element binding protein 1, and fatty acid synthase, and enhanced expression of vitellogenin and proopiomelanocortin.

The above-mentioned *ci* mutant with reduced orange pigments made the mutant males unattractive to females. This observation prompted experiments where somatolactin was overexpressed (Fukamachi et al. 2009). It could be shown that

somatolactin overexpression enhanced orange pigmentation and simultaneously male attractiveness. These findings support the role of somatolactin in pigmentation and reproduction. It should also be mentioned that the somatolactin receptor was not found to be modulated in tilapia when transferred from seawater to freshwater although GH receptors were increasingly expressed and prolactin receptors reduced (Breves et al. 2010).

4.5.1.4 Phylogeny

Somatolactin is fish specific. In some species, two different somatolactin genes are present that are most probably due to the third karyotype duplication which happened only in fish.

4.5.2 Prolactin-Releasing Peptide

Fact sheet 4.24: Prolactin-releasing peptide (PRLRP)

Gene:	Chromosome 2; locus 2q37; two exons.
Sequence:	Fig. 4.30.
Synthesis and target	PRLRP is formed in the brain and the hypophyseal pars media, and perhaps in other tissues, too. PRLRP receptors have been identified in the anterior pituitary as well as in other tissues. How PRLRP can target the anterior pituitary is unknown.
Function:	Release of prolactin.
Receptor:	The GPCR hGR3 specifically binds PRLRP.

4.5.2.1 Introduction

Prolactin-releasing peptide (PRLRP) is the most promising candidate for the long-term evasive hormone that triggers prolactin release from lactotropic pituitary cells (Hnasko et al. 1997). PRLRP has been described as formed in the hypothalamus of different species but it is (if at all) an unusual releasing hormone: liberation of the mature peptide from the precursors requires PC2 instead of PC1 because an **KR** motif is lacking. In the rat brain, remarkably, there were no PRLRP neurons in the median eminence which strongly argues against a normal hypothalamic pituitary transport.

4.5.2.2 Structure and Genes

The RNA transcribed from the gene on chromosome 2 gives rise to an 87 amino acid precursor protein. After the removal of the 21 amino acid long signal peptide the precursor is further processed by PC2 cleaving at the **RR** motif. Thus the 32 amino acid long PRLRP is made. Alternatively the N-terminal **SRTHRHSMEIR** can be removed by a thus far unknown endopeptidase that results

Fig. 4.30 Primary structure of prolactin-releasing peptides (PRLRP). The precursor protein has 87 amino acids including the signal peptide with 21 amino acids (on *gray background*). Prohormone convertase-2 cleaves after the **RR** motif; the C-terminus of the free peptide *bold, uppercase on blue backround* is amidated. An alternatively shortened PRLRP has only 20 amino acids (on *lighter blue background*) (Source: Genbank P81277)

in the **TPDINPAWYASRGIRPVGRFGRR**. Both peptides are C-terminally amidated and are stored in granules. PRLRP was found to be expressed preferentially in the medulla oblongata, then in the hypothalamus and the pituitary pars media.

4.5.2.3 Physiology

PRLRP was identified as the product of cell lines that originate from the intermediate lobe of the pituitary pars media, Hnasko et al. (1997)). Very soon the role of PRLRP as a PRL-releasing hormone was questioned. PRLRP is expressed in the hypothalamus. But one structural requirement for a releasing hormone acting in the pituitary is not met: axons of PRLRP neurons do not reach the median eminence, and the peptide thus cannot get into the portal system and reach the pituitary (Maruyama et al. 1999). In addition the PRL-releasing activity of PRLRP is much less than that of TRH. It remains doubtful whether PRLRP is a veritable PRL-releasing hormone. The doubts are supported by findings in tilapia where PRLRP did not stimulate PRL secretion (Watanabe and Kaneko 2010).

The most remarkable phenotype of PRLRP knockout mice is obesity; this strongly suggests that PRLRP might be of broader or other activity than previous thought (Mochiduki et al. 2010). Confirming these results in mice, in goldfish PRLRP was found to reduce food uptake after intracerebral or intraperitoneal injection (Kelly and Peter 2006).

4.5.2.4 Phylogeny

PRLRP has been observed only in vertebrates.

4.6 Hormones of the Posterior Pituitary: Oxytocin and Vasopressin

4.6.1 Introduction

Oxytocin (OXT) and vasopressin (AVP) are the two hormones of the neurohypophysis (posterior pituitary (PoP); *lobus nervosus*). Unlike the anterior pituitary hormones they are not transcribed in the pituitary, but in magnocellular neurons

Fact sheet 4.25: Oxytocin (OXT)

Gene:	Chromosome 20 (20p13).
Sequence:	Table 4.3.
Synthesis and target:	Magnocellular neurons in the supraoptic nucleus and in the paraventricular nucleus generate oxytocin which after axonal transport is released in the posterior pituitary. OXT is made in the **CL!**[a] as well. OXT acts on the OXT receptor on smooth muscle cells.
Physiology:	Oxytocin is required for relaxation of the pelvis muscles during birth. In addition it acts on the heart. Oxytocin induces brood care in mammalians.
Receptor:	Oxytocin receptor is a heptahelical GPCR.

[a] **CL!**.

of the supraoptic nucleus or of the paraventricular nucleus. Axons of these neurons reach the PoP via the infundibulum (Fig. 10.2). Prohormones are axonally transported into the PoP. During the transport the precursors are processed to the mature peptides. Vasopressin's role as regulator of blood osmolarity is known from invertebrates; an oxytoxic-related peptide has been found in echinoderms, that is, invertebrates; the OXT role during birth, however, is restricted to placental mammals. Receptors for both hormones are of early evolutionary origin.

Table 4.3 Sequences of oxytocin/vasopressin analogues and their occurrences

Name	Sequence	Taxon
Arginine vasopressin	CY**F**QNCP**R**G-NH_2	Most mammals
Lysine vasopressin	CY**F**QNCP**K**G-NH_2	Pigs, marsupials
Oxytocin	CY**F**QNCP**L**G-NH_2	Mammals
Mesotocin	CY**I**QNCP**I**G-NH_2	Birds, marsupials, reptiles
Arginine vasotocin	CY**I**QNCP**R**G-NH_2	All nonmammalian vertebrates
Isotocin	CY**I**SNCP**I**G-NH_2	Fish
Glumitocin	CY**I**SNCP**Q**G-NH_2	Fish
Valitocin	CY**I**QNCP**V**G-NH_2	Fish
Aspartocin	CY**I**NNCP**L**G-NH_2	Fish
Inotocin	CL**I**TNCP**R**G-NH_2	Insects
Arginine conopressin	CI**I**RNCP**R**G-NH_2	Snails
Lysine conopressin	CF**I**RNCP**K**G-NH_2	*Hirudo*, snails
Anetocin	CF**V**RNCP**T**G-NH_2	Insects
Cephalotocin	CL**I**RNCP**I**G-NH_2	Octopus
Octopressin	CF**W**TSCP**I**G-NH_2	Octopus

4.6.2 Structure and Genes

OXT and AVP are cyclic peptides with an intramolecular disulfide bridge.

The human genes of OXT and AVP are closely linked on chromosome 20 (p13)[22] with reversed orientations. In humans the distance is 12 kb, and in mice on the syntenic region of chromosome 2 3 kb.[23]

Fact sheet 4.26: Vasopressin/adiuretin (AVP)

Gene:	Chromosome 20 (20p13).
Sequence:	Table 4.3.
Synthesis and target:	Magnocellular neurons in the supraoptic nucleus and in the paraventricular nucleus generate vasopressin that, after axonal transport, is released in the posterior pituitary. AVP acts on AVP receptors in the adrenal cortex.
Physiology:	Vasopressin is the regulator of water homeostasis.
Receptor:	AVP receptor is a heptahelical GPCR.

Despite distinct promoters transcriptional activation of OXT and AVP genes is similar with respect to osmotic stimuli. OXT and AVP bind to the OXT-R as well as to AVP-R. OXT thus might be substituted for by AVP which has been formally proven in OXT knockout mice discussed below.

Apart from OXT and AVP the precursor proteins contain additional associated polypeptides called neurophysins. During maturation and axonal transport in acidified vesicles OXT and AVP are complexed to these neurophysins. On release in a more basic environment, hormones and neurophysins are separated. The AVP prohormone bears a third glycoprotein of unknown function.

Structural analyses have found that OXT/AVP association onto neurophysins depends on the pH of the secretory granula. OXT and bovine neurophysins crystallize in a complex of two neurophysins plus one OXT.

The importance of the AVP neurophysin interaction for proper axonal transport and regulated AVP release is evident from "Brattleboro"-rat which harbors a mutation of neurophysin II with the consequence of disturbed osmolarity regulation and water uptake. Such mutations have been found in families with diabetes insipidus patients.

[22] http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?TAXID=9606&QSTR=5020[gene_id]&chr=20&maps=ugHs-r,genes-r&beg=2950000&end=3130000&links=off&verbose=on&compress=off&width=350&size=30

[23] Syntenic chromosomal region exhibits similar sequences of genes and their orientations.

4.6.3 Physiology of Oxytocin

OXT is the hormone of birth induction. Shortly before parturition uterus OXT receptors are massively increased. OXT is released in large amounts directly prior to birth. It is the arrival of the amnion in the cervix that triggers this sudden burst of preformed oxytocin (and vasopressin). This is called the Ferguson reflex. By intramuscular OXT injection by an obstetrician or a midwife birth is initiated. However, during pregnancy OXT antagonists block the receptor and maintain tocolysis. The prepartal OXT burst overcomes tocolysis.

The uterus muscles are additionally protected from preterm labor by the enzyme oxytocinase. Only the strong prepartal burst of OXT can overcome the enzymatic degradation and let sufficient molecules reach the OXT-R on the myometrium.

In the mammary gland this OXT burst triggers contraction of myoepithelial cells around milk-producing alveolar cells and thus presses the milk into the milk ducts (milk ejection reflex).

The complexity of these actions is further confirmed by the analysis of OXT knockout mice: these mice do not have any difficulties in giving birth. Their pregnancy period of 18.5 ± 1 days does not differ from wildtype mice. Obviously OXT triggers can be induced by other mediators.

OXT knockouts were fully fertile and gave birth to viable offspring, however, it turned out that the knockout foster mice could not provide milk to their offspring. The defect could be overcome by injection of external OXT demonstrating that milk ejection is strongly dependent on OXT.

Apart from the direct effects on the parturition OXT induces in animals social behavior patterns that secure care of the newborn: nest building, carrying the litter together, licking the brood. Such patterns could be induced by OXT in rats and in wild mice which would usually kill foreign offspring. OXT antagonists could inhibit brood care even in suckling animals. However, had brood care been triggered once it could no longer be inhibited.

OXT is not restricted to females but made by males as well. It could be shown (at least in rats) that OXT injection led to erection and ejaculation. OXT has been found together with ANP[24] to regulate natriuresis and kaliuresis in the heart .

Owing to its role as an antistress hormone, OXT facilitates pairing. The full image of OXT effects, in particular its regulation by steroids, merits many further efforts.

4.6.4 Physiology of Vasopressin (AVP, Adiuretin)

Osmotic stimuli—changes in the salt concentration of blood—are the most important regulators of vasopressin release. The magnocellular hypothalamic neurons of the supraoptic nucleus themselves are osmoreceptors. In response to changes

[24] Atrial natriuretic peptide.

in osmolarity they change the openings of their potassium channels. AVP release can also be triggered by blood pressure and blood volume changes. AVP neurons, however, are more sensitive to changes of osmolarity than to those of pressure or volume.

The prominent role of AVP is regulation of the water balance by diuresis (secretion in the kidneys) reflected by the alternative term adiuretin. AVP on the one hand and the kidney, both together with thirst, are *the* regulators of this homeostasis. If increased osmolarity leads to vasopressin release, then kidney channels retain water. The kidney may excrete between 0.5 and 201 of water per day without influencing homeostasis. If the kidney retains the maximal amount of water and water loss still occurs, for example, by transpiration, then the brain induces thirst to input more water for maintaining the osmolar optimum.

In parallel to osmoregulation vasopressin acts as a neurotransmitter. Magnocellular neurons from the supraoptic nucleus and the paraventricular nucleus not only project into the PoP[25] but into the anterior pituitary, too, and into other parts of the brain. In the anterior pituitary AVP facilitates ACTH release.

4.6.5 Phylogeny

The phylogeny of OXT and AVP reaches back to invertebrates. AVP analogues have been observed in locusts and earthworms (Proux et al. 1987; Oumi et al. 1994). In echinoderms (sea stars), early deuterostomes, octopressin and cephalotocin proteins are already associated with neurophysins having 14 cysteines as in vertebrates.[26] The locupressin (inotocin) from *L. migratoria*[27] exists (with identical amino acid sequence and so far unknown neurophysin to date) in two peptide variants: one is the cyclic peptide as in deuterostomes, the other a peptide dimer where two intermolecular disulfide bridges couple two peptide monomers. The bridges are between cysteine residue on position 1 of the one monomer to the cysteine on position 6 of the other (Proux et al. 1987).

Fish isotocin and vasotocin genes are already on the same chromosome, but not yet in the immediate neighborhood. Agnathans only have a single vasotocin gene. In coelacanths the GnRH2 gene is already linked to the two neuropeptide genes. This conformation is maintained in frogs and chicken. In the mammalian genome there is the change to vasopressin. In marsupials a selective duplication of vasopressin and mesotocin has taken place with one VP bearing a [Lys8] variant, which can be seen in pigs as well (Gwee et al. 2008). The change from mesotocin to oxytocin and the inversion of the OXT gene is characteristic of the clade Eutheria.

[25] Posterior pituitary.

[26] GenBank: BAD93372 und BAD93373.

[27] Locusta migratoria.

Vasotocin and vasopressin have been found in all analyzed species to be functionally active in osmolarity regulation.[28] The roles of mesotocin, oxytocin, and isotocin, in contrast, are more divergent.

In nonmammalian species especially in birds and marsupials oxytocins are important for reproduction: In chicken arginine vasotocin is required for egg laying; marsupials need mesotocin during birth, for example, in *Macropus eugenii* (tammar wallaby) (Siebel et al. 2005). In *Trichosurus vulpecula* (common brushtail possum) mesotocin regulates the seasonal size changes of the prostate (Fink et al. 2005). Analogues are listed in Table 4.3.

4.7 Regulators of Sugar and Energy Metabolism

4.7.1 Insulin

Fact sheet 4.27: Insulin (INS)

Gene:	Chromosome: 11; locus: 11q5 ; three exons.
Sequence:	Fig. 4.32.
Synthesis and target:	Human insulin is formed inβ cells of Langerhans islets and is used by insulin receptors on almost any cell for glucose transport therein.
Function:	Insulin regulates the usage of glucose in liver and other cells. In the liver it stimulates sugar storage and amino acid synthesis. In muscle and adipose tissue the incorporated glucose is used for ATP production and fatty acid synthesis.
Receptor:	Insulin receptor is a transmembrane receptor with tyrosine kinase activity. Because the insulin receptor does not have SH domains, an INS receptor substrate called a molecule is required for the initiation of a signal cascade.

4.7.1.1 Introduction

Insulin, the hormone of β cells in Langerhans islets of the pancreas, is indispensable for sugar metabolism.

4.7.1.2 Biochemistry and Genes

The mature hormone consists of two peptide chains called A and B that are linked via two intermolecular disulfide bridges. Proinsulin bears a C-peptide linking the A and B chains. The name of the chains had already been established before the gene was cloned and it was found that the B chain lies before the A chain (Fig. 4.31). By

[28] Vasotocin regulates oocytes maturation and ovulation in fish. (Joy KP, Chaube R (2015) Vasotocin – A new player in the control of oocyte maturation and ovulation in fish. Gen Comp Endo 221:54–63)

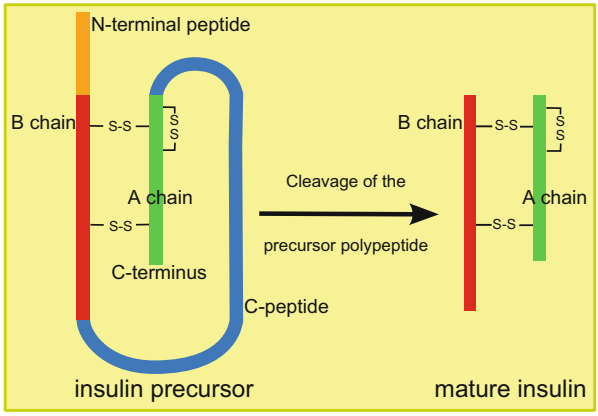


Fig. 4.31 Insulin structure

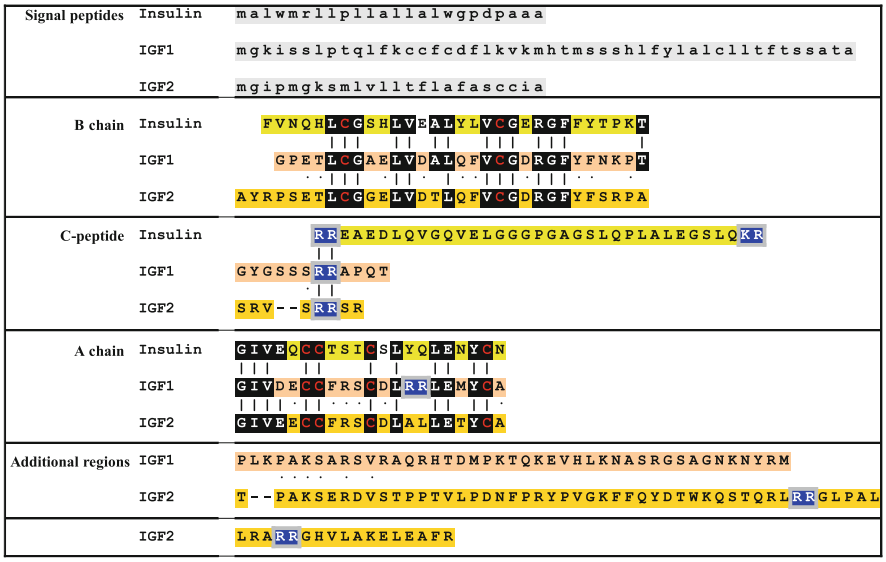


Fig. 4.32 Sequence comparison of insulin and insulin-like growth factors (IGF) (Source: GenBank: NP_000198, NP_000609.1, NP_000603.1)

peptidases (e.g., carbopeptidase-H or PC2) the C-peptide is cleaved, whereas the A and B chains remain linked by the disulfide bridges. These enzymes furthermore remove the N-terminus of proinsulin..

The insulin gene on chromosome 11 is closely linked to tyrosine hydroxylase and IGF2. These genes are oriented in the same direction. Apart from primary insulin

and IGF2 transcripts a mixed insulin/IGF2 transcript has been found expressed in limbs and eyes.[29]

Coded for by the same locus there is an antisense IGF2 transcript potently suppressing placental expression of IGF2. On the gene map[30] it is evident that several inherited diseases have been linked to this region. For some time it has been known the 500-kb section on chromosome 11 between the IGF2 gene and CDKN1C (cyclin-dependent kinase inhibitor 1C) is regulated by imprinting: IGF2 and insulin, but not tyrosine hydroxylase, are only transcribed from the paternal allele, not from the maternal one. CDKN1C and genes in its neighborhood are transcribed only from the maternal, but not from the paternal allele. In some diseases, for example, in the case of Wilms tumor, imprinting does not work correctly and genes are transcribed from both alleles. Some insight into the development of imprinting exemplified using the IGF2-CDKN1C chromosomal section can be found from Ager et al. (2008a,b).

4.7.1.3 Physiology

Insulin influences several key reactions of carbohydrate, protein, and lipid metabolism (see Sect. 11.4):

Glucose uptake: In adipose and muscle cells binding of insulin to insulin receptor triggers the transport of preformed glucose transporters into the membrane. This leads directly to an increased glucose uptake into the cells.

Storage of glucose into glycogen: Triggered by insulin, glucose is stored as glycogen.

Glucose oxidation: By the oxidation of glucose to carbon dioxide energy in the form of adenosine triphosphate (ATP) is gained. This ATP is a general energy carrier in cells and forces movements, intracellular transport along the cytoskeleton, ion transports across membranes and many other reactions. ATP is the fuel on which the cell depends.

Anabolism of lipids: In adipose cells insulin triggers mobilization of the glucose transporter GlcT; glucose is utilized for fatty acid synthesis which gives rise to lipids. The lipid formation again is triggered by insulin.

Amino acid and protein synthesis: Protein synthesis from amino acids is enhanced by insulin. Furthermore insulin stimulates amino acid synthesis from liver cells to reach, for example, muscle cells and their usage in translation into proteins.

In general we can note that due to insulin triggering glucose is used for fatty acid synthesis, for storage as glycogen, for protein synthesis, and for ATP formation.

[29] http://www.ncbi.nlm.nih.gov/projects/sviewer/?id=NC_000011.8&v=2105322..2140619

[30] http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?TAXID=9606&CHR=11&MAPS=ugHs-r%2Cgenes-r&QSTR=5020[gene_id]&BEG=2M&END=2%2C300K&oview=default

Fact sheet 4.28: IGF1

Gene:	Chromosome 12; locus 12q22; five exons.
Sequence:	Fig. 4.32, two variants due to alternative splicing.
Synthesis and target	IGF1 synthesis preferentially in the liver; IGF1 receptors are ubiquitous on all cells.
Function:	IGF1 is the major mediator of insulin after birth and a regulator of growth, too.
Receptor:	IGF1-R triggers anabolic metabolism of carbohydrates and proteins; it enhances cellular proliferation.

4.7.1.4 Phylogeny

Insulin is member of a family of related proteins. Among these are *androgenic gland factor* of crustaceans, insect bombyxins (silkworm, mosquito, hawk moth, and others), insulin-like proteins from *D. melanogaster*, and relaxins from vertebrates, also further IGF. Insulin has already been isolated from sponges (Porifera). Insulin, IGF1, and IGF2 differ by the fact that the C peptide is cleaved from the insulin precursor whereas the IGF retains the entire sequence. Insect bombyxin, however, as well as the sponge insulin are built of two chains. In humans (as in other mammals) there are additional insulin-like (two chain) proteins: insulin-like 3 (INSL3; chromosome locus 19p13), insulin-like 4 (chromosome locus 9p24), insulin-like 5 (chromosome locus 1p31), insulin-like 6 (chromosome locus 9p24), as well as IGF-like (one chain) proteins: IGF-like 1, 2, 3, and 4 (all on chromosome 19). Trying to prepare a tree showing evolutionary dependencies we failed due to very low bootstrap values which indicate low significance. We therefore have resigned showing such a tree of the insulin/relaxin/IGF family. Using only the sequences of the A and B peptide, Perillo and Arnone (2014) have shown a tree that included echinoderms, hemichordates, cartilaginous and bony fish, birds, and mammals. No protostome sequence was included.

The receptors form insulin and IGF of nonvertebrates and vertebrates are, as far as analyzed, tyrosine kinase receptors.

4.7.1.5 Insulin-Like Hormone: IGF1 und IGF2

Insulin-like growth factors (IFG) differ from insulin by retaining the C peptide due to the lack of the **KR** cleavage site for PC1 or PC2 (Fig. 4.32). Cysteine residues are conserved; in the B peptide are 14 of 30 amino acids between insulin and IGF1 and 12 of 30 amino acids between insulin and IGF2 conserved; in the A peptide 11 of 21 for IGF1 and 12 of 21 for IGF are identical.

The similarity of the molecules lets insulin and IGF bind to the same receptors. Preferentially insulin binds to an insulin receptor and IGF binds to IGF-R. With reduced avidity insulin binds to IGF-R, like the binding of IGF to an insulin receptor. Combination of one extracellular chain of insulin receptor with one chain of IGF-R lets insulin and IGF bind with similar avidity to this recombinant receptor (Jones and Clemmons 1995).

IGF influences several functions in different cells. Some of these are described in Sect. 11.6. For IGF there are binding proteins IGFBP that act as transport proteins

Fact sheet 4.29: IGF2

Gene:	Chromosome 11; locus 11q5; eight exons.
Sequence:	Fig. 4.32.
Synthesis and target:	IGF2 is expressed in many different cells and binds to insulin receptor or IGF1 receptor.
Function:	IGF2 is a growth stimulus. Its regulation by imprinting and antisense transcription inhibits overshooting growth and transformation. IGF2 preferentially regulates prenatal growth.
Receptor:	IGF2 binds to IGF1-R as well as to the IGF2-R, also known as mannose 6-phosphate receptor. Growth-promoting signals are mediated by IGF1-R; the role of IGF2-R appears to be IGF2 regulating.

and help to keep IGF in the bloodstream. With the help of the binding proteins, binding to the extracellular matrix is possible. Receptor interaction is very often possible once the binding proteins are digested. There are special IGFBP proteases for this digestion. These different proteins establish a regulatory system with many ways of fine tuning (Baxter 2000).

4.7.2 Glucagon

Fact sheet 4.30: Glucagon

Gene:	Chromosome 2; locus 2q36; six exons.
Sequence:	Fig. 4.33.
Synthesis and target:	Glucagon is made in the α cells of pancreatic islets and released into the circulation. It acts preferentially on glucagon receptors on liver cells.
Function:	Glucagon stimulates glucose release from glycogen stores in the liver cells.
Receptor:	Glucagon receptor is a GPCR.

4.7.2.1 Introduction

The preproglucagon precursor contains apart from glucagon three additional peptides: glicentin-related peptide (GRPP) and the glucagon-like peptide 1 and 2 ((GLP1 and GLP2).

4.7.2.2 Structure and Genes

In the section on prohormone convertases (Sect. 4.2), we discussed preferential cleavage after the amino acids **KR**, **RR**, **RK** and **KK** with PC1 preferentially cleaving after **KR**. In the glucagon precursor there are several such arginine–lysine combinations: 51/52 **KR**, 69/70 **RR**, 82/83 **KR**, 89/90 **KR**, 128/ 129 **RR**, 144/145

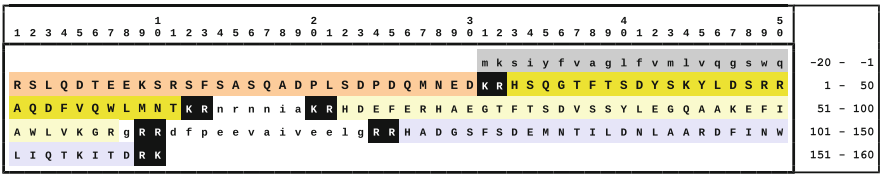


Fig. 4.33 Primary sequence and organization of the glucagon precursor. In α-cells of the pancreas the preproglucagon is cleaved into the glizentin-related peptide (GrPP, amino acids 1–30), glucagon (amino acids 33–61) and the two glucagon-like peptides GLP1 (amino acids 78–108) and GLP2 (amino acids 126–158); in the brain and in the intestinal tract, however, glizentin (amino acids 1–69) and the glizentin cleavage products GrPP and oxyntomodulin (amino acids 33–69) as well GLP1 and GLP2 are formed. The prohormone convertase motifs are *inverted*. Those unused are shown in *light gray* (Source NP_002045)

RR, and 179/180 **RK**. By PC1 only glucagon 53–81, is released. Other prohormone convertases generate the other three peptides. GLP-1 is C-terminally amidated.

4.7.2.3 Physiology

After binding to its GPCR glucagon acts via G-proteins on the adenylate cyclase and stimulates protein kinase A. Thus phosporylase kinase and in turn phosphorylase are stimulated, the latter triggering glycogen degradation whereby glucose is made available. Additionally, gluconeogenesis is induced by activating phosphoenolpyruvate carboxykinase and Glc6Pase. The glucagon actions are exclusively directed to liver cells (see Sect. 11.4.6).

GLP-1 equally excised from the glucagon precursor, is postprandially released in endocrine cells of the intestine. It stimulates insulin release and insulin transcription, and suppresses glucagon synthesis and release.

GLP-2 is most probably a paracrine stimulator of intestinal crypts (Sherwood et al. 2000).

4.7.2.4 Phylogeny

Glucagon belongs to the superfamily of PACAP/secretin/glucagon peptides. Earliest genes for PACAP have been discovered in tunicates, glucagon is described in the earliest vertebrates. In humans apart from PACAP, secretin, glucagon, GLP-1 and GLP-2, GIP, GRF, PHM, and VIP belong to this family. The glucagon sequence is identical in humans, monkey, swine, sheep, and cow; two glucagon genes have been found in some fish species (Sherwood et al. 2000).

4.8 Regulators of Food Intake

4.8.1 Leptin

Fact sheet 4.31: Leptin

Gene:	Chromosome 7; locus 7q13.3; three exons.
Sequence:	Fig. 4.34.
Synthesis and target:	Leptin is made in adipose cells. It binds to brain and peripheral leptin receptors.
Function:	Leptin inhibits food intake and stimulates energy consumption.
Receptor:	Leptin receptor belongs to the cytokine-like receptor family.

4.8.1.1 Introduction

Leptin (Fig. 4.34; from the Greek *leptos*: thin) is the hormone of adipose cells. Using leptin these cells signal their fat content. The more fat is stored, the larger the leptin amounts released. The leptin level in blood is a measure ofthe state of nutrition.

4.8.1.2 Structure and Genes

The protein leptin resembles the growth hormone in its structure. Both belong to the family of tetrahelical cytokines such as the granulocyte-colony-stimulating factor (G-CSF).

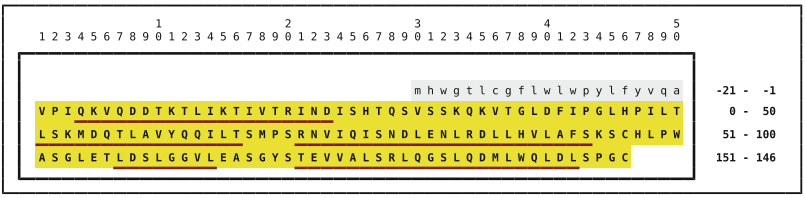
4.8.1.3 Physiology

Leptin exhibits two major functions. First, it antagonizes hunger. Hunger is centrally mediated, for example, by neuropeptide-Y (NPY). With NPY applied or intrinsically released there is a demand for food intake. This NPY-mediated demand is blocked by leptin. Second, enhanced leptin stimulates oxygen consumption for glucose metabolism, enhancement of body temperature, and depletion of fat depots in adipose cells.

Leptin is additionally secreted by myofibroblast in order to induce surfactant production in type 2 alveolar cells. There leptin acts in a paracrine manner (Torday and Rehan 2007).

4.8.1.4 Phylogeny

Leptin has only been identified in vertebrates (Huising et al. 2006). The evolutionary tree reflects the majority view of vertebrate evolution. Some teleosts possess two leptin genes.



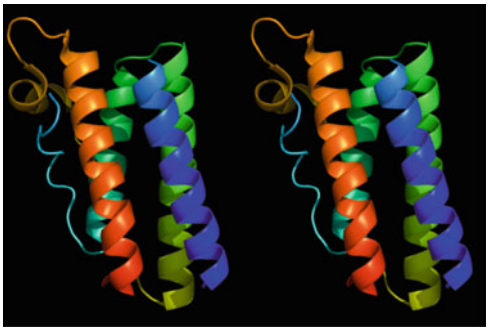


Fig. 4.34 Sequence and three-dimensional structure of leptin. *Upper:* the signal peptide is highlighted *gray*, the mature peptide *yellow*; helices are *red underlined* (Source: Protein Data Bank entry 1AX8 with PyMOL)

4.8.2 Ghrelin

Fact sheet 4.32: Ghrelin

Gene:	Chromosome 3; locus 3p26; four exons.
Sequence:	Fig. 4.35.
Synthesis and target	Ghrelin is released in the stomach and in hypothalamic neurons. It is involved in control of GH secretion in pituitary somatotropic cells.
Function:	Ghrelin stimulates the transcription of the pit-1 transcription factor required for GH transcription.
Receptor:	Ghrelin receptor (growth hormone secretagogue receptor) is a heptahelical GPCR.

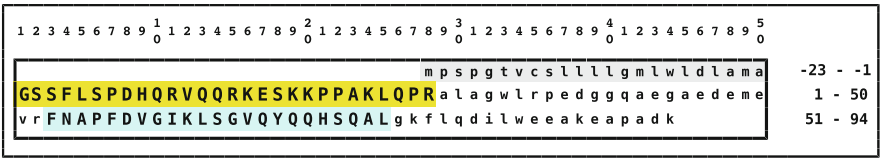


Fig. 4.35 The primary sequence of ghrelin. The signal peptide (on *gray background*) is followed by the ghrelin sequence (*bold* on *yellow background*). On the same precursor there is an additional peptide with antagonistic actions at the ghrelin receptor: obestatin (on *light blue background*) (Zhang et al. 2005)

4.8.2.1 Introduction

Small synthetic peptides and other nonpeptide molecules had been reported for quite some time to stimulate GH release from the pituitary. The receptor that transfers the signals is called the growth hormone secretagogue receptor GHS-R1. In 1999, the endogenous ligand for this receptor was discovered by a Japanese team: ghrelin (Kojima et al. 1999).

4.8.2.2 Structure and Genes

Ghrelin is a peptide of 28 amino acids (Fig. 4.35) with an octanoic acid ester group on serine in position 3 for maximal functional activity. The esterification by octanoic acid is without precedent in the literature. The analogue des-gln14-ghrelin, by alternative splicing lacking a glutamine residue at position 14, once esterified by octanoic acid is as fully active as ghrelin. The first five amino acids including the esterified serine in position 3 appear sufficient for ghrelin activity.

The O-acyl transferase adding an octanoyl group to ghrelin has recently been described (Yang et al. 2008; Gutierrez et al. 2008).

The majority of ghrelin molecules, however, are not esterified. According to a recent publication, such a desacyl ghrelin is an ghrelin antagonist in mice (Asakawa et al. 2005).

There is a ghrelin-associated peptide cleaved from the common precursor and which may give rise to another peptide with potential C-terminal amidation. Whether this peptide called obestatin is an ghrelin antagonist, as well, is debated.

4.8.2.3 Physiology

Octanoyl-ghrelin is synthesized in endocrine cells of the GI tract, preferentially in the X/A-like stomach cells. This ghrelin release precedes food intake. Postprandially, ghrelin is suppressed.

Ghrelin's role as regulator of food intake is amplified by the fact that certain neurons in between hypothalamic nuclei also release ghrelin, which then triggers release of NPY and/or AgRP from neurons of the arcuate nucleus. These NPY and AgRP are those mediators eliciting hunger.

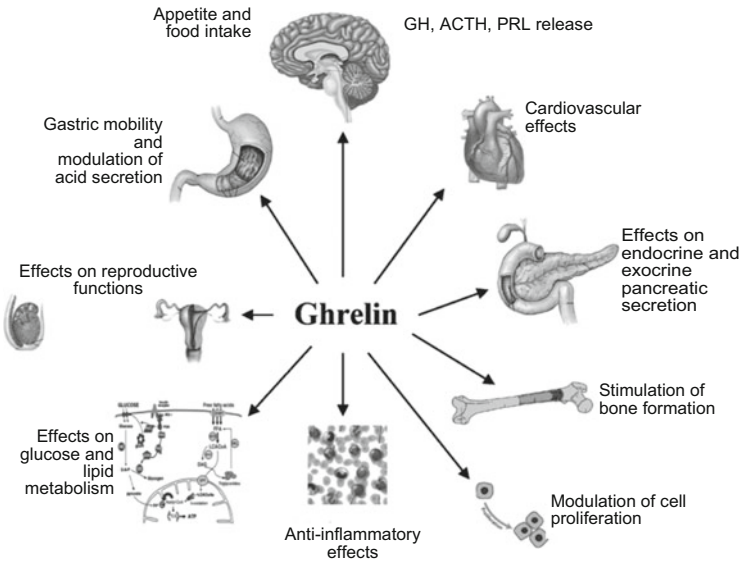


Fig. 4.36 Known effects of ghrelin (From De Vriese and Delporte (2008); Ghrelin: a new peptide regulating growth hormone release and food intake. Int J Biochem Cell Biol 40:1420–1424; with permission of Elsevier)

De Vriese and Delporte review in 2008 different actions of ghrelin: enhancement of GH, ACTH and PRL release, effect on endocrine and exocrine pancreas functions, increase in bone formation, influence on cellular division, anti-inflammatory effects, action of glucose and fat metabolism, stimulus of reproductive activity, triggering gut mobility and acid secretion, appetite stimulus, and boosting of food intake. Because ghrelin was only discovered 12 years ago, further actions might be discovered. Some the actions of ghrelin are sketched in Fig. 4.36.

Totally surprising was the observation that ghrelin knockout mice neither exhibit strong defects, nor are these mice especially obese (Kim et al. 2015).

4.8.2.4 Phylogeny

Until now, ghrelin is only found in vertebrates. In teleosts, too, ghrelin is a stomach hormone triggering pituitary GH and PRL release. In eels ghrelin is esterified by octanoic as well as by decanoic acid; in the cichlid *Tilapia oreochromis* it is exclusively esterified by decanoic acid (Kaiya et al. 2003b,a). A ghrelin-like peptide from shark is likewise acylated; whereas all other vertebrate ghrelin analyzed possess the N-terminal sequence **GSSF**, shark have **GVSF**. Such a peptide was able to enhance intracellular calcium in hamster cells expressing GHS-R. With respect to invertebrates, it has been found that the GHS-R is related to pheromone-biosynthesis–activating neuropeptide (PBAN); PBAN, however, does not show any homology to ghrelin (Choi et al. 2003).

The obestatin peptide from the ghrelin precursor has also been found in teleosts.

4.9 Nonsteroidal Regulators of Bone Formation: Calcitonin/Calcitonin-Gene–Related Peptide

Fact sheet 4.33: Calcitonin (CT)

Gene:	Chromosome 11; locus 11p15.2; six exons.
Sequence:	Fig. 4.37.
Synthesis and target:	Calcitonin from the thyroid gland acts on bone cells.
Function:	Calcitonin controls the estradiol-dependent bone storage of calcium and blocks bone catabolism.
Receptor:	Calcitonin receptor is a heptahelical GPCR.

4.9.1 Introduction

Calcitonin (CT) is a product of thyroid C cells. It is a major regulator of calcium metabolism. CGrP (calcitonin-gene–related peptide) transcribed from the same gene but derived by alternate splicing, however, is a neuropeptide. Similar peptides such as CGrP are the amylin from pancreatic β cells and adrenomedullin.

4.9.2 Structure and Genes

The primary calcitonin transcript of the *CALCA* gene on chromosome 11 encodes a second peptide, the "calcitonin-gene–related peptide" (CGrP). Owing to alternative splicing the primary transcript is modified either to a calcitonin or CGrP mRNA (Fig. 4.37). The precursor sequences coded for by the exons 2, 3, and 4 are identical; those from exon 5 are calcitonin specific, and those from exon 6 are CGrP specific (Fig. 4.38). The processing of the calcitonin gene is taken as a model for alternative splicing (Lou and Gagel 1998).

Almost in the direct neighborhood of the *CALCA* gene on chromosome 11 the *CALCB* which gives rise only to an additional CGrP due to the lack of the CT exon.[31] There is a *CALCP* pseudogene closely linked on chromosome 11. The gene for amylin (aka islet amyloid polypeptide; IAPP) with its three exons is located on chromosome locus 12p12.1 on the antisense strand and the intron of a anion transport gene; the gene for adrenomedullin with four exons is on chromosome locus 11p15.4 (5 megabases distant from *CALCA*/*CACLB*).

Calcitonin is cleaved from the precursor by PC1, shortened by endopeptidases, and amidated by PAM. CGrP is only cleaved from its precursor by PC2 because the

[31] NCBI mapview: http://www.ncbi.nlm.nih.gov/mapview/maps.cgi?TAXID=9606&CHR=11&MAPS=genes-r,genec[868.93%3A878.71]-r&QSTR=114130[MIM]&QUERY=uid(14176198,12718486)&GOTO=874.75human%3A11%3AISCN&rsize=9.780000000000086

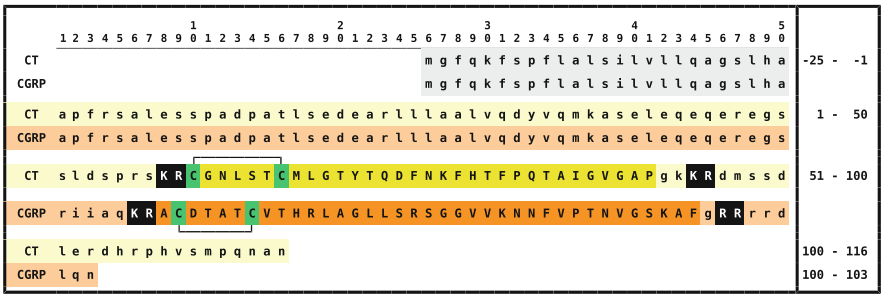


Fig. 4.37 Sequences of calcitonin and calcitonin-gene–related peptide (CGrP) (According to Broad et al. (1989); source: AAA58403)

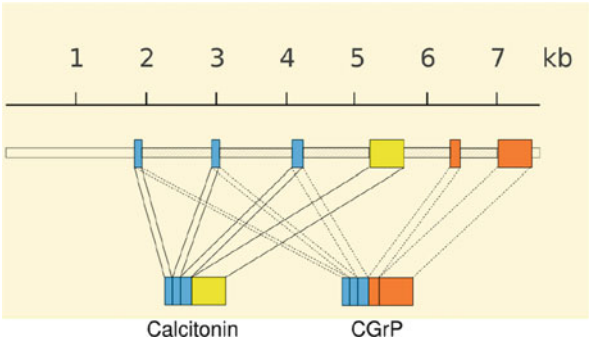


Fig. 4.38 Organization of the calcitonin/calcitonin-gene–related peptide (CGrP) gene (According to HSCALCAC, GenBank X15943)

C-terminal **KR** motif for PC1 is lacking. Both peptides have an N-terminal disulfide bridge in common.

CTs and CGrPs action are mediated by specific receptors. The CT receptor is CALC-R, a heptahelical GPCR, like the CGrP receptor. The receptor activity modulating protein (RAMP-1) associates with CALC-R and can bind to CGrP-R. Amylin binds to the complex of CALC-R and RAMP-1 or RAMP-3. Association with RAMP-1 is necessary for an efficient membrane expression of CGrP. Without RAMP-1 the CGrP-R is not functionally active.

Recently it had been found that the serum concentrations of the CT precursor ProCT (amino acids 3–116) are characteristically enhanced during bacterial infections (Muller et al. 2001). ProCT might possibly be a more specific marker for bacterial infections than, for example, C-reactive protein (CRP) (Schuetz et al. 2011).

4.9.3 Physiology

Calcitonin biosynthesis is controlled by estrogens. For the treatment of osteoporosis as a consequence of postmenopausal estradiol deficiency calcitonin is also used.

CT inhibits further bone resorption. CT is further required for the maintenance of adequate calcium levels during stress, pregnancy, or lactation (Wimalawansa 1996).

CGrP is the neuropeptide most active for dilatation. An important role of CGrP is in the heart where CGrP secreted by neurons enhances heartbeat frequency, atrial contraction, and in summary the coronary circulation. The renal blood flow and that of other organs could equally be stimulated by CGrP.

Calca-knockout mice were viable, fertile, and showed enhanced bone mass and bone formation more than wildtype mice. Although $Calca^{+/+}$ mice reduced bone mass after estradiol depletion, the bone mass remained constant in *Calca*-knockout mice (Hoff et al. 2002). The fact that CT knockouts do not exhibit deficiencies led to the conclusion of CT redundancy in humans and rodents. The activity of human CT is considerably reduced compared with, for example, fish CT. Due to the development of parathyroid glands and of the parathormone the action of CT seemed to be confined to bone protection during stress (Hirsch and Baruch 2003).

4.9.4 Phylogeny

Calcitonin and CGrP are vertebrate peptides. In invertebrates only homologues to the calcitonin receptor have been discovered in tunicates, echinodermates, insects, and nematodes.

4.10 Digestive Tract Hormones

In the GI tract there are more than 30 polypeptides, some of them controlling digestion as hormones, others as neurotransmitters. The majority of these hormones are released by specialized GI tract cells. In the stomach, gastrin and somatostatin are released, and in the duodenum somatostatin, cholecystokinin, secretin, and GIP. In the pancreas there are cells that secrete insulin, glucagon, somatostatin, or the pancreatic polypeptide. In the ileum, there is somatostatin, enteroglucagon, and neurotensin release.

4.10.1 Gastrin

4.10.1.1 Introduction

Gastrin is a peptide from so-called G cells in the pyloric antrum, close to the duodenum. The hormone is secreted into the blood in an endocrine way and not exocrine into the gut lumen like digestion enzymes, acid, or carbonate. Gastrin, like cholecystokinin, was discovered almost 100 years ago in animals. Recently its role in triggering gastric ulcer under infection by *Helicobacter pylori* has attracted attention.

Fact sheet 4.34: Gastrin

Gene: Chromosome 17; locus 17q21; three exons.

Sequence:

Gastrin-71 **SWKPRSQQPD APLGTGANRD LELPWLEQQG PASHHRRQLG PQGPPHLVAD PSKKQGPWLE EEEEAYGWMD F**-NH_2

Gastrin-34 **QLG PQGPPHLVAD PSKKQGPWLE EEEEAYGWMD F**-NH_2

Gastrin-17 **QGPWLE EEEEAYGWMD F**-NH_2

Gastrin-6 **YGWMD F**-NH_2

Gastrin-17, the major active molecule, may be sulfated on the tyrosine residue (**Y**). The N-terminal glutamine of gastrin-17 could be cycled to pyroglutamate.

Synthesis and target: Gastrin is released from G-cells of the stomach antrum and acts on gastrin receptors on parietal cells of the stomach epithelium and on the central nervous system.

Function: Gastrin-34 and gastrin-17 stimulate gastric acid release from stomach parietal cells. It is further required for cellular differentiation of the gastric epithelium.

Receptor: Gastrin receptor (cholecystokinin receptor-2) is a heptahelical GPCR. It is expressed in many brain cells.

4.10.1.2 Structure and Genes

The active gastrin-34 and gastrin-17 are cleaved from a precursor. For the formation of Gastrin-17 PC2 is required which is expressed in G cells and can effectively cleave at the **KK** motif. Sulfate substitution of the tyrosine residue might enhance PC2 recognition. Sulfated and nonsulfated gastrins are equally active. Gastrin-34 has a prolonged half-life in the circulation compared with gastrin-17 (Dockray 1999).

4.10.1.3 Physiology

Gastrin is formed in G cells of the stomach and duodenum. It enhances gastric acid release from parietal stomach epithelium cells. Gastrin-gene–defective mice lack maturation of the stomach mucosa. Although acid synthesis and release can be maintained with gastrin synthesis blocked by histamine or acetylcholine, epithelial cells do not mature at all without gastrin.

The gastrin-releasing peptide (bombesin, Sect. 4.11.3) stimulates enhancement of gastrin levels in serum and of acid release in the stomach. Whether bombesin is functional active is not known inasmuch as bombesin is a brain neuropeptide and any secretion into the blood has not been found in humans.

4.10.1.4 Phylogeny

See cholecystokinin (next section).

4.10.2 Cholecystokinin

Fact sheet 4.35: Cholecystokinin (CCK)

Gene: Chromosome 3; locus 3p22.1; three exons.

Sequence:

CCK-83	QPVPPADPAG SGLQRAEEAP
	RRQLRVSQRT DGESRAHLGA LLARYIQQAR
	KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF-NH_2
CCK-58	VSQRT DGESRAHLGA LLARYIQQAR
	KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF-NH_2
CCK-39	YIQQAR
	KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF-NH_2
CCK-33	KAPSGRMSIV KNLQNLDPSH RISDRDYMGW MDF-NH_2
CCK-22	NLQNLDPSH RISDRDYMGW MDF-NH_2
CCK-08	DYMGW MDF-NH_2

Synthesis and target: The majority of CCK is released from I cells in the duodenum and jejunum and acts on gallbladder muscles, pancreatic acinar cells, and parietal cells of the stomach. CCK is widely found in the CNS.

Function: By CCK bile release and pancreatic enzyme production are increased, gastric acid synthesis reduced, and (centrally) appetite reduced.

Receptor: The CCK receptor CCK-R1 is a heptahelical GPCR; CCK-R2 is the gastrin receptor.

4.10.2.1 Introduction

Cholecystokinin (CCK) is released in the duodenum and the jejunun. Due to the common C-terminal **WMDF**amide sequence a close phylogenetic relation to gastrin had been postulated for some time. CCK is probably the original hormone.

4.10.2.2 Structure and Genes

CCK is cleaved from a precursor shortened by PC2 and amidated by PAM. After the release of CCK-33 an endopeptidase might degrade CCK to CCK-8 (Moran and Kinzig 2004). In serum CCK-33 is most abundant (Rehfeld et al. 2001). The tyrosin of CCK-08 is often sulfated. The CCK gene is located on the short arm of chromosome 3.

The preferred CCK receptor is CCK-R1 present on pancreatic acinar cells, on gallbladder muscles, on smooth muscle cells, on D cells of the gut mucosa and on nerve cells of the central and peripheral nervous system.

4.10.2.3 Physiology

CCK-8 was observed as the hormone triggering gallbladder contractions. The action on acinar cells might facilitate the development of pancreatic tumors. Applied into animals CCK reduces food intake, whereas a receptor antagonist increases food intake. CCK is therefore regarded as a satiation signal (Moran and Kinzig 2004).

Table 4.4 Peptides of the gastrin/CCK family

Name	Sequence
Cholecystokinin-8	**DYMGWMDF**-NH_2
Gastrin-17	**QGPWLEE EEEAYGWMDF**-NH_2
Caerulein[a]	p**EQDYTGWMDF**-NH_2
Phyllocaerulein[b]	p**EQYTGWMDF**-NH_2
Cionin[c]	**NYYGWMDF**-NH_2

Tyrosines (**Y**) are frequently sulfated
[a]From the Australian green tree frog (*Litoria caerulea*), Anastasi et al. (1967, 1970)
[b]From the waxy monkey frog (*Phyllomedusa sauvagii*), Anastasi et al. (1969)
[c]From *Ciona intestinalis*, Johnsen and Rehfeld (1990)

4.10.2.4 Phylogeny of the Gastrin Peptide Gene Family

Anti-CCK have been found to bind to nonvertebrate tissues, however, the only peptide identified is cionin from *Ciona intestinalis* which shares the C-terminal sequence with gastrin and CCK. In frog caeruleins with the same sequence have been discovered (see Table 4.4). CCK and gastrin have been found in all vertebrates analyzed: fish, amphibians, reptiles, birds, and mammals (Johnsen 1998).

4.10.3 Secretin

Fact sheet 4.36: Secretin

Gene:	Chromosome 11; locus 11p15; five exons.
Sequence:	**HSDGTFT SELSRLREGA RLQRLLQGLV**-NH_2.
Synthesis and target:	Duodenal S cells stimulated by gastric acid release secretin which acts on the secretin receptor on pancreatic acinar cells and on stomach cells.
Function:	Secretin controls gastric acid neutralization by stimulating bicarbonate, water, and salt release from pancreas and gut. It inhibits gastric acid release from parietal stomach epithelium cells.
Receptor:	Secretin receptor is a heptahelical membrane GPCR.

4.10.3.1 Introduction

Secretin, discovered on January 16, 1902,[32] passes as the prototype of peptide hormones.

4.10.3.2 Structure and Genes

Human secretin was cloned and sequenced only in 2000 (Whitmore et al. 2000). It is released classically from a precursor protein; most probably PC2 is the active enzyme recognizing the **RR** motif in front and the **KR** motif after the secretin sequence. The C-terminal glycine is amidated.

The human gene for secretin is located on the telomeric end of the short arm of chromosome 11 (11p15) close to dopamine receptor-D4.

4.10.3.3 Physiology

Secretin is released from cells in the duodenum and in the jujenum into the blood. It facilitates water, salt, and enzyme release from the pancreas, stomach, and gut cells into the lumen thus neutralizing gastric acid; secretin inhibits endocrine gastrin and somatostatin release; it advances insulin release. Contractions of stomach and gut are inhibited.

Secretin is preferentially expressed in the jejunum. The expression in other cells, the brain included, is reduced compared to the jejunum. In cells of the gallbladder it triggers membrane fusion of vesicles with preformed aquaporin thus enhancing water pumping into the bile.

4.10.3.4 Phylogeny

Bacterial secretins responsible for export of proteins only share the name with the vertebrate hormone secretin. Secretins have been isolated and cloned but from mammals and birds, not from fish. The secretin receptor family (GPCR-B), however, has been observed in arthropods, nematodes, and tunicates. The protein has not been found in the fully sequenced genome of *Ciona intestinalis* (tunicates) (Burke et al. 2006).

[32] I happened to be present at the discovery. In an anesthetized dog, a loop of jejunum was tied at both ends and the nerves supplying it dissected out and divided so that it was connected with the rest of the body only by its blood vessels. On the introduction of some weak HCl into the duodenum, secretion from the pancreas occurred and continued for some minutes. After this had subsided a few cubic centimeters of acid were introduced into the enervated loop of jejunum. To our surprise a similarly marked secretion was produced. I remember Starling saying: "Then it must be a chemical reflex." Rapidly cutting off a further piece of jejunum he rubbed its mucous membrane with sand in weak HCl, filtered it, and injected it into the jugular vein of the animal. After a few moments the pancreas responded with a much greater secretion than had occurred before. It was a great afternoon (Martin 1927).

4.10.4 VIP

Fact sheet 4.37: Vasoactive intestinal peptide (VIP)

Gene:	Chromosome 6; locus 6q25.2; seven exons.
Sequence:	**HSDAVFTD NYTRLRKQMA VKKYLNSILN**-NH_2.
Synthesis and target:	VIP is made by neurons and mostly acts as a neurotransmitter.
Function	In the GI tract VIP acts to relax smooth muscles. VIP blocks gastric acid synthesis and stimulates water transport into bile, as well as bicarbonate, enzyme, and chloride secretion in the gut.
Receptor:	VCAP receptors 1 and 2 are heptahelical membrane GPCR. They bind VIP and PACAP.

4.10.4.1 Introduction

As are the neuromedins or secretin vasoactive intestinal peptide (VIP) is an member of the evolutionary old glucagon/PACAP protein family already found in tunicates (Sherwood et al. 2000). VIP actions are not restricted to the GI tract, they include brain activity, neuroendocrine functions, heart muscle activity, breathing, and sexual activity. In addition to its expression in the duodenum VIP is made in many brain cells as well as in other organs.

4.10.4.2 Structure and Genes

VIP is released from a precursor by PC1 (or PC2) and C-terminally amidated by PAM. There is an additional peptide on the same precursor, called peptide–histidine–methionine in humans and peptide–histidine–isoleucine in other mammals.

4.10.4.3 Physiology

VIP is a neurotransmitter and neuromodulator of the enteric nervous system (ENS; see Fig. 11.7). VIP is synthesized in neurons, and also in mast cells and granulocytes. VIP makes the smooth muscle cells of the gut relax; it blocks gastric acid formation and stimulates water transport in the bile, as well as release of bicarbonate and pancreatic enzymes and chloride from the gut (Boushey and Drucker 2003).

Because VIP passively transfuses through the blood–brain barrier, VIP from the periphery might act centrally. Recent results suggest that VIP is decisive for light-dark adaption of the biological clock. Mice with a defect of the VCAP2 receptor could not maintain a 24-h rhythm and got arrhythmic (in the dark; Colwell et al. 2003). Furthermore, triggered by VIP, glia cells secrete neuroprotective factors: interleukin-1 (IL-1), IL-6, neurotrophin-3, nexin-1, RANTES and MIP chemokines, activity-dependent neurotrophic factor (ADNF) and activity-dependent neuroprotective protein (ADNP) (Dejda et al. 2005)

In addition, VIP is an important regulator of the immune system and of dendritic cell maturation. VIP exhibits potent anti-inflammatory actions and is efficient against rheumatoid arthritis (Delgado et al. 2004).

4.10.4.4 Phylogeny

VIP has exclusively been found in vertebrates: sharks, bony fishes, and in the other vertebrate classes. The VCAP receptor, too, belongs to an ancient receptor family, but is not older than vertebrates. in sharks it has been observed that VIP stimulates chloride channels.

4.10.5 GIP

Fact sheet 4.38: Glucose-dependent insulinotropic polypeptide, gastroinhibitory polypeptide (GIP)

Gene:	Chromosome 17; locus 17q21.32; six exons.
Sequence:	Fig. 4.39.
Synthesis and target:	GIP is mainly a product of endocrine GI tract cells and acts on insulin or glucagon secreting pancreatic islet cells.
Function:	GIP triggers (dependent on glucose) insulin release, inhibits glucagon synthesis, and blocks glucagon-induced lipolysis.
Receptor:	GIP receptor is a GPCR from the secretin protein family.

4.10.5.1 Introduction

The glucose-dependent insulinotropic polypeptide (aka gastroinhibitory polypeptide (GIP)), originally identified as a regulator of gastric acid release, of the major gastric enzyme pepsin and of gastrin, is first of all a major insulin formation and release stimulus.

4.10.5.2 Structure and Genes

The *GIP* gene with its six exons is located on chromosome 17 close to an IFG2 binding protein (IGF2BP1). After removal of the signal peptide the remaining 132 amino acid long precursor is cleaved at two furin recognition sites by PC1 giving rise to the mature hormone. The cleavage is, surprisingly, affected by PC1, as has been demonstrated in PC1-defective mice (Ugleholdt et al. 2006).

```
                  1                   2                   3                   4                   5
1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
                                                m v a t k t f a l l l l s l f l a v g l g  -21 -  -1
e k k e g h f s a l p s l p v g s h a k v s s p q p r g p r Y A E G T F I S D Y S I A M D K I H Q Q    1 -  50
D F V N W L L A Q K G K K N D W K H N I T Q r e a r a l e l a s q a n r k e e e a v e p q s s p a k   51 - 100
n p s d e d l l r d l l i q e l l a c l l d q t n l c r l r s r                                       101 - 132
```

Fig. 4.39 Primary gastroinhibitory peptide (GIP) sequence. The 153 amino acid long protein bears a 21 amino acid long signal peptide. By the prohormone convertase 1 (!see text!) the precursor is cleaved at the *inverted* arginine residues and the mature polypeptide (*in uppercase*) released (Source: GenBank NP_004114)

4.10.5.3 Physiology

GIP is classified as an incretin, that is, a stimulator of insulin production. It is rapidly inactivated in serum by the dipeptidyl peptidase IV. In diabetes mellitus type 2 patients, a prolongation of GIP (or GLP1, another incretin) presence in blood is tried as an option to enhance insulin release.

4.10.5.4 Phylogeny

Until now, the protein library only contains vertebrate GIP sequences: fish, frogs, birds, and mammals. The GIP from *D. melanogaster* appears to be an isomerase with no relation to vertebrate GIP.

4.10.6 PNP, NPY, PYY

Fact sheet 4.39: Pancreatic polypeptide (PNP)

Gene:	Chromosome 17; locus 17q21.31; four exons.
Sequence:	Fig. 4.40: PNP.
Synthesis and target:	PNP is the product of pancreatic islet PP cells. PNP receptors are present on pancreatic acinar cells.
Function:	PNP acts on exocrine pancreatic secretion. It is furthermore involved in blockade of food intake.
Receptor:	The PNP receptor is a heptahelical membrane GPCR.

Fact sheet 4.40: Peptide tyrosine tyrosine (PYY)

Gene:	Chromosome 17; locus 17q21.31; five exons.
Sequence:	Fig. 4.40: PYY.
Synthesis and target:	PYY is released from endocrine cells in the ileum and colon. It binds to PNP receptor 1 (PNP-R1, aka NPY-R4) which is expressed in brain, coronary arteries, and gut and to NPY-R2 expressed preferentially in the nervous system.
Function:	PYY has a role in signaling between the enteric nervous system and the central nervous system. PYY signals decrease appetite.
Receptor:	Receptors belong to the heptahelical membrane GPCR class.

Fact sheet 4.41: Neuropeptide Y (NPY)

Gene:	Chromosome 7; locus 7p15.3; four exons.
Sequence:	Fig. 4.40: NPY.
Synthesis and target:	NPY is secreted by neurosecretory cells. In the hypothalamus NPY signals appetite. NPY receptors are expressed in brain, spleen, ileum, kidney, testis, placenta, and in aortic smooth muscles.
Function:	Apart from its appetite-regulating function NPY acts anxiolytically. NPY defects impair development of the olfaction. NPY can control growth of neuron precursors in adults.
Receptor:	Receptors are heptahelical membrane GPCR.

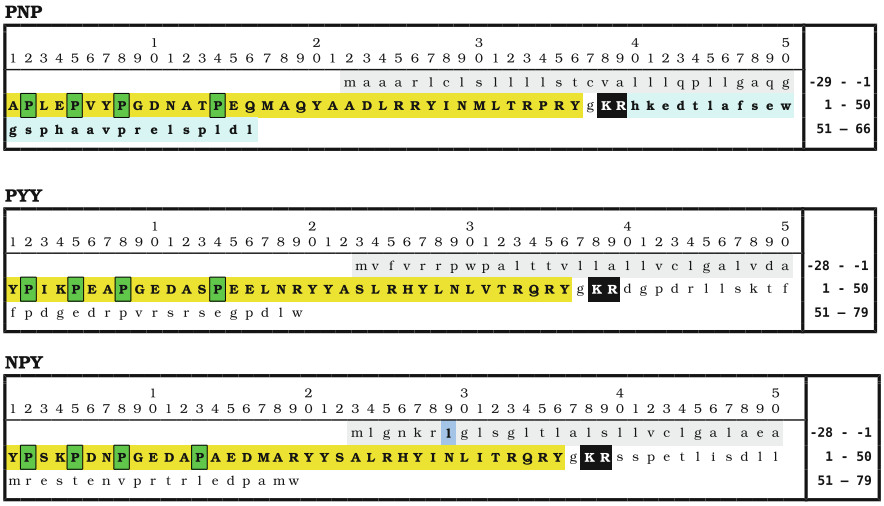


Fig. 4.40 Primary sequences of pancreatic polypeptide (PNP), protein tyrosyl-tyrosine (PYY), and neuropeptide-Y (NPY): Precursor proteins contain a 29 or 28 amino acid long signal peptide (on *gray* background). Prohormone convertase-1 cleaves the precursors at **KR** (position 38/39; shown *inverted*); endopeptidases shorten the peptides till **G** (37) which is finally amidated. The mature peptide is shown highlighted *yellow*. The four prolines determining the polyprolin (PP)-fold are shown on *green* background. The associated peptide in the PNP precursor highlighted (*light blue*) is called pancreatic icosapeptide (Sources: GenBank NP_002713 (PNP), NP_004151.3 (PYY), NP_000896.1 (NPY))

4.10.6.1 Introduction

Pancreatic polypeptide (PNP), neuropeptide Y (NPY), and peptide tyrosine tyrosine (PYY) share a common three-dimensional structure, the so-called PP-fold a polyproline coil of amino acids 1–8 (*lower part* in Fig. 4.41), an amphipathic helix with a polar and a hydrophobic side (aa 15–30; *upper part* of Fig. 4.41. Both parts are linked by a β-turn called sequence (*right side* in the figure). The three-dimensional structure is tightened by hydrophobic interactions between prolines and the hydrophobic side of the helix.

4.10.6.2 Structure and Genes

Two peptides (PNP and PYY) are coded for by closely linked genes on the long arm of chromosome 17 (17q21). The *NPY* gene, however, is located on chromosome 7 (7p15). *PNP2* and *PYY2* (arisen by gene duplication) are on chromosome 17 in 17q11.2. Products of these genes are shortened due to frameshift mutations and are not active as hormones.

The three precursors are cleaved by the signal-peptidase and furthermore by PC1 and finally amidated, giving rise to 36 amino acid long hormones. Characteristic features are the prolines (*boxed* in Fig. 4.40 at the start of the sequence causing the proline coil and the particular PP-fold (Fig. 4.41). Whether these peptides are always present in the PP-fold has been debated (Bettio et al. 2002).

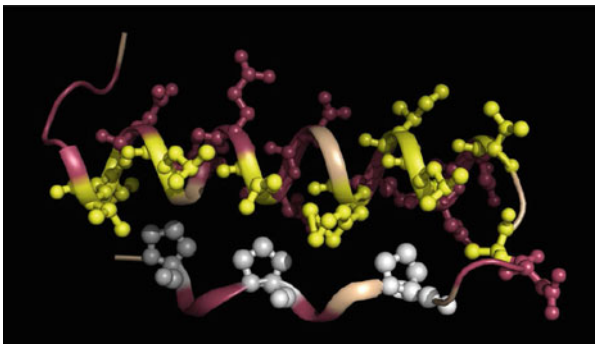


Fig. 4.41 Common structure of pancreatic polypeptide (PNP), protein tyrosyl-tyrosine (PYY), and neuropeptide-Y (NPY). The polyproline (PP) -fold is composed of an amphipathic helix (*upper part*) with polar (*red*) amino acids on one side (*facing upwards and to the back*) and hydrophobic (*yellow*) amino acids on the other side (*facing to the front and downwards*), paired with a proline coil (*lower part*). The hydrophobic helical side interacts with the equally hydrophobic proline residues *white* of the proline coil which due to the inflexible structure of the proline rings form an extended helix (Source: Lerch et al. 2004; PDB: 1PPT (RasMol))

4.10.6.3 Physiology: PNP

Pancreatic islet PP cells form PNP (see Sect. 10.5). PNP acts back on the pancreas blocking its activity, in addition to gut contractions. PNP influences gluconeogenesis and decreases fat levels. PNP receptors are present in the brain, too.

4.10.6.4 Physiology: NPY

NPY is one of the most abundant neuropeptides in the human organism. NPY once released signals hunger. For this action NPY is expressed in the brain (arcuate nucleus and nearby areas; see Sect. 4.3), in the GI tract, and other organs (see also Sect. 11.5). In the brain NPY acts as neurotransmitter, usually together with noradrenaline (see Chap. 7).

In addition, NPY is required for normal development of the olfactory sense: postpartum NPY supports cellular division and development of olfactory neurons. NPY-deficient mice have only half of the olfactory cell precursors as wildtype mice and develop fewer mature olfactory neurons.

NPY neurons in the arcuate nucleus interact with POMC neurons and their MSH products for regulation of fertility and energy homeostasis. In older postmenopausal women NPY expression is enhanced compared to the POMC expression.

Because NPY is anxiolytically active, individuals with the ability to release larger amounts of NPY deal better with stress. Moreover, NPY counteracts the usage of alcohol. Persons with a Leu→Pro exchange at amino acid 7 of the NPY signal peptide (in Fig. 4.40 *bold* and highlighted *blueish*) consumed significantly more alcohol than those with the prominent sequence.

Table 4.5 Affinities of neuropeptide-Y (NPY) receptors for pancreatic polypeptide (PNP), protein tyrosyl-tyrosine (PYY), and NPY (Berglund et al. 2003)

Receptor	Relative affinities of different ligands
NPY-R1	NPY = PYY > NPY[2-36][a] > NPY[3-36][b] ≥ PNP
NPY-R2	NPY ≥ NPY[2-36] = NPY[3-36]
NPY-R4	PNP > PYY ≥ NPY > NPY[2-36]
NPY-R5	NPY = PYY = NPY2-36 > hPNP[c] > rPNP[d]
NPY-R6	??[e]

From Berglund et al. (2003)
[a]NPY[2-36] lacks the N-terminal amino acid of NPY (at the proline coil).
[b]NPY[3-36] lacks 2 N-terminal amino acids of NPY (at the proline coil)
[c]hPNP: human PNP
[d]rPNP: rat PNP
[e]NPY-R6 is (in humans, swine, or guinea pigs) due to mutations C-shortened and thus afunctional as receptor. Because NPY-R6 is strongly expressed in heart- and skeletal muscles, a new function is supposed

4.10.6.5 Physiology: PYY

PYY was originally isolated from the small intestine. Its actions are comparable to those of PNP. In addition, PYY acts on salt release and blood support by regulating the diameter of capillaries. PYY is part of the communication between the ENS and the CNS: PYY receptors are present on vagus nerve endings in the GI tract.

4.10.6.6 Physiology: Receptors for PNP, PYY, and NPY

The three peptides bind with similar, but divergent, affinity to the different NPY receptors (Table 4.5). As a result, the NPY-R2 appears to be NPY specific; NPY-R4, however, to be PNP specific. PYY does not preferentially bind to any of these receptors. PYY binds both to NPY-R1 and to NPY-R5 with equal affinity as does NPY. Therefore, PYY may induce similar actions as NPY.

4.10.6.7 Phylogeny

The most ancient PYY and NPY genes and peptides were discovered in *Lampetra fluvialis*, *Petramyzon marinus*, and related species. PYY and NPY genes are closely linked in the vicinity of a so-called hox gene; hox genes are responsible for time and spatially controlled gene expression. In fish these hox genes are duplicated, however, without duplicated NPY and PYY genes. As the zebrafish genome is almost completely sequenced, the duplicates appear to have been lost.

In molluscs and insects NPY peptides have been described. The peptide homology is only scarce. Using a ClustalW analysis these peptides are less related to vertebrate NPY than are, for example, human galanin or FSH sequences which usually are not regarded as NPY related (Fig. 4.42). A proline coil is only partially present; the feature appears accidentally.

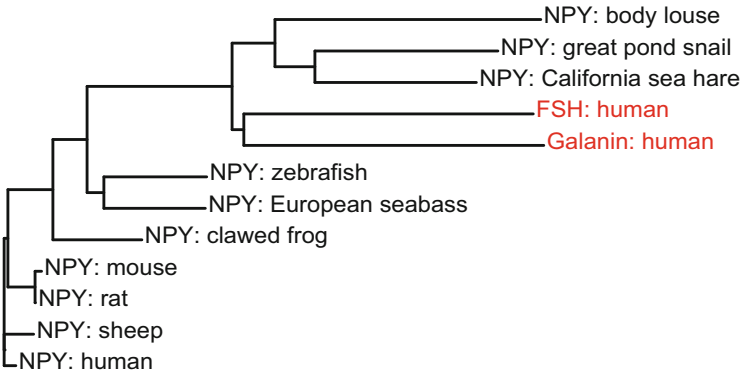


Fig. 4.42 Potential relation of NPY peptides of vertebrates and nonvertebrates

The *PNP* gene located in the direct vicinity of *PYY* is most probably derived from a local gene duplication shortly before or at the time of amphibian evolution. PNP is only active in the tetrapod pancreas. In fish a PY peptide is expressed, for example, in European seabass *Dicentrachus labrax* **YPPKPESPGS NASPEDWAKY HAAVRHYVNL ITRQRY** (amino acids identical to the human PYY are *underlined*). Such a peptide is expressed in the pancreas and brain. The gene might stem from a local duplication of the *Pyy* gene (Conlon 2002).

4.11 Neuropeptides in the Enteric Nervous System

4.11.1 Endorphins and Enkephalins

Endorphins and enkephalins are endogenous opioids. They act via opioid receptors. β-Endorphin is a processed peptide of POMC (see Sect. 4.4). Neoendorphins are cleaved from the prodynorphin, and enkephalins from the proenkephalin A and from prodynorphin (Figs. 4.43 and 4.44).

These neuropeptides equally undergo maturation by prohormone convertases. Thus far we got used to dibasic peptide motifs as recognition sites for these enzymes. However, here, an additional enzyme, cathepsin L is active, which cleaves after furin-like recognition sites too. Similarities between proenkephalin A and prodynorphin A are not only restricted to similar peptide sequences and active prohormone convertases. Whereas the *PENK* gene possesses two exons and the *PDYN* gene four, the exon 1 still codes for the signal peptide and the region with the six cysteines equally spaced in both sequences. This analogy is shown by drawing similar disulfide bridges in prodynorphin without formal proof in the literature (Fig. 4.44).

In the GI tract, endorphins and enkephalins increase the chyme's staying in the stomach and intestine by inhibiting motor activity on the gut musculature after binding to opioid receptors.

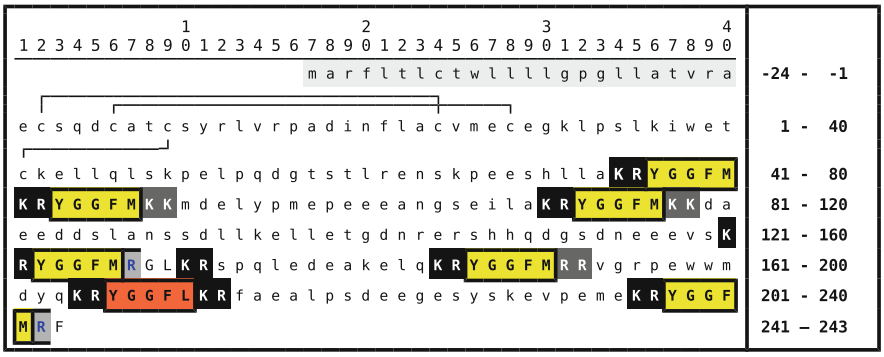
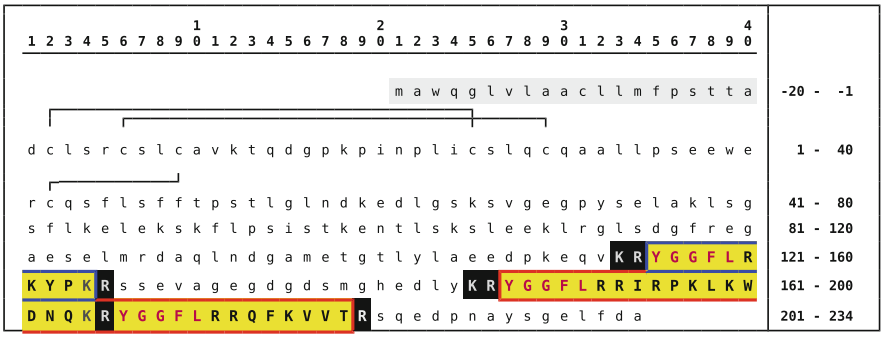


Fig. 4.43 Primary sequence of proenkephalin A. The precursor protein comprises six copies of met-enkephalin and a single copy of leu-enkephalin. According to Yasothornsrikul et al. (2003) cathepsin L is the active prohormone convertase recognizing dibasic dipeptides (such as prohormone convertase 1 and 2) but singular amino acids **R**, too; met-enkephalin is equally excised from the **YGGFMRGL** (162–169) and **YGGFMRF** (237–243) (Source: GenBank P01210)



leu-enkephalin: **YGGFL** (three copies)
alpha-neoendorphin: **YGGFLRKYPK** (155-164)
beta-neoendorphin: **YGGFLRKYP** (155-163)
dynorphin (1-32): **YGGFLRRIRPKLKWDNQKRYGGFLRRQFKVVT**
dynorphin A(1-17): **YGGFLRRIRPKLKWDNQ**
dynorphin A(1-13): **YGGFLRRIRPKLK**
dynorphin A(1-8): **YGGFLRRI**

Fig. 4.44 Primary sequence of prodynorphins (proenkephalin B). Those products of peptide maturation isolated are shown below the primary sequence. Due to the mixture of utilized dibasic and monobasic motifs, a cathepsin L endopeptidase or a similar enzyme must be assumed to be acting as prohormone convertase (Source: GenBank P01213)

4.11.2 Tachykinins: Substance P, Neurokinin, and Endokinin

Tachykinins are characterized by a C-terminal pentapeptide: **FxGLM**-NH_2, with x = **V** or **F** (Figs. 4.45 and 4.46). Among others neurokinin A, neurokinin B, and substance P belong to these neuropeptides.

These peptides, excluding endokinins, all 10 to 12 amino acids long, are neurotransmitter acting via neurokinin receptors. In the GI tract substance P stimulates

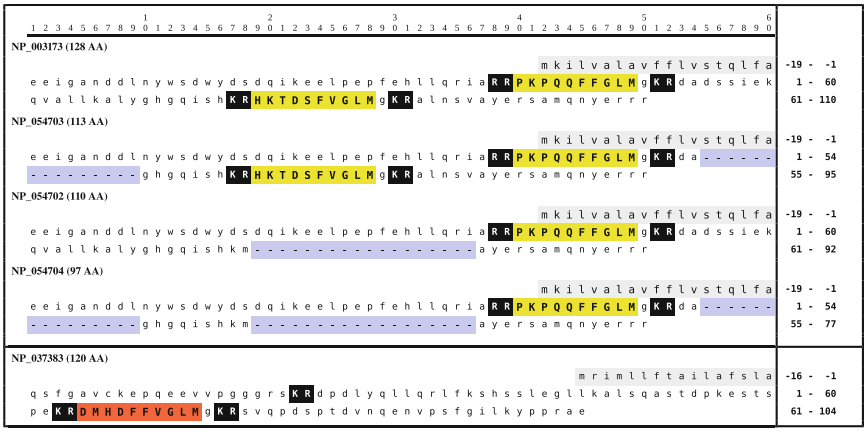


Fig. 4.45 See Fig. 4.46

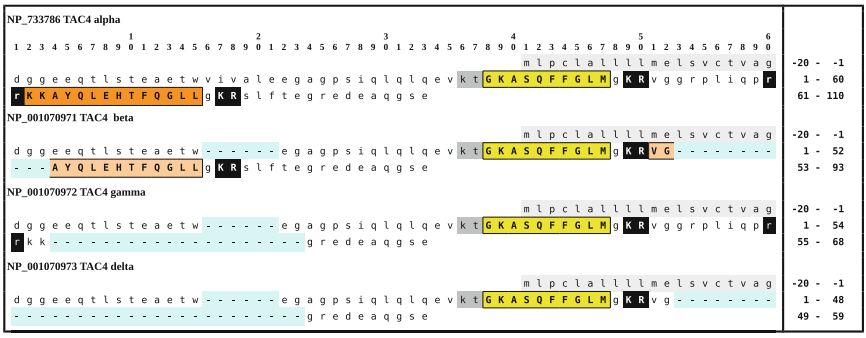


Fig. 4.46 Primary sequence of different protachykinins. The four tachykinin 1-3 (Fig. 4.45) and the four tachykinin 4 isoforms (this figure) differ by exon loss due to alternate splicing. Lacking amino acid from missed exons is indicated by single *dashes on light blue*. Protachykinin 1 gives rise to substance P (**PKPQQFFLGM**-NH_2; highlighted *yellow*) and neurokinin A (**HKTDSFVLGM**-NH_2; *green*), the sole product from tachykinin B is neurokinin B (**DMHDFFVLGM**-NH_2; *orange*; *above* NP_733786, fifth sequence). PC1 or PC2 are the likely prohormone convertases. From tachykinin 4 endokinin A (**GKASQFFGLM**; *yellow*) and endokinin C (**KKAYQLEHTFQGLL**-NH_2; *orange*) or endokinin D (**VGAYQLEHTFQGLL**-NH_2; *light orange*) are derived. The endokinin peptides have be isolated, however, the enzyme that recognizes **KT** has still to be identified (Source: GenBank *no. in the first column*)

muscle contraction and saliva production (see Sect. 11.5). Tachykinins stimulate vascular muscles, stomach secretion, and kidney functions.

Endokinin is the product of the tachykinin 4 gene (Fig. 4.46). The murine hemokinin 1 is the analogue of human endokinin A. In contrast to the other vertebrates analyzed, the human tachykinin 4 gene lacks the dibasic endopeptidase motif in front of the endokinin A obscuring the cleavage site.

Receptors for all tachykinins are the neurokinin receptors (NKR 1–3). Tachykinin 1 expression was preferentially found in the brain, marrow, and GI tract, where tachykinin 4 was not found to be expressed; the latter is preferentially

found in placenta. The different splice variants of tachykinin 4 exhibit differential expressions (Page et al. 2003). In contrast to neurokinins and substance P which act as neurotransmitters, Page et al. (2003) found that endokinins act in an endocrine manner. They stimulate mostly lymphocyte development.

4.11.3 Gastrin-Releasing Peptide/Bombesin

Fact sheet 4.42: Gastrin-releasing peptide (GRP)/Bombesin)

Gene	Chromosome 18; locus 18q21; three exons.
Sequence	Fig. 4.47.
Synthesis and target	GRP is made in the lungs and brain and acts on pancreatic islet cells.
Function	GRP stimulates insulin and glucagon release and influences anxiety behavior.
Receptor	GRP receptor is a heptahelical GPCR.

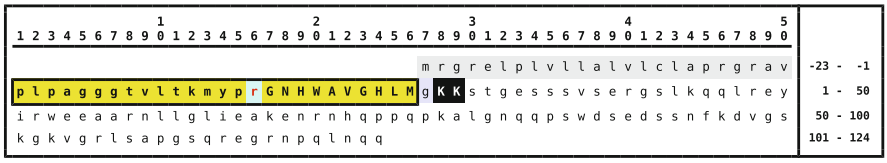


Fig. 4.47 Primary sequence of gastrin-releasing peptide (GRP)-bombesin. From GRP (*boxed on yellow*) the decapeptide neuromedin C (*uppercase*) can be cleaved by an endopeptidase cutting after arginine. Because splice-variants do not change the GRP sequence, they are omitted here (Source: NP_001012531)

4.11.3.1 Introduction

Gastrin-releasing peptide (GRP) is the human analogue of the toad bombesin. Bombesin had originally been isolated from toads and frogs (Erspamer et al. 1972) (hence the name; for an early review, see Erspamer 1971).

4.11.3.2 Structure and Genes

GRP is released from a precursor by the action of the signal-peptidase and PC2. A furin-like enzyme can excise the neuromedin-C (Fig. 4.47).

4.11.3.3 Physiology

GRP is expressed mostly in neuroendocrine cells of the lungs. Injected into humans it enhances gastrin, GIP, PNP, glucagon, and insulin levels in blood similarly to bombesin (**PQRLGNQWAVGHLM**-NH_2) from the toad *Bombina bombina*.

The C-terminal decapeptide neuromedin C (amino acids 18–27) is active as an neurotransmitter. This peptide exhibits strong homology to neuromedin B (Fig. 4.47).

In mice it was found that GRP is expressed in the lateral nucleus of the amygdala where Pavlovian learned fear associations are formed. The GRP receptor was found in interneurons of the lateral nucleus (Shumyatsky et al. 2002). The results demonstrated a negative feedback function of GRP regulating fear and a relationship between the GRP receptor, long-term potentiation, and the amygdala-dependent memory for fear.

Studying the angiogenic effect of adrenomedullin and its associated peptide PAMP, which had been shown to bind to GRP receptor, Martínez et al. probed GRP for angiogenic activity and could confirm that GRP has potent angiogenic capacity, for example, in tumors (Martínez 2006; Martínez et al. 2005).

The latest addition to the bouquet of GRP features stems from Sakamoto (2011), who has shown expression of GRP in the spinal cord and its relation to male sexual functions (for a review, see Sakamoto 2011).

4.11.3.4 Phylogeny

A GRP receptor analogue has already been found in the California purple sea urchin (*S. purpurata*; echinoderms), however, GRP or bombesin could not be detected in the fully sequenced genome (Burke et al. 2006). Thus, GRP is most probably restricted to vertebrates.

4.12 Nonsteroidal Regulators of Reproduction

4.12.1 Activin/Inhibin

Fact sheet 4.43: Activin/Inhibin

Gene:	Inhibin-α: Chromosome 2; locus 2q35; two exons . Inhibin-β-A: Chromosome 7; locus 7p14.1; three exons . Inhibin-β-B: Chromosome 2; locus 2q14.2; two exons.
Sequences:	Fig. 4.48.
Synthesis and target:	Dimerization of two inhibin-β-chains gives rise to activins (A,B,AB); association of a α- to a β-chain generates inhibin. Activin-A binds to different serine/„'threonine kinase receptors, that is, on pituitary gonadotropic cells: inhibin binds to an inhibin-binding protein and to β-glucan.
Function:	Activins and inhibins are paracrine regulators in endocrine glands and in organs of reproduction; in other tissues and organs they act as cytokines
Receptor:	Activin-A receptors are serine/threonine kinases. Cell surface receptors of inhibins have not yet been found; inhibins bind to β-glucan and to inhibin-binding proteins.

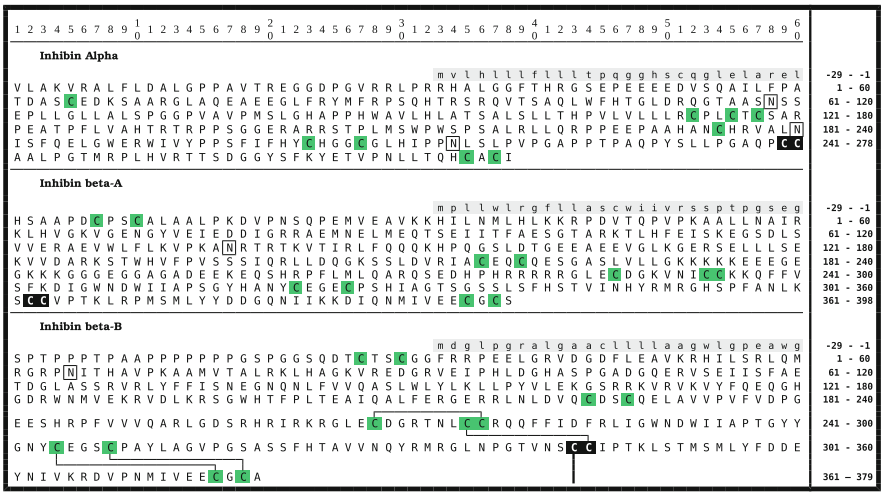


Fig. 4.48 Primary sequence of activin/inhibin. Three polypeptides are cysteine knot proteins as can be seen in the figure for inhibin-β B. Every β chain binds to another β chain generating thus an activin dimer or to an α chain giving rise to an inhibin. The dimers are stabilized by a single or multiple cysteine disulfide bridges. At least the first cysteine of last CC motif (*inverted*) is involved. (Source: inhibin alpha: NP_002182; inhibin beta A: NP_002183; inhibin beta B: NP_002184); disulfide bonds of inhibin dimers have not yet been provided for by analysis or X-ray structure; only activins have been crystallized thus far. The cysteine knot of the beta A chain has been crystallized (2ARV)

4.12.1.1 Introduction

As with follistatin the activins and inhibins are not only required for feedback regulation from gonads to the pituitary; activin-A at least is a regulator of dental development and a potential autocrine stimulator in placenta.

4.12.1.2 Structure and Biochemistry

The three different polypeptides give rise to five dimeric proteins: activin A, AB, or B, and inhibin A or B (Fig. 4.49). The chains contain, as does any TGF-β family member, a cysteine knot. There is a differential expression of β-A and β-B genes. Defects in β-A are neonatally lethal; those of β-B are not (Thompson et al. 2004).

Receptors for activins are serine/threonine membrane kinases signaling via SMAD proteins, a common TGF-β and bone morphogenic factor (BMF) signal transduction pathway. There are activin receptors and activin-like receptors, the latter having been stimulated by other TGF family proteins.

Receptors for inhibins are largely unknown. Today β-glucan and an inhibin-binding protein are regarded as mediators of inhibin action. Together with β-glucan, inhibin binds to the activin receptor type-II (ActRII).

With β-glucan binding to activin, activin can no longer interact with an activin receptor. Its function is blocked. Likewise the actions of follistatin are blocked by activin (Florio et al. 2004).

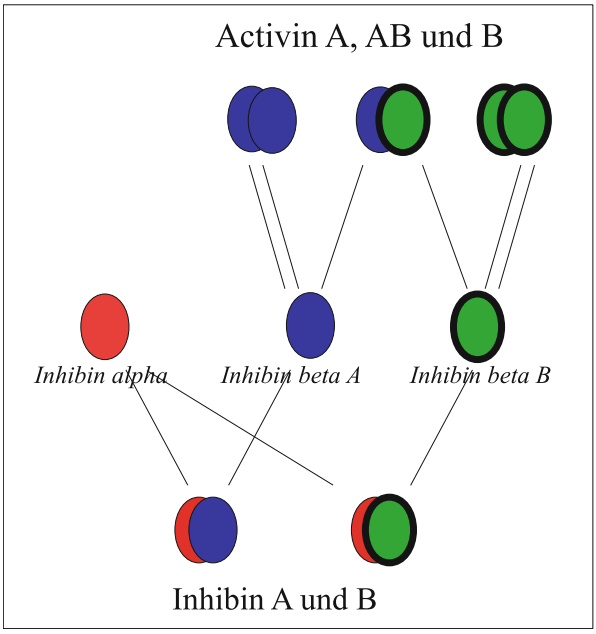


Fig. 4.49 Model of inhibin chain dimerization. Three proteins might dimerize to five distinct proteins. Beta chains dimerizing to each other give rise to activins; a beta chain binding to an alpha chain generates an inhibin

4.12.1.3 Physiology

Inhibins and activins were discovered for their control on FSH release. Additionally they act as regulators in ovary and testis. Furthermore they are called cytokines. Each chain is expressed separately and the expression is tissue, sex, and time controlled. In adult rhesus monkeys, only inhibin-B is present in serum.

Differences in β-A and β-B expression found in animals during uterine development could not be confirmed in humans: both subunits were equally present in brain, spinal cord, liver, kidney, or adrenal (Thompson et al. 2004).

During the estrous cycle β-chain expression is modulated in any animal species analyzed. The α-chain is expressed in any follicular stage: in antral follicles the β-B chain is more abundantly found; in later stages the β-A chain is prevalent, and thus there is an apparent switch in β-chain expression. The potentially formed activin-A is, however, not present in the serum, but mostly bound to follistatin. Activin-A controls expression of the FSH receptor on granulosa cells and activates steroidogenesis.

In the pituitary both beta chains are made in gonadotropic cells: the A-chain also in somatotropic and lactotropic cells, and the B-chain additionally in thyrotropic cells. A paracrine (possibly autocrine) stimulation of FSH by activin-B in rats has been confirmed. Cultured pituitary cells secrete activin-B, but not activin-A. Once activin-B is neutralized by antibodies in these cell cultures, FSH secretion is

stopped. These antibodies blunt, *in vivo* applied, the preovulatory FSH surge in rats (Thompson et al. 2004, and references).

In his review on paracrine effects Denef (2008) summarizes the paracrine actions of inhibins/activin as well as follistatin on gonadotropin secretion.

4.12.1.4 Phylogeny

Inhibin genes and activin receptors were found together with other TGF-β family members in insects which demonstrates their early metazoic origin.

4.12.2 Follistatin

Fact sheet 4.44: Follistatin (FST)

Gene:	Chromosome 5; locus 5q11.2; six exons.
Sequence:	Fig. 4.50; follistatin has been crystallized: 2B0U.
Synthesis and target:	Follistatin is preferentially made in folliculostellate pituitary cells, in ovarian follicles, and in the kidney. It binds to proteins of the TGF-β family.
Function:	Follistatin controls *i.a.* by binding to activin/inhibin FSH biosynthesis. In addition it controls the activity of other TGF-β family members.
Receptor:	Follistatin binds to heparan sulfate proteoglucans.

4.12.2.1 Introduction

Since it could be established that FSH and LH are made in the same gonadotropic cells, regulators that provide for differential expression of LH and FSH synthesis get into the focus. Follistatin is one of such regulators. Being first isolated from the ovarian follicular fluid, today the folliculostellate pituitary cells are also known to secrete follistatin as well. Follistatin is considered to be an endocrine as well as paracrine regulator of FSH synthesis.

4.12.2.2 Structure and Biochemistry

Follistatin is member of the transforming growth factor-β family of proteins. By alternative splicing two isoforms are generated with similar, only C-terminally differing, sequences: FS-288 and FS-315 (Fig. 4.50).

Follistatin inhibits action of other TGF-β proteins by forming heterodimers with these proteins; among those are bone morphogenetic protein (BMP), activin and inhibin, as well as α_2-macroglobulin. Two follistatin molecules bind to one activin dimer and the inhibin-α/β-dimer binds only one follistatin polypeptide. This leads to the conclusion that follistatin bind to the β-activin subunit. Whether the follistatin–inhibin interaction is biologically relevant, is not yet conclusively analyzed.

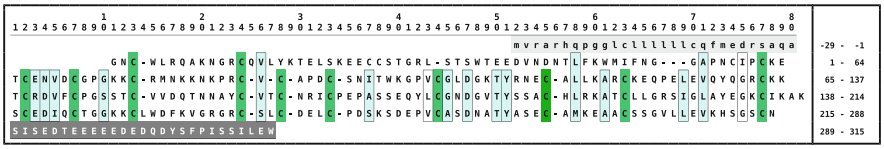


Fig. 4.50 Primary sequence of follistatin. Following the signal peptide (*light gray*) the protein exhibits four domains (*one per row*), characterized by identical cysteine positions and conservative amino acid exchanges. The (*inverted*) C-terminal sequence distinguishes the two follistatin isoforms (Source: NP_037541, Shimasaki et al. 1988)

4.12.2.3 Physiology

Follistatin blocks in a paracrine manner the action of activin and other proteins of the TGF-β family. It is made by pituitary folliculostellate and ovarian granulosa cells. Whereas activins stimulate the maturation of FSH-dependent antral follicles and block the generation of the corpus luteum, follistatin is expressed only in the final state follicle and facilitates corpus luteum generation as an activin antagonist (Lin et al. 2003).

It is not yet evident whether follistatin is endocrine active, that is, whether it is transported via the blood to distant target organs. As a paracrine regulator it is active not only in gonads, but in a variety of other organs, too (Schneider et al. 2000).

4.12.2.4 Phylogeny

Like inhibins, follistatin is an "ancient" metazoic protein found in common precursors of vertebrates and arthropods.

4.12.3 Antimüllerian Hormone

Fact sheet 4.45: Antimüllerian Hormone (AMH), Müllerian Inhibitory Factor (MIF)

Gene:	Chromosome 19; locus 19p13.3; five exons.
Structure:	Glycoprotein of the TGF-β family.
Synthesis and target:	AMH is expressed in fetal and postnatal Sertoli cells; it acts on the AMH receptor in fetal müllerian duct cells. In women AMH is made by granulosa cells of preantral and antral follicles up to the moment when its synthesis is blocked by FSH.
Function:	AMH acts as a testicular hormone on the müllerian ducts and induces their regression. A postnatal role has yet to be found. AMH in serum or seminal fluid is used to determine functional reproduction capacity of men and women in assisted reproductive technology.
Receptor:	AMH receptor is a membrane serine/threonine kinase.

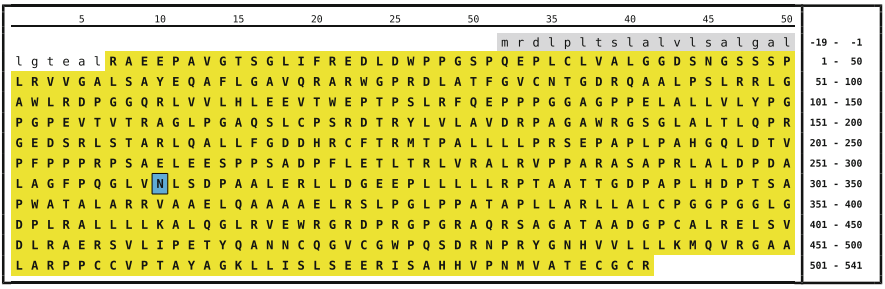


Fig. 4.51 Antimüllerian hormone (AMH). The protein is cleaved from a precursor. The three-dimensional structure has not yet been determined, thus disulfide bridges cannot be assigned. The asparagine (**N**; on *blue background*) is glycosylated (Source: Swiss-Prot 03971)

4.12.3.1 Introduction

In the nineteenth century Johannes Müller (1801–1858) described the müllerian ducts as the female analogue to the male wolffian ducts found by Caspar E. Wolff (1733–1794). Experiments by A. Jost in the middle of the twentieth century could only be interpreted that in males a protein induces regression of müllerian ducts. Thus male fetuses do not develop ovaries and ovarian ducts. The development of wolffian ducts into seminiferous tubules requires testosterone that female fetuses do not yet have.

Therefore, in the female sex development the müllerian ducts evolve which due to the missing AMH do not regress, whereas wolffian ducts are not developed due to the lack of testosterone.

The hormonal nature of AMH was proven by A. Jost while analyzing the calf of Freemartin: two calf fetuses of different sexes share a placenta. This situation induces in the female calf the occurrence of male sex characteristics. The effect had to be induced via the placenta and the blood; thus AMH has been proven to act in an endocrine manner (Teixeira et al. 2001).

4.12.3.2 Structure and Gene

AMH is a member of TGF-β growth factors, the *AMH* is coded for in the short arm of chromosome 19 in locus 19p13.3 and contains five exons. It has not yet been crystallized. Which enzyme releases the N-terminal hexapeptide is not known.

The structure cannot be found in the Protein Data Bank, however, there is a presumably deduced structure on the Internet when you look for “AMH structure”.

4.12.3.3 Physiology

AMH is already expressed in (male) Sertoli cells during fetal development; in women AMH is expressed in preantral and antral follicles. AMH in male fetuses induce the müllerian ducts to regress via apoptosis. Postpartum AMH is measurable until puberty and is downregulated when testosterone levels increase. The expression in girls and women is arrested by LH of the menstrual cycle (Teixeira et al. 2001).

The AMH receptor, AMHRII, is a serine/threonin kinase of type 2 in the cell membrane. The protein recruits a type 1 receptor kinase. Together these proteins phosphorylate SMAD proteins that induce further signals and gene activations (Teixeira et al. 2001).

AMH defects leave the müllerian ducts intact and their development while concomitantly male sex characteristics develop (müllerian duct persistence syndrome) characterized first by maldescendant testes. Mutations of AMH and AMH receptor can both be responsible for the syndrome (Josso et al. 2005)

4.12.3.4 Phylogeny

AMH has been identified in mammals, birds, reptiles, amphibians, and fish. Although female birds show the heterogamete type ZW and males are homogamete ZZ, genes such as *sry*, *sox9*, *sf-1*,and *dax1* together with *amh* have a sex-determining role. In reptiles sex determination depends more on environmental temperature (Thurston and Korn 2000). In fishAMH is a growth factor of early germ cell without influence on any müllerian ducts because they are lacking in fish (Shiraishi et al. 2008)

4.13 Angiotensins and Renin

Fact sheet 4.46: Angiotensin-II

Gene:	Chromosome 1; locus 1q42.2; five exons.
Sequence:	**DRVYIHPF** (Fig. 4.52).
Synthesis and target:	Angiotensinogen is a liver protein, renin a renal enzyme, and angiotensin-converting enzyme (ACE) a lung enzyme. Renin cleaves from the angiotensinogen the angiotensin I (highlighted in Fig. 4.52) which is later converted by ACE into the active angiotensin-II.
Function:	Angiotensin-II stimulates in the adrenal the synthesis (and release) of aldosterone. Angiotensin- is a major regulator of blood pressure, water, and electrolyte homeostasis.
Receptor:	The angiotensin receptor (AGT-R) is a heptahelical GPCR with two splice variants: AGT receptor 1 (AGT-R1) signals stimulation of aldosterone synthesis; in contrast, AGT-R2, a heptahelical GPCR, too, acts to reduce aldosterone.

4.13.1 Introduction

Angiotensin is released by renin from the angiotensinogen precursor. This precursor is a liver protein. In the juxtaglomerular renal cells (Fig. 10.12) blood pressure is estimated. With decreasing blood pressure or with increasing osmolarity, that is, increasing ion content in the blood, these juxtaglomerular cells release renin. The only known substrate of renin is angiotensinogen (Sect. 11.8.7).

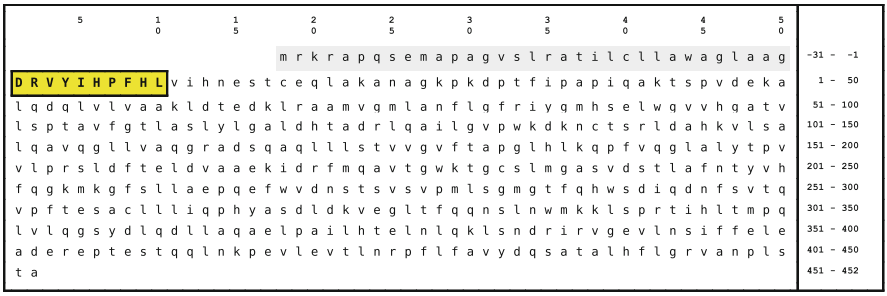


Fig. 4.52 The angiotensinogen precursor. Renin cleaves angiotensin I (highlighted *yellow*) from the angiotensinogen precursor. The Angiotensin-converting enzyme removes two C-terminal amino acids and generates the active angiotensin II (**DRVYIHPF**) (Source: NP_000020.1)

4.13.2 Structure and Genes

Renin cleaves from the precursor the angiotensin-I: **DRVYIHPFHL** (highlighted *yellow* in Fig. 4.52). Angiotensin-I circulates and is converted by angiotenin-converting enzyme (ACE) into Angiotensin-II: **DRVYIHPF**.

Angiotensin-II can additionally be formed (without renin and ACE) by the endopeptidase chymase, which is relevant for the pathophysiology of hypertension, atherosclerosis, and diabetic renal insufficiency (Miyazaki and Takai 2006).

In the brain, however, renin and ACE are expressed and the local neuronal angiotensin-II formation appears in the classical way (Saavedra 1992).

4.13.3 Physiology

Angiotensin-II triggers in the adrenal synthesis of the mineralocorticoid aldosterone (see Fig. 11.15). This action is signaled by AGT-R1.

Pathophysiological actions of angiotensin-II are mostly related to reactive oxygen radicals, so-called ROS. In addition, metalloproteinases, the PDGF receptor, and an EGF receptor set off a cascade that leads to vasoconstriction, fibrosis, hypertrophy, or inflammation. However, with the AGT-R2 receptor expressed, the tissue is protected against these symptoms.

The central actions of angiotensin-II target baroreflex, blood pressure control, and control of water intake/thirst are discussed in Sect. 11.8.

4.13.4 Phylogeny

Angiotensin-I has been found in fish and later vertebrates. ACE is a very old enzyme with an existence reported in insects and bacteria, as well. Renin, however, is not found earlier than fish. This leaves angiotensin-II formation restricted to vertebrates. In *Ciona intestinalis* and later in fish the AGT-R1 has been found.

4.14 Atrial Natriuretic Peptides

Fact sheet 4.47: ANP — BNP — CNP

Gene:	ANP; Chromosome 1; locus 1p36.2; three exons.
Gene:	BNP; Chromosome 1; locus 1p36.2; three exons.
Gene:	CNP; Chromosome 2; locus 2q37; two exons.
Sequence:	Fig. 4.53.
Synthesis and target:	ANP is formed in atrial myocytes in reaction to elevated blood pressure. It acts on renal NP receptors. BNP is likewise made in the heart (and in the brain). It acts on cardiac NP receptors to prevent fibrosis in heart muscle cells. CNP is a brain peptide.
Function:	ANP is an endocrine regulator of water homeostasis and a major regulator of cardiac development. BNP appears to be a paracrine organizer of cardiac tissue, whereas CNP participates in bone formation and prolongs ossification.
Receptor:	The ANP and BNP receptor is a membrane guanylate cyclase. The CNP receptor is a similar guanylate cyclase. CNP binds additionally to the NP clearance receptor NP-R3.

4.14.1 Introduction

Since 1984 the hormone from the myocytes of the right atrium has been known which controls volume and electrolyte balance. Its origin helped to name it atrial natriuretic peptide (ANP). A second related peptide was later found in the brain (and after that in the heart, too) which was labeled brain natriuretic peptide (BNP). When the third peptide was found in the brain, it was simply named CNP.

4.14.2 Structure and Genes

The genes for ANP and BNP are closely linked on the short arm of chromosome 1 (1p36.2).[33] CNP is coded for on chromosome 2, on the long arm (2q36).

ANP is synthesized in right atrial myocytes, BNP in the ventricle and in brain. CNP, apart from being made in the brain, is formed in circular endothelia.

The characteristic feature is the intramolecular disulfide bridge that generates a cyclic peptide. In this cyclic part the homology of the three peptides is obvious (Fig. 4.54).

[33] http://www.ncbi.nlm.nih.gov/projects/mapview/maps.cgi?TAXID=9606&CHR=1&MAPS=genes-r&QSTR=600295[MIM]&QUERY=uid%2812721970%29&BEG=11%2C780K&END=11%2C900K&oview=default

Fig. 4.53 Primary sequences of the three atrial natriuretic peptides ANP, BNP, and CNP. The three peptides, ANP, BNP, and CNP, are generated from precursors, which are cleaved by the signal peptidase and different endopeptidases to release the C-terminal natriuretic peptides. The cyclic nature of these peptides due to disulfide bridges is indicated by connecting the cysteine residues (Source: GenBank AAA35529.1 (ANP), P16860.1 (BNP), BAA14351.1 (CNP))

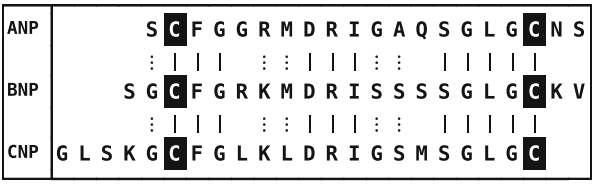


Fig. 4.54 Sequence homologies of atrial natriuretic peptides. Identical amino acids are labeled by |, similar amino acids by ⋮, cysteines are shown *inverted*

The prohormone convertase for ANP is corin, a type II membrane endopeptidase with several characteristic regions: trypsin-like peptidase domain, LDL receptor domain, scavenger receptor domain, and two cysteine-rich FZ domain (for frizzled involved in signal transduction of the Wnt protein). Defects of corin are similar to those of ANP. The CNP precursor is cleaved by furin (Wu et al. 2003a). The CNP precursor has a PC2 motif, too (Fig. 4.53; aa 88–89).

The receptor for natriuretic peptides are membrane-located guanylate cyclases NP-R1 and NP-R2.

4.14.3 Physiology

ANP is released from strongly stretched atrial myocytes. The mechanoreceptors that signal this stretching are nonselective cation channels (Zhang et al. 2008b). ANP acts on renal, cardiac, and adrenal NP receptor 1. Due to the increase in cGMP the sodium transport into the urine is accelerated in the kidney. In addition aquaporins augment water excretion. In the adrenal aldosterone synthesis is inhibited.

ANP is a major regulator of heart development. ANP is already expressed in the very first stages of the heart anlage in humans and rodents (Chuva de Sousa Lopes et al. 2006). Two heart-specific transcription factors, GATA-4 and Nkx2-5, activate the ANP gene promoter.

BNP, originally isolated from the brain, but later identified as a hormone from the heart like ANP, acts similarly inasmuch as it also binds to NP-R1. A mutant in the BNP promoter is causal for diminished bone density and an especially dramatic loss of bone mineralization in postmenopausal women. The BNP serum levels are doubled in these patients (Takeishi et al. 2007). Further cause-and-effect relations have not been identified.

In BNP knockout mice pathological changes in heart tissue were evident: increase and hardening of connective tissue (Tamura et al. 2000). $BNP^{-/-}$ mice did not exhibit–compared with wildtype mice–alterations of potassium serum levels, aldosterone levels, urine sodium or potassium excretion, which led to the conclusion that BNP does not or only marginally affects metabolic parameters. In contrast to ANP's endocrine action, BNP appears as a paracrine effector (Ogawa et al. 2001).

The role of CNP was estimated using CNP knockout mice: Chusho et al. (2001) demonstrated that these animals develop very long bones because ossification is retarded (see Sect. 11.6; Chusho et al. 2001). Whether human variants of achondrodysplasia are due to CNP mutants has not been analyzed thus far. CNP might act as drug in these bone growth defect patients.

4.14.4 Phylogeny

Although in hagfish (*Eptatretus burgeri*) only a single NP gene has been found (Kawakoshi et al. 2003), cartilaginous and bony fish and later vertebrates have three and more NP genes (Houweling et al. 2005). There are up to four CNP genes. CNP-4 is regarded as the primordial gene of cyclostomes (Kawakoshi et al. 2006).

As before for other hormones there are some reports that antibodies against human or rodent NP bind to snail, crab, or even protozoa specifically. Any biochemical support for a hormone or cloning and sequencing has, however, not been provided. Takei thus rejected in 2001 these reports as lacking proof. Since that time any further confirmation for expression of NP in invertebrates is missing.

Guanylate cyclase receptors have been found in many divergent vertebrates and invertebrates, however, the NP-R1 and NP-R2 are vertebrate specific (Fitzpatrick et al. 2006). NP-R3, the NP-clearance receptor is equally restricted to vertebrates.[34]

4.15 Additional Neuropeptides

4.15.1 Parathormone

Fact sheet 4.48: Parathormone (PTH)

Gene:	Chromosome 11; locus 11p15.2; three exons.
Sequence:	Fig. 4.55.
Synthesis and target:	PTH is synthesized and released by parathyroid glands as a response to low serum calcium concentrations. PTH acts on the PTH receptor on bone cells and in the kidney.
Function:	PTH induces decalcification of bone. Simultaneously it inhibits renal calcium excretion.
Receptor:	PTH receptor is a heptahelical GPCR.

4.15.1.1 Introduction

Parathormone (PTH; Fig. 4.55) is the product of the parathyroid gland (pars parathyroidea or glandula parathyroidea). Very sensitive calcium sensors estimate the free calcium concentration ($[Ca^{2+}]_{free}$) in blood. When $[Ca^{2+}]_{free}$ rises parathormone release is stopped and calcitonin release initiated; when $[Ca^{2+}]_{free}$ declines PTH is released and calcitonin release inhibited.

4.15.1.2 Structure and Genes

The *PTH* gene is located on chromosome 11, about 1.5 megabase pairs (MBp) away from *CALCA*. After removing the signal peptide the furin endopeptidase acting, for example on the somatostatin precursor (Sect. 4.3.5), removes the N-terminal

```
         1         2         3         4         5
1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0
                                    m i p a k d m a k v m i v m l a i c f l t k s d g  -25 - -1
k s v k k r S V S E I Q L M H N L G K H L N S M E R V E W L R K K L Q D V H N F V A L G A P L A P R  1 - 50
D A G S Q R P R K K E D N V L V E S H E K S L G E A D K A D V N V L T K A K S Q  51 - 90
```

Fig. 4.55 Sequence of human parathormone. The signal peptide is highlighted *light gray*, the mature peptide highlighted *yellow* and in *uppercase* (Source: GenBank NP_000306.1)

[34] http://www.ncbi.nlm.nih.gov/sites/entrez?Db=homologene&Cmd=ShowDetailView&TermToSearch=699

hexapeptide. Although a **KR**-motif is present, PC1 is not used; these cells obviously do not express PC1.

A parathormone-related peptide (PTHrP) is not made in the parathyroid gland, but in bone by chondrocytes (see Sect. 11.6.3) and by tumor cells.

4.15.1.3 Physiology

PTH is required for control of free calcium ion concentration ($[Ca^{2+}]_{free}$) in the blood. In the PTH forming chief cells in the glandula parathyroidea and in the kidney the calcium-sensing receptor (CASR[35]) is expressed, a heptahelical membrane GPCR. Its expression is *i.a.* stimulated by calcitriol (dihydroxy vitamin D_3). The signal transduction cascade from CASR is directed to the PTH promoter.

Sufficiently high $[Ca^{2+}]_{free}$ is required, for example, to achieve a fast transport in signals with calcium influx. The function of many proteins requires complexes with calcium, such as integrins that do not bind to their ligands on cells or in the extracellular matrix without calcium.

Several hereditary diseases are linked to mutants in the PTH protein/gene or in the PTH receptor (see NCBI:OMIM:PTH[36]): chondrodysplasia or enchondromatosis, as well as hyper- and hypoparathyroidisms.

4.15.1.4 Phylogeny

The phylogeny of PTH is related to calcium sensing by CASR. In fish there are PTH-like polypeptides. These are expressed but in gills. Okabe and Graham (2004) demonstrated that gills and parathyroid glands are functionally related: the controlling factor for expression of PTH and CASR in gills and parathyroid glands is in fish, chicken, and mammals GCM2 (glial cell missing) already found in *D. melanogaster* and which is the only transcription factor whose expression is restricted to the parathyroid gland. Whereas fish take calcium from the water, tetrapods got independent of this source by developing the parathyroid gland but use the same hormone for calcium homeostasis (Okabe and Graham 2004).

No PTH could be identified in invertebrates. There are anecdotal descriptions of PTH actions on neurons and ganglia of snails without molecular proof. Antimammalian-PTH antibodies could bind to snail tissue, but the molecule(s) recognized have thus far escaped identification (Hull et al. 2006).

4.15.2 Stanniocalcin

4.15.2.1 Introduction

Stanniocalcin (STC) is the hormone of the corpuscles of Stannius, that is, endocrine fish glands associated with teleost kidney. At elevated $[Ca^{2+}]_{free}$ STC is released which blocks calcium uptake in the gill. Thus fish regulate their calcium levels. In

[35] http://omim.org/entry/601199

[36] http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=168468

Fact sheet 4.49: Stanniocalcin (STC)

Sequence:	Fig. 4.56 (Coho salmon).
Synthesis and target	STC is formed and released in the corpuscles of Stannius in fish. It acts on the calcium gill transporter and kidney phosphate transporters.
Function:	STC blocks calcium transporters in gills to reduce calcium uptake. Kidney phosphate transporters, in contrast, are stimulated.
Receptor:	(unknown; July 2008/October 2011).

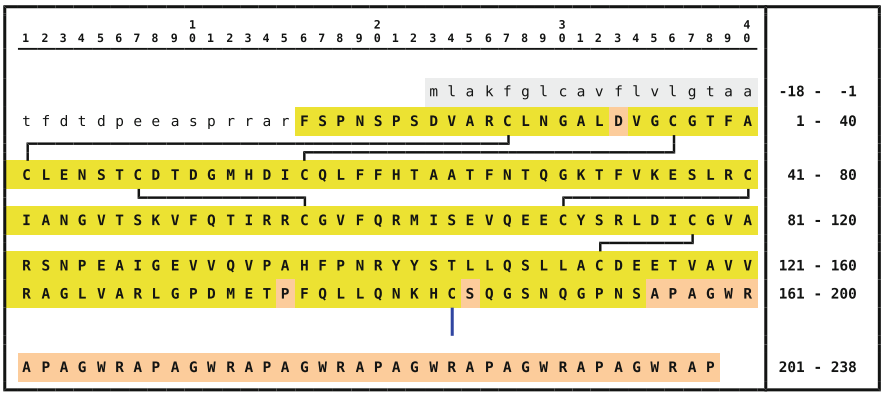


Fig. 4.56 Sequences of stanniocalcin from *Oncorhynchus kisutch* (Coho salmon/silver salmon) and *Oncorhynchus keta* (chum salmon). The precursor from −18 to 238 is from *O. kisutch*. The *O. keta* sequence (highlighted *yellow*) differs from the *O. kisutch* sequence in three amino acids (highlighted *light orange*). We superimposed these two sequences because for *O. keta* the disulfide bonds have been determined (shown by *black* lines connecting the cysteine residues). The cys residue 184 serves as a intermolecular disulfide bond linking two stanniocalcins in a homodimer (Source: Swiss-Prot P43647.2 (*O.keta*) and GenBank AAB26419.1 *O.kisutch*)

addition STC stimulates renal resorption of phosphate which leads to more calcium incorporated into bone and reduces $[Ca^{2+}]_{free}$ as well.

4.15.2.2 Structure and Genes

Stanniocalcin is generated from a precursor that is cleaved by the signal peptidase and a furin-like enzyme, inasmuch as PC1 or PC2 would degrade the STC peptide further at their recognition motifs (Fig. 4.56; PC1: amino acids 227–228) and PC2: (amino acids 12–13; 94–95; 226–227). The molecule exists as a disulfide-linked homodimer.

In humans (as well as in rodents) two stanniocalcin genes have been found: on chromosome 8 (8p21) and on chromosome 5 (5q35.2).

4.15.2.3 Physiology

The calcium sensor receptors CaSR in gills and corpuscles of Stannius induce at elevated $[Ca^{2+}]_{free}$ STC release from the corpuscles, but according to recent findings, from gills and other tissues, too. Calcium transport from water through the gills into the circulation is blocked by STC. STC augments, in addition, renal phosphate resorption. By reducing uptake and forcing bone deposition as calcium phosphate serum calcium concentrations are reduced.

Recently STC have been found in rodents and humans. Apart from some speculation on the role of STC in mammals, it has been shown that STC is a SUMO E3 ligase: SUMOylation adds small ubiquitin-like molecules covalently to other proteins (Small Ubiquitin-like MOdifier = SUMO). In contrast to modification by ubiquitin, this sumoylation does not tag proteins for degradation. STC appears as a ligase to couple SUMOs to other proteins in leukemias and breast tumor cells (Daniel and Lange 2009; dos Santos et al. 2011). Additional functional characterization of STC in humans awaits further analysis.

4.15.2.4 Phylogeny

Stanniocalcins have only been reported in vertebrates. A stanniocalcin-like named molecule in snails does not show any relation to STC of mammals and fish.[37] Gills are responsible for gaseous exchanges and ion uptake from cartilaginous or bony fish on. Obviously for calcium uptake regulation some protein should exist in older classes; the presence of STC-related polypeptides, however, cannot be excluded.

4.15.3 Erythropoietin

Fact sheet 4.50: Erythropoietin (Epo)

Gene:	Chromosome 7; locus 7q21; five exons.
Sequence:	Fig. 4.57; the protein has been crystallized: 1EER; there is structural homology to GH, PRL or leptin.
Synthesis and target:	Epo is synthesized in oxygen-sensing cells of juxtaglomerular kidney cells.
Function:	Epo stimulates the proliferation of reticulocytes which mature into erythrocytes.
Receptor:	The receptor belongs to the family of interleukin growth factors such as GHRH-R or PRL-R.

[37] Our ClustalW analysis

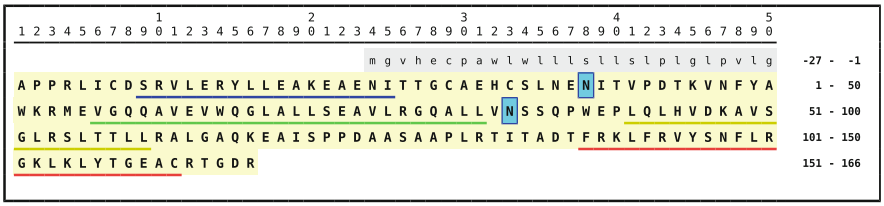


Fig. 4.57 Primary sequence of erythropoietin. The signal peptide is in *lowercase* highlighted *gray*. The protein is highlighted *yellow*; helical regions are underlined in different colors

4.15.3.1 Introduction

The endocrine role of erythropoietin has somehow escaped our attention. Although not being part of central control via the hypothalamus and pituitary, it is a sensu stricto hormone made in the kidney, transported via the blood, and acting in the bone marrow on reticulocytes. Even structurally it is closely related to other hormones, for example, GH, PRL, or leptin.

4.15.3.2 Structure and Genes

The *EPO* gene on chromosome locus 7q21 has five exons. The gene is activated in kidney cells when the oxygen concentration in the medullary cortex drops. While in normoxic cells hypoxia-inducible factor (HIF) is oxidized by reactive oxygen species (ROS); in hypoxic cells, HIF is stable and can bind to the *EPO* promoter and stimulate transcription.

The translated protein is a tetrahelical glycoprotein hormone such as GH, PRL, or leptin. The helical regions are underlined with different colors in Fig. 4.57, the N-glycosylation sites are boxed. There are additional O-glycosylation sites not indicated.

The receptor is very similar to the GH receptor belonging to the CSF-receptor family (see Sect. 8.5. It is expressed in small numbers (some 1,000 molecules per cell) on erythroid cells. The EPO-R is the earliest molecule to distinguish the erythroid from the myeloid lineage.

4.15.3.3 Physiology

EPO transcription is controlled by the oxygen content in the renal medullary cortex. The REPOS cells (Wenger and Hoogewijs 2010) are characterized by HIF (hypoxia-induced factor) which is under normoxic situation oxidized and thus degraded. When the oxygen content drops due to reduced oxygen partial pressure in blood, the production of reactive oxygen species (ROS) is blocked and HIF remains stable. Together with co-factors, HIF induces transcription of the *EPO* gene. After transport via the blood, EPO acts in the bone marrow on erythropoiesis by stimulating cell division of reticulocytes.

The receptor interaction of EPO and the EPO-R has been crystallized. Figure 8.6 exemplifies such an interaction for the highly homologous GH and GH receptor. A picture for EPO and its receptor appeared very similar.

The available literature does not discuss a mechanism whereby EPO would be released by a release triggering signal. On the contrary, there is no evidence for storage of EPO in secretory granules (personal communication by R. Krstić, author of Human Microscopic Anatomy, see Chap. 10). In an experiment to analyze EPO secretion as a requirement for autocrine growth stimulation of EPO transfected cells, Villeval et al. (1994) transfected the UT-7 pluripotent cell line with an EPO vector and got an EPO secretion that stimulated UT-7 growth. Anti-EPO antibodies blocked UT-7 proliferation, likewise an additional retention signal at the C-terminal end of the EPO sequence. This shows that normally EPO is not retained, at least in these cells.

Because EPO belongs to the cytokine family of proteins, it should be noted that cytokines and protein/peptide hormones use different secretory pathways. Cytokines are constitutively secreted whereas secretion of hormones is regulated. The signals whether a peptide/protein is sorted into either of the two pathways seem to be generated by the protein/peptide sequence itself as has been recently shown in the case of gastrin (Bundgaard and Rehfeld 2008). Comparing EPO, GH, PRL, and leptin and, for example, generating hybrid sequences should answer the question of which part of a molecule is required for the regulated pathway.

EPO, once released, can enter the bloodstream using the fenestrated peritubular ascending arterial vas rectum (Krstić 1991, plate 154).

EPO acts on cells of the erythroid lineage. These are present in the bone marrow. The EPO receptor (EPO-R) has been found to be the earliest marker distinguishing the erythroid and the myeloid lineage. The stimulation of erythroid precursor cells enhances reticulocyte and erythrocyte production. Absence of EPO results in lack of erythrocyte renewal and thus in anemia. Injection of EPO can substitute for the failure of endogenous EPO production.

Being available as a drug, EPO has been used for nonnormal stimulation of erythropoiesis. Such an enhancement of erythrocytes might facilitate thrombosis which may be fatal. Because not all EPO preparations are biosimilars, detection of doping is possible but difficult. EPO doping with the unchanged human sequence is impossible to track. For this reason, athletes with an abnormally increased hematocrit are prevented from competition for a certain period, but not challenged for doping (see Chap. 15).

4.15.3.4 Phylogeny

GenBank contains erythropoietin entries from primates, humans, orangutans, guenons, and rodents. There is more work required to clarify the phylogeny of EPO.