

The Hypothalamic–Pituitary–Adrenal Axis in the Neuroendocrine Regulation of Food Intake and Obesity: The Role of Corticotropin Releasing Hormone

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The aim of this paper is to review the present knowledge on the role of the hypothalamic-pituitary-adrenal axis in the control of food intake and the pathogenesis of obesity and to discuss, on the basis of available literature, the interactions between other neurosystems and this hormonal axis. Food intake is influenced by a system of physiologic signals and behavioral controls consisting of positive and negative sensory feedback mechanisms. It is regulated by a complex neuroendocrine system consisting of peripheral signals (cortisol, leptin) in constant interplay with central neurosystems such as the cocaine-amphetamine-regulated transcript system. In these neurosystems, corticotropin-releasing hormone, pro-opiomelanocortin, melanin-concentrating hormone and neuropeptide Y are actively involved. The corticotropin-releasing hormone system is widely distributed throughout the brain, but it is particularly abundant in the medial parvocellular division of the paraventricular nucleus. Within the brain corticotropin-releasing hormone with its two receptor types, its binding protein and its closely related peptide urocortin forms a network of neuronal pathways capable of interacting with other circuitries controlling food intake and sympathetically-mediated thermogenesis. A defect in the synthesis and release of corticotropin-releasing hormone has been implicated in the development of obesity in laboratory animals. This condition is alleviated by exogenous corticotropin-releasing hormone treatment. The relationship between the neuropeptide Y system and the hypothalamic-pituitary-adrenal axis is complex and seems to include positive feedback between neuropeptide Y and corticosteroids and negative feedback between corticotropin-releasing hormone and neuropeptide Y. Leptin is involved in the regulation of energy balance by interacting with the hypothalamic-pituitary-adrenal axis. In the past, we have shown by cross-correlation analysis, that under physiological conditions cortisol

and plasma leptin levels are related to each other in a time-related negative and positive fashion over 24 h.

Keywords: CRH; HPA; Food intake; Leptin; Obesity; NPY

NEUROENDOCRINE REGULATION OF FOOD INTAKE (FIG. 1)

There are three primary neuroendocrine components that control the regulation of food intake (Lustig 2001): the afferent peripheral system that is stimulated in response to a meal; the central nervous system (CNS) food intake integrating unit; and the efferent system.

The afferent system involves numerous hormonal and neural signals. In the periphery, the hormones insulin (secreted by the endocrine pancreas in proportion to body adiposity), leptin (secreted by adipocytes) and ghrelin link the control of peripheral energy metabolism to the CNS feeding behavior integrating unit by modulating short term signals that determine meal initiation and termination as well as energy balance. In contrast to the timing of meal initiation, which may be influenced by several variables (emotional factors, palatability of foods), meal termination tends to be a more biologically controlled process (Schwartz *et al.*, 2000). Satiety signals include gut hormones (bombesin, cholecystokinin) while signals that provide short-term information about hunger and satiety include gut hormones, such as glucagon-like peptide 1, cholecystokinin.

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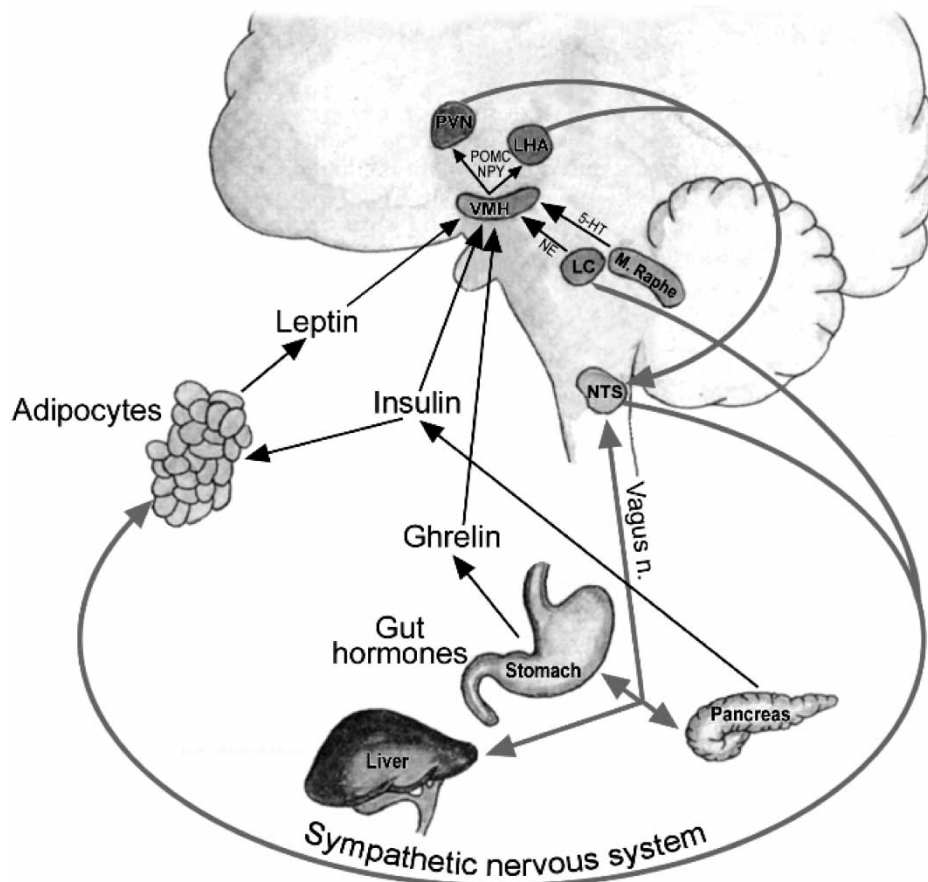


FIGURE 1 Neuroendocrine regulation of energy balance. Afferent neural and hormonal signals are generated from the liver, gut, pancreas and adipose. These signals are interpreted by the nucleus tractus solitarius (NTS) and the central nervous system food intake integrating unit located in the ventromedial hypothalamus (VMH). These signals are integrated in the paraventricular nucleus (PVN) and lateral hypothalamus (LHA). Efferent signals from these areas in turn stimulate either the sympathetic nervous system to expend energy by activating β_3 -adrenergic receptors in peripheral adipose tissue or the parasympathetic nervous system (efferent vagal) to increase insulin secretion, with resultant adipogenesis and energy storage (LC, locus ceruleus; 5-HT, serotonin; NE, norepinephrine).

ghrelin and peptide YY₃₋₃₆ (Korner and Leibel 2003). Furthermore, leptin may potentiate the action of other short-term satiety signals (Lusting 2001). These signals are transmitted through the vagus nerve and sympathetic fibers to the nucleus of the solitary tract (NTS). Endocrine signals are integrated through neural pathways in specific regions of the hypothalamus and the brain stem.

The CNS food intake integrating unit is located in the ventromedial hypothalamus (VMH) (comprising the ventromedial and arcuate nuclei), the lateral hypothalamus (LHA) and the paraventricular nucleus (PVN). The VMH receives and integrates information from many sites of the brain resulting to coordination of feeding behavior and energy balance. The arcuate nucleus is the site of pro-opiomelanocortin (POMC) and α -melanocyte-stimulating hormone (α -MSH) (a cleavage product of POMC) production, as well as of cocaine-amphetamine-regulated transcript (CART) pericarya. The binding of α -MSH to the melanocortin receptor (MCR) 4 in the PVN induces anorexia while the intracerebroventricular (icv) administration of

MCR4 antagonists stimulates feeding (Lusting 2001). Similarly, the intrahypothalamic infusion of CART has anorexigenic effects. Neuropeptide Y (NPY) and Agouti-related protein (AgRP) have an orexigenic effect in the hypothalamus and they lead to increased food intake. Neuropeptide Y and AgRP neurons colocalize with Ghrelin receptor within the arcuate nucleus suggesting that Ghrelin might exert orexigenic effects by binding to receptors on NPY/AgRP neurons. Furthermore, other neurotransmitters such as dopamine and gamma-aminobutyric acid (in the dorsomedial, arcuate nuclei and posterior hypothalamus), and neuropeptides such as met-enkephalin, melanin-concentrating hormone (MCH) and galanin (in the PVN and LHA) have been shown to influence both food intake and energy balance (Schwartz *et al.*, 2000).

The efferent system is represented by a complex of effectors organized into appetite/satiety, autonomic, thermogenic and motor components. A defect in the synthesis or release of various neuropeptides or disruptions in any of the three components can lead to the development of obesity (Rothwell 1990). It has

been shown that lesion in the LHA (feeding center) causes aphagia, and lesion in the VMH (satiety center) causes hyperphagia and obesity (Hetherington and Ranson 1940; Anand and Brobeck 1951).

CORTICOTROPIN-RELEASING HORMONE (CRH)

Corticotropin-releasing Hormone and the Hypothalamic–pituitary–adrenal (HPA) Axis

Corticotropin-releasing hormone (CRH) is a 41-amino acid peptide, which shows considerable interspecies homology at the amino terminal region and acts as the major physiologic secretagogue of corticotropin hormone (ACTH) (Rivier *et al.*, 1982, Vale *et al.*, 1983). There are two types of G protein-coupled CRH receptors (R) subtypes; type 1 and 2 (CRH-R1 and CRH-R2, respectively) with seven transmembrane domains each. Their genes are located on chromosome 17q12. The CRH-R1 has higher affinity to CRH and is mainly found in the pituitary and the cerebral cortex (Hillebrand *et al.*, 2002). The CRH-R2 is primarily found in the limbic regions and has at least three splice variants (CRH-R2 α , -R2 β and -R2 γ) which have lower affinity to CRH (Hillebrand *et al.*, 2002). Both receptors are located in the cerebellum, brainstem and hypothalamus. Corticotropin-releasing hormone belongs to a family of peptides with similar activity, such as sauvagine, urotensin I and urocortin (Spiess *et al.*, 1981, Shibahara *et al.*, 1983, Donaldson *et al.*, 1996). This new family of neuropeptides has been identified recently in the rat brain and termed CRH family. Urocortin (UCN) I consists of 40 aminoacids and it is differently distributed in the CNS from CRH (Vaughan *et al.*, 1995). It is expressed in the Edinger–Westphal nucleus, the lateral superior olive, the LHA, but also in the digestive system and endocrine organs. Urocortin I binds to both CRH receptors and has more affinity for the CRH-R2 than CRH itself (Vaughan *et al.*, 1995). Urocortin II is also a member of the CRH family and consists of 38 aminoacids. Similar to CRH, UCN I and II decrease food intake and increase energy expenditure. The single CRH and UCN genes are located in humans on chromosomes 8 and 2, respectively. Initially CRH is synthesized as a larger precursor molecule (191 amino acids in humans) from which it is cleaved at flanking basic amino acid pairs (Swanson *et al.*, 1983). Corticotropin-releasing hormone is synthesized by neurons of the parvocellular hypothalamic PVN and is secreted along with other ACTH secretagogues, such as arginine vasopressin (AVP), cholecystokinin, met-enkephalin and dynorphin into the hypophyseal portal blood via their projecting axons to the median eminence. Corticotropin releasing hormone is also

synthesized by anterior pituitary corticotroph cells where it stimulates ACTH secretion in an autocrine or paracrine fashion (Giraldi and Cavagnini 1998). The magnocellular PVN neurons, which project to the posterior pituitary, also contain CRH-synthesizing neurons (Sawchenko *et al.*, 1984). Moreover, CRH is distributed in the brain and spinal cord. The plasma half-life of CRH in human is 4 min (Schurmeyer *et al.*, 1984). Another protein which is involved in CRH signaling is CRH binding protein (CRHbp) (Richard *et al.*, 2000). This protein inactivates CRH by binding with it. Corticotropin releasing hormone binds to type 1 CRH receptor of the anterior pituitary corticotrophs (Chen *et al.*, 1993), resulting in adenylcyclase activation. The result is the secretion of ACTH and other POMC-derived peptides within a few seconds (Watanabe *et al.*, 1987), while increased POMC gene transcription and POMC biosynthesis ensue (Lundblad and Roberts 1988). The number of CRH-R in corticotroph cells may modulate the ACTH response. Immobilization stress, adrenalectomy, the administration of CRH, AVP or glucocorticoids (Wynn *et al.*, 1985) reduce the number of CRH-R in the anterior pituitary, though not in the brain. An additive effect of CRH and AVP on CRH-R expression has been noted (Hauger and Aguilera 1993).

In anterior pituitary ACTH released by CRH leads in its turn to stimulation of the adrenals and secretion of cortisol and other adrenal steroids, such as dehydroepiandrosterone (DHEA) and, transiently, aldosterone (Conaglen *et al.*, 1984). Although no sex or age differences in plasma ACTH or cortisol response to CRH have been noted (Pavlov *et al.*, 1986), plasma DHEA response is reduced in elderly men. In obese subjects plasma cortisol response is blunted (DeCherney *et al.*, 1985). Additionally, although ACTH response to CRH is not influenced by the hour of day, the corresponding cortisol response is maximized in late afternoon. The plasma ACTH response to CRH varies inversely with the basal plasma cortisol concentration.

The HPA Axis and the Stress Response

The term stress describes the state of the organism under the influence of external or internal forces, or stressors, threatening to alter its dynamic equilibrium or homeostasis. The adaptive changes occurring in response to stressors are both behavioral and physical. Once a certain threshold has been exceeded, a systemic reaction takes place that involves the “stress system” in the brain along its peripheral components, the HPA axis, and the autonomic sympathetic system (Chrousos 1992). The central players of these components of this system are CRH and AVP neurons in the PVN and other brain areas and the locus ceruleus (LC)/norepinephrine (NE) and central autonomic sympathetic system in the brainstem.

The principal central nervous system regulators of the HPA axis are CRH and AVP. The secretion of CRH is regulated by inputs from higher centers, on which the effects of the circadian pacemaker, stress and glucocorticoid negative feedback are superimposed (Mastorakos and Ilias 2003). The glucocorticoid negative feedback acts at pituitary, hypothalamic and higher levels, such as the hippocampus (Chrousos, 1995). Corticotropin-releasing hormone and CRH receptors were found in parts of the limbic system and the LC/NE-sympathetic system in the brain stem and spinal cord. Except from the axons of CRH neurons that terminate in the median eminence and secrete CRH into the hypophyseal portal system there are axons of these neurons that terminate in the LC/NE-sympathetic system neurons in the brainstem. Neurons of this system send projections, mostly noradrenergic, to the PVN. Thus, there are reciprocal interactions between the CRH neurons and those of the LC/NE-sympathetic system with CRH. There is also parallel regulation of both of the central components of the stress system by both stimulatory and inhibitory neurotransmitters and modulators (Tsigos and Chrousos 1994). Serotonin, acetylcholine, catecholamines ($\alpha 1$ receptors) and NPY, in other words, serotonergic and cholinergic systems, stimulate CRH, AVP and noradrenergic neurons, whereas γ -aminobutyric acid (GABA)/benzodiazepine system and endogenous opioid peptides system of the brain inhibit them. Centrally secreted substance P inhibits hypothalamic CRH neurons but not AVP neurons and stimulates the central noradrenergic system (Larsen *et al.*, 1993). Glucocorticoids released from the adrenal cortex in response to ACTH exert negative feedback effects not only on the pituitary ACTH secretion but also on the hypothalamic CRH neuron and the LC/NE-sympathetic system. Regulatory opioid peptides are produced by the arcuate nucleus POMC neurons that produce ACTH, α MSH and β -endorphin, all of which are inhibitory to CRH secretion and by CRH and AVP neurons which co-secrete dynorphin along with CRH and AVP.

ROLE OF CRH IN THE REGULATION OF FOOD INTAKE AND OBESITY

Central Effects of CRH on Food Intake

Corticotropin-releasing hormone influences food intake, cardiovascular and gastrointestinal function as well as the inflammatory process. Corticotropin-releasing hormone is a potent anorexigenic peptide and stimulates sympathetically-mediated thermogenesis and lipolysis (Hillebrand *et al.*, 2002). Series of experiments in rats have shown that chronic icv administration of CRH suppresses food intake via

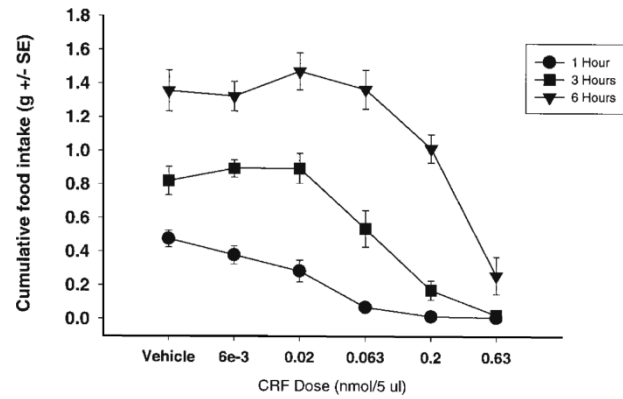


FIGURE 2 Effects of CRH (0.006–0.6 nmol/5 μ l; intracerebroventricularly) on cumulative food intake in 16-h food-deprived female CD1 mice 1, 3, and 6 h after injection (with permission from Pellemounter *et al.* (2000)).

stimulation of the synthesis and secretion of POMC-related peptides in the pituitary and suppresses food intake (Hotta 1991). Intracerebroventricular administration of CRH at non-pharmacological doses into the PVN, in female CD-1 mice, inhibits nighttime and fasting-induced feeding (Pellemounter *et al.*, 2000) (Fig. 2). The inhibition is significant from 0.063 nmol/5 μ l CRH icv.

Corticotropin-releasing hormone R2 have recently been implicated in the appetite suppressant effects of CRH. It seems that CRH-R2 has a central role on food intake. Treatment with CRH-R2 antisense attenuates the effects of CRH on food intake whereas treatment with the CRH-R1 antagonist NBI-27914 does not alter the appetite-suppressing effects of CRH (Smagin *et al.*, 1998). The CRH-R2 selective antagonist antisauvagine-30 attenuates CRH-induced appetite suppression as well as the behaviors associated with CRH-induced appetite suppression when administered to female CD-1 mice (Pellemounter *et al.*, 2000). Other studies questioned the CRH-R2 role in the food intake regulation attributing the anorexigenic effects of CRH to CRH-R1 activation (Richard *et al.*, 2000).

Corticotropin-releasing Hormone and the HPA Axis in Obesity

There are findings suggesting that obesity is associated with decreased hypothalamic CRH. The administration of a CRHbp inhibitor to obese fa/fa zucker rats can efficiently reduce weight gain via unbound-CRH increase (Heinrichs *et al.*, 1996). A reduction in the expression of the CRH-R2 α mRNA within the VMH has been also reported in obese fa/fa zucker rats (Timofeva and Richard 1997). However, it is not clear whether this reduction is translated into a reduction of CRH activity that could reduce energy expenditure. Other authors have proposed that in the obese mutant Zucker rat,

the activity of the hypophysiotropic CRH neurons is increased inducing an increased activation of the HPA axis and increased levels of corticosterone, whereas that of a CRH subsystem within the brain more restrictedly involved in the regulation of energy balance seems to be reduced (Richard *et al.*, 2000). However, the mechanisms by which the activity of the CRH system could be reduced in obesity remain to be elucidated. It must be mentioned, though, that the Zucker rat has other known deficits which might intervene and obscure the interpretation of these studies.

On the other hand, there is increasing evidence that, in humans, abdominal obesity phenotype may be characterized by a hyperactivation or hyperresponsiveness of the HPA axis (Vicennati *et al.*, 2002). There are data in obese women indicating an association between urinary and serum cortisol and the waist-to-hip ratio (WHR) (Marin *et al.*, 1992). If hypercortisolemia results to abdominal obesity as it is the case in endogenous hypercortisolism (Cushing syndrome) one might incriminate environmental and other stressors as potential factors in abdominal obesity. It has been reported that, when adipocytes retrieved from sc fat from healthy nonobese individuals, were incubated *in vitro* with CRH, 11 β -hydroxysteroid dehydrogenase (11 β -HSD)-1 activity was downregulated reducing cortisone to cortisol conversion and reducing adiposity. Thus, it seems that CRH, acting as a hypothalamic mediator, inhibits adipose tissue 11 β -HSD-1 activity. This enzyme is responsible for conversion and activation of cortisone to cortisol. Hypothalamic obesity caused by damage of the hypothalamus might be related to CRH deficiency leading to enhanced conversion of cortisone to cortisol (Friedberg *et al.*, 2003). Therefore, it seems, that the CRH system in the CNS is anorexigenic, it increases energy expenditure and adipose tissue oxidation, whereas cortisol as an effector of the HPA axis activity is orexigenic (via central NPY stimulation). Furthermore, it inhibits hypothalamic CRH and it facilitates the adipose tissue deposition.

Based on other than the Zucker rat models such as dietary induced obesity or models involving HPA axis and the CRH system Dallman *et al.*, have proposed a new model of chronic corticosteroid effects in intact and adrenalectomized rats. They have suggested that direct chronic actions (duration over 24h) of corticosteroids on brain are stimulatory on the HPA axis and that the negative feedback inhibition of this axis results from the metabolic effects of glucocorticoids increasing abdominal energy stores. According to the authors, the mesenteric fat stores provide a to-date unidentified feedback signal to the brain to reduce HPA axis activity. It seems that the abdominal energy generated unidentified signal inhibits catecholamine

neurons in the NTS. The decreased catecholamine action is translated to decreased CRH synthesis and secretion in the PVN. Thus, there is a powerful metabolic feedback control of CRH in the PVN whereas the abdominal generated signal does not appear to affect CRH in the amygdala (Dallman *et al.*, 2003).

In addition, the same study demonstrated that stress levels of corticosterone treatment in adrenalectomized rats specifically increase consumption of palatable foods "comfort foods" which when is nutritious increases mesenteric but not subcutaneous fat depots. Similar effects occur in intact rats exposed to the chronic stressor of cold (Bell *et al.*, 2002).

This model of the effect of chronic stress in food intake and the HPA axis activity may apply to humans. It is possible that people with eating disorders and depression consume comfort food in an attempt to feel better by reducing hypothalamic CRH activity, by increasing their abdominal metabolic signal (Dallman *et al.*, 2003).

NPY AND HPA AXIS

Neuropeptide Y is an orexigenic neuropeptide playing a central role in the regulation of food intake. Its relationship to HPA axis is complex. The important role of corticosteroids in the control of NPY activity is well known. Neuropeptide Y stimulates the HPA axis primarily at the hypothalamic level and it leads to increased CRH synthesis in the PVN and CRH release in the median eminence (Krysiac *et al.*, 1999). A negative feedback exists between CRH and NPY. On the other hand, there is a positive feedback between adrenal corticosteroids and NPY. It has been shown that in obese Zucker rats or humans with eating disorders or exposed to stress, the positive feedback between corticosteroids and NPY may produce a "vicious circle" of events. Increased plasma corticosterone concentrations in obese Zucker rats results in increased NPY mRNA and NPY concentrations in the arcuate and paraventricular nuclei leading to increased NPY release (Guillaume-Gentil *et al.*, 1990). In turn, NPY increases via positive feedback plasma corticosterone concentrations. Adrenalectomy, or hypophysectomy in these rats reduce most symptoms of obesity (Powley and Morton 1976).

LEPTIN ACTIONS IN THE HYPOTHALAMUS (FIG. 3)

Leptin is a 167 aminoacid protein isolated in 1994, secreted in a pulsatile fashion by adipocytes. Its secretion demonstrates a nycthemeral rhythm (Zhang *et al.*, 1994). Leptin mediates information to the VMH about the size of peripheral adipocyte

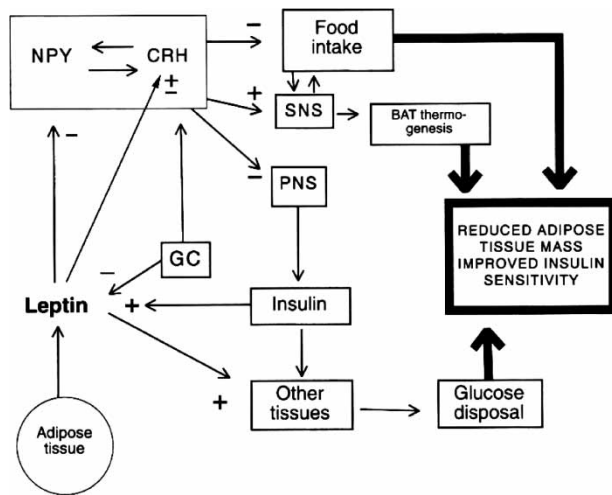


FIGURE 3 Heuristic representation of the leptin, NPY, CRH interplay with peripheral tissues and mechanisms involved in energy expenditure. The \pm symbol on the arrow between leptin and CRH indicates the discrepancy of literature data between laboratory animals and humans regarding the action of leptin on CRH. (NPY, neuropeptide Y; CRH, corticotropin-releasing hormone; GC, glucocorticoids; SNS, sympathetic nervous system; PNS, parasympathetic nervous system; BAT, brown adipose tissue).

energy stores. Food intake or increased adiposity increases serum leptin concentrations resulting in reduced appetite and increased energy expenditure. Low serum leptin concentrations during the post-absorptive (fasting) state or during weight loss result in increased appetite and food intake and in reduced energy expenditure (Roemmich and Rogol 1999). Accumulated evidence strongly suggests that leptin exerts its action by modifying the synthesis and secretion of a large number of both anorexigenic and orexigenic neuropeptides. Leptin sensitive neurons include those that produce NPY, AgRP, MCH, neurotensin (NT), CART, CRH and α MSH. In similar to CRH way, leptin reduces energy intake and stimulates thermogenesis, which is consistent with the possibility that in rodents the effects of leptin on energy balance are mediated by the CRH system (Richard *et al.*, 2000). It seems that leptin in mice increases CRH mRNA expression and CRH release in the PVN (Burdakov *et al.*, 2003). The anorexigenic effect of leptin is attenuated by the administration of the CRH antagonist α -helical CRH₉₋₄₁ (α CRH) (Ahima *et al.*, 1996; Huang *et al.*, 1998; Masaki *et al.*, 2003) or by administration of CRH antibody (Gardner *et al.*, 1998). Recent demonstration of the attenuating effects of α CRH on leptin-induced c-fos expression in the PVN and VMH supports the importance of CRH in leptin signaling in the hypothalamus (Masaki *et al.*, 2003). More evidence of the role of CRH in mediating leptin action comes from the study of another neuropeptide, NT. Neurotensin is an anorectic signal localized in hypothalamic areas implicated in the regulation of food intake and energy balance. Recent

experiments by Sahu *et al.*, have demonstrated that daily icv injection of leptin significantly increased NT expression in the hypothalamus (Sahu 1998a,b). Other studies have shown that leptin induced NT gene expression in a hypothalamic cell line (Cui and Belsham 2003). Interestingly, NT stimulates CRH neurons activity (Nicot *et al.*, 1997). Furthermore, we have recently shown that cortisol and leptin are related to each other in a time-related negative and positive fashion (Ghizzoni *et al.*, 2001). More specifically, we have examined the interactions between leptin and cortisol, we analyzed and time-cross-correlated their spontaneous 24 h secretion in 12 short normal prepubertal children of both sexes (6 females and 6 males). Time-cross-correlation analyses demonstrated that the negative correlation with cortisol leading leptin by 4 and 3 h for boys and girls, respectively, might reflect the stimulatory effect of CRH on the sympathetic system, which inhibits leptin secretion, while the positive correlation with leptin leading cortisol by 6 and 5 h for boys and girls, respectively, might reflect a direct effect of leptin on CRH secretion in the hypophyseal portal system. Thus, it seems that under baseline physiologic conditions, the HPA axis has a prevailing inhibitory effect on leptin secretion, while leptin seems to exert a positive effect on the HPA axis. In total, this evidence strongly suggests that the interplay of CRH and leptin has an important role in food intake regulation.

Depending on the nutritional state of the animal, leptin increases CRH expression and the activity of the CRH neurons in the fed state and decreases them in the fasted state. The administration, however, of replacement doses of r-metHuLeptin, a leptin agonist, to healthy human volunteers in acute fasting did not modify the mild activation of the HPA axis suggesting species-specific differences in leptin regulation of the HPA axis as compared with rodents (Chan *et al.*, 2003).

Neuropeptide Y is one of the most important neuronal systems implicated in mediating leptin actions in the hypothalamus. Leptin decreases hypothalamic NPY gene expression (Sahu 1998a,b) and it opposes the action of NPY on feeding (Sahu 1998a,b). Neuropeptide Y neurons also express AgRP and, like NPY, AgRP overexpression results in obesity. Leptin decreases NPY and AgRP mRNA levels in the hypothalamus and by this mechanism may also enhance its anorectic effects (Ebihara *et al.*, 1999).

In rodents, leptin influences energy balance by modulating the activity of hypothalamic neurotransmitters and neuropeptides. Proopiomelanocortin producing neurons express leptin receptor (Cheung *et al.*, 1997). Leptin infusion induces the synthesis of POMC and α -MSH in the arcuate nucleus. Ob/ob mice have reduced POMC mRNA levels and leptin administration reduces this finding (Mizuno *et al.*, 1998).

α MSH is considered one of the most potent anorectic signals implicated in the control of food intake. It binds with high affinity to melanocortin receptors 3 and 4 (Cone *et al.*, 1996) which are highly expressed in the hypothalamus. Proopiomelanocortin neurons also express CART a potent inhibitor of food intake. CART mRNA is reduced in the hypothalamus in the leptin-deficient *ob/ob* mice and leptin normalizes CART mRNA in these animals (Kristensen *et al.*, 1998). However, chronic sc leptin infusion had no effect on POMC mRNA in *ad lib* fed rats (Ahima *et al.*, 1999). Other experiments have shown a small decrease (Sahu 1998a,b) or no change (Van dijk *et al.*, 1999) in hypothalamic POMC mRNA levels following central administration of leptin in *ad lib* fed rats. Furthermore, during fasting, systemic leptin administration increases the reduced levels of thyrotropic releasing hormone (TRH) gene expression in the PVN (Blake *et al.*, 1992; Legradi *et al.*, 1998). The synthesis and secretion of TRH in hypophysiotropic neurons in the PVN is affected by nutritional status. Moreover, icv infusion of α MSH recapitulates the effect of leptin on hypophysiotropic TRH neurons, and also reactivates CRH gene expression in the PVN of fasting animals (Fekete *et al.*, 2000). It has been proposed by Sarcar *et al.*, that the activation of melanocortin receptors by the administration of α MSH to fasting animals results in the phosphorylation of the transcription factor, (cAMP response element binding protein) CREB in the PVN. TRH as well as CRH promoters contain a consensus cAMP response element (CRE) suggesting that these genes are regulated by binding of CREB (Sarkar *et al.*, 2002). Recent *in vitro* evidence indicates that phosphorylated CREB (PCRRB) binds to the CRE in TRH promoter and activates the gene (Harris *et al.*, 2001).

Leptin exerts its actions by activation of the leptin receptor which is a member of the cytokine receptor superfamily on target VMH neurons. Four receptor isoforms of the leptin receptor (Ob) are formed by differential splicing; of these only one (Ob-Rb) is involved in signal transduction. This receptor is mainly expressed in the arcuate, in the VMH, PVN and in the dorsomedial (DMH) nuclei of the hypothalamus. The Ob-R is a cytokine receptor lacking intrinsic tyrosine kinase activity but activates a cytoplasmic janus kinase (JAK) which phosphorylates a tyrosine moiety on signal transduction and transcription (STAT) proteins of a family called STAT family resulting in alterations of gene transcription (e.g. POMC) (Kishimoto *et al.*, 1999). Among several STAT proteins leptin only activates STAT₃ in the hypothalamus (McCowen 1998). However, leptin can also influence neural activity by the induction of an insulin-like signaling pathway involving P13K-dependent activator of a phosphodiesterase (PDE 3B) and eventual reduction in cAMP levels (Zhao *et al.*, 1997). It has been suggested that a crosstalk between JAK2-STAT3 and P13K-PDE3B-cAMP

pathways might be critical for normal leptin signaling in the hypothalamus (Sahu 2003).

However, leptin can also influence neural activity by opening ATP-sensitive potassium channels which hyperpolarize glucose-responsive neurons in the hypothalamus (Spanswick *et al.*, 1997). Mutations of leptin receptor have been documented (Clement *et al.*, 1998) in obese patients. However, most obese patients have high leptin levels but do not have mutations. It has been proposed that in these patients might exist a form of leptin resistance (Banks *et al.*, 1996). There is evidence suggesting that central leptin resistance contributes to the development of diet induced obesity and aging-associated obesity. Among the possible mechanisms, a defective STAT3 signaling and or a defect in other pathways of leptin signaling, such as P13K-PDE3B-cAMP pathway have been suggested (Sahu 2003).

Insulin is the other hormone that fulfils the criteria for being an adiposity signal which acts in the hypothalamus to stimulate catabolic effector pathways. Insulin receptors are expressed by brain neurons involved in energy intake (Baskin *et al.*, 1988). Administration of insulin directly into the brain reduces food intake (Woods *et al.*, 1979). Different mechanisms underly the association of insulin and leptin with body fat content. The β -subunit of the insulin receptor has intrinsic tyrosine kinase activity, and activates intracellular signaling proteins—which are present in neurons and peripheral tissues—by phosphorylating them on tyrosine residues (Cheatham and Kahn 1995). Mutations of genes that encode neuronal proteins may result in obesity (Schwartz *et al.*, 2000). It has been suggested that energy homeostasis is regulated by both a lipostatic (adiposity signal) as well as glucostatic factors. It has been also suggested the involvement of “metabolic sensing neurons” in the CNS in the control of food intake and the possibility that the underlying etiology of obesity could be the result of environmental factors that alter the “sensing” of stored fuel or currently available fuel, or the integration of these two types of signal (Seeley and Woods 2003).

CONCLUSIONS

Many pathways served by neuropeptides and their receptors are interconnected within the brain to integrate the regulation of food intake. Food intake is influenced by a system of physiologic signals and behavioral controls consisting of positive and negative sensory feedback mechanisms. It is regulated by a complex neuroendocrine system consisting of peripheral signals (cortisol, leptin) in constant interplay with central neurosystems. The latter comprise CRH, POMC, MCH, NPY and the CART system. It seems, that the CRH system in the CNS is anorexigenic, it increases energy expenditure and

adipose tissue oxidation, whereas cortisol as an effector of the HPA axis activity is orexigenic (via central NPY stimulation). Leptin is involved in the regulation of energy balance by interacting with HPA axis. Chronic action of leptin in the hypothalamus inhibits POMC and CART production leading, thus, to a decrease of food intake. In addition, we have recently shown that cortisol and leptin are related to each other in a time-related negative and positive fashion. The relationship between the NPY system and the HPA axis is complex and seems to include positive feedback between NPY and corticosteroids and negative feedback between CRH and NPY.

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