

Review

# Neuropeptides, food intake and body weight regulation: a hypothalamic focus

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## Abstract

Energy homeostasis is controlled by a complex neuroendocrine system consisting of peripheral signals like leptin and central signals, in particular, neuropeptides. Several neuropeptides with anorexigenic (POMC, CART, and CRH) as well as orexigenic (NPY, AgRP, and MCH) actions are involved in this complex (partly redundant) controlling system. Starvation as well as overfeeding lead to changes in expression levels of these neuropeptides, which act downstream of leptin, resulting in a physiological response. In this review the role of several anorexigenic and orexigenic (hypothalamic) neuropeptides on food intake and body weight regulation is summarized.

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## 1. General aspects of neuropeptides

The history of neuropeptides started in the 1970s when the term ‘neuropeptides’ was first introduced [51]. Neuropeptides were defined as fragments of peptide hormones without the activity of the parent hormone, but capable of

*Abbreviations:* ACTH, adrenocorticotropic hormone; AgRP, agouti-related protein; ARC, arcuate nucleus of the hypothalamus; BB, bombesin receptor; BBB, blood brain barrier; BSTL, bed nucleus of the stria terminalis; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CCKA-R, cholecystokinin receptor A; CNS, central nervous system; CRH, corticotrophin-releasing hormone; Db, diabetic gene, leptin receptor; DMH, dorsomedial nucleus of the hypothalamus; DMV, dorsomotor nucleus of the vagus; GALP, galanin-like peptide; Gal-R, galanin receptor; GLP, glucagon-like peptide; GPCR, G-protein coupled receptor; HPA axis, hypothalamus–pituitary–adrenal axis; HPT axis, hypothalamus–pituitary–thyroid axis; IAPP, islet amyloid polypeptide; Ins-Rb, insulin receptor; JAK, janus kinase; LC, locus coeruleus; LHA, lateral hypothalamic area; MC, melanocortin; MCH, melanin-concentrating hormone; MC-R, melanocortin receptor; ME, median eminence; MSH, melanocyte-stimulating hormone; NAcc, nucleus accumbens; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; Ob, obese gene, leptin; Ob-Rb, leptin receptor; Ox-R, oxytocin receptor; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus of the hypothalamus; PYY, peptide YY; RAMP, receptor-activity-modifying protein; SON, supraoptic nucleus; STAT, signal transduction and transcription; TRH, thyrotrophin-releasing hormone; TSH, thyroid-stimulating hormone; UCP, uncoupling protein; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area; Y, NPY/PYY receptor; ZI, zona incerta

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producing behavior effects on their own [51]. Currently, over 40 neuropeptide precursors are known and the definition of neuropeptides has extended. Neuropeptides are peptides or fragments of peptides, which are synthesized in cells via large inactive precursor proteins. The neuropeptide precursors can contain several copies of the same peptide (for example, preprothyrotrophin-releasing hormone), but can also hold several different neuropeptides (for example, pro-opiomelanocortin). The regulation of neuropeptide expression is a cell-specific phenomenon. Different processing of these precursors can lead to the generation of novel neuropeptide fragments with novel biological activities. Neuropeptides act via G-protein coupled receptors (GPCRs) and modulate neuronal activity in conjunction with the co-localized neurotransmitters [3]. In recent years, several receptors have been discovered for many neuropeptides in the brain, however, not all effects of these peptides on the central nervous system (CNS) can be explained by the receptor sites known so far.

## 2. Regulation of energy homeostasis: hypothalamic neuropeptides

Physiological functions are regulated by multiple factors. This certainly holds for the regulation of food intake and body weight, which appears to be affected by a bewildering number of endogenous compounds. For clinical

implications, for instance, the rising prevalence of obesity and anorexia nervosa, a better understanding of the mechanism involved in the regulation of food intake and body weight is required. Consequently, literature of studies on this subject is expanding [126,195].

Food intake involves several aspects of different behaviors, like hunting for food and decision-making. The complexity of feeding behavior is reflected in the amount of brain areas involved. For example, the orbitofrontal cortex is involved in sensory-specific satiety [216], while the amygdala is implicated in the evaluation of taste palatability [214]. Hence, feeding behavior can be divided into different phases, such as the appetitive phase, which involves the searching for food, and a consummatory phase, which involves the real eating of the food. Besides the more central effects mentioned above, feeding behavior is also influenced by a peripheral system, which uses, for instance, sensory and gastrointestinal cues to terminate ingestion. The modulation of food intake by the central as well as the peripheral system is amongst others achieved by neuropeptides. Regarding the different behaviors involved, it is logical that the neuropeptides controlling feeding behavior are not only implicated in feeding behavior, but also modulate other processes like, memory and analgesia [195]. Despite the large number of brain areas involved in feeding behavior, the hypothalamus is still regarded as the main feeding center of the brain. In this review, we will focus on the role of hypothalamic neuropeptides on the regulation of food intake and body weight.

Already in the beginning of the 20th century, the hypothalamus was thought to play an important role in feeding behavior. Experiments in the early forties led to the development of the ‘Dual Center Model’ for regulation of feeding, in which the lateral area of the hypothalamus served as the feeding center and the ventral medial part of the hypothalamus as the satiety center [105]. Although this model has been questioned several times, the hypothalamus still appears to be the most important area for regulation of food intake and body weight homeostasis in the brain. The hypothalamus consists of several nuclei involved in food intake, including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the lateral hypothalamic area (LHA), the ventromedial nucleus (VMH), and the dorsomedial nucleus (DMH). ARC neurons are located at the bottom of the hypothalamus around the third ventricle and are called ‘first order neurons’ because of their ‘direct’ contact with peripheral satiety factors like leptin and insulin. This is due to the fact that in the median eminence (ME), which overlies the ARC, the blood brain barrier (BBB) is not present and ARC axons terminals are in direct contact with the bloodstream. However, the neuronal cell bodies in the ARC are protected by the BBB and thus are not in direct contact with the bloodstream [229]. The ARC contains at least two distinct groups of neurons controlling energy balance (see Fig. 1), e.g. neurons that contain the orexigenic neuropeptides agouti-gene-related protein (AgRP) and neuropeptide Y (NPY), and neurons that contain the anorexigenic neuropeptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript

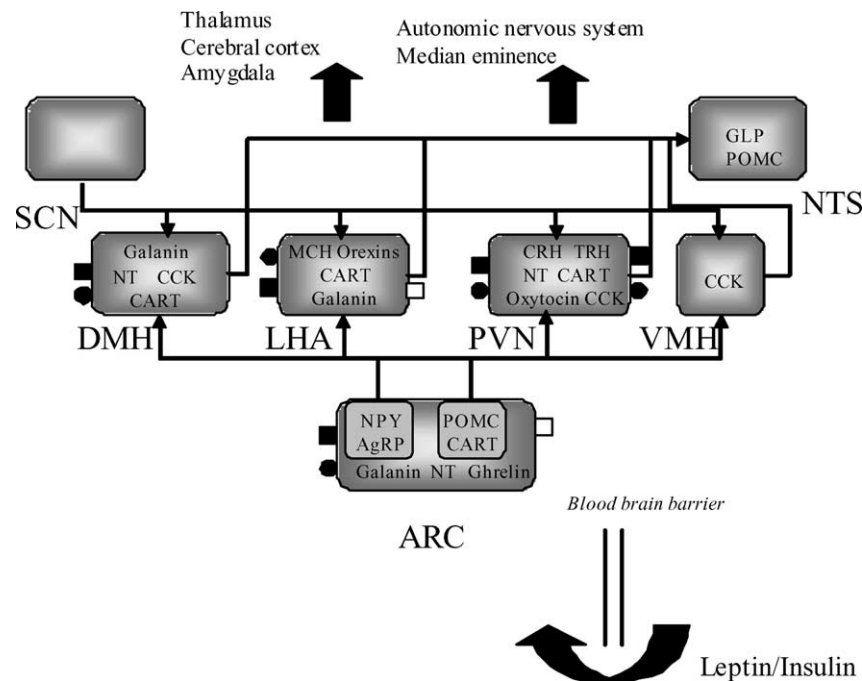


Fig. 1. Simplified schematic drawing of the hypothalamic regions involved in food intake. First order neurons in the arcuate nucleus (ARC) are activated by leptin and insulin and project to second order neurons in the paraventricular nucleus (PVN), dorsomedial nucleus (DMH), lateral hypothalamic area (LHA), and ventromedial nucleus (VMH) of the hypothalamus. The suprachiasmatic nucleus (SCN) as well as the nucleus of the solitary tract (NTS) also connect with these hypothalamic feeding centers. Leptin receptors (■), insulin receptors (●), and melanocortin receptor 4 (□).

(CART). From the ARC, neurons project to ‘second order neurons’ in the PVN, VMH, DMH, and LHA [257]. ‘Second order neurons’ project amongst others to the nucleus of the solitary tract (NTS) in the brainstem and the dorsomotor nucleus of the vagus (DMV). This communication between hypothalamic pathways and the caudal brainstem, responding to meal-related satiety signals, is essential for the long-term regulation of energy homeostasis.

It has been suggested that firing of hypothalamic neurons is dependent on local glucose levels. These neurons would use glucose sensors, just like pancreatic  $\beta$ -cells. Evidence exists that around 40% of the ARC and VMH neurons are glucose-responsive neurons, expressing glucokinase (that phosphorylates glucose to glucose 6-P) and GLUT2 (a glucose transporter). These two proteins are important for glucose sensing in  $\beta$ -cells and might most likely exert the same function in neurons [210,252]. The ARC, PVN, and VMH contain glucose-stimulated neurons, while the LHA consists of glucose-inhibited neurons. The glucose responsiveness of the DMH is yet unknown [242].

Since the discovery of leptin in 1994 [324], the understanding of the role of hypothalamic neuropeptides in feeding behavior has greatly expanded. Studies on the role of leptin and downstream acting peptides on energy homeostasis brought to light many interesting aspects of this complicated neuroendocrine control system. Not only the existence of numerous neuropeptides involved in food intake and body weight regulation adds to the complexity of the system; the various anorexigenic and orexigenic neuropeptides signaling pathways comprise an interconnected (possibly partly redundant) network as well. Besides, anorexigenic and orexigenic peptides are often co-localized and released, and also their receptor localization overlaps.

In this review the most important hypothalamic neuropeptides that play a role in food intake and body weight regulation are discussed. Hence, we will focus on the role of the hypothalamus and hypothalamic neuropeptides on food intake and body weight regulation. We are aware of the fact that several other brain areas and other (non)-peptidergic compounds, like norepinephrine and serotonin and dopamine [183,308], are involved in these processes. However, the role of these compounds on food intake and body weight regulation is beyond the scope of this review.

### 3. Leptin and insulin

The understanding of the neuropeptidergic regulation of body weight and food intake has gained enormous by the discovery of leptin. Its existence was already hypothesized in 1969, but only in 1994 the 167 amino acids containing protein from the obese gene (*Ob*) was cloned [324]. Leptin is the peripheral satiety signal arising from adipose tissue, while it can also be produced (in smaller amounts) in the placenta, and gastric parietal cells. Leptin rapidly influences the activity of ARC neurons in the hypothalamus through

entrance by the ME, but it can also cross the BBB to exert direct actions at, for instance, the PVN. Leptin levels correlate with adipose mass. As well, there are diurnal changes in plasma leptin levels, which are regulated by the biological clock, the suprachiasmatic nucleus (SCN) of the hypothalamus [127]. Recently, several reviews on the role of leptin in reducing food intake have been published [68,78,172,257].

Intracerebroventricular (icv) administration of leptin decreases food intake (by affecting meal size) and body weight without inducing malaise [5,228,281]. This decrease in food intake does not lead to the normally associated reduction in energy expenditure, and rise in free fatty acids and ketones [294]. Icv administration of leptin also influences adiposity by affecting thermogenesis (uncoupling protein (UCP) expression) and in-place utilization of fat [47,250] and has an effect on reproduction [27,173]. The decrease in food intake by leptin is associated with inhibition of glucose-responsive neurons in the LHA, and stimulation of glucose-responsive neurons in the VMH and PVN [263]. The involvement of hypothalamic nuclei is also demonstrated by activation of the immediately early gene *c-fos* in the ME, ARC, DMH, and parvocellular part of the PVN following leptin administration [66,295]. In addition, literature also shows the involvement of the caudal brainstem on leptin induced decreases in food intake and body weight. Fourth ventricle and DMV administration of leptin results in a decrease in food intake and body weight to the same extent as lateral ventricle or VMH administration of leptin [93].

Leptin exerts its actions by activation of the leptin receptor which is the product of the diabetes (Db) gene [40,280]. Five alternatively spliced forms of the leptin (*Ob*) receptor (*Ob-R<sub>a-e</sub>*) are produced of which only one, *Ob-R<sub>b</sub>*, has a long cytoplasmic region that is required for signal transduction. This receptor is mainly expressed in the hypothalamic ARC, VMH, PVN, and DMH, where it is involved in the weight reducing effects of leptin [67,184,255]. During fasting, the expression and number of binding sites of this receptor is upregulated in hypothalamic areas [16]. The *Ob-R* is a member of the family of cytokine receptors, which lack intrinsic tyrosine kinase activity, but have docking sites for janus kinases (JAK) that are involved in cytokine signaling. Binding of these kinases to the active receptor results in phosphorylation of signal transduction and transcription (STAT) proteins resulting in alterations of gene transcription. However, leptin can also influence neuronal activity by opening ATP-sensitive potassium channels, thus hyperpolarizing glucose-responsive neurons in the hypothalamus [84,266,292]. The existence of natural leptin and leptin receptor mutations in rodents, like the obese and hyperphagic *ob/ob* mice (leptin deficient), the *db/db* mice (defective leptin signaling), and the homologue *fa/fa* Zucker rat strain, have highly contributed to broaden our knowledge on the role of leptin and neuropeptides in energy homeostasis.

Another adiposity signal is insulin, which is the controller of blood glucose levels and is secreted by the  $\beta$ -cells of the pancreas under control of glucose levels. A rise in blood

glucose, amino acids, or glucagon, as well as stimulation of sympathetic ( $\alpha$ ) and parasympathetic (cholinergic) innervation, leads to insulin release. Insulin release is diminished by decreased blood glucose levels, by stimulation of sympathetic ( $\beta$ ) innervation, and by stress (reviewed in [254,314]). Insulin is the major activator of energy storage in adipose tissue. Chronically high levels of insulin consequently lead to obesity (reviewed in [138]). Injections of insulin in the third ventricle (or ARC) of rodents lead to a decrease in food intake and body weight [256]. However, the difficulties of creating a proper experimental setup and achieving data of physiological relevance make research on the role of insulin in food intake complicated. This is especially the case in human studies in which insulin is peripherally administered. The resulting changes in food intake are most often caused by secondary metabolic effects (the strong decrease in glucose levels) of insulin [39]. Besides its 'role' in food intake, insulin does also play a role in neuropeptide release by causing a rise in intracellular  $\text{Ca}^{2+}$  release [121], and in neuronal survival and differentiation [238]. Insulin can rapidly cross the BBB by a receptor-mediated mechanism. In the brain it can bind to the brain insulin receptor, which is expressed in the VMH and ARC, as well as the striatum and choroid plexus, where its function is still unknown [17,174]. Insulin receptors (Ins-R<sub>b</sub>) are receptor tyrosine kinases that phosphorylate and activate intracellular signaling proteins (Irs). Ins-R<sub>b</sub> differs from the peripheral receptors in size, glycosylation, and insulin-binding specifics (reviewed in [253]). Activation of Ins-R<sub>b</sub> also leads to phosphorylation of the neuronal *tub* gene protein in the hypothalamus. A loss of function mutation of this gene in mice (Tubby  $-/-$  mice) results in an obese phenotype, linking impaired insulin signaling with obesity at the level of the hypothalamus [131,274]. Mice with a neuron-specific deletion of the insulin receptor (NIRKO mice) have a normal survival, but also an obese phenotype with increased leptin levels, insulin levels, and reproductive impairments [29,274]. Hence, this observation corresponds with the anorexigenic response of central insulin administration as mentioned above.

From the above it is clear that leptin and insulin are important regulators of energy homeostasis, acting in the same hypothalamic areas. Interaction between leptin and insulin is important for an integrated response concerning food intake. It has recently been shown that such an interaction exists at the level of the hypothalamus. That is, insulin induces JAK2 tyrosine phosphorylation, and also increases STAT3 phosphorylation and activation in the presence of leptin [37].

#### 4. Hypothalamic neuropeptides involved in food intake and body weight

##### 4.1. Anorexigenic neuropeptides

##### 4.1.1. Anorexigenic neuropeptides in first order neurons

*Melanocortins* (MC) are peptides that are cleaved from the

precursor molecule POMC that is amongst others expressed in the pituitary gland, ARC, NTS, and peripheral tissue. Processing of POMC is a cell-specific phenomenon in which prohormone convertase 1 (PC1) and 2 (PC2) are involved [45]. POMC is processed into adrenocorticotrophic hormone (ACTH<sub>(1–39)</sub>) and  $\beta$ -lipotropic hormone. ACTH<sub>(1–39)</sub> is further converted into  $\alpha$ -melanocyte-stimulating hormone (MSH<sub>(1–13)</sub>), ACTH<sub>(18–39)</sub>/corticotropin-like intermediate peptide (CLIP), while  $\beta$ -lipotropic hormone can be further processed into  $\gamma$ -lipotropic hormone,  $\beta$ -endorphin<sub>(1–31)</sub>, and  $\beta$ -MSH. The N-terminal part of POMC contains the sequences for  $\gamma$ <sub>1–3</sub>-MSH. In addition, ACTH<sub>(1–39)</sub> is further converted into smaller fragments like ACTH<sub>(1–16)</sub>, ACTH<sub>(4–16)</sub>, and ACTH<sub>(7–16)</sub>, which are responsible for behavioral effects (see Table 1). The central and peripheral (pharmacological) effects of melanocortins are frequently described; stimulation of pigmentation, adrenal steroidogenesis, lipolysis, grooming, effects on feeding, enhanced motivation and attention processes, modulation of blood pressure and heart rate, anti-inflammatory actions, influences on pain, temperature, and nerve regeneration [20,107,301,309]. The role of melanocortins in feeding behavior is strengthened by the presence of POMC neurons expressing the Ob-R<sub>b</sub> leptin receptor in the ARC. When leptin levels are high, POMC neurons in the ARC are stimulated which leads to a decrease in food intake and increase in sympathetic activity that is prevented by melanocortin antagonists [46,98,188]. Injections of ACTH<sub>(1–24)</sub> in the lateral ventricle and in the VMH of fasted rats decreased food intake significantly (see Table 2) [300]. The same effect was found after central injection of  $\alpha$ -MSH and  $\beta$ -MSH [133]. It is interesting to note that the POMC gene also encodes the orexigenic peptide  $\beta$ -endorphin, beside the anorexigenic MC mentioned above [288]. The decrease in food intake by MC is associated with a reduction of basal insulin release and an increase of fasting blood glucose, indicating that the MC system not only influences food intake but is also independently involved in glucose homeostasis [71]. In addition, deletion of the POMC gene in vivo results in an obese phenotype due to hyperphagia, and also results in defects in pigmentation and adrenal development (see Table 3) [322].

The effects of melanocortins are mediated by melanocortin receptors (MC-Rs) of which currently five are cloned, which all stimulate adenylate cyclase activity. All natural melanocortins can bind to the melanocortin 1 receptor (MC1-R) that is expressed in melanocytes [198]. ACTH is the only melanocortin ligand that can bind to the melanocortin 2 receptor (MC2-R) located in the adrenal cortex [150,235]. Melanocortin 3 and 4 receptors (MC3-R and MC4-R) are mainly distributed in the brain, where they interfere with food intake [55,79,80] while the melanocortin 5 receptor (MC5-R) is found in many peripheral tissues like skeletal muscle, skin, adrenal gland, and spleen [81,92]. MC3-R, MC4-R, and MC5-R can bind all natural melanocortins, although with different affinities. Recently, a fundamental role for the MC4-R in feeding and body

Table 1  
Overview of amino acid sequences of the neuropeptides discussed in this review

Gene family	Neuropeptide	Amino acids (source, amount)
Pro-opiomelanocortin	$\alpha$ -MSH	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val (rat, 13)
	$\beta$ -MSH	Ala-Asp-Gly-Pro-Tyr-Arg-Val-Glu-His-Phe-Arg-Trp-Gly-Asn-Pro-Pro-Lys-Asp (rat, 18)
	$\gamma_1$ -MSH	Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe (rat, 11)
	$\gamma_2$ -MSH	Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly (rat, 12)
	$\gamma_3$ -MSH	Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-Arg-Arg-Asn-Gly-Ser-Ser-Ser-Gly-Val-Gly-Ala-Ala-Gln (rat, 25)
	ACTH	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Val-Pro-Asn-Val-Ala-Glu-Asn-Glu-Ser-Als-Glu-Ala-Phe-Pro-Leu-Glu-Phe (rat, 39)
	$\beta$ -Endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Val-His-Lys-Lys-Gly-Gln (rat, 31)
	CLIP	Pro-Val-Lys-Val-Tyr-Pro-Asn-Val-Ala-Glu-Asn-Glu-Ser-Ala-Glu-Ala-Phe-Pro-Leu-Glu-Phe (rat, 21)
	CART	CART
		Met-Glu-Ser-Ser-Arg-Leu-Arg-Leu-Leu-Pro-Val-Leu-Gly-Ala-Ala-Leu-Leu-Leu-Leu-Leu-Pro-Leu-Leu-Gly-Ala-Gly-Ala-Gln-Glu-Asp-Ala-Glu-Leu-Gln-Pro-Arg-Ala-Leu-Asp-Ile-Tyr-Ser-Ala-Val-Asp-Asp-Ala-Ser-His-Glu-Lys-Glu-Leu-Pro-Arg-Arg-Gln-Leu-Arg-Ala-Pro-Gly-Ala-Val-Leu-Gln-Ile-Glu-Ala-Leu-Gln-Glu-Val-Leu-Lys-Lys-Leu-Lys-Ser-Lys-Arg-Ile-Pro-Ile-Tyr-Glu-Lys-Lys-Tyr-Gly-Gln-Val-Pro-Met-Cys-Asp-Ala-Gly-Glu-Gln-Cys-Ala-Val-Arg-Lys-Gly-Ala-Arg-Ile-Gly-Lys-Leu-Cys-Asp-Cys-Pro-Arg-Gly-Thr-Ser-Cys-Asn-Ser-Phe-Leu-Leu-Lys-Cys-Leu (rat, 129)
Corticotropin-releasing hormone	CRH	Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-Gly-Lys (mouse, 43)
	Urocortin I	Asp-Asp-Pro-Pro-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg-Thr-Gln-Ser-Gln-Arg-Glu-Arg-Ala-Glu-Gln-Asn-Arg-Ile-Ile-Phe-Asp-Ser-Val (mouse, 40)
	Urocortin II	Val-Ile-Leu-Ser-Leu-Asp-Val-Pro-Ile-Gly-Leu-Leu-Arg-Ile-Leu-Leu-Glu-Gln-Ala-Arg-Tyr-Lys-Ala-Ala-Arg-Asn-Gln-Ala-Ala-Thr-Asn-Ala-Gln-Ile-Leu-Ala-His-Val (mouse, 38)
	Urocortin III	Phe-Thr-Leu-Ser-Leu-Asp-Val-Pro-Thr-Asn-Ile-Met-Asn-Ile-Leu-Phe-Asn-Ile-Asp-Lys-Ala-Lys-Asn-Leu-Arg-Ala-Lys-Ala-Ala-Ala-Asn-Ala-Gln-Leu-Met-Ala-Gln-Ile (mouse, 38)
Tyrotropin-releasing hormone	TRH	Glu-His-Pro (rat, 3)
	TRH <sub>(160--169)</sub>	Ser-Phe-Pro-Trp-Met-Glu-Ser-Asp-Val-Thr (rat, 10)
	TRH <sub>(178--199)</sub> (CRIF)	Phe-Ile-Asp-Pro-Glu-Leu-Gln-Arg-Ser-Trp-Glu-Glu-Lys-Glu-Gly-Glu-Gly-Val-Leu-Met-Pro-Glu (rat, 22)
Oxytocin	Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly (rat, 9)
Neurotensin	Neurotensin	Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu (mouse, 13)
Cholecystokinin	CCK <sub>(1--58)</sub>	Ala-Val-Leu-Arg-Pro-Asp-Ser-Glu-Pro-Arg-Ala-Arg-Leu-Gly-Ala-Leu-Leu-Ala-Arg-Tyr-Ile-Gln-Gln-Val-Arg-Lys-Ala-Pro-Ser-Gly-Arg-Met-Ser-Val-Leu-Lys-Asn-Leu-Gln-Gly-Leu-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe (rat, 58)
GLP	GLP1	His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (rat, 37)
	GLP2	His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Thr-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp (rat, 33)
	Glicentin	Asn-Arg-Asn-Asn-Ile-Ala (rat, 6)
	Oxyntomodulin	His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Gln-Phe-Leu-Lys-Tyr-Leu-Leu-Asn-Val-Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala (guinea pig, 37)
Bombesin	Bombesin	Gln-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met (amphibian, 14)
	Neuromedin B	Gly-Asn-Gln-Trp-Ala-Ile-Gly-His-Phe-Met (amphibian, 10)
Amylin	Amylin	Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-Arg-Ser-Ser-Asn-Asn-Leu-Gly-Pro-Val-Leu-Pro-Pro-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr (rat, 37)
AgRP	AgRP	Met-Leu-Thr-Ala-Ala-Val-Leu-Ser-Cys-Ala-Leu-Leu-Leu-Ala-Leu-Pro-Ala-Thr-Arg-Gly-Ala-Gln-Met-Gly-Leu-Ala-Pro-Met-Glu-Gly-Ile-Arg-Arg-Pro-Asp-Gln-Ala-Leu-Leu-Pro-Glu-Leu-Pro-Gly-Leu-Gly-Leu-Arg-Ala-Pro-Leu-Lys-Lys-Thr-Thr-Ala-Glu-Gln-Ala-Glu-Glu-Asp-Leu-Leu-Gln-Glu-Ala-Gln-Ala-Leu-Ala-Glu-Val-Leu-Asp-Leu-Gln-Asp-Arg-Glu-Pro-Arg-Ser-Ser-Arg-Arg-Cys-Val-Arg-Leu-His-Glu-Ser-Cys-Leu-Gly-Gln-Gln-Val-Pro-Cys-Cys-Asp-Pro-Cys-Ala-Thr-Cys-Tyr-Cys-Arg-Phe-Phe-Asn-Ala-Phe-Cys-Tyr-Cys-Arg-Lys-Leu-Gly-Thr-Ala-Met-Asn-Pro-Cys-Ser-Arg-Thr (rat, 132)

Table 1 (Continued)

Gene family	Neuropeptide	Amino acids (source, amount)
Neuropeptide Y	NPY <sub>(1–36)</sub>	Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr (rat, 36)
	PYY <sub>(1–36)</sub>	Tyr-Pro-Ala-Lys-Pro-Glu-Ala-Pro-Gly-Glu-Asp-Ala-Ser-Pro-Glu-Glu-Leu-Ser-Arg-Thr-Thr-Ala-Ser-Leu-Arg-His-Tyr-Leu-Asn-Leu-Val-Thr-Arg-Gln-Arg-Tyr (rat, 36)
Melanin-concentrating hormone	MCH	Asp-Phe-Asp-Met-Leu-Arg-Cys-Met-Leu-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Gln-Val (mouse, 19)
	Neuropeptide Glu-Ile Neuropeptide Gly-Glu	Glu-Ile-Gly-Asp-Glu-Glu-Asn-Ser-Ala-Lys-Phe-Pro-Ile (mouse, 13) Gly-Ser-Val-Ala-Val-Phe-Pro-Ala-Glu-Asn-Gly-Val-Gln-Asn-Thr-Glu-Ser-Thr-Gln-Glu-Lys (mouse, 21)
Prepro-orexin	Orexin A	Gln-Pro-Leu-Pro-Asp-Cys-Cys-Arg-Gln-Lys-Thr-Cys-Ser-Cys-Arg-Leu-Tyr-Glu-Leu-Leu-His-Gly-Ala-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Leu (rat, 33)
	Orexin B	Arg-Pro-Gly-Pro-Pro-Gly-Leu-Gln-Gly-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Ala-Asn-Gly-Asn-His-Ala-Ala-Gly-Ile-Leu-Thr-Met (rat, 28)
Galanin	Galanin	Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-His-Ala-Ile-Asp-Asn-His-Arg-Ser-Phe-Ser-Asp-Lys-His-Gly-Leu-Thr (mouse, 29)
	Galanin-like peptide	Ala-Pro-Val-His-Arg-Gly-Arg-Gly-Gly-Trp-Thr-Leu-Asn-Ser-Ala-Gly-Tyr-Leu-Leu-Gly-Pro-Val-Leu-His-Pro-Pro-Ser-Arg-Ala-Glu-Gly-Gly-Gly-Lys-Gly-Lys-Thr-Ala-Leu-Gly-Ile-Leu-Asp-Leu-Trp-Lys-Ala-Ile-Asp-Gly-Leu-Pro-Tyr-Pro-Gln-Ser-Gln-Leu-Ala-Ser (rat, 60)
Ghrelin	Ghrelin	Gly-Ser-Ser-Phe-Leu-Ser-Pro-Glu-His-Gln-Lys-Ala-Pro-Pro-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg (mouse, 28)

weight homeostasis was demonstrated. This receptor is amongst others expressed in the PVN and LHA and holds high affinity to  $\alpha$ -MSH. Indeed infusion of  $\alpha$ -MSH in the brain results in a decrease in food intake and increase in energy expenditure in rodents [76], which can be prevented by administration of a MC3-R/MC4-R antagonist [1,259]. In addition, MC4-R  $-/-$  mice are obese, hyperphagic,

and have an increased somatic growth [116]. Not only in rodents, but also in man, mutations of the MC4-R are associated with an obese phenotype [291,323]. The role of the MC3-R in food intake regulation is less clear. MC3-R has highest affinity to  $\gamma$ -MSH. However,  $\gamma$ -MSH does not influence food intake, but influences blood pressure and heart rate via a non-MC receptor mechanism [213,293].

Table 2

Overview of effects on food intake and body weight in wildtype (wt) rodents and leptin deficient ob/ob mice (compared to wt) after administration of the neuropeptides discussed in this review

Neuropeptide (precursor)	Neuropeptide	Wild type mouse/rat		Reference
		Food intake	Body weight	
POMC	$\alpha$ -MSH	↓	↓	[133,182]
	$\beta$ -MSH	↓		[128]
	$\gamma$ -MSH	–	–	[293]
	$\beta$ -Endorphin	↑		[288]
CART	CART	↓	↓	[156]
	CRH	↓	↓	[5,104,115]
CRH	Urocortin I–III	↓	↓	[5,104,115]
	TRH	↓	–	[158,162]
Neurotensin	Neurotensin	↓	–	[159,168]
CCK	CCK	↓		[85,265]
GLP	GLP1	↓	–	[289,296]
Bombesin	Bombesin	↓		[86]
Amylin	Amylin	↓	↓	[169,244,245]
AgRP	AgRP	↑	↑	[242,265]
NPY	NPY	↑	–	[269]
MCH	MCH	↑	–	[12,24,119,233]
Orexin	Orexin A	↑	–	[263]
	Orexin B	↑/–	–	[103]
Galanin	Galanin	↑	–	[154]
Ghrelin	Ghrelin	↑	↑	[207]

Changes are depicted with arrows.

Table 3  
Overview of available knockout and transgenic rodents of the neuropeptides discussed in this review

Neuropeptide	Transgene	Observed changes		Knockout	Observed changes		References
		Body weight	Food		Body weight	Food	
POMC				POMC	↑	↑	[322]
				MC4-R	↑	↑	[116]
				MC3-R	–	–	[33]
CART				CART	–	–	[15]
CRH	CRH	↑	–	CRH	–	–	[201,273]
				CRH-1R	–/↓	–	[26,283]
				CRH-2R	–	–	[13,141]
TRH				TRH	–	–	[320]
				TRH-α	–	–	[36,310]
				TRH-β	–	–	[77]
Oxytocin				Oxytocin	–	–	[215]
Neurotensin				Neurotensin	–	–	[56]
CCK				CCK-AR	↑/–	↑/–	[190,219,258]
				CCK-BR	–	–	[205]
GLP				GLP-1R	–	–	[171,258]
Bombesin				BB1-R	–	–	[217]
				BB2-R	–	–	[100]
				BB3-R	↑	↑	[218]
Amylin	Amylin	–		Amylin	↑	–	[48,83]
AgRP	Agouti	↑	↑				[30]
	AgRP	↑					[89,205]
NPY	NPY	–/↑	–/↑	NPY	–	–/↓	[14,70,124]
				NPY-Y1R	↑ (F)	–/↓	[31,130,205]
				NPY-Y2R	↑	↑	[211]
				NPY-Y5R	↑ (M)	↑	[175]
MCH	MCH	↑	↑	MCH	↓	↓	[167]
Orexin				Orexin	↑	↓	[102]
Galanin	Galanin	–	–	Galanin	–	–	[25,109,136,272,317]

Observed changes in body weight and food intake are depicted with arrows. F: female, M: male.

Nevertheless, MC3-R is expressed in POMC and AgRP neurons in the ARC, suggesting the existence of a negative autoregulation of POMC and AgRP signals [6,22]. MC3-R  $-/-$  mice have an increased adipose mass, while not significantly obese. Thus, this genotype could be described as a metabolic syndrome, without hyperphagia and without increased somatic growth [33].

*Cocaine- and amphetamine-regulated transcript* (CART) is another first order neuropeptide that inhibits food intake. In the rat, splicing of the precursor results in two products consisting of 116 and 129 amino acids [57]. Only the 116 amino acid containing product is found in man. The two splice products are tissue specifically processed into smaller peptides. After processing, CART peptides are packed into dense core vesicles as neurotransmitters. Several forms of CART peptides are presently known: CART<sub>(55–86)</sub>, CART<sub>(54–102)</sub>, CART<sub>(55–102)</sub>, CART<sub>(61–102)</sub>, and CART<sub>(62–102)</sub> [282]. CART was first identified in the striatum, where it was dramatically upregulated after administration of psychoactive drugs [57]. However, CART

mRNA is more highly expressed in several parts of the hypothalamus (ARC, PVN, DMH, LHA) [82] as well as in the ME, pituitary, and adrenal medulla [151]. CART mRNA and CART peptide are co-localized with POMC in the ARC, with melanin-concentrating hormone (MCH) (but not with orexins) in the LHA, and with TRH, galanin, vasopressin, and oxytocin in the PVN [151]. Thus, CART is co-localized with orexigenic as well as anorexigenic neuropeptides.

Central administration of CART peptide leads to an inhibition of normal (acute) and NPY-induced food intake in rodents [148,155]. In addition, chronic administration of CART decreases food intake and body weight [156], while injections of CART antibodies increase food intake in rodents [148,155]. CART expression is upregulated after leptin administration, suggesting that CART is under leptin control [62,304]. Food deprived rodents as well as ob/ob mice and fa/fa rats show a decrease in CART mRNA in the ARC (see Table 4).

Icv injections of CART not only influence food intake, but also induce activation of the hypothalamus–pituitary–adrenal

Table 4

Overview of changes in expression levels of anorexigenic and orexigenic neuropeptides in the leptin deficient ob/ob mice compared to wildtype mice

Neuropeptide	Expression changes in ob/ob mice	Reference
POMC	↓	[188]
CART	↓	[148]
CRH	–	[115]
NT	↓/–	[311,312]
CCK	–	[221]
Bombesin	–	[69]
AgRP	↑	[264]
NPY	↑	[311]
MCH	↑	[233]
Orexin	↓	[321]
Galanin	–	[137]

Upregulation or downregulation is depicted with arrows.

axis (HPA axis), as shown by an increased activity of central corticotropin-releasing hormone (CRH) neurons in the PVN, and increased secretion of corticosterone from the adrenal gland [271]. In addition, CART injections in the PVN result in increased expression of UCPs I, II, III in adipose tissue and muscle, suggesting an important role for CART in energy metabolism [305]. The mechanisms that mediate CARTs effects are still poorly understood. Studies with recombinant CART peptide showed increased *c-fos* expression in the PVN, DMH, supraoptic nucleus (SON), and ARC as well as in the central nucleus of the amygdala, bed nucleus of the stria terminalis (BSTL), and NTS. These regions could thus be possible sites of action of CART in the CNS [204]. However, until now no receptor has been identified.

Despite the effects of CART on food intake mentioned above, CART knockout mice have a normal body weight and food intake but appear to be less sensitive to pain [15]. This certainly suggests a role of this neuropeptide beyond the regulation of food intake.

#### 4.1.2. Anorexigenic neuropeptides in second order neurons

*Corticotropin-releasing hormone* (CRH) is a 41 amino acid peptide first isolated from the ovine hypothalamus and best known for its initiating role in the HPA axis. On top of that, CRH is widely distributed in the brain and serves as an integrator of adaptive responses to stress, influences food intake, gastrointestinal function, cardiovascular processes, and inflammatory processes. CRH cell bodies are mainly found in the PVN and in the central nucleus of the amygdala, from where projections arise to the hypothalamus, brainstem, NTS, and locus coeruleus (LC) [90,91,250]. The effects of CRH are mediated by two GPCRs, which are both positively coupled to adenylate cyclase. Both receptors share homology but have distinct binding characteristics and differ in their anatomical distribution. The CRH2-receptor (CRH2-R) exists of three spliced forms, the CRH2 $\alpha$ , the CRH2 $\beta$ , and the CRH2 $\gamma$  [146], which have low affinity to

CRH [164,165]. The CRH1-receptor (CRH1-R) has higher affinity to CRH and is mainly found in the pituitary and the cerebral cortex. The CRH2-R is primarily found in limbic regions. Both receptors are located in the cerebellum, brainstem and hypothalamus [38,165,232]. Another protein which is involved in CRH signaling is CRH binding protein. This protein is located in both the CNS and periphery, and has important modulating actions on CRH. Namely, it binds free CRH and thereby reduces its free concentration in plasma [44,132].

CRH is a potent anorexigenic peptide, acting downstream of leptin. Central administration or direct administration into the PVN inhibits nighttime and fasting-induced feeding. Depending on the nutritional state of the animal, leptin increases CRH expression and the activity of CRH neurons (fed state) or decreases CRH expression and activity of CRH neurons (fasted state) [5,115]. The anorexic effect of leptin is attenuated by administration of the CRH antagonist  $\alpha$ -helical CRH<sub>(9–41)</sub>. This antagonist inhibits endogenous CRH activity and enhances the feeding response to exogenous NPY, implying that CRH is a secondary anorexigenic neuropeptide acting downstream of NPY [104,290]. Co-administration of CRH and a MC4-R antagonist (HS014) does not change the effect of CRH on food intake and grooming, indicating that these CRH effects are downstream of the MC4-R pathway [299]. Besides the effect on food intake, CRH increases sympathetic nervous system activity, thereby increasing thermogenesis, energy expenditure, and lipolysis.

CRH knockout mice do not have a distinctive phenotype despite the disruption of their HPA axis. Their body weight is normal as well as their fertility and longevity [201]. CRH1-R knockout mice show an increased exploratory behavior and decreased anxiety-related behavior in addition to a disruption of the circadian distribution of food intake [202,283]. In addition, CRH2-R knockout mice show normal basal feeding, weight gain, decreased food intake following food deprivation, and anxiety-like behavior independent from the HPA axis [13]. This further underscores that the effects of CRH on food intake are context and state dependent.

Another endogenous member of the CRH family of neuropeptides is urocortin I (UCNI). UCNI was originally identified in the rat brain and consists of 40 amino acids [297]. UCNI is differently distributed in the CNS from CRH; its expression is mainly found in the Edinger–Westphal nucleus, the lateral superior olive, the LHA, and the SON, but also in the digestive system (stomach and colon) and endocrine organs [220]. UCNI binds to both CRH receptors, being a more potent ligand for the CRH2-R than CRH itself. Central and peripheral administration of UCNI influences satiety and reduces meal size [267]. The effects of UCNI on food intake are larger than the effects of CRH, and are not related to effects on anxiety, oxygen consumption or adrenal steroids. Peripheral UCNI can enter the brain through the BBB where interactions with leptin lead to an enhanced entrance [134]. Urocortin II (UCNII) is also a member of the CRH family and consists of 38 amino acids. The human

form of the peptide is also referred to as urocortin-related peptide (URP). UCNII is expressed in cell populations in the ARC, PVN, SON, LC, and motor nuclei of the brain stem. In the mouse brain UCNII binds with high selectivity to the CRH2-R [237]. UCNII inhibits food intake in the dark phase in rodents without an effect on gross motor activity and behavioral activation [237]. A third urocortin peptide has recently been identified. This peptide, urocortin III (UCNIII) also consists of 38 amino acids and again binds preferentially to the CRH2-R. UCNIII expression is found in hypothalamic nuclei, brainstem, lateral septum, bed nucleus of stria terminalis, and peripherally in the small intestine and skin [160]. Physiological effects of UCNIII have not been reported yet.

*Thyrotropin-releasing hormone* (TRH) is an anorexigenic peptide that plays a major role in the hypothalamus–pituitary–thyroid (HPT) axis by stimulating the release of thyroid-stimulating hormone (TSH). In addition, TRH also holds CNS effects. TRH is produced in neurons of the medial PVN [158,185,262], which project to the ME and pituitary, and in neurons of the anterior, dorsal, and ventral PVN, that influence parasympathetic and sympathetic centers in the brainstem. The hypothalamic DMH projects strongly to TRH neurons in the PVN and regulates the HPT axis by changing the sensitivity of TRH neurons to feedback effects of thyroid hormone. It also directs autonomic activity through projections to the brainstem and spinal cord [157]. In addition, the DMH is probably involved in circadian variations in TSH levels and TRH expression levels via its connections to the SCN [128]. TRH neurons in the PVN are also influenced by neurons of the medulla oblongata via its catecholaminergic projections [262].

Processing of preproTRH (255 amino acids) leads to several new fragments like preproTRH<sub>(160–169)</sub> and preproTRH<sub>(178–199)</sub> besides the five copies of the real mature TRH [319]. PreproTRH<sub>(160–169)</sub> is also called TRH potentiating peptide since it enhances TRH stimulated TSH release and potentiates TRH-induced gastric acid secretion when injected into the DMV [243]. PreproTRH<sub>(178–199)</sub> is an endogenous inhibitor of ACTH release and is abundant in the external zone of the ME and the PVN [236]. This corticotropin release-inhibiting factor (CRIF) inhibits ACTH synthesis as well as its release in vivo and holds anxiolytic activities. Within the CNS, the tripeptide TRH is degraded by proglutamate aminopeptidase into a cyclic dipeptide cHis–Pro. This peptide is even more potent than mature TRH in reducing food intake [196].

The effects of TRH are mediated by two TRH receptors, which have distinct anatomical distributions. The TRH-R1 is mainly found in hypothalamic regions, while the TRH-R2 is located in the thalamus, in the cerebral and cerebellar cortex, and throughout the reticular formation. The latter is in keeping with the effects of TRH on higher cognitive functions, arousal, and pain perception [106]. TRH inhibits food intake acting downstream of the leptin–melanocortin pathway. Fasting levels of leptin lead to a reduction of cir-

culating thyroid hormones, followed by a reduction in TRH synthesis in the PVN and TSH secretion to conserve energy until refeeding is possible again. Not only leptin, but also MC, like  $\alpha$ -MSH, influence TRH expression and secretion through POMC fibers connecting to TRH neurons in the PVN [158]. Studies with hypothalamic explants show that MC indeed increase TRH release, while antagonists of the MC3/MC4-R block this expansion [139].

TRH is an important stimulator of energy expenditure through an increase in thermogenesis [276,302]. Therefore, TRH also serves as a controller of body temperature, since cold exposure leads to rising levels of TRH and thyroid hormones to stimulate thermogenesis [325]. It could therefore be that the anorexigenic effect of TRH is mediated by increased sympathetic outflow and increased metabolic rate. In addition, TRH and thyroid hormones are also connected to energy homeostasis in another way. Thyroid hormones regulate *tub* gene expression in rats and in neuronal cells, linking thyroid status with *tub*-gene associated obesity [145].

*Oxytocin* is expressed in magnocellular neurons and parvocellular neurons in the hypothalamus. Magnocellular neurons are mainly found in the SON and the PVN, and project to the posterior pituitary where excretion of oxytocin takes place. Parvocellular neurons are mainly localized in the PVN from where projections arise to central brain regions and to the external zone of the ME.

Oxytocin is cleaved from a precursor, which also contains the sequence of neurophysin I and signal peptide. After cleavage, neurophysin I becomes a carrier for oxytocin in secretory granules [157].

Oxytocin peptide (nine amino acids) has central anorexic effects, influences gastric emptying, and motility [188,202]. It also plays a role in fluid and electrolyte homeostasis through central and peripheral actions [298], but is best known for its actions during parturition and lactation. Central administration of oxytocin inhibits food intake, which can be prevented by icv pretreatment with oxytocin receptor (Ox-R) antagonists [10,223]. Oxytocin also has peripheral effects on the gastrointestinal tract, that is, injections of oxytocin in the DMV influence gastric motility [240].

Its effects are mediated by the oxytocin receptor, which is amongst others expressed in the hypothalamus, brainstem, basal ganglia, limbic system, and peripherally in the reproductive system [140]. The oxytocin system is probably linked to the CRH-system. This is shown by expression of CRH1- and CRH2-receptors in oxytocin neurons in the PVN and SON [9], and by secretion of oxytocin by the pituitary following icv CRH administration [28]. In addition, pretreatment with oxytocin antagonists blocks the effect of CRH on food intake [224], suggesting that oxytocin is a mediator of CRH-induced hypophagia.

*Neurotensin* is a 13 amino acids containing neuropeptide that is released during a meal. It is produced in neurons of the ARC, PVN, and DMH as well as in the periphery, where it acts on the digestive and cardiovascular system. Neurotensin

is cleaved from the precursor preproneurotensin, which also encodes neuromedin N and neuromedin N-like peptide [142]. Systemic administration of neurotensin leads to rapid degradation of the peptide. Only the six amino acids of the C-terminal end are necessary for biological activity. However, this fragment (NT<sub>(8–13)</sub>) has so far never been found in vivo. The biological activity of neurotensin is mediated by binding to one of the three neurotensin receptors (NT1-R, NT2-R, and NT3-R) cloned. All three receptors recognize the C-terminal fragment [178,179,278].

Central as well as peripheral administration of neurotensin in rats results in a decrease in food intake [159,168]. Hypothalamic neurotensin expression is upregulated after central injections of leptin resulting in a decrease in food intake [246]. In addition, the neurotensin gene is downregulated in obesity models like the ob/ob mice and the Zucker (fa/fa) rat [19]. Neurotensin does not only influence energy metabolism by affecting food intake, but also reduces body temperature [22]. In addition, it exerts influence on the dopaminergic system and has strong antinociceptive effects [42].

*Cholecystokinin* (CCK) is one of the best studied peptides of the (peripheral) satiety system, which strongly affects meal size by influencing satiety upon nutrient stimulation (especially long chain fatty acids) [87]. CCK is a gastrin-like peptide and is called a gut-brain peptide, due to its presence and actions in both gut and brain. It is synthesized in enteric neurons and endocrine cells (of the mucosa) of the small intestine following nutrient stimulation, as well as in the cortex, hippocampus, VMH, PVN and thalamus [117]. Several forms of endogenous CCK exist, including CCK<sub>(1–58)</sub>, CCK<sub>(1–39)</sub>, CCK<sub>(1–38)</sub>, CCK<sub>(1–33)</sub>, CCK<sub>(1–22)</sub>, CCK<sub>(1–12)</sub>, CCK<sub>(1–10)</sub>, CCK<sub>(1–9)</sub>, CCK<sub>(1–5)</sub>, and CCK<sub>(1–8)</sub>. The latter is the predominant form in the brain and is found either in sulfated (active CCK<sub>(1–8S)</sub>) or desulfated (CCK<sub>(1–8NS)</sub>) form (reviewed in [12]). The existence of the biological active CCK<sub>(1–4)</sub> (gastrin<sub>(1–4)</sub>) fragment in the brain is still questioned.

CCK possesses a wide variety of behavioral effects, which are mediated by two CCK receptors. The CCKA-receptor (CCKA-R) is found in the pancreas, stomach, enteric neurons, nervous vagus and NTS. It has high affinity to CCK<sub>(1–33)</sub> and CCK<sub>(1–8S)</sub>, but less for CCK<sub>(1–8NS)</sub>, CCK<sub>(1–4)</sub>, and gastrin [58]. Blockade of this receptor attenuates the satiety effects of CCK [191]. The CCKB-R is widely distributed in the brain, amongst others in the NTS, PVN, and VMH, but also peripherally in the stomach. CCKB-R binds all CCK fragments and has high affinity to gastrin [193]. Cholecystokinin affects food intake, when administered in the periphery as well as in the CNS, and also results in behavioral changes affecting sleep, grooming, pain, anxiety, memory, and conditioned taste aversion [8,72,85,227].

Peripheral administration of CCK stimulates the gallbladder (this refers to the name cholecystokinin), decreases gastric emptying, and gastric distention, and thereby results in decreased food intake, without an effect on fluid intake

through afferent fibers of the nervous vagus to the DMV and NTS [192].

In general, central CCK administration is less effective in decreasing food intake than peripheral administration. However, this can differ between species. Most researchers report an anorexigenic response of CCK, when administered into the lateral ventricle, VMH or PVN [227,239]. However, others find no effect [152]. The effects of central CCK administration could be caused by interactions with neuro-modulators/neuropeptides like noradrenalin, dopamine and bombesin [204]. The short-term effects of CCK on food intake can be potentiated by leptin [306]. Co-administration of CCK and leptin (icv or intraperitoneal (ip)) enhances vagal afferent activity relaying information to the NTS and PVN, and also leads to elevated levels of *c-fos* in the PVN and NTS [69]. In addition, leptin and CCK can also interact directly at the level of the PVN. Thus, the leptin deficient ob/ob mice and fa/fa Zucker rats are relatively insensitive to meal-terminating effects of peripherally administered CCK [12]. Although the role of CCK in feeding behavior has been demonstrated, the relative importance of this neuropeptide is questionable. Knockout mice of both the CCKA-R and CCKB-R have a normal body weight and food intake [190]. In contrast, rats that lack the CCKA-R (OLETF) are genetically diabetic, obese and have satiety deficits [187]. In addition, OLETF rats also show spatial memory deficits, hypoactivity and anxiety [161]. However, it cannot be excluded that the lack of CCKA-R is the sole genetic defect in OLETF rats causing this phenotype.

*Glucagon-like peptide 1 and 2* (GLP1 and GLP2) are two neuropeptides which share 50% amino acid identity with glucagons. Both peptides are involved in nutrient assimilation and energy homeostasis. GLP1 and GLP2 (33 amino acids) are derived from pancreatic proglucagon, which is proteolytically cleaved into the 29 amino acids peptide glucagon and a proglucagon fragment. This proglucagon fragment is further cleaved into GLP1, GLP2, as well as glicentin, and oxyntomodulin [18,225]. GLP1<sub>(7–36)</sub> is synthesized in L-cells of the jejunum, ileum, colon, in neurons of the NTS, in the medulla of the caudal brainstem, and to a lesser extent in the hypothalamus.

GLP1 inhibits gastric emptying [209] and glucagon secretion, stimulates glucose-dependent insulin secretion and biosynthesis, and has short-term effects on the regulation of feeding behavior in the CNS [211]. Icv administration of GLP1 results in a decrease in food intake and water intake, while administration of exendin, a GLP1 receptor antagonist that shares sequence homology with GLP1, results in the opposite effect [289]. The expression of GLP1 in the hypothalamus is decreased following food restriction, which is prevented by icv administration of leptin [88].

Unlike GLP1<sub>(7–36)</sub>, other processing products like GLP1<sub>(8–36)</sub>, GLP1<sub>(9–36)</sub>, and GLP1<sub>(11–36)</sub> have no effect on food intake and locomotor activity at doses up to 100 µg [289]. The effect of GLP1 on food intake is acute, which can be explained by the short half time in vivo. The N-terminal

(active) part is degraded within 2 min, leaving GLP1<sub>(9–36)</sub> amide, which is biologically inactive [52]. Regarding the rapid degradation of GLP1, chronic administration of GLP1 has no effect on food intake or body weight [50].

GLP1 exerts its actions via the GLP1-receptor (GLP1-R) that is widely expressed in the olfactory bulb, temporal cortex, amygdala, ARC, and PVN. The exact mechanism by which GLP1 influences feeding behavior is not yet known. GLP1 could indirectly act as an anorexigenic agent by influencing NPY signaling. It has been reported that GLP1 inhibits NPY-induced feeding, while the GLP1 antagonist increases NPY-induced feeding [79,248]. This interruption of the NPY signaling then probably takes place at the level of the PVN, thus affecting NPY release [289]. However, there is also some evidence that the anorexic response of GLP1 is a consequence of food aversion [296]. Even though studies indicate a role for GLP1 in food intake, it does not seem to be essential for the physiological control of nutrient intake and body weight regulation. For example, GLP1-R knockout mice show normal food intake and body weight regulation, although their glucose metabolism, and behavioral and neuroendocrine responses to stress are impaired [171,258].

Like GLP1, GLP2 is produced in the gut as well as in the NTS, reticular nucleus and hypothalamus by post-translational processing of proglucagon [60,101]. GLP2 decreases (on the short-term) food intake and (on the long-term) body weight, when administered centrally without inducing taste aversion [61,286].

The effects of GLP2 effects are mediated via the GLP2-receptor (GLP2-R) that shares sequence homology with the glucagon receptor and GLP1-R. The GLP2-R is expressed in the gut, DMH, thalamic regions, hippocampus, and cortical regions [203]. Recent research shows that exendin<sub>(9–39)</sub>, which was always thought to be a true GLP1-R antagonist, is also an antagonist of the GLP2-R [279]. Literature shows that GLP2 is expressed in the NTS, whereas GLP2-R is expressed in the DMH. Combined with the fact that central injections of GLP2 lead to *c-fos* activation in the DMH [279], this suggests that the effect of GLP2 on food intake and body weight is probably mediated by GLP2 containing neurons connecting the NTS and the DMH [279]. Beside the role of GLP2 on food intake, it has trophic and cytoprotective effects on the gastrointestinal tract and influences on gastric motility [59].

*Bombesin-like peptides* are widely distributed in the mammalian gastrointestinal tract and CNS. Bombesin itself was originally isolated from the frog skin, while the bombesin-like peptides gastrin-releasing peptide and neuromedin B are mammalian peptides [181]. Bombesin-like peptides have many actions similar to CCK on gut visceral function. They are responsible for a wide variety of effects when injected in rats, concerning inhibition of food intake, thermoregulation, smooth muscle contraction, and exocrine and endocrine processes, such as release of CCK and gastrin [166]. Icv and peripheral administration of gastrin-releasing peptide as well as bombesin results in an inhibition of feed-

ing in deprived as well as non-deprived animals [54,86]. In addition, fourth ventricle injections of bombesin in decerebrated and intact rats show that not only the hypothalamus, but also the caudal brainstem is involved in bombesin induced inhibition of feeding [75]. As well, local injections of bombesin in the NTS decrease food intake [120]. Peripheral or central administration of bombesin results in activation of the HPA axis, activation of the sympathetic nervous system through CRH neurons [88], increased grooming behavior and decreased exploratory behavior [45,74].

Currently, four bombesin-like peptide receptors have been cloned: neuromedin B receptor (BB1), gastrin-releasing peptide receptor (BB2), bombesin-receptor subtype 3 (BB3), and bombesin-receptor subtype 4 (BB4) [72,135,268,303]. Although its name would suggest otherwise, bombesin has only low affinity to the (bombesin) BB3 receptor, and the natural ligand for the BB3 receptor is still unknown. Knock-out studies show that the different receptors play independently a role in food intake and body weight regulation. The BB1 knockout mouse is not obese and reacts normally to injections of gastrin-releasing peptide [217], while the BB2 knockout mouse also has a normal body weight but does not respond to bombesin injections [100]. In contradiction, mice lacking the BB3-receptor are obese, hyperphagic, show a reduced metabolic rate, and have an impaired glucose metabolism [218].

*Islet amyloid polypeptide* (IAPP), also called amylin, is a 37 amino acid containing peptide that is co-secreted with insulin in the pancreatic  $\beta$ -cell in response to food intake [34]. Amylin is a family member of calcitonin, calcitonin gene-related peptide (CGRP) and adrenomedullin. Although amylin is not produced in the hypothalamus, it can rapidly cross the BBB and enter the hypothalamus, area postrema and NTS, where numerous amylin binding places are located [260]. This peptide acts as a satiety factor, controlling blood glucose and the rate of stomach emptying on the short term. However, amylin also has true anorexigenic actions. Peripheral administration of amylin decreases food intake (partly) due to a reduction in meal size [170] and chronic administration leads to a reduction in body weight [11]. Central third or lateral ventricular injections of amylin also lead to inhibition of food intake in the short and long term [165]. In addition, chronic central amylin infusion decreases fat pad weight and body weight without inducing conditioned taste aversion in rats [245], while central infusion of a specific amylin antagonist leads to increased food intake and adiposity in rats [244].

IAPP knockout mice have normal basal insulin and glucose levels. However, male IAPP knockout mice have a higher body weight than their wildtype controls at 18 weeks of age and show increased insulin secretion and rapid glucose decline in the intravenous glucose tolerance test [83].

The receptor that mediates the anorectic effect of amylin is probably located in the NTS and hypothalamus, which carry amylin binding sites [199]. Recently, it was shown that the amylin receptor is constituted of a calcitonin receptor (CR) gene product associated with a receptor-activity-modifying

protein (RAMP) [181,200]. The interaction of the calcitonin receptor and RAMP1 or RAMP3 creates a specific amylin binding site [200].

Nowadays, amylin receives much attention through its involvement in diabetes mellitus type II. The change in  $\beta$ -cell function in diabetics, probably results in a change in production, processing or secretion of IAPP. This leads to the formation of amyloid fibrils in islets, which severely affect the islets [125].

## 4.2. Orexigenic neuropeptides

### 4.2.1. Orexigenic neuropeptides in first order neurons

Agouti protein is a paracrine-signaling molecule that affects pigmentation by antagonism of the MC1R. Expression of agouti is normally limited to the skin, where overexpression in mice results in a yellow fur. Agouti yellow ( $A^y$ ) mice ubiquitously express the agouti gene, and show an autosomal dominant obese phenotype with insulin resistance and a yellow fur [30]. In 1997, a murine and a human gene were isolated, encoding a protein with nearly identical size and structure as agouti [222]. In contradiction to agouti, this *agouti-related protein* (AgRP, 132 amino acids) is mainly expressed in the ARC and the adrenal medulla. In the ARC neurons, AgRP is inhibited by leptin and stimulated after fasting (lack of leptin), thus AgRP acts downstream of leptin. AgRP acts as a high affinity antagonist of the MC4-R and MC3-R, which are predominantly expressed in the brain. Recently, it was found that AgRP, in fact, is an inverse agonist of the MC4-R, which is constitutively active [212].

Icv administration of the C-terminal fragment of AgRP<sub>(83–132)</sub> stimulates feeding and increases body weight in rats [242,265]. Just like agouti, overexpression of AgRP in mice results in an obese phenotype [186]. As well, AgRP expression is increased in ob/ob mice, db/db mice, and in fasted wildtype mice. The orexigenic response of AgRP can be increased by co-administration of NPY, which is another orexigenic peptide co-expressed with AgRP in ARC neurons [99]. AgRP shows both acute and long-term effects on food intake. The acute effect of AgRP probably involves the opioid system, since the opioid receptor antagonist naloxone blocks acute AgRP-induced food intake [96]. However, the long-term effect of AgRP is most likely not mediated by opioid receptors [97]. AgRP influences energy expenditure and thermogenesis by connections with the TRH system. Administration of the C-terminal part of AgRP<sub>(83–132)</sub> leads to a decrease in plasma TSH and total T4 in rats [139].

Beside the effect on food intake and thermogenesis, AgRP also seems to be involved in food selection. AgRP enhances the intake of specifically high fat content diets, but not of low fat content diets in rodents [84]. It also selectively increases food intake in rats given ad libitum access to a 20% sucrose solution versus rats with standard rat chow [97,313].

Recently, two mutations (760 G  $\rightarrow$  A and the silent 526 G  $\rightarrow$  A in complete linkage disequilibrium) in the human AgRP gene were identified. These mutations appeared more

frequently in a population of anorexia nervosa patients as compared to a control group [201]. It might be that these mutations cause a functional deficit in the AgRP protein, resulting in a loss of function. In addition, a polymorphism (38 C  $\rightarrow$  T) in the AgRP promoter was identified. The C/C genotype was associated with high body mass index and type II diabetes in Africans and also resulted in a higher promoter (luciferase) activity, when transiently transfected into CHO cells [177]. Thus, these recent findings suggest once more the involvement of AgRP in (human) body weight homeostasis.

*Neuropeptide Y* (NPY) is a 36 amino acid peptide member of the pancreatic polypeptide family. It is one of the most abundant peptides found in the CNS, with high concentrations in the hypothalamus and LC in the brainstem [6]. NPY is regarded as the most potent orexigenic peptide. It is also involved in activation of the HPA axis, regulation of growth, and cardiovascular functioning [270]. NPY expression follows a daily rhythm, which is probably regulated by the circadian clock [318]. NPY-producing neurons in the ARC are co-localized with AgRP and galanin, and express the leptin Ob-R<sub>b</sub> receptor [99]. From the ARC, which is itself also innervated by NPY neurons arising from the brainstem [247], NPY projections run to the PVN, VMH, and DMH. From there on, NPY fibers are connected to the NTS and dorsal vagal complex.

Processing of the pro-NPY gene results in the formation of several NPY fragments, amongst others NPY<sub>(2–36)</sub>, NPY<sub>(13–36)</sub>, NPY<sub>(20–36)</sub>, NPY<sub>(22–36)</sub>, and NPY<sub>(26–36)</sub>.

The effect of NPY on food intake is mediated by the intact peptide (NPY<sub>(1–36)</sub>), while it is suggested that the fragments elicit effects on memory processes [73].

Central administration of NPY<sub>(1–36)</sub> into the cerebral ventricles or directly into the PVN increases food intake, decreases energy expenditure, reduces sympathetic outflow to brown adipose tissue, and increases lipogenesis by stimulating the expression of lipogenic enzymes in white adipose tissue [21,269]. Thus, NPY administration leads to a state of positive energy balance and increases fat storage. The effect on food intake is dramatic and takes place in the dark as well as in the light phase. During fasting (when leptin levels are low) and in the leptin deficient ob/ob mouse, expression levels of NPY are increased [263]. In contrast, in a positive energy state, leptin as well as insulin, decrease NPY expression and NPY levels via their arcuate leptin/insulin receptors [149,256]. Interestingly, studies show that administration of NPY increases sucrose solution intake, while it decreases intraoral sucrose solution intake. This suggests that NPY is an orexigenic peptide that is merely important in directing attention to the food, thus playing a role in the appetitive phase of food intake [7]. Beside leptin and insulin, the NPY system is also influenced by peripheral glucocorticoids. Glucocorticoids stimulate glucocorticoid receptors (GR) on NPY neurons (direct influence) or block CRH expression, which abolishes its inhibitory effect on NPY-expression (indirect influence) [99].

Although we focus on the role of NPY in the hypothalamic regulation of food intake, literature shows that not only the hypothalamus is involved in NPY induced food intake. Results of fourth ventricular administrations of NPY show that also the hindbrain is involved in NPY-induced feeding [43]. In addition, results from double injections of NPY in the PVN and naloxone in the NTS suggest that hypothalamic NPY may stimulate feeding through opioidergic pathways in the hindbrain [147].

Six different NPY receptors have been cloned, which mediate the effects of NPY. The influence of NPY on feeding is predominantly mediated by the postsynaptic NPY1-receptor and the NPY5-receptor, which are highly expressed in the PVN [114]. Better phenotypic analysis of NPY knockout mice and NPY-receptor knockout mice might provide more insight into the role of NPY on food intake and autonomic functions. Initially it was found that the NPY  $-/-$  mouse did not have a characteristic phenotype. NPY  $-/-$  mice showed no differences when compared to heterozygote mice in body weight, food intake, and endocrine parameters in the fed or fasted state [70]. In NPY  $-/-$  mice, peripheral leptin administration for 2 days led to a stronger inhibition of feeding and suppressed refeeding after fasting as compared to NPY  $+/+$  mice. However, the response of NPY  $-/-$  mice to other anorexigenic peptides like CRH and the MSH analog MTII was unaltered [108]. In contrast to the above, another group showed that NPY  $-/-$  mice do have a reduced food intake compared to wildtypes in response to fasting, and also have an anxiogenic-like behavior as compared with wildtype controls [14]. The discrepancy in phenotypic analysis of the NPY  $-/-$  mouse may be explained by slight differences in experimental setup or genetic background. NPY1-R knockouts have reduced energy expenditure and are obese, although they have a reduced food intake as compared to controls. Fasted NPY1-R  $-/-$  mice show hyperinsulinemia and glucose turnover, which is prevented by leptin infusion [31]. In addition, NPY5-R knockout mice are also obese and respond normally to exogenous applied leptin and to fasting [175]. Thus, although NPY has always been considered the major orexigenic neuropeptide, knockout studies now question this dominant role of NPY in food intake. Probably other orexigenic peptides are as important in food intake regulation and compensate for the loss of NPY.

Another member of the pancreatic polypeptide family (although encoded by a different gene) with potent dose-related orexigenic actions is *peptide YY* (PYY). PYY consists of 36 amino acids and is localized in endocrine cells of the ileum and large intestine and in smaller amounts in cervical spinal cord, NTS, medulla oblongata, and hypothalamus [43]. Hence, the anatomical distribution differs from NPY [4,6]. The orexigenic effect of PYY and NPY is mediated by the same receptors. However, activation of the Y1, Y2 and Y5 receptor by PYY results in a more potent orexigenic response [197]. Results from feeding studies show that PYY is involved in ingestive and gastrointestinal regulation of the feeding drive, but also affects other (non-feeding) behavior

like sleep and exploratory behavior [43,95]. Administration of PYY in the cerebroventricles but also in the hippocampus induces hyperphagia by affecting several components of food intake, for example, appetitive, reward and cognitive factors [95].

#### 4.2.2. Orexigenic neuropeptides in second order neurons

*Melanin-concentrating hormone* (MCH) is an orexigenic cyclic 19 amino acid peptide ( $MCH_{(146-165)}$ ), originally isolated from salmon pituitaries, and produced by neurons of the LHA and the zona incerta (ZI). MCH neurons receive input from ARC neurons [64], and make widespread connections throughout the cerebrum to several brain areas, which are involved in integrating inputs related to taste olfaction and visceral sensations [23]. The preproMCH also encodes for preproMCH<sub>(132-144)</sub> (neuropeptide–glutamic acid–isoleucine amide) and preproMCH<sub>(110-128)</sub> (neuropeptide–glycine–glutamic acid) [206]. These two fragments do not play a role in food intake.

In 1996 SLC-1, an orphan GPCR, was discovered which exhibits 40% amino acid identity to somatostatin receptors [144]. A few years later, this receptor was identified as the MCH receptor. MCH-R expression is found in the hypothalamic VMH, DMH, ARC, in the hippocampal formation, in olfactory regions, in the medial nucleus accumbens (NAcc) [249]. Peripherally, it is expressed in the eye and skeletal muscle [248]. Recently, a second receptor has been identified, the MCH-R2, which shares about 38% amino acid identity with MCH-R1 [8,248]. MCH-R2 is highly expressed in the hypothalamus (VMH, LHA, ARC), in the cerebral cortex as well as in the hippocampus, olfactory regions, and NAcc. This suggests a possible role of this receptor in the regulation of food intake by olfactory processes and reinforcement [249].

MCH plays an important role in food intake behavior and also stimulates the activity of the HPA axis by increasing ACTH release [78]. Icv administration of MCH to mice or rats induces hyperphagia in the light and dark phase [233,249] and decreases energy expenditure [167,233]. MCH expression in the LHA is increased after fasting [233]. Inhibition of MCH neurons results in hypophagia and leanness, as shown by bilateral lesions of the LHA. MCH is therefore thought to regulate feeding by acting downstream of the leptin and melanocortin pathway, also because the long isoform of the leptin receptor is only sparsely expressed in the LHA [24]. MCH knockout mice have a reduced body weight and are lean due to hypophagia. Surprisingly, these mice show an increased metabolic rate accompanied by decreased leptin and arcuate NPY expression levels [224].

*Orexins (or hypocretins)* are orexigenic neuropeptides, which have been first discovered in 1999 in the perifornical area and the LHA of the rat brain [251]. The precursor prepro-orexin, a 130 amino acids peptide is processed to orexin A (33 amino acid peptide) and orexin B (28 amino acid peptide) [251]. Orexins are implicated in food intake

and temperature regulation [118,153]. They are produced in neurons of the LHA and perifornical area that project to areas important in feeding behavior, sleep, neuroendocrine homeostasis, and autonomic regulation, including the NTS and the ventral tegmental area (VTA) [50]. Orexins are also found in peripheral organs like the liver and heart [208,230].

Two types of GPCRs related to the NPY2 receptor (26% homology) have been discovered, which bind with differing degree to the ligands [285]. These receptors are expressed throughout the whole brain, with the orexin 1 receptor (Ox1-R) mainly expressed in the VMH and LHA, and the orexin 2 receptor (Ox2-R) mainly expressed in the parvocellular part of the PVN and VTA [65,285]. Orexin A has equal affinity to both receptors, while orexin B has greater affinity to Ox2-R than Ox1-R [251]. The activity of orexin neurons is influenced by feeding status, thus increased by low glucose levels and decreased by signals related to nutrient ingestion [163]. Orexin neurons (as well as MCH neurons) in the LHA receive innervation from the arcuate POMC and NPY/AgRP neurons, and consequently express their receptors as well as Ob-R<sub>b</sub>. In addition, orexin neurons project back to POMC and NPY neurons where they, just like leptin, inhibit POMC neurons and activate NPY neurons [65,233,251]. As well, central injection of orexin A leads to *c-fos* activation in the ARC and PVN. Given the role of orexins in food intake, their expression levels rise in dark (feeding) period, following a circadian rhythm [241].

The orexigenic effect of orexin A has been frequently described [63,263], while the role of orexin B on food intake is more often questioned [103,275]. Orexin A has an acute effect on (deprived) food intake and water intake [153,277]. Beside the regulation of food intake, orexin A also seems to play a role in temperature regulation. Icv orexin A administration results in a decrease of body temperature, which is probably (just like the effect on food intake) mediated by NPY [118]. The combination of these two effects, imply that orexin A is more involved in the regulation of the energy status of the body. Orexin A is probably mainly involved in short-term regulation of energy homeostasis, since expression is not changed during chronic food restriction and hypoglycemia when food is available, but after 48 h of fasting and after acute hypoglycemia when food is withheld [251]. Besides their role in energy homeostasis, orexins influence the HPA axis probably via the release of NPY [153], stimulate motor activity [122], and more importantly play a role in the regulation of sleep and narcolepsy [94,231]. This is illustrated by prepro-orexin knockout mice that are narcoleptic [251] and hypophagic, although they show a normal growth.

*Galanin* is a 29 amino acid neuropeptide that was originally identified in the porcine gut, but is also (species specifically) widely expressed in the CNS [35]. Pro-galanin is processed to galanin and galanin-like peptide (GALP 60 amino acids). Galanin and GALP are mainly found in the ARC, DMH, LHA, PVN, ME, and infundibular stalk [123]. They are expressed in neurons that possess leptin recep-

tors [241]. Galanin stimulates feeding, especially fat intake [41], inhibits insulin secretion, and induces hyperglycemia in the periphery [180]. Its expression in the ARC and PVN is (indirectly) influenced by leptin and insulin [26]. The orexigenic effect is augmented at the start of the dark period and is only short-term [154]. Central administration of galanin in the hypothalamus, amygdala or NTS results in an increase in food intake. The multiple actions of galanin are mediated by three galanin receptors; galanin receptor 1 (GalR1), galanin receptor 2 (GalR2), and galanin receptor 3 (GalR3), which share homology but have different distribution patterns [32,113,307]. GalR1 is abundantly expressed in the CNS, while GalR2 and GalR3 are also expressed in the periphery. However, the exact mechanism of the increase in food intake is still unclear. One possibility could be the linkage to the NPY system. It is suggested that galanin (just like  $\beta$ -endorphin) mediates NPY-induced feeding and is also linked to POMC neurons in the ARC [111,112]. Galanin-induced food intake could also be mediated via the release of NA or activation of neurons of the NAcc that mediate rewarding properties of food consumption [234].

Icv administration of GALP stimulates food intake 10-fold compared to galanin and has (just like galanin) anxiogenic actions [176,189]. Of the receptors known, GALP has highest affinity to the GalR2. However, since galanin and GALP show equal affinity to this receptor, it is not known whether this is receptor implicated in GALP-induced hyperphagia. It might be that another unknown receptor is involved [176].

*Ghrelin* is a 28 amino acids acylated orexigenic peptide, first isolated from the stomach, which stimulates the release of growth hormone from the pituitary, distinct from the regulation via growth hormone-releasing hormone (GHRH) [143]. Ghrelin is produced in the oxyntic glands of the stomach, and to a lesser extent in the pancreas, kidney, hypothalamus (ARC), and pituitary [49,194]. In the periphery, ghrelin influences pancreatic function, glucose metabolism, gastric motility, and gastric acid secretion [49]. The release of ghrelin is decreased in a situation of a positive (obesity) energy balance, and increased during fasting (anorexia nervosa) [226,284,287]. Central chronic administration of ghrelin to rats results in an increase in cumulative food intake and body weight. It stimulates NPY and AgRP expression in the hypothalamus, without affecting glucose and leptin levels [77,129,200,315]. Central ghrelin administration also results in an increase of fat mass without affecting longitudinal skeletal growth and a decrease in energy expenditure [110,287]. In addition, ghrelin administration leads to a rapid increase of ACTH and a decrease in plasma TSH levels [316]. Ghrelin is the endogenous ligand for growth hormone secretagogues 1A receptor (GHSR), which is expressed in various regions of the brain and is co-localized with NPY in the ARC [143]. Further actions of ghrelin on food intake are probably mediated by functional antagonism of leptin action through the activation of the NPY1-receptor pathway [207,261].

## 5. Concluding remarks

All neuroendocrine and behavioral responses, including food intake, are the result of an integrated response of neural circuits using a variety of signaling molecules. In this review we gave an overview of the main neuropeptides involved in the food intake and body weight regulation. We focused on peptides in the hypothalamus. The hypothalamus contains an integrated neural circuit that uses partly redundant orexigenic and anorexigenic pathways. It is the main brain-controlling center of energy homeostasis. However, it should be clear that far more signaling molecules and brain areas are involved in the regulation of food intake and body weight than discussed in this review.

Feeding behavior appears to be a complicated phenomenon involving several aspects like looking for food/hunting, rewarding properties, motivation and selection. We summarized that several hypothalamic neuropeptides play a role in this complex behavior. Due to its complexity, feeding behavior can be divided into (at least) two phases; the appetitive phase, which involves searching for food and approaching the food, and the consummatory phase, which involves the real eating. Consequently, the way two orexigenic peptides stimulate food intake can be totally different. For instance, NPY initiates food intake immediately, while AgRP does not. Caution has to be taken in analyzing data on food intake, since reduced food intake can easily be caused by inexplicit side effects. Visceral illness including nausea and stress can easily reduce food intake. However, this does not necessarily mean that sick rats do not eat. Sick rats display pica, meaning that they repetitively eat inedible compounds.

In this review we focussed on the role of the hypothalamus in food intake and body weight regulation. The identification of leptin, and its downstream signaling pathways, provided the hardware underlying regulation of food intake. POMC and CART are the main hypothalamic anorexigenic neuropeptides, which are co-localized in the ARC, and project to secondary anorexigenic neuropeptides in the PVN, like TRH and CRH. NPY and AgRP are the most important hypothalamic orexigenic neuropeptides, which are also co-localized in the ARC (Fig. 1). These neurons project amongst others to secondary neurons in the PVN and LHA, which contain the orexigenic MCH, orexins, and galanin.

We reviewed that several neuropeptides either stimulate food intake or decrease food intake (Table 2). The availability of knockout mice of several neuropeptides and neuropeptide receptors could lead to a better understanding of the hypothalamic regulation of food intake and body weight. However, in some cases (e.g. the NPY  $-/-$  mouse) knocking out important genes did lead to unexpected intriguing results (Table 3). With the idea in mind that the hypothalamus contains several (orexigenic) neuropeptides, it could be possible that the brain adapts to the knockout situation and that other (redundant) peptides take over the role of the specifically deleted neuropeptide. Therefore, the application

of conditional knockouts may clarify the role of some neuropeptides in feeding behavior in future.

Future research should be focused on a further characterization of the role of these peptides in feeding behavior. It appeared that several orexigenic peptides enhanced all aspects of food intake, but the real difference between these orexigenic peptides is unknown. Therefore, food intake needs to be investigated by specifically altering the circumstances to understand the role of the individual neuropeptides on food intake. For instance, by presenting an enriched environment, by introducing stressful situations, by using different methods of food deprivation prior to food access or by changing the type of food (palatability, taste).

A more precise phenotypic analysis of data during the experiment could teach us much more about the exact role of these neuropeptides in feeding behavior in future. Therefore, we suggest concentrating on *context and state, interactions between signaling molecules and functional neuroanatomy* in future research.

Thus, when examining the effect of neuropeptides on feeding behavior, *context and state* of the organism should be taken into account. The effects of each signaling molecule depend on these two characteristics. For instance, the effects of leptin on CRH are dependent on the nutritional state of the animal [4,115]. As well, the orexigenic NPY is more involved in the appetitive phase of food intake than the consummatory phase of food intake [6].

Another issue to take into account is that neuropeptides often display their effect by means of an *interaction with other signaling molecules*. For instance, the acute effect of AgRP on food intake is blocked by the opioid receptor antagonist naloxone [96]. Recently, it was described that the melanocortin agonist MTII potentiates amphetamine reward [35]. This effect is most likely caused by direct interactions between the melanocortin system and dopamine [35]. Thus, the melanocortin system is linked to both the opioid system and the dopamine system.

Another area, which may clarify the role of neuropeptides, is *functional neuroanatomy*. By linking neuroanatomy to behavior, the integrated response of food intake regulation may be better understood. By using modern techniques like viral (pseudorabies) retrograde neuronal tract tracing [53], neuronal (hypothalamic) circuits could be further unraveled. In addition to the above, the neuroanatomical overlap between the projections of the melanocortin system and of the dopamine system [2] could be used to design experiments directed towards understanding the rewarding properties of food intake. The application of inducible knockout strategies, that enables one to address questions like: “What happens when leptin receptors are only absent in NPY-ergic neurons?” or “What is the role of MC4-receptors on CRH-producing cells?”, will provide an important step forward in unraveling how neuropeptides influence food intake and energy balance.

Clearly, another important contribution to this field of research will come from the Human Genome Project. We

expect that people will be identified that carry variants of neuropeptide and neuropeptide receptor genes that strongly affect phenotype. These individuals can be investigated not only physically but also psychologically. It will be tremendously important to discover how individuals carrying mutations in the NPY gene or the MC4 gene deal with their body weight and appetite. In this way, information on the ‘real’ players in the control of food intake and body weight might be obtained, which will lead to a better understanding of the complexity of energy homeostasis. In the end this may provide us the instruments for the prevention of disorders like obesity and anorexia nervosa.

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