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Recent Insights into Body Weight Control: From Physiology to Pathology

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Abstract: Over the past several years, new modulators of feeding and body weight have been discovered, and our knowledge of the mechanisms and neurohumoral interactions between anorexigenic and orexigenic compounds has increased dramatically. This review aims to summarize the present knowledge of the role of leptin and several hypothalamic neuropeptides, such as neuropeptide Y (NPY), corticotropin-releasing hormone (CRH) and melanocortins, in the regulation of appetite and body weight. It also presents the progress made in the design of interactions between leptin and hypothalamic peptides in the regulation of feeding. The role of these compounds in the pathogenesis of obesity in animals and humans, and their potential usefulness in the treatment of this disorder, are discussed.

Keywords: leptin; neuropeptide Y (NPY); corticotropin-releasing hormone (CRH); melanocortins; feeding; body weight; obesity

INTRODUCTION

Obesity is one of the most common and most important health problems in civilized societies because it is conducive to many diseases, such as diabetes mellitus, arterial hypertension, dyslipidaemias, alimentary and respiratory disorders, and some types of cancer, all of which decrease life expectancy and the quality of life [1]. The prerequisite for the development of effective antiobesity therapies is the understanding of the pathomechanism of obesity. The available data contradict the presence of a single system regulating energy homeostasis and they indicate that many endogenous compounds, which act both centrally and peripherally, are involved in this process [2]. The hypothalamus, a brain structure that plays the most important role in body weight control, is the main area where these compounds exert their actions. Disturbances of the hypothalamic processes regulating food intake and energy expenditure seem to be a major factor responsible for excess body weight [3]. The normal functioning of the hypothalamus depends on its response to changes in the energy balance of the body, the process being mediated mainly by intestinal peptides and the satiety factor leptin which is released by adipose tissue and was isolated in 1994 [4]. Although for many years bioamines were thought to play a key role in the hypothalamic regulation of hunger and satiety, recent studies have been focused on peptides [3,5].

This review discusses the role of leptin and hypothalamic peptides in the regulation of body weight in mammals. Special attention is paid to

Abbreviations: AgRP, agouti-related protein; ASP, agouti signalling protein; CARTs, cocaine- and amphetamine-regulated transcript peptides; CRH, corticotropin-releasing hormone; CRH-BP, CRH binding protein; GLP-1, glucagon-like peptide; MCH, melanin concentrating hormone; NPY, neuropeptide Y; POMC, Pro-opiomelanocortin; SOCS-3, suppressor of cytokine signalling-3.

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BIOGRAPHIES

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All authors work in the Department of Clinical Pharmacology, Medical University of Silesia. Robert Krysiak (see photograph) is concerned with the physiological role of neuropeptides in the central nervous system and their potential contribution in the mechanism of action of antiobesity and psychotropic drugs. Both Boguslaw Okopień M.D. and Andrzej Madej are interested in the treatment of obesity and metabolic disorders. Dariusz Belowski is engaged in studies on the role of various peptides, especially cytokines, in pathogenesis and the treatment of mental disorders. Zbigniew S. Herman is the Head of the Department.

neuropeptide Y, corticotropin-releasing hormone and melanocortins, whose role in the regulation of food intake and energy expenditure and in the development of obesity is best known, and which, in the light of recent studies, are likely to become targets for new antiobesity drugs.

LEPTIN

Leptin is a 167 amino acid hormone synthesized mainly in white fat tissue and to a lesser degree in brown adipose tissue, the placenta and the stomach [6,7]. It derives its name from the Greek word 'leptos', which means 'lean'. The synthesis of leptin is dependent on the expression of the *ob* gene regulated by many endogenous compounds. Insulin and glucocorticosteroids decrease *ob* gene expression, while norepinephrine, epinephrine and the artificial catecholamine, isoprenaline, increase it by stimulating the β_3 receptor [7].

The most important effect of leptin is the inhibition of appetite by acting on the hypothalamus. Disturbances of leptin transmission underlie the most important animal models of obesity, such as *ob*/*ob* mice, *db*/*db* mice, obese Zucker rats (*fa*/*fa*) and Koletsky corpulent rats [8]. In *ob*/*ob* mice, leptin is not synthesized due to a lack of *ob* gene expression [6,9]. Koletsky corpulent rats have no leptin receptor [10]. In *db*/*db* mice, the absence of the cytoplasmic component of the leptin receptor disturbs signal transmission at the second messenger level [11]. In obese Zucker rats, a point mutation alters the structure of the extracellular domain of the leptin receptor, thus decreasing the hypothalamic receptor's response to leptin [12].

The mechanism of leptin action also comprises the stimulation of the sympathetic system and potentiation of its effector action [13]. According to the MONA LISA (Most Obesities Known Are Low In Sympathetic Activity) hypothesis, which suggests that most obesity cases result from low sympathetic activity, the peripheral action of leptin may play an important role in body weight control [13].

These experimental data are only partly confirmed by clinical studies. A hitherto unexplained finding is that obese persons usually have increased plasma leptin levels, which correlate with the fat content and body mass index (BMI) [6]. This does not seem to result from an increased plasma protein-bound leptin fraction, because obese persons have more free leptin than controls [14]. Moreover, plasma leptin levels are generally lower in men than in women, probably due to the role of sex hormones, especially testosterone, in the regulation of *ob* gene expression [6,15].

Leptin deficiency seems to be a rare cause of obesity in humans. Nevertheless, such cases have been reported [16], and one can expect that leptin would be highly effective in these patients. It is most likely that the more common cause of obesity in humans is hypothalamic leptin resistance, which is thought by some authors to account for 5% of cases of obesity [15]. Drawing an analogy between leptin resistance and insulin resistance and taking into account the fact that obese persons probably show partial leptin resistance, it may be supposed that eventually high doses of leptin may be effective in these cases. This suggestion is supported by the fact that high intracerebroventricular injections of leptin effectively decrease the body weight and hypothalamic NPY levels in obese Zucker rats [12]. As with other cytokine receptors, stimulation of the leptin receptor induces the expression of a protein that inhibits leptin signalling. This protein is termed 'suppressor of cytokine signalling-3' (SOCS-3) [17]: its role in acquired leptin resistance is currently under intensive study.

Caro *et al.* [18] have found that despite a great difference in plasma leptin levels between obese and lean persons (about 300%), the leptin levels in the cerebrospinal fluid of obese persons were only slightly higher (by about 30%) than those in lean persons. This fact suggests that changes

in the permeability of the blood–brain barrier for leptin may also play a part in the pathogenesis of obesity [15]. Provided this is true, low-molecular weight lipid-soluble leptin analogues could be used for obesity therapy. Recent studies indicate that leptin acts as a sensor regulating the energetic balance of the body, and that when given with other drugs in the initial phase of antiobesity therapy, it may improve the efficacy of the therapy by inhibiting compensatory changes [15].

It should be stressed that leptin exerts its effect via hormonal and metabolic mechanisms, e.g. it inhibits the peripheral action of insulin and release of this hormone by β-pancreatic islet cells. Moreover, leptin decreases the secretion of glucocorticosteroids, and enhances fatty acid oxidation in muscle tissue [19].

Although the role of leptin in the pathogenesis of obesity is far from being completely understood because of the high costs of leptin therapy, a randomized double-blind study including 73 obese patients has shown that recombined leptin effectively reduces their body weight and fat mass [20]. There are high expectations that newly synthesized leptin analogues will be more stable and will penetrate the CNS better than does the native hormone.

NEUROPEPTIDE Y

Neuropeptide Y (NPY) is a 36 amino acid member of the pancreatic polypeptide family [21]. The hypothalamus is the brain structure in which the NPY concentration is the highest. Hypothalamic NPY is found mainly in interneurons whose perikarya are located in the arcuate nucleus, while their terminals are located in the periventricular nucleus. A small amount of NPY has been colocalized with norepinephrine in neurons projecting from the brainstem [21]. Among the many functions of NPY, such as the regulation of food intake, hormone (corticotropin-releasing hormone, thyroid-stimulating hormone, growth hormone, reproductive hormones, insulin, melatonin) synthesis and release, antianxiety and antiepileptic action, and the central regulation of the cardiovascular system [21], appetite control is the best documented. NPY is the most potent regulator of food consumption [22]. When compared with catecholamines, NPY exerts its action at a much lower range of doses, and the effect appears later and lasts much longer [23]. NPY stimulates mainly carbohydrate intake [24]. Its orexigenic effect was observed in both hungry and satiated animals, and it continued also when NPY was given on a long-term basis, indicating a lack of tolerance of this peptide [23]. The increased appetite in rats in which hypothalamic NPY release was increased by KCl administration [25] and the loss of appetite in rats devoid of NPY due to antisense oligonucleotide administration [26] or immunoneutralization [27] indicate that NPY is a endogenous orexigenic compound and that impaired NPY transmission may affect the appetite.

The periventricular nucleus and perifornical region [3,28] are the most important hypothalamic structures mediating the effect of NPY on the appetite. Increased NPY mRNA levels in the arcuate nucleus and increased extracellular and total NPY levels in the periventricular nucleus and other hypothalamic structures are observed in starved animals and in experimental models of obesity [23,28]. Circadian fluctuations in hypothalamic NPY system activity seem to be responsible for the circadian rhythmicity of appetite in rats [24]. Hypothalamic NPY system activity is stimulated by glucocorticosteroids and inhibited by insulin [24,29,30] and leptin [30]. In turn, NPY increases glucocorticosteroid and insulin levels [24,29,30], indicating that the interaction between glucocorticosteroids and NPY is a positive feedback loop, while the interaction between insulin and NPY makes a negative feedback loop.

NPY stimulates specific receptors, five of which have been cloned, namely Y_1, Y_2, Y_4, Y_5 and Y_6 [3]. Despite extensive research, it has been not unequivocally determined which receptor subtype is responsible for the orexigenic effect of NPY. Most data support the involvement of Y_1 and Y_5 receptors [31]. However, the effect of NPY on appetite cannot be explained exclusively by either Y_1 or Y_5 receptor stimulation. This indicates that feeding behaviour may be mediated by more than one receptor subtype or by a receptor subtype that has not been cloned yet [31]. Mice devoid of the NPY gene, and consequently, of NPY protein, show normal eating behaviour, indicating that NPY is not the only appetite-regulating system in the hypothalamus [32].

NPY-induced body weight gain results not only from alterations in appetite and hormone synthesis and release, but also from inhibition by NPY of the stimulation of the sympathetic system in brown adipose tissue, which inhibits thermogenesis. NPY also increases the activity of the lipogenic enzyme acetyl-CoA carboxylase and the *de novo* synthesis of fatty acids in the liver and white adipose tissue. The latter effect seems to be responsible

for the increase in triglyceride levels following NPY treatment. Moreover, NPY increases both glucose expenditure and lipoprotein lipase activity in white adipose tissue, thus stimulating lipid accumulation in adipocytes [9,23,28,30].

Much less is known about the role of NPY in appetite disorders in humans. The available data contradict the presence of Y_1 and Y_5 receptor gene polymorphism in persons of different body weights [33] and in the obese [34]. The complex nature of obesity, the relatively small numbers of cases studied, and the lack of data on other activities of NPY have resulted in the role of NPY in the pathogenesis of human obesity remaining obscure.

CORTICOTROPIN-RELEASING HORMONE

Corticotropin-releasing hormone (CRH) is a 41 amino acid peptide, whose the best known and most extensively studied function is the regulation of the hypothalamic–pituitary–adrenal axis [35].

When given intracerebroventricularly or into the paraventricular nucleus of the hypothalamus, CRH strongly inhibits appetite [36]. CRH abolishes increased appetite in rats with obesity caused by either genetic mutation or damage to the ventromedial hypothalamus [2]. Body weight loss in CRH-treated animals results also from increased energy expenditure due to increased sympathetic system activity [13]. The level of CRH mRNA in the periventricular nucleus increases in starved animals, while this parameter is decreased when the energy balance is positive [36]. Recently, a CRH-like peptide that reacts with CRH receptors, especially with CRH_2, has been discovered. This peptide, called urocortin, diminishes appetite and reduces body weight when given at doses that produce no CRH-related adverse effects [37]. There is evidence that CRH interacts with the serotoninergic system, NPY system and leptin to regulate body weight [38].

Decreased CRH_2 mRNA levels in the hypothalamus of starved or obese rats [38], inhibition of appetite by CRH and its agonists in mice lacking CRH_1 receptor gene expression [38], and inhibition of the anorexigenic effect of CRH by antisense oligonucleotides directed against CRH_2 mRNA [39], all indicate that this receptor mediates the effect of CRH on appetite, and plays a role in the pathogenesis of obesity.

A major problem associated with the use of CRH or its analogues in antiobesity therapy is that increased CRH activity is accompanied by increased levels of both adrenocorticotropic hormone (ACTH) and corticosteroids as well as behavioural changes, such as anxiety and depression, since endogenous CRH plays an important role in stress, anxiety and depression [35].

A new approach in the search for compounds that produce an anorexigenic effect by acting on the CRH system is the study of corticotropin releasing hormone binding protein (CRH-BP) [37]. This protein, when bound to CRH or urocortin, blocks some of their biological functions (including their influence on appetite), while it does not alter the influence of CRH on the activity of the hypothalamus–pituitary–adrenal axis, heart rate and blood pressure [40]. CRH-BP inhibitors increase the pool of the free CRH, but they do not change the level of total CRH, and they therefore inhibit appetite without giving any of the adverse effects of CRH [40].

MELANOCORTINS

Pro-opiomelanocortin (POMC) is a macromolecular glycoprotein that is converted by specific enzymes to biologically active compounds, such as ACTH, α-melanotropic hormone (α-MSH) and β-endorphin. The best known effect of α-MSH is skin pigmentation by stimulating the MC1 receptor [41]. It has recently been shown that α-MSH and its artificial analogue Melanotan II strongly inhibit appetite in mice and rats [42]. The increased appetite caused by the MC4 receptor antagonists SHU9119 [42], HS014 and HS024 [43], inhibition of the anorexigenic effect of Melanotan II by SHU9119 [42] and obesity-inducing hyperphagia in mice lacking MC4 receptor gene expression [44] prove that the MC4 receptor mediates the effect of α-MSH and other melanocortins on appetite. Since mice, in which MC4 receptor gene expression is blocked on only one chromosome, show increased appetite and body weight [44], it seems that even partial inhibition of the function of this receptor may play a role in the pathogenesis of obesity.

The melanocortin system is a rare example of a system in which endogenous antagonists are produced in physiological conditions. The best known antagonist is the so-called *agouti* protein, which when overproduced, induces hyperphagia and obesity accompanied by yellow-coloured skin and fur in mice [36]. Its human counterpart is the so-called *agouti signalling protein* (ASP) [45]. However, the utmost attention has been paid recently

to another melanocortin called *agouti-related protein* (AgRP) [5], which when given intracerebroventricularly, increases appetite, and its single-dose effect continues for several days [46]. In normal conditions, this protein can be found in the neurons of the arcuate nucleus of the hypothalamus, where it is colocalized with NPY [5]. This fact, increased AgRP expression in the arcuate nucleus following starvation and in *ob*/*ob* and *db*/*db* mice [47], and hyperphagia/obesity in transgenic mice showing increased AgRP gene expression [48], indicate that AgRP is involved in the physiological regulation of appetite and body weight, and that AgRP interacts with other compounds while regulating appetite and body weight.

In contrast to *agouti* protein overproduction, mice with increased AgRP gene expression show no changes in pigmentation, because AgRP is an MC3 and MC4 receptor antagonist, and it has no effect on the MC1 receptor [36].

It seems that a peripheral mechanism may be also involved in the melanocortinic regulation of body weight, because *agouti* protein stimulates fatty acid production and triglyceride accumulation in adipocytes [49].

As with other neuropeptides, the role of the melanocortin system in the pathogenesis of obesity is poorly understood. However, clinical studies seem to support its role in the regulation of appetite in humans. Decreased POMC gene expression induces pathological appetite, resulting in early obesity accompanied by red-coloured hair and adrenal cortex insufficiency [50]. Decreased conversion of POMC due to prohormone convertase 1 deficiency results in childhood obesity associated with adrenal insufficiency, hypogonadotropic hypogonadism, impaired glucose tolerance and reactive hypoglycaemia [51,52]. The fact that MC4 receptor mutation leads to severe obesity [53] and the obese, compared with the lean, show differences in MC4 alleles detected by restriction fragment length polymorphism analysis [41], indicates that this receptor is involved in the anorexigenic effect of melanocortins in humans.

OTHER PEPTIDES

Of the compounds that regulate body weight by affecting appetite in the hypothalamus, the cocaine- and amphetamine-regulated transcript peptides (CARTs), melanin concentrating hormone (MCH) and the orexins deserve special attention.

When given centrally to mice and rats, CARTs produce a strong anorexigenic effect and block the stimulating effect of NPY on appetite [54]. Hypothalamic CART levels change in starvation or experimental hyperphagia, and are regulated by leptin in the same manner as is POMC [55], which seems interesting in the light of the colocalization of CARTs and POMC in the lateral hypothalamus [5].

In contrast, intracerebroventricular injection of MCH produces a strong orexigenic effect. Hypophagia observed in mice lacking MCH gene expression and increased synthesis of MCH caused by starvation, leptin deficiency and obesity indicate the role of MCH as a physiological regulator of appetite [5,41].

Like MCH, orexins A and B (hypocretins I and II) stimulate appetite. The orexigenic effect of intracerebroventricular doses of orexins is probably caused by the stimulation of specific receptors located in the lateral hypothalamus. Prepro-orexin gene expression in the lateral hypothalamus increases during starvation, and decreases when appetite is inhibited by leptin [56].

INTERACTIONS OF LEPTIN WITH HYPOTHALAMIC PEPTIDES IN THE REGULATION OF APPETITE

Since the discovery of leptin an effector system whose activity in the hypothalamus is responsible for the anorexigenic effect of leptin has been investigated. Most attention has been paid to NPY. Administration of leptin to food-deprived rats normalizes increased NPY mRNA and NPY levels in the hypothalamus [36]. A similar effect of leptin was observed in *ob*/*ob* mice but not in *db*/*db* mice and obese Zucker rats [36]. The fact that the cross-breeding of *ob*/*ob* mice and NPY knockout mice decreases leptin deficiency-induced hyperphagia and obesity [57] indicates that the NPY system is involved in the effect of leptin on appetite. However, the inhibition of food intake by leptin in NPY-knockout mice [32] suggests that NPY is not the only hypothalamic system regulated by leptin. In recent years special attention has been paid to the melanocortin system. Leptin receptors have been found in many arcuate nucleus neurons containing POMC mRNA [58]. POMC expression in the arcuate nucleus is decreased both in hypoleptinaemia

(starvation and *ob*/*ob* mice) and in leptin resistance (*db*/*db* mice). Leptin administration slows down the decrease in POMC mRNA levels in hypoleptinaemic mice [59]. Of particular interest is the fact that the MC4 receptor antagonist SHU9119 blocks the anorexigenic effect of leptin [60]. Apart from NPY and melanocortin, many other endogenous compounds mediate the anorexigenic effect of leptin. These compounds seem to form two effector pathways, anabolic and catabolic, whose hypothalamic activities are regulated by leptin [9,36]. Leptin inhibits the anabolic effector system, thus inhibiting the synthesis and release of hypothalamic appetite-stimulating compounds, such as NPY, MCH, AgRP and galanin [36]. Moreover, the effect of leptin on appetite involves stimulation of the catabolic effector system, which increases the synthesis and release of melanocortins and other appetite inhibitors, such as CRH, CARTs and glucagon-like peptide 1 (GLP-1), in the hypothalamus [7,9,36]. When the appetite-promoting systems are inhibited and the appetite-inhibiting systems are stimulated, the anorexigenic properties of leptin appear [9,36].

The colocalization of NPY and AgRP and of POMC and CARTs in the hypothalamus [5], the presence of Y_1 receptors and CRH receptors on the POMC-containing neurons of the arcuate nucleus [31,38] and the presence of synaptic connections between NPY-containing neurons and CRH-containing perikarya in the periventricular nucleus [23] suggest that there are interactions between the peptides in the hypothalamus. Behavioural studies in which the anorexigenic effect of CRH was blocked by NPY [24] and the inhibition of the stimulating effect of NPY on appetite by α-MSH and CARTs [31,54] prove that these interactions play a role in the regulation of appetite and body weight. Thus the mechanism of action of a peptide seems to consist not only in its direct effect, but also in its indirect effect resulting in the modulation of other hypothalamic systems. The presence of numerous appetite-regulating systems in the hypothalamus and the possibility of numerous interactions between them result in precise response to energetic balance changes, and consequently in precise regulation of organism homeostasis. This fact, although favourable in normal conditions, may hamper the efficacy of the treatment of body weight disorders because the pharmacological manipulation of the activity of a single system alters the activity of the other systems, thus neutralizing the therapeutic effect achieved.

POSSIBLE USE OF LEPTIN AND HYPOTHALAMIC PEPTIDES FOR THE TREATMENT OF OBESITY

Recent years have witnessed a change in the strategy for treating obesity. Most antiobesity drugs have been withdrawn from the market (adrenomimetics, fenfluramine) or their use has been drastically limited (fluoxetine, ephedrine) because of minimal or lack of efficacy, numerous adverse events and the risk of drug dependence [1,2,61]. The recently used antiobesity drugs sibutramine and orlistat, although significantly safer and more effective than the previous ones, produce only a moderate body weight loss [37,61]. Moreover, their adverse effects limit their use in all patients and can require cessation of the therapy [61]. The high prevalence of obesity and the unsatisfactory results of antiobesity therapies require an intensive search for novel effective and safe antiobesity agents. As a consequence, the endogenous appetite- and body weight-regulating systems arouse deep interest as potential targets for new pharmacological agents. Studies of leptin, NPY, CRH, melanocortins and their analogues (Y_1 or/and Y_5 receptor antagonists, CRH_2 receptor agonists, CRH-BP inhibitors, MC4 receptor agonists) seem to give particularly interesting results [2]. It should be stressed that for the time being these studies are mainly experimental in nature, and their results will not necessarily be confirmed by clinical data. These compounds are usually long polypeptide chains characterized by poor or lack of penetration into the CNS and therefore they have to be given centrally. This is why great efforts are being made now to synthesize their analogues and synthetic receptor ligands which will be well absorbed in the alimentary tract, will cross the blood–brain barrier and will show a high affinity for native compound-specific receptors in the CNS. Only then, when these analogues are synthesized, we will be able to assess their efficacy in the treatment of obesity.

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