Hypothalamic Neuropeptide Systems as Targets for Potential Anti-Obesity Drugs

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Abstract: Food intake and energy homeostasis are controlled by peripheral humoral signals, afferent neuronal pathways to the brain and central signals, represented, in particular, by neuropeptides. This review reports the status of development of novel compounds targeting some hypothalamic neuropeptide systems which are currently viewed as potential targets to treat obesity.

Key Words: NPY, AgRP, MCH, orexins, galanin, POMC, CART, CRH.

INTRODUCTION

The control of food intake and energy metabolism is an extremely complicated process which depends on the brain ability to receive, interpret and integrate a wide range of signals indicating the organism's nutritional state and energy level, and to make appropriate adjustments in food intake, energy expenditure and metabolism [1]. The central regulation of food intake is organized by a long-loop mechanism involving humoral signals and afferent neuronal pathways to the brain, obligatory processing in hypothalamic neuronal circuits, and descending commands through vagal and spinal neurones to the body [2]. Despite the large number of brain regions involved in the control of energy homeostasis, the hypothalamus has long been regarded as the "feeding control centre" of the body, as also suggested by early lesional studies [3, 4]. However, the hypothalamus-centered concept of food intake regulation has recently been complemented by a broader physiological vision, which considers the hypothalamus to be an integrator of signals from central and peripheral structures linked by a bidirectional network. In particular, receptors sensitive to glucose metabolism, body fat reserves, distension of the stomach, neuropeptide and cannabinoid receptors as well as neural transmitters and modulators synthesized and secreted within the brain itself have been identified and localized in the hypothalamus [2, 5].

The prevalence of obesity among modern communities increases dramatically, achieving the characteristics of an epidemic. This has prompted researchers into a better understanding of the aetiology of obesity, focusing, in particular, on the mechanisms which regulate appetite and energy balance, and involve appetite centres in the hypothalamus and hormonal signals of energy status released by the periphery. This review reports information on the current status of development of novel compounds targeting some hypothalamic neuropeptide systems which are currently viewed as potential targets to treat obesity and/or other nutritional disorders.

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1. BRAIN LOCALIZATION OF NEUROPEPTIDE SYSTEMS

Five groups of cells in different hypothalamic areas - arcuate (ARC), paraventricular (PVN), ventromedial (VMH) and dorsomedial nuclei (DMH), and the dorsolateral hypothalamic area (LHA) - contain neurones with either orexigenic or anorexigenic actions (Fig. (**1**)). The ARC, enclosing the base of the third ventricle (3V) and lying immediately above

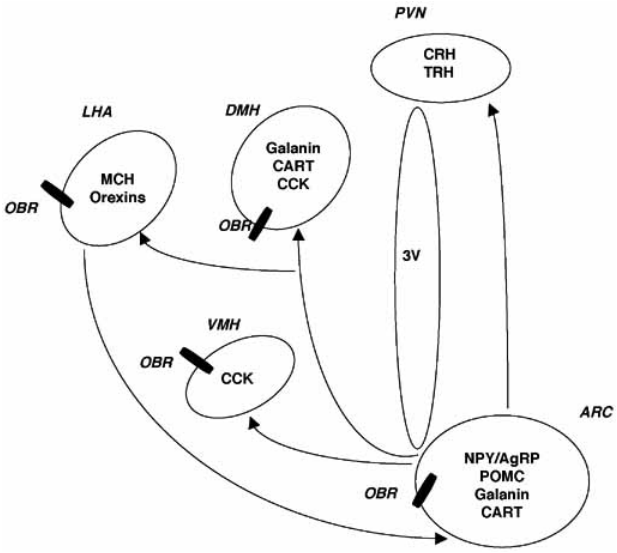

Fig. (1). Schematic rappresentation of the hypothalamic nuclei involved in in the regulation of food intake.

the median eminence, contains populations of neurones that express the orexigenic neuropeptide Y (NPY) and the agouti gene-related peptide (AgRP), and neurones containing the anorexigenic neuropeptides pro-opiomelanocortin (POMC) and cocain- and amphetamine-regulated transcript (CART). The PVN is a site where numerous neuronal pathways implicated in energy balance converge, including major projections from the NPY neurones of the ARC and others containing orexins, the POMC derivative α-melanocyte-stimulating

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hormone (α-MSH) and the appetite-stimulating peptide galanin. The VMH has been identified as a key target for leptin, which acts on the hypothalamus to inhibit feeding and stimulate energy expenditure. The DMH, which has extensive connections with other medial hypothalamic nuclei and the lateral hypothalamus, is thought to serve as an integrative tool in processing information from neuronal populations in these sites. The LHA has a lower density of cell bodies than the obvious nuclei, but includes neurones expressing melanin-concentrating hormone (MCH) and the orexins. It also contains numerous fibre systems projecting to and from the medial hypothalamus and glucose-sensitive neurones that are stimulated by hypoglycaemia (mainly indirectly by pathways ascending from the brainstem). This network is crucial in mediating the marked hyperphagia which is normally induced by hypoglycaemia. The perifornical part of the LHA, surrounding the longitudinal fibre bundle of the fornix, contains a high density of NPY receptors, notably the NPY Y5 receptor subtype (Y5-R), thought to be the NPY 'feeding' receptor, and, like the adjacent PVN, is highly sensitive to the hyperphagic effect of locally-injected NPY (see ref. in [1]).

2. OREXIGENIC NEUROPEPTIDES

2.1. Neuropeptide Y

NPY, a 36-amino acid neuropeptide belonging to the pancreatic polypeptide family, is widely distributed in both the central (CNS), including the hypothalamus, and the peripheral nervous system (PNS), and is one of the most abundant neuropeptides known [6]. NPY is implicated in a broad variety of physiological effects, but we will focus on the role played by NPY and NPY receptors in the regulation of energy homeostasis. Under normal conditions, NPY-expressing neurones in the rodent hypothalamus are essentially confined to the ARC and they send dense projections to other hypothalamic nuclei, particularly the PVN, DMH and LHA [7, 8]. The hypothalamus also receives NPY inputs which originate from cell groups in the medulla that express catecholamines as well as NPY. A subpopulation of the ARC NPY neurones expresses the long isoform of the leptin receptor (OB-Rb) and these cells appear to respond specifically under conditions in which circulating leptin levels are altered [9]. The ARC NPY neurones are therefore potential hypothalamic targets for leptin; inhibition of the synthesis (and presumably release) of the powerfully orexigenic NPY seems to explain, at least in part, the ability of leptin to induce hypophagia and weight loss [10, 11]. Insulin has the same central actions as leptin on energy balance and, before the discovery of leptin, was attributed an important role as a signal that reflects fat mass [12]. In addition to these circulating factors, NPY neurones have extensive neural routes of communication with other hypothalamic regions and specific neuronal populations involved in energy homeostasis [1], including reciprocal connections with systems that inhibit feeding (*e.g.,* corticotrophin-releasing factor (CRH) neurones in the PVN, POMC neurones in the ARC and serotonergic neurones in the raphe nuclei of the midbrain), and other systems that stimulate eating (*e.g.*, the MCH and orexin cell populations of the LHA). The recent demonstration that most of the NPY neurones of the ARC also express AgRP, an endogenous antagonist at the melanocortin (MC) 4 receptor (MC4-R), that mediates the appetite-suppressing action of α-MSH released from POMC neurones, underlines the complexity of the mechanisms placed over feeding behaviour.

2.1.1. Neuropeptide Y and Energy Homeostasis

NPY is a powerful stimulator of feeding when injected into the PVN and perifornical LHA of rodents; indeed, it and its C-terminal derivatives (*e.g.*, NPY_{3-36}) are amongst the most potent orexigenic substances yet identified [13, 14]. Interestingly, hyperphagia is accompanied by inhibition of the sympathetic outflow to brown adipose tissue and other thermogenic tissues, leading to a fall in energy expenditure and a net shift towards positive energy balance [15]. Further evidence that NPY is involved in driving feeding under at least some conditions derives from the finding of increased hypothalamic NPY neuronal activity in a range of conditions characterized by weight loss and increased hunger, such as food restriction and fasting, insulin-deficient diabetes and lactation, especially when mild food restriction is superimposed [1, 16]. ARC NPY neuronal activity is increased in the leptin deficient *ob/ob* mouse and in the *fa/fa* Zucker rat, similarly to the changes seen in states of energy deficits, in which leptin levels fall and fat mass is depleted [17-19]. By contrast, dietary obesity induced by voluntary overeating of a highly-palatable diet is not accompanied by obvious increases in the activity of the ARC NPY neurones; indeed, there is some evidence that aspects of their activity are inhibited, perhaps suggesting an attempt to restrain overeating of palatable food [20, 21]. The possible contribution of NPY to dietary obesity, and to overeating which is driven by the hedonic attractions of palatable food, rather than the physiological consequences of energy deprivation, remains to be elucidated.

2.1.2. Neuropeptide Y Receptors

NPY elicits its physiological effects by interacting with six different G protein-coupled receptors designated Y1-R, Y2-R, Y3-R, Y4-R, Y5-R and y6-R [22]. The Y3-R was originally characterized pharmacologically in the bovine and subsequently in the rat [22], but there are no reports of this receptor in humans and the receptor has not yet been cloned, whereas all the other human receptors have been cloned. The y6-R was found to be a functional receptor gene in mice and rabbit, but is a non-functional pseudogene in rabbits and primates and has not been detected in rats [23]. These receptors have distinct amino acid sequences, a unique pharmacological profile and a distinct tissue localization.

The Y1-R and Y2-R are abundantly expressed in many rat and human brain regions, including hypothalamic centres controlling energy homeostasis [24, 25]. The Y4-R is probably a pancreatic polypeptide receptor rather than a NPY receptor; its mRNA is sparsely expressed in brain and low levels have been found in the hypothalamus [26]. The Y5-R mRNA is discretely localized in rat and human brain and also in the hypothalamus [26]; Y5-R binding sites have also been detected in these regions, although some groups failed to detect Y5-R binding in the hypothalamus [21, 27]. The available data suggest a primary, direct role for the Y1-R and Y5-R in mediating the effects of NPY on energy homeostasis [28]; in particular, the Y5-R type is considered to mediate the hyperphagic and obesity-promoting effects of NPY. The

Y5-R is expressed at relatively high levels in the LHA, close to the site where NPY acts most potently to stimulate feeding [29]. It has been shown that reduction in Y5-R availability, either by using antisense oligodeoxynucleotides targeted against Y5-R mRNA [30] or with a synthetic high-affinity Y5-R antagonist [31], can decrease spontaneous feeding and the hyperphagia induced by central injection of exogenous NPY. Interestingly, however, the Y5-R 'knockout' mouse shows no reductions in either feeding or weight [32]. This finding may reflect the general difficulties of applying the 'knockout' approach to systems such as energy homeostasis, which are regulated by several systems that interact to a considerable degree.

2.1.3. Non-Peptidic Neuropeptide Y Receptor Antagonists

Within the pharmaceutical industry, there has been great interest in the development of Y1-R and Y5-R antagonists as potential drugs for obesity management.

Y1-R Antagonists

Initially reported Y1-R antagonists failed to cross the blood-brain barrier, and only recently orally brain-penetrant Y1-R antagonists have been developed. The first potent and selective non-peptidic Y1-R antagonist, BIBP3226, designed to mimic the C-terminal region of NPY, was shown to have high affinity for human and rat Y1-R, to be inactive at Y2-R [33], to have no significant affinity for Y4-R and Y5-R [29] and to not penetrate blood-brain barrier (Fig. (**2**)). Administration of BIBP3226 intracerebroventricularly [34, 35] or into the paraventricular nucleus [36] was reported to block NPY-induced food intake. However, toxicity in CNS was observed upon administration of BIBP3226 by these routes and these observations have cast doubt on the specificity of these effects [36, 37]. A structurally related analogue, BIBO 3304, has high affinity for cloned rat and human Y1-R, no significant affinity for the other NPY receptor subtypes and is claimed to cause less CNS toxicity than BIBP3226 (Fig. (**2**)) [38]. The substituted indole derivative LY-357897 [39] was the first Y1-R antagonist with sub-nanomolar potency (Fig. (**2**)). Unfortunately, the compound could not be evaluated systemically due to the lack of oral and subcutaneous bioavailability. J-104870 and J-115814, derived from a diaminopyridine series [40, 41], are potent and selective Y1-R antagonists (Fig. (**2**)). J-104870 is the first reported potent, orally bioavailable, brain-penetrant Y1-R antagonist with high affinity for cloned rat and human Y1-R, no significant affinity for human Y2-R, Y4-R, and Y5-R. The related compound J-115814 showed high affinity for human, rat and mouse Y1-R, while much lower affinity was seen at human Y2-R, Y4-R and Y5-R, as well as mouse y6-R [41]. CP-671, 906 is an orally bioavailable Y1-R antagonist that crosses the blood-brain barrier (Fig. (**2**)). The compound showed high

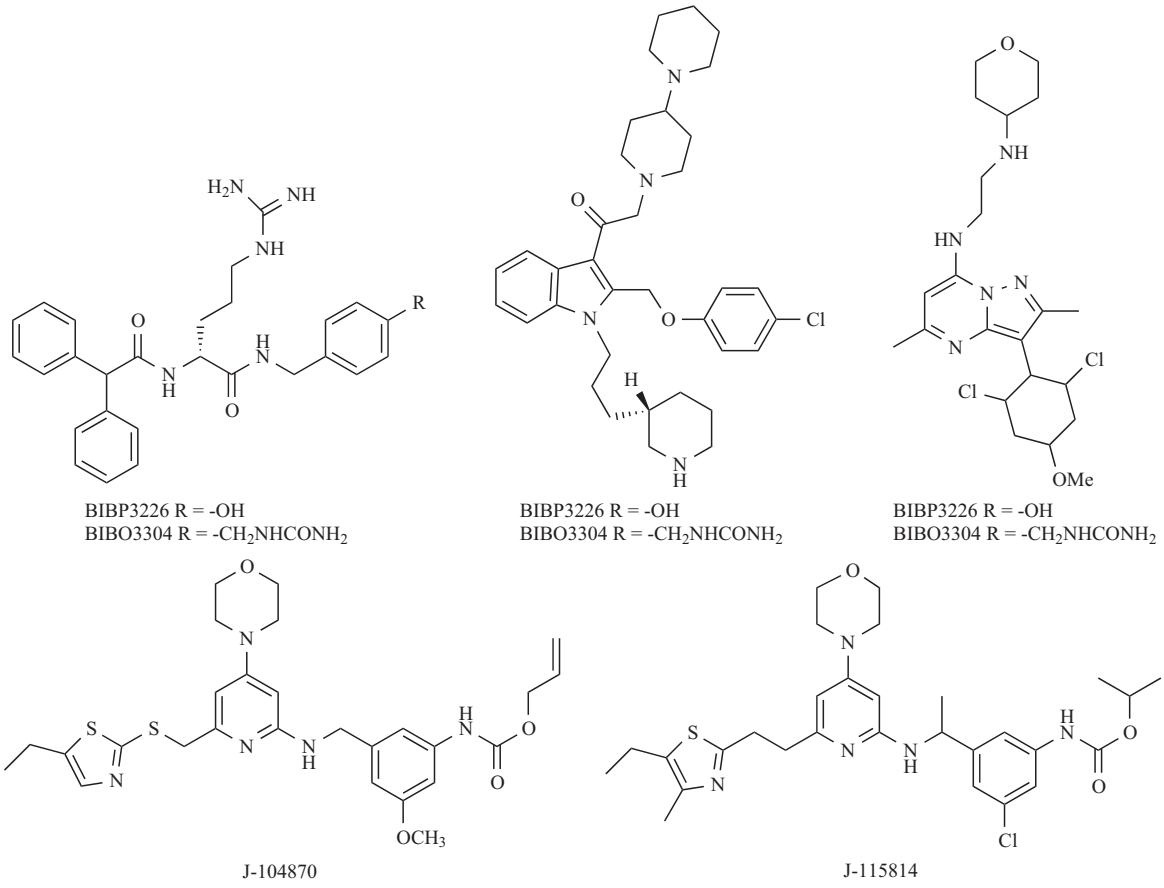

Fig. (2). NPY Y1 receptor antagonists.

affinity for cloned rat and human Y1-R, with no significant affinity for Y2-R, Y5-R and more than 35 other receptors, enzymes and ion channels (see ref. in [28]).

In general, data with Y1-R antagonists support a role for the Y1-R in the modulation of energy homeostasis. Orally active, brain-penetrant Y1-R antagonists have only recently been developed, but the compounds reported thus far are not very potent *in vivo* [28]. Likely it will be necessary to develop compounds with greater *in vivo* potency and/or a lower incidence of adverse effects.

Y5 Receptor Antagonists

CGP 71683A was the first potent and selective Y5-R antagonist discovered through a process of combinatorial chemistry and traditional medicinal chemistry (Fig. (**3**)) [31]. CGP 71683A has high affinity for the rat and human Y5-R and low affinity for human Y1-R, Y2-R and Y4-R. A combination of intraperitoneally administered CGP 71683A and the Y1-R antagonist BIBO3304 was recently reported to produce anorectic effects in lean rats, *fa/fa* Zucker rats, and *ob/ob* mice at doses where either agent alone was ineffective [42]. Indeed, it was reported that CGP 71683A has also a potent affinity for the 5-hydroxytryptamine reuptake recognition site and muscarinic receptors [43] and that the anorectic effects of CGP 71683A are identical in wild type and Y5-R-deficient mice (see ref. in [28]). Thus, the possibility that CGP 71683A produces anorectic effects through mechanisms other than Y5-R blockade cannot be ruled out. Compounds indicated in (Fig. (**3**)) represent a novel series of NPY Y5-R antagonists based on a β-aminotetralin scaffold [44]. Compounds 1 and 2 bind with high affinity to human Y5-R and after intraperitoneal administration to fasted rats reduce food consumption. A structurally distinct pyrazole series, represented by compounds 3 and 4 were also described (Fig. (**3**)) [45, 46]. These compounds do not bind significantly to human Y1-R or Y2-R; no data confirming the antagonistic properties of these compounds at Y5-R was reported. L-152,804 is an orally bioavailable, brain-penetrant Y5-R antagonist with reasonable affinity for human and rat Y5-R and low affinity for human Y1-R, Y2-R, and Y4-R ((Fig. (**3**)) [47]. Administration of L-152,804 alone had no effect on food intake, and was reported to induce no overt behavioural changes, indicating that the anorectic effect of the compound is Y5-R-specific. When administered orally to rats, L-152,804 also inhibited food intake elicited by intracerebroventricular bovine pancreatic polypeptide. However, L-152,804 administered either intracerebroventricularly or orally failed to inhibit NPY-stimulated food intake. Based on these observations, it was concluded that activation of the Y5-R does not substantially contribute to food intake in rodents. Further studies with functionally more potent NPY

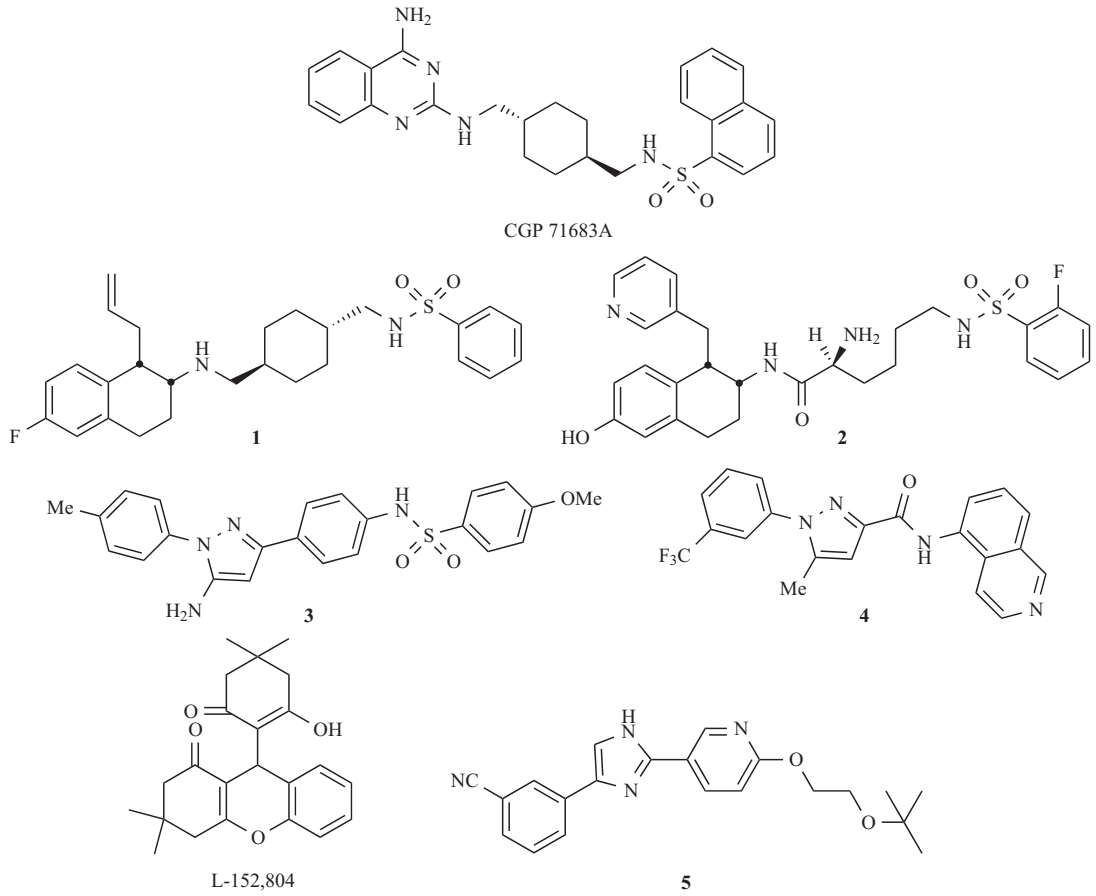

Fig. (3). NPY Y5 receptor antagonists.

Y5-R antagonists therefore would address this more definitively. Compound 5 has been reported as a more potent, orally bioavailable, brain-penetrant Y5-R (Fig. (**3**)). The compound did not have significant affinity for human Y1-R or Y2-R or for over 50 other receptors (see ref. in [28]). Further studies of the Y1 and Y5 receptor antagonists are needed to clarify their potential as antiobesity agents.

2.2. Agouti Gene-Related Protein

Agouti protein is a paracrine-signaling molecule that affects pigmentation by antagonism at the melanocortin receptor 1 (MC1-R). Expression of agouti is normally limited to the skin, where overexpression in mice results in a yellow fur. In 1997, a murine and a human genes were isolated, encoding a protein with nearly identical size and structure as agouti. This AgRP (132 amino acids) is mainly expressed in the ARC, where it is inhibited by leptin and stimulated after fasting. 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 is, actually, an inverse agonist of the MC4-R, which is constitutively active. AgRP expression is increased in *ob/ob* mice, *db/db* mice, and in fasted wildtype mice (see ref. in [48]). 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. However, the long-term effect of AgRP is most likely not mediated by opioid receptors (see ref. in [48]). AgRP also influences energy expenditure and thermogenesis and seems to be involved in food selection, specifically enhancing the intake of high fat content diets (see ref. in [48]). Thus, these findings suggest the possible involvement of AgRP in body weight homeostasis and in the development of obesity. The hypothesis that the interaction of AgRP and MC4-R modulates feeding behaviour in humans has stimulated the synthesis of conformationally constrained peptides and small molecules in efforts to identify novel compounds in the clinical treatment of obesity and related eating disorders. The cysteine-rich, C-terminal domain of AgRP has been shown to be a nanomolar antagonist of the mouse MC4-R that is equipotent to the full-length protein. Indeed the Arg-Phe-Phe motif is a critical element in conferring the observed antagonistic behaviour [49]. Structure-function studies of the hAgRP(109-118) decapeptide, Tyr-c[Cys-Arg-Phe-Phe-Asn-Ala-Phe-Cys]-Tyr-NH2, obtained by replacing the 26-membered disulfide Cys2-Cys9 ring with lactam bridges resulted in the identification of a novel antagonist for the peripheral skin MC1-R and the brain MC4-R, with no observable pharmacology at the MC3-R or MC5-R [50].

2.3. Melanin-Concentrating Hormone

MCH is an orexigenic cyclic 19 amino acid peptide produced by neurones of the LHA and the zona incerta, which receive input from ARC. The expression of MCH receptors (MCH-R1 and MCH-R2) is found in some hypothalamic nuclei and in other brain regions. MCH-R2 shares about 38% amino acid identity with MCH-R1. MCH plays an important role in food intake behaviour and also stimulates the activity of the hypothalamus-pituitary-adrenal (HPA) axis by increasing the release of the adrenocorticotropic hormone (ACTH). Intracerebroventricular administration of MCH to mice and rats induces hyperphagia in the light and dark phase and decreases energy expenditure. Inhibition of MCH neurones results in hypophagia and leanness. MCH knockout mice have a reduced body weight and are lean due to hypophagia (see ref. in [48]). The compelling genetic and pharmacological evidence implicating MCH-1R signalling in the regulation of food intake and energy expenditure has generated a great interest for the discovery of MCH-1R antagonists. Preclinical studies showing hypophagia and weight loss with MCH-1R antagonists in rodents are encouraging and suggest that the identification of clinical candidates as antiobesity drugs will be forthcoming [51].

2.4. Orexins

Orexins or hypocretins are orexigenic neuropeptides. 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). Orexins are implicated in food intake and temperature regulation and they are produced in neurones of the LHA and perifornical area that project to areas important in feeding behaviour and neuroendocrine homeostasis. Two types of G-protein coupled receptors (GPCRs) related to the Y2-R (26% homology) have been discovered: Ox2-R and Ox1-R. Orexin A has equal affinity to both receptors, while orexin B has greater affinity to Ox1-R than Ox2-R. The activity of orexin neurones is influenced by the feeding status, being increased by low glucose levels and decreased by signals related to nutrient ingestion. Orexin neurones in the LHA receive innervation from the POMC and NPY/AgRP neurones of the ARC, express their receptors, as well as OB-Rb, and project back to POMC and NPY neurones, where they, just like leptin, inhibit POMC neurones and activate NPY neurones. The role of orexin B on food intake is more often questioned. Orexin A has an acute effect on food and water intake and also seems to play a role in temperature regulation and in short-term regulation of energy homeostasis (see review in [48]). It is only by antagonising the orexin system that the contribution of endogenous orexins to the normal regulation of energy balance can be fully addressed. Central injection of orexin-A antibodies reduces food intake in fasted rats, and there is a preliminary report that an antibody to the orexin-1 receptor also reduced feeding (see review in [52]). More extensive studies have been conducted with the non-peptide orexin-1 receptor selective antagonist SB-334867, which reduced orexin-A-driven feeding and feeding stimulated by an overnight fast in rats [53]. The combined anorectic and thermogenic effect of SB-334867 caused a reduction in body fat gain in the *ob/ob* mice. Moreover, fasting blood glucose and insulin were both suppressed, indicating that orexin-1 receptor antagonists may have potential as antidiabetic as well as anti-obesity agents [52].

2.5. Galanin

Galanin is a 29 amino acid neuropeptide widely expressed in the CNS. Pro-galanin is processed to galanin and galanin-like peptide (GALP, 60 amino acids). Galanin and GALP are mainly found in the ARC and LHA areas and they are expressed in neurones that possess leptin receptors. Galanin stimulates feeding, especially fat intake, inhibits

insulin secretion, and induces hyperglycemia in the periphery. Central administration of galanin in the hypothalamus 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. The exact mechanism of the increase in food intake induced by galanin is still unclear: one possibility might reside in the linkage to the NPY system. It is suggested that galanin (just like β-endorphin) mediates NPY-induced feeding and is also linked to POMC neurones in the ARC. Intracerebroventricular administration of GALP stimulates food intake 10-fold compared to galanin and has (just like galanin) anxiogenic actions. GALP has highest affinity to the Gal2R. However, since galanin and GALP show equal affinity to this receptor, it is not known whether this receptor is implicated in GALP-induced hyperphagia or another unknown receptor is involved (see review in [48]). Since GALP is involved in the control of food intake and energy balance, it is possible that it plays an important role in the development of obesity. At the moment no galanin and GALP antagonists have been studied.

3. ANOREXIGENIC NEUROPEPTIDES

3.1. Melanocortins and the Melanocortin-4 Receptor

The MC peptides are derived from the precursor molecule POMC, which is posttranslationally processed by prohormone convertases to generate the MC agonists α, β, γ melanocyte-stimulating hormones (MSH) and adrenocorticotropin (ACTH) [54]. The role of the MC pathway in the regulation of food intake gained significant importance with the characterization of five distinct MC-R subtypes [55, 56]. Amongst the five MC-R, the MC3-R and MC4-R are referred to as the central MC-Rs and their expression was reported in several hypothalamic nuclei [56, 57]. A fundamental role for MC4-R in feeding and body weight homeostasis was demonstrated [48]. This receptor is expressed in the PVN and LHA and holds high affinity to α-MSH. Indeed, infusion of α-MSH in the brain results in a decreased food intake and increased energy expenditure in rodents, which can be prevented by administration of MC3-R/MC4-R antagonists [58, 59]. These findings have led to the development of selective MC3-R and MC4-R agonist or antagonist in order to define the exact role of MC3-R and MC4-R in the regulation of food intake.

3.1.1. Synthetic Cyclic Analogues

Linear peptides including α-MSH are rapidly degraded. Extensive structure-function studies of the melanocortin peptide α-MSH resulted in the identification of a more potent and enzyme resistant analogue called NDP-MSH, which has a D-Phe instead of L-Phe at position 7 [60]. Substitution of α-MSH and NDP-MSH at the 4 and 10 positions resulted in identification of a potent Cys4-Cys10 MSH derivative [61]. Subsequently, a cyclic lactam derived from the (4–10) fragment of NDP-MSH, melanotan II (MTII) was discovered (Fig. (**4**)) [62]. MTII was found to be a potent, non-selective agonist at the MC1-R, MC3-R, MC4-R and MC5-R [63]. Substitution of D-Phe with a bulky hydrophobic amino acid, DNal (2') in position 7 of MTII, yielded an antagonist for the MC3-R (also partial agonist) and MC4-R, known as SHU9119 (Fig. (**4**)) [63]. Several disulfide α-MSH analogues (HS014 and HS024) have also been reported as selective antagonists for the MC4-R versus the MC3-R [64, 65].

3.1.2. Linear Tetrapeptide Analogues

Several linear peptides, containing the melanocortin receptor "message" His-Phe-Arg-Trp tetrapeptide, have been synthesized, with one goal to identify tetrapeptides with improved MC4-R versus MC3-R selectivity [66, 67]. Recently, two tetrapeptides JRH420-12 (Ac-Anc-DPhe-Arg-Trp-NH2) and JRH322-18 [Ac-His-(pI)DPhe-Arg-Trp-NH2] possessing unique pharmacology have been identified [68, 69]. In the mouse, JRH420-12 is a potent agonist at the MC4-R, a weak MC3-R micromolar antagonist, and possesses >4700-fold agonist selectivity for the MC4-R versus the MC3-R. JRH322-18 is a full nanomolar agonist at the MC1-R and MC5-R, a MC3-R partial agonist with potent antagonist activity and is also a potent agonist at the MC4-R [68, 69]. Therefore, it can be concluded that the highly selective MC4-R agonist JRH420-12 is very efficacious in reducing food intake as compared to non-selective MC3-R/MC4-R agonists or compounds with mixed pharmacology at the central MC3-R and MC4-R.

3.1.3. Small Molecule MC4R Agonists and Antagonists

Analogs containing the His-Phe-Arg-Trp tetrapeptide core unit, modified by an additional amino acid and hydrophobic N-terminus, have shown improved MC4-R selectivity. In the human, two such compounds Ro27-3225 and Ro27-4680 demonstrated, *in vitro* receptor pharmacology, having selectivity at the MC4-R with little or no activity at the MC3-R and MC5-R [66]. The compound 1,2,3*R*,4-tetrahydro-3-isoquinolinecarboxylic acid [1*R*-(4-chloro-benzyl)-2-(4-cyclohexyl-4-[1,2,3]triazol-1-ylmethyl-piperidin-1-yl)-2-oxo-ethyl]-amide (THIQ) is a potent orally MC4-R agonist with >1300-fold selectivity over MC1-R, >1100-fold selectivity over MC3-R and >350-fold selectivity over MC5-R [70]. These findings clearly implicate that MC4-R is involved in food intake regulation. Future studies with MC3-R and MC4-R selective compounds will result in understanding the roles played by the central MC-Rs in regulating energy homeostasis.

3.2. Cocaine- and Amphetamine-Regulated Transcript

CART is another neuropeptide that inhibits food intake. Splicing of the precursor results, in the rat, in two products consisting of 116 and 129 amino acids, while only a 116 amino acid product is found in man. The splice products are processed into smaller peptides, $CART_{(55\text{-}86)}$, $CART_{(54\text{-}102)}$, $CART_{(55\text{-}102)}$, $CART_{(61\text{-}102)}$ and $CART_{(66\text{-}102)}$ [71]. CART mRNA is more expressed in different hypothalamic nuclei (ARC, PVN, DMH, LHA) [72]; CART mRNA and peptide are co-localized with POMC in the ARC, with MCH in the LHA and with galanin in the PVN, thus showing co-localization with orexigenic and anorexigenic neuropeptides. Central administration of CART peptide leads to an inhibition of normal and NPY-induced food intake in rodents; in addition, chronic administration of CART decreases food intake and body weight, while injections of CART antibodies increase food intake in rodents. Moreover, food depriva-

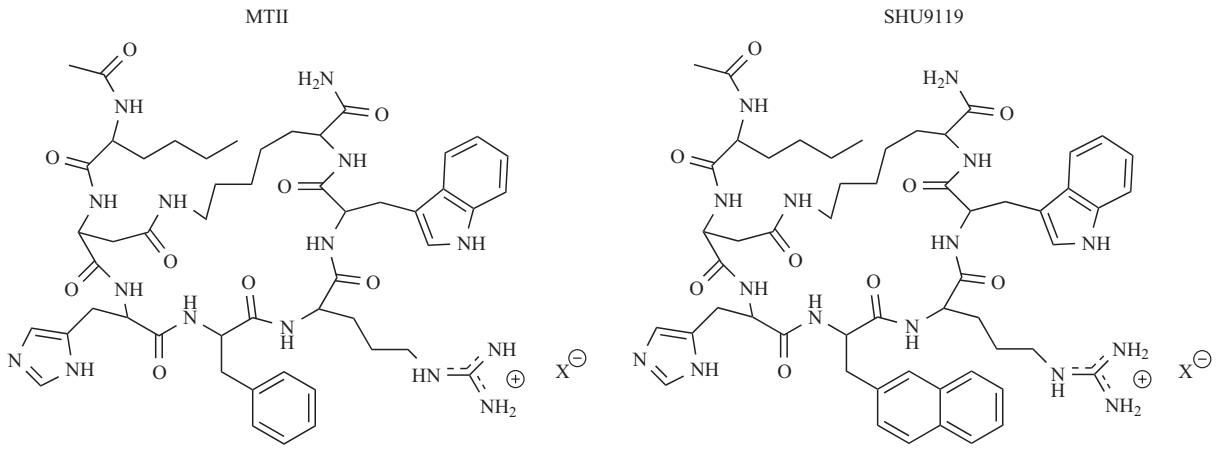

Fig. (4). Cyclic melanocortin analogues.

tion induced a decrease in CART mRNA, while leptin administration upregulated CART expression. (see review in [48]). Several genetic findings link CART to human obesity. The human CART gene is a positional candidate for obesity, because it maps to human chromosome 5q13–14 [73, 74], which has been shown to be a susceptibility locus for obesity [75]. Animal models also support a role for CART in obesity. As mentioned above, mice that have a targeted deletion of the CART gene become more obese when fed a high-fat diet from weaning than wild-type littermates [76]. Interestingly, CART-deficient animals did not show an increase in body weight until after the 14th week of the high-fat diet, suggesting that the role of CART in obesity requires interactions with the environment or with developmental events. A confluence of findings shows that CART has a substantial role in the regulation of feeding and body weight. CART represents a definite target for the treatment of obesity in these genetically determined cases and might represent a useful drug target for the treatment of human eating disorders in general (see review in [77]). No doubt future work in the field, including identification of a receptor or receptors for CART, will increase our understanding of feeding behaviour and the aetiology of obesity.

3.3. Corticotropin-Releasing Hormone

CRH is a 41 amino acid peptide best known for its role in the HPA axis. CRH is widely distributed in the brain and serves as an integrator of adaptive responses to stress and influences food intake, gastrointestinal, cardiovascular and inflammatory function. CRH is a potent anorexigenic peptide, acting downstream of leptin. Central administration or direct administration into the PVN inhibits nighttime and fasting-induced feeding. Leptin increases CRH expression and CRH neurones activity in fed state, while decreases CRH expression and CRH neuron activity in fasted state (see review in [48]). The administration of the CRH antagonist α-helical CRH (9-41) inhibits endogenous CRH activity and enhances the feeding response to exogenous NPY, indicating that CRH is a secondary anorexigenic neuropeptide acting downstream of NPY [78]. The effects of CRH are mediated by two GPCRs, which are both positively coupled to adenylate cyclase.

CONCLUSIONS

Hypothalamic neuropeptide systems, due to their pivotal involvement in the control of normal and pathological energy metabolism, are the target of intensive research, with the aim of developing novel therapeutic molecules for obesity and related disorders. It appears evident that the redundancy and complexity of this system (*i.e.,* multiple receptor isoforms and ligands) makes difficult to reach this task with selective and specific agents.

ABBREVIATIONS

ARC	=	Arcuate nucleus
PVN	=	Paraventricular nucleus
DMH	=	Dorsomedial nucleus
LHA	=	Dorsolateral hypothalamic area
3V	=	Third ventricle
NPY	=	Neuropeptide Y
AgRP	=	Agouti gene-related protein
MCH	=	Melanin-concentrating hormone
POMC	=	Proopiomelanocortin
CART	=	Cocaine- and amphetamine-regulated transcript
CRH	=	Corticotropin-releasing hormone
MSH	=	Melanocyte-stimulating hormone
Y-Rs	=	Neuropeptide Y receptors
CNS	=	Central nervous system
PNS	=	Peripheral nervous system
OB-Rb	=	Long isoform of leptin receptor
MC	=	Melanocortin
MC-Rs	=	Melanocortin receptors
Ox-Rs	=	Orexins receptors
GALP	=	Galanin-like peptide

GalRs = Galanin receptors

ACTH = Adrenocorticotropin hormone

GPCRs = G-protein coupled receptors

HPA = Hypothalamus-pituitary-adrenal axis.

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