The hypothalamus and the control of energy homeostasis
Different circuits, different purposes

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Abstract

The hypothalamus regulates many aspects of energy homeostasis, adjusting both the drive to eat and the expenditure of energy in response to a wide range of nutritional and other signals. It is becoming clear that various neural circuits operate to different degrees and probably serve specific functions under particular conditions of altered feeding behaviour. This review will discuss this functional diversity by illustrating hypothalamic neurones that express neuropeptide Y (NPY), the melanocortin-4 receptor (MC4-R) and the orexins. NPY neurones in the arcuate nucleus (ARC) release NPY, a powerful inducer of feeding and obesity, in the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA). ARC-NPY neurones are inhibited by leptin and insulin and become overactive when levels of these hormones fall during undernutrition. They may function physiologically to protect against starvation. With disruption of the inhibitory leptin signals due to gene mutations, the NPY neurones are overactive, which contributes to hyperphagia and obesity in the ob/ob and db/db mice and fa/fa Zucker rat. The MC4-R is activated by α-melanocyte-stimulating hormone [α-MSH; a cleavage product of pro-opiomelanocortin (POMC), which is expressed in the other ARC neurones] and inhibits feeding. This effect is antagonised by agouti gene-related peptide (AGRP), which is coexpressed by the ARC-NPY neurones only. Activation of MC4-R, possibly mediated by blockade of AGRP release, appears to restrain overeating of a palatable diet. This response may be programmed by a transient rise in leptin soon after presentation of palatable food, and rats that fail to do this will overeat and become obese. Orexin-A and -B (corresponding to hypocretins 1 and 2) are expressed in specific LHA neurones. These have extensive reciprocal connections with many areas involved in appetite control, including the nucleus of the solitary tracts (NTS), which relays vagal afferent satiety signals from the viscera. Orexin neurones also have close anatomical connections with LHA glucose-sensitive neurones. Orexin-A induces acute feeding but does not cause obesity. Orexin neurones are stimulated by hypoglycaemia partly via the NTS and inhibited by food ingestion. These neurones may therefore be involved in the severe hyperphagia of hypoglycaemia and short-term control of feeding.

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1. Introduction

The brain regulates many aspects of energy homeostasis, adjusting both the drive to eat and the expenditure of energy in response to a wide range of nutritional and other signals. This process is highly complex and involves several brain regions ranging from cortex to brainstem, but most interest has focused on the hypothalamus.

During the past decade, our knowledge about the specific mediators and neuronal networks that regulate food intake and body weight has increased dramatically. Important milestones in understanding hypothalamic mechanisms include the characterisation of several novel hypothalamic peptides and their receptors and the elucidation of the fundamental defects of several long-recognised but enigmatic rodent models of genetic obesity.

It has become clear that various neural circuits operate to different degrees and probably serve specific functions under particular conditions of altered energy balance. The aim of this article is to illustrate this functional diversity, which we believe is fundamental both to the understanding of physiology and to the process of drug discovery in the field of obesity and nutrition.

1.1. Anatomy of the hypothalamus

Numerous neural circuits are located in the hypothalamus. In the early 1940s, lesions or electrical stimulation of
specific nuclei within the hypothalamus were shown to alter feeding behaviour. Accordingly, some of these nuclei were regarded as “feeding” or “satiety” centers. As our knowledge of the neuronal networks and their ramifications has expanded, the view of functional “centers” has been replaced by that of discrete neuronal populations, expressing specific neurotransmitters that mediate particular effects on food intake and/or energy expenditure and that are regulated by specific signals of nutritional state.

The basic neuroanatomy of the rat hypothalamus is shown in Fig. 1. The arcuate nucleus (ARC), situated around the base of the third ventricle, lies immediately above the median eminence. The ARC is an elongated collection of neuronal cell bodies occupying nearly one-half of the length of the hypothalamus and is apparently subdivided into several functional domains. For example, neuropeptide Y (NPY) and agouti gene-related protein (AGRP), both potent stimulators of food intake, are colocalised in a population of neurones in the ARC [1,2], while pro-opiomelanocortin (POMC; the precursor of α-melanocyte-stimulating hormone (α-MSH)) and cocaine- and amphetamine-regulated transcript (CART), which induce an anorectic response, are colocalised in an adjacent subset of ARC neurones [3,4]. These two populations interact with each other (see below). The ARC also has extensively reciprocal connections with other hypothalamic regions, including the paraventricular nucleus (PVN), dorsomedial hypothalamic nucleus (DMH), ventromedial hypothalamic nucleus (VMH) and lateral hypothalamus (see below). Capillaries in the underlying median eminence lack tight junctions: this region therefore effectively lies outside the blood–brain barrier [5], so that the ARC neurones are readily accessible to circulating messengers including leptin and insulin. These and other signals (e.g., glucose) may also gain access to the ARC by diffusion across the ependyma from the cerebrospinal fluid (CSF) in the third ventricle [6].

The PVN lies beside the top of third ventricle in the anterior hypothalamus. The PVN is an integrating center, on which converge many neural pathways that influence energy homeostasis, and is richly supplied by axons projecting from the ARC-NPY/AGRP and POMC/CART neurones and from the orexin neurones of the lateral hypothalamus [7,8]. The nucleus is rich in terminals containing numerous appetite-modifying neurotransmitters, including NPY, α-MSH, serotonin (5-HT), galanin, noradrenaline and the opioid peptides, and the PVN is particularly sensitive to these neurotransmitters’ effects on feeding and energy expenditures. Corticotrophin-releasing factor (CRF) is expressed by neurones in the PVN that project to the median eminence [9] and may act to inhibit the NPY neurones of the ARC-PVN projection.

The VMH, one of the largest nuclei of the hypothalamus, was long considered to be a “satiety center.” Stimulation of the VMH inhibits feeding, whereas a lesion in this region causes overeating and weight gain [10]. Recent studies have shown high abundance of leptin receptors (long form: Ob-Rb) in neurones of the VMH, and evidence indicates that this region may be an important target for circulating leptin [11]. The VMH has direct connections with the PVN, the lateral hypothalamus and the DMH.

The DMH, located immediately dorsal to the VMH, has extensively direct connections with other hypothalamic nuclei such as the PVN, the lateral hypothalamus and the brainstem. The VMH and the lateral hypothalamus have no direct connections but connect indirectly through the DMH and the PVN (Fig. 1). The PVN and the DMH may cooperate functionally as a unit, which is involved in initiating and maintaining food intake [12]. The DMH contains plentiful insulin receptors as well as leptin receptors (Ob-Rb). Some ARC-NPY/AGRP neurones also terminate in the DMH [13].

The lateral hypothalamic area (LHA) is vaguely defined and comprises a large, diffuse population of neurones including define subpopulations that express orexins and melanin-concentrating hormone (MCH), both peptides that stimulate food intake. NPY terminals are abundant in the LHA, in contact with orexin and MCH cells [2,14], while the perifornical part of the LHA contains a high density of NPY-“Y5” receptors thought to mediate the appetite-stimulating effects of NPY [15]. The LHA was viewed classically as the “feeding center.” Stimulation of this nucleus increases food intake, while its destruction attenuates feeding and causes weight loss. This nucleus also contains large numbers of glucose-receptive neurones that respond to circulating glucose levels, probably mainly via pathways ascending from the hypothalamus [16]. These glucose-sensing neurones are discussed further below.

1.2. Connections to extrahypothalamic regions involved in appetite control

The integration of energy homeostasis involves multiple brain areas both within and outside the hypothalamus. For example, meal-related satiety information is conveyed to the nucleus of the tractus solitarius (NTS) in the medulla, on
Table 1

<table>
<thead>
<tr>
<th>Hypothalamic neurotransmitters implicated in the control of feeding</th>
<th>Regulated by adiposity signals</th>
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<tr>
<td><strong>Increase food intake</strong></td>
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<td>NPY</td>
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<td><strong>Reduce food intake</strong></td>
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<td>α-MSH</td>
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<td>5-HT</td>
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Arrows indicate direction of effect of the adiposity signals, leptin and/or insulin.

1.3. Access of adiposity signals leptin and insulin to the hypothalamus

Insulin and leptin are hypothesised to be “adiposity signals” for the long-term regulation of body weight by the brain. Insulin circulates at levels that parallel fat mass and enters specific brain regions in proportion to its plasma levels [22]. Insulin receptors are abundant in the mediobasal hypothalamus, including the ARC-median eminence area [23,24], while intracerebroventricular (icv) administration of insulin induces hypophagia and weight loss [25].

Leptin, the ob gene product secreted by adipocytes [26], also circulates at concentrations that are broadly proportional to fat stores [27]. Its specific entry to the mediobasal hypothalamus appears to take place across the modified blood–brain barrier of the ARC-median eminence. Also, it is transported into the CSF via the choroid, from where it can enter the hypothalamus across the ependyma [28,29,30].

Leptin inhibits food intake and increases energy expenditure through interactions with its specific receptors in the hypothalamus. The Ob-Rb isoform, fully capable of transducing leptin signals, has been colocalised with NPY/AGRP and POMC/CART in the ventromedial and ventrolateral ARC and with MCH and orexins in the lateral hypothalamus, suggesting that these neurotransmitters are mediators of leptin’s action in the hypothalamus [11,31–33].

2. Hypothalamic neuronal populations implicated in energy homeostasis

Numerous neurotransmitters found in the hypothalamus affect feeding or body weight when injected centrally or if their activity is enhanced or blocked by pharmacological or genetic manipulation (Table 1). Functional studies suggest that NPY, AGRP, POMC, CART, MCH and orexins are all important regulators of food intake and body weight (Table 1).

In this review, we have elected to describe in some detail NPY, melanocortins, orexins and glucose-receptive neurones. We will focus mainly on the current understanding of these neuronal populations’ involvement in energy homeostasis, with particular emphasis on the specific
aspects of feeding behaviour and energy expenditure, which they may control.

3. NPY

NPY, a 36-amino acid neurotransmitter [34] belonging to the pancreatic polypeptide (PP) family, is one of the most abundant and widely distributed neurotransmitters in the mammalian brain, including man [35]. Like other members of the PP family, it has a characteristic hairpin tertiary structure [36].

3.1. Neuroanatomy

The ARC is the major hypothalamic site of NPY expression of NPY, where 90% of the NPY neurones also contain AGRP [2]. The ARC-NPY/AGRP neurones project dorsally and anteriorly into the perifornical LHA, PVN, DMH and medial preoptic area (MPO) and ventrally to the median eminence: the ARC-PVN projection is particularly dense (Fig. 2) [37,38]. There are also short projections that terminate within the ARC [39], and NPY released locally may affect the activity of specific ARC neurones, including the NPY neurones themselves and the POMC neurones. NPY may regulate ARC-NPY/AGRP neurones by acting on inhibitory autoreceptors. These cells possibly express the NPY may regulate ARC-NPY/AGRP neurones by acting on inhibitory autoreceptors. These cells possibly express the NPY-Y2/Y4 receptor [40]. Indeed, recent data show that NPY release from hypothalamic blocks in vitro is inhibited by NPY-Y2 and -Y4 receptor activation [41,42].

Other hypothalamic regions containing substantial NPY levels include the periventricular area surrounding the third ventricle, the supraoptic nucleus (SON) and the suprachiasmatic nucleus (SCN). The latter receives a dense NPY projection from the lateral geniculate body, which may be implicated in circadian rhythm regulation [43,44].

In addition to the NPYergic projections from the ARC, the PVN and DMH also receive afferent NPY-containing fibres from catecholaminergic nuclei in the brainstem (Fig. 2B) [37,45]. It is likely that there exist functionally different ARC-NPY/AGRP neurones, as states of negative energy balance increase NPY mRNA expression only in the midportion of the ARC that projects to the DMH [38,46]. This subdivision of ARC-NPY/AGRP neurones may play a dominant role in regulating food intake.

3.2. Effects of NPY on energy balance

NPY has a formidable list of experimental actions, ranging from memory processing and retention to the regulation of blood pressure and circadian body temperature [47] body temperature [48], but we focus here on its potent ability to stimulate feeding, reduce energy expenditure and induce obesity [49,50].

The robust feeding effects of NPY can be elicited by nanomolar doses injected either into the cerebral ventricles or various hypothalamic areas, notably the PVN and the perifornical LHA. NPY is about 500-fold more potent than noradrenaline (on a molar basis) in stimulating feeding [51,52] and can increase food intake acutely by several folds. Repeated or chronic central admission of NPY continues to stimulate feeding, demonstrating that NPY is capable of overriding both short- and long-term mechanisms of satiety and body weight regulation [53]. The development of obesity following multiple NPY injections is also attributable to NPY’s antithermogenic properties and insulin secretatogogue actions, which are mediated via the autonomic nervous system [54].

Overall, NPY produces a shift to positive energy balance by increasing food intake, by reducing energy expenditure via a reduction in nonshivering thermogenesis in brown adipose tissue (BAT) and also by facilitating triglyceride deposition through increased insulin levels.

3.3. Regulation of ARC-NPY neurones

NPY synthesis and release in the hypothalamus are regulated by several factors, including leptin and insulin (inhibitory) and glucocorticoids (stimulatory) [49].

Leptin reduces appetite and increases energy expenditure when injected either peripherally or intracerebroventricularly, and doses of leptin that elicit these effects also reduce NPY mRNA levels in the ARC and PVN [55]. Leptin probably acts directly on the NPY neurones, as it readily enters the mediobasal hypothalamus and ARC [56], where a subset of the ARC-NPY/AGRP neurones express the OB-Rb isoform of the leptin receptor [57,58].

Insulin has similar effects to leptin on energy homeostasis, in that it inhibits feeding, stimulates thermogenesis and induces weight loss, and these actions are probably mediated at least in part through inhibition of NPY neurones. The ARC is rich in insulin receptors [46], and overactivity of the ARC-NPY/AGRP neurones is therefore consistent with falls in plasma insulin concentrations in states of negative energy deficit, such as starvation and insulin-deficient diabetes. Supporting this notion, various groups have shown that insulin inhibits NPY neurones in the hypothalamus [59].

3.4. NPY receptor subtypes and feeding

Currently, six different NPY receptors have been characterised, and there is some evidence for further subtypes [60,61]. Only five have been identified in the rat brain, namely the NPY-Y1, -Y2, -Y3, -Y4 and -Y5 receptors with the sixth (characterized in the mouse) being truncated and inoperative in rat and human [62]. Cloning studies have confirmed the existence of NPY-Y1, -Y2, -Y4 and -Y5 receptors as distinct entities in the hypothalamus [63–69], while pharmacological evidence alone suggests the presence of a NPY-Y3 subtype, located mainly in the NTS [70].
The multiple NPY receptor subtypes located in the hypothalamus are proposed to be involved in the regulating food intake. The initial notion was that Y\textsubscript{1} receptors mediate the hyperphagic effect of NPY [71,72], [\textsuperscript{3}P]NPY, a full Y\textsubscript{1} receptor agonist, stimulates feeding [60], while the potent and selective Y\textsubscript{1} receptor antagonist, BIBO3304, can significantly reduce the hyperphagia induced by NPY or 24-h fasting [73].

The Y\textsubscript{1} receptor is apparently regulated by nutritional state. Immunohistochemistry and in situ hybridization studies have revealed that fasting decreases the number and area of NPY-Y\textsubscript{1} receptor-immunoreactive neurones in the ARC and reduced NPY-Y\textsubscript{1} receptor mRNA levels [74]. Intriguingly, this response was attenuated by supplementing the drinking water with 10% glucose in these animals [74], pointing to a link between energy status and circulating glucose levels and the possible involvement of glucose-receptive neurones [75,76].

However, there is still ambiguity as to how much the NPY-Y\textsubscript{1} receptor contributes to NPY’s stimulation of food intake. For example, NPY(2–36), human PP, and human PYY(3–36) are all robust inducers of feeding, despite being weak or inactive NPY-Y\textsubscript{1} receptor agonists in vitro [60,69,77,78]. Much attention has focused recently on the NPY-Y\textsubscript{5} receptor subtype, whose pharmacological profile—notably its high affinity for human NPY, PYY, PP, [\textsuperscript{3}P]NPY, PYY(3–36) and NPY(2–36)—has led the suggestion that this is the “feeding” receptor [69,79,80]. Moreover, the NPY-Y\textsubscript{5} receptor antagonist, CGP71683A, could inhibit both NPY-induced hyperphagia and the hyperphagia present in some genetically obese models (e.g., the ob/ob mouse and fa/fa Zucker rats), which is thought to be NPY mediated [81]. Furthermore, administration of antisense oligonucleotides directed against the NPY-Y\textsubscript{5} receptors has been shown to inhibit NPY-induced feeding and the hyperphagia in ob/ob mice and Zucker rats [82,83]. The importance of NPY-Y\textsubscript{5} receptors is reinforced by indirect studies of the hyperphagia caused by fasting, in which there was a selective decrease in the density of NPY binding sites specifically in the perifornical LHA, a critical target area involved in feeding, which also contains NPY-Y\textsubscript{5} receptor mRNA [84]. We hypothesise that decreased NPY binding represents down-regulation on NPY “feeding” receptors, secondary to increased basal release of the peptide NPY-Y\textsubscript{5} receptor mRNA [84].

However, inconsistencies remain. As already mentioned, the selective NPY-Y\textsubscript{1} receptor antagonist, BIBO3304, which has low affinity for NPY-Y\textsubscript{5} receptors, antagonises hyperphagia induced by NPY, [\textsuperscript{3}P]NPY (a Y\textsubscript{1} agonist) and NPY(3–36) (a Y\textsubscript{5} > Y\textsubscript{2} agonist) [72]. Additionally, the potent and selective NPY-Y\textsubscript{5} receptor antagonist, L-154,804, failed to inhibit NPY-induced feeding but decreased that induced by bovine PP (a potent NPY-Y\textsubscript{5} receptor agonist) [85]. More recent studies revealed that antisense oligonucleotides targeting the Y\textsubscript{5} receptor [86] only caused a significant decrease in food intake after 10 h following the initial feeding response induced by NPY or NPY(3–36). As an antisense oligonucleotide targeting the Y\textsubscript{5} receptor had no effect on either spontaneous 2-h or NPY-, NPY 3–36- or PP-stimulated 2-h food intake in the same studies, it seems that the Y\textsubscript{5} receptor may act to maintain feeding but is not involved in the initial feeding response elicited by NPY.

Finally, autoreceptors (Y\textsubscript{2} and Y\textsubscript{4}) on the ARC-NPY/AGRP neurones themselves may modulate feeding by acting to inhibit NPY release from these cells in the PVN, LHA and other sites. The NPY-Y\textsubscript{1} receptor antagonist, BW1229U91, inhibits NPY-induced feeding but not that induced by bPP [85]. It has been shown that BW1229U91 also binds with high affinity to Y\textsubscript{4} receptors, at which it acts as an agonist [87,88]. This suggests that the NPY-Y\textsubscript{4} receptor may act to decrease feeding and body weight. We recently showed that NPY release from the hypothalamus in vitro was inhibited by both NPY-Y\textsubscript{2} and -Y\textsubscript{4} receptor activation [41,42]. Consistent with the possible self-inhibitory role of Y\textsubscript{2} receptors, transgenic mice that lack the NPY-Y\textsubscript{2} receptor are hyperphagic and obese [89].

### 3.5. Physiological role of ARC-NPY neurones

The primary physiological role of the ARC-NPY/AGRP neurones may be to sense and respond to states of negative energy balance. These neurones becomes overactive following a critical fall in the body’s energy stores, and the experimental effects of NPY suggest that they initiate appropriate behavioural and metabolic responses to restore energy balance. Such states of negative energy balance include starvation, insulin-dependent diabetes mellitus (IDDM) and lactication. All are characterised by increased hunger and active food seeking at expense of other behaviours and by reduced thermogenesis. These effects mirror the peptide’s central actions [90]. ARC-NPY/AGRP neuronal activity is increased in all of these states with rises in NPY expression in the ARC and increased NPY levels in the ARC and sites of release (PVN, DMH and LHA). Elevated NPY release in the PVN has been confirmed directly by stereotactic sampling in starvation and insulin-deficient diabetes [50,91]. The suggestion that NPY drives hyperphagia in these conditions is supported by the observations that NPY gene expression and peptide level increase in the hypothalamus of streptozotocin-diabetic rats before any rise in food intake occurs [92] and that diabetes-induced hyperphagia can be attenuated by the injection of anti-NPY monoclonal antibodies (intracerebroventricularly) [93].

Overall, these data indicate that the ARC-NPY/AGRP neurones act homeostatically to restore normal energy balance and body fat stores under conditions of energy deficit. The signals registering the fall in body fat probably include the falls in circulating leptin and/or insulin that occur in these conditions, which would be predicted to disinhibit the ARC-NPY/AGRP neurones through the mechanisms described above.
3.6. Role of NPY neurones in obesity

Inappropriate and unrestrained overcapacity of the ARC-NPY neurones leads to obesity in the genetic rodent models of obesity, which are due to interruption of leptin’s normal inhibitory effects on these neurones. Such models include the ob/ob mouse (which has no biologically active leptin) [26,94] and the db/db mouse and fa/fa fatty Zucker rat (both have loss of function mutations in the leptin receptor) [95–97]. Recalling the experimental effects of NPY, obesity in these genetic models results from a combination of hyperphagia and decreases in BAT activity and in whole body thermogenesis. In all these models, raised NPY mRNA levels have been demonstrated together with raised NPY levels in specific hypothalamic nuclei, including the ARC, PVN and DMH. Such results parallel those observed in normal rodents subjected to conditions of energy deficit, such as fasting and IDDM [72]. Moreover, down-regulation of NPY receptors, specifically of non-NPY-Y1 receptors (putatively NPY-Y5 receptors) in the perifornical LHA, has been shown in the fatty Zucker rat [72,84,90].

Interestingly, the normal inhibitory effect of insulin on the ARC-NPY/AGRP neurones is also attenuated in fa/fa Zucker rats, concomitantly with insensitivity of metabolic targets to the hormone. This “central insulin resistance” may develop as a consequence of chronic, gross hyperinsulinaemia, which may down-regulate hypothalamic insulin receptors and/or insulin transport into the brain [46].

All these observations strengthen the hypothesis that overactivity of the ARC-PVN projection plays a role in the hyperphagia and reduced energy expenditure that leads to obesity in these models.

By contrast, dietary-induced obesity leads to a fall in hypothalamic NPY mRNA levels and up-regulation of the non-NPY-Y1 receptors in the LHA [84]. This suggests that the hypothalamic NPYergic activity, particularly of the ARC-PVN neurones, does not drive the hyperphagia associated with exposure to a palatable diet [84]. Indeed, these same neurones appear to be inhibited, perhaps in an attempt to limit hyperphagia and weight gain [84].

3.7. Interactions with other neuronal systems

There is much potential for the NPY/AGRP neurones to interact with other neuronal populations that control energy balance. Some of the interactions with POMC, orexin and glucose receptive neurones are discussed below.

Recent studies in NPY knockout (− / − ) mice, which surprisingly grow normally and manifest a normal hyperphagic response to fasting [98], have highlighted the ability of alternate pathways to compensate for the lack role of NPY in the control of energy homeostasis. One factor is apparently AGRP, which is coexpressed in most of the ARC-NPY neurones and is an endogenous antagonist at the MC4-R that mediates the appetite-suppressing action of α-MSH (released from POMC neurones; discussed below). AGRP, like NPY, stimulates feeding when administered centrally, and its expression is significantly elevated in ob/ob mice [99]. Moreover, the ubiquitous overexpression of AGRP in transgenic mice causes obesity [99]. Interestingly, AGRP mRNA and immunoreactivity are up-regulated with fasting in NPY (− / − ) knockout mice [100], suggesting that AGRP compensates for the lack of NPY in this model and that these neurones retain their ability to sense and respond to energy deficits.

4. Melanocortins and the MC4-R

Convincing evidence implicates the hypothalamic melanocortin neuronal system in the regulation of food intake and body weight. These neurones produce peptides derived from a common precursor, POMC, of which α-MSH is considered to be the most important regulator of feeding. POMC is synthesized is specific neurones in the ARC and the NTS. Discrete α-MSH-containing pathways project from the ARC neurones to numerous brain regions, particularly within the hypothalamus. Several pontine and medullary regions receive projections from both the ARC and NTS systems, suggesting functional integration of the two neuronal populations.

Three melanocortin receptors (MC-R: MC3-R, MC4-R and MC5-R) have been identified within the brain, and both MC3-R and MC4-R are expressed within discrete hypothalamic nuclei implicated in energy homeostasis, including the VMH, DMH and the ARC-median eminence [18,101,102].

The role of melanocortins in energy homeostasis was strengthened by the discovery of a 131-residue protein termed agouti, which was found to antagonise the actions of α-MSH [103]. This 13-residue peptide is generally assumed to be the endogenous ligand at the CNS MC3-R and MC4-R, as administration of it or potent analogues, such as MT-II, markedly reduces food intake [104]. This advance was prompted by observations of the obese yellow (A') mouse, a long-recognised rodent model of obesity now known to be caused by a mutation within the promoter region of the agouti gene. This results in ectopic expression of agouti in numerous sites, including the hypothalamus. (Agouti expression is usually restricted to the hair follicle, where it blocks α-MSH action at MC1-R, thus inhibiting production of black melanin and so turning the fur pale.) In the hypothalamus, MC3-R and MC4-R antagonism leads to hyperphagia, reduced energy expenditure and ultimately obesity [103]. Thus, it was suggested that α-MSH acts tonically at sites within the hypothalamus to limit food intake.

However, controversy persists over whether MC3-R or MC4-R mediate the hypophagic action. Recent studies firmly place MC4-R rather than MC3-R at center stage of this regulatory pathway. The MC4-R knockout mouse generated by Huszar et al. [105] displays obesity like the A' mouse (but without yellow fur). Additionally, powerful modulators of
feeding are more selective for MC4-R than MC3-R: for example, the potent and selective MC4-R antagonists (HS014 and Ro27-3225) induced sustained hyperphagia and obesity [106,107], while the highly selective MC4-R agonist Ro27-4680 exerts the opposite effect on feeding [107]. By contrast, the reasonably selective MC3-R agonist γ2-MSH has been found to have no effect on food intake [108]. Recently, we have shown that hypothalamic MC4-R, notably within the ARC-median eminence, VHM and DMH, are selectively down-regulated (implying increased receptor activation in response to elevated exposure to ligand) in rats that develop diet-induced obesity. Moreover, MC4-R are up-regulated (indicating decreased receptor activation) in food-restricted rats. By contrast, the density of MC3-R does not alter in either of these conditions[102].

However, a role for MC3-R has been implied by the recent observation that the MC3-R knockout mouse is obese but with reduced food intake. This phenotype is attributed to greater feeding efficiency [109]. Also, mice lacking both MC3-R and MC4-R demonstrate greater obesity than that caused by MC4-R deficiency alone [109]. This is consistent with functions for both receptors in the regulation of the body weight, although which one is responsible for mediating the melanocortin-induced activation of metabolic rate is still unclear.

4.1. Modulation of melanocortins and their receptors

The melanocortin system is responsive to physiological inputs from peripheral signals of nutritional status, notably leptin. Additionally, it is known to interact at various levels with other appetite-regulating factors, particularly AGRP, NPY and mahogany.

4.1.1. Actions of leptin

Leptin appears to stimulate the melanocortin system, consistent with the fact that they both inhibit feeding, and melanocortin neurones are likely to mediate some of leptin’s central actions. In the ARC, approximately 30% of the POMC-expressing neurones also carry the OB-Rb [32]. Intraperitoneal leptin administration increases hypothalamic POMC mRNA levels [110], while conditions associated either with reduced leptin levels (e.g., fasting) or loss of the leptin signal (ob/ob mouse and fa/fa Zucker rat) show reduced POMC mRNA levels [111]. Also, pharmacological blockade of MC4-R impairs the ability of leptin to reduce food intake and body weight [112–114]. Leptin therefore appears to stimulate the POMC neurones, with the increase in POMC expression presumably resulting in elevated α-MSH production and release and thus reduced food intake via its interactions with MC4-R and/or MC3-R.

We recently investigated the relationship between leptin and hypothalamic MC4-R and have suggested that their interaction determines individual susceptibility to dietary obesity in rats presented with palatable food [115]. We found that an early rise in plasma leptin, soon after exposure to palatable food and preceding weight gain, predicts a lesser weight gain some weeks later. Leptin may therefore program the hypothalamus in some way to resist overeating in the long term. Intriguingly, this imprinting appears to involve specifically MC4-R within the VMH, as a significant correlation was identified between receptor density here at 8 weeks and plasma leptin levels at 1 week after the presentation of palatable food [115]. Therefore, we suggest that those animals able to elevate plasma leptin, which presumably influences a specific subset of neurones projecting to impact on MC4-R in the VMH, are relatively protected against overeating and developing obesity.

The involvement of the melanocortin system in the control of feeding is unquestionable, but its precise position in the chain of control is presently unclear. The expression of leptin receptors on POMC neurones suggests that MC4-R lie downstream of leptin. Furthermore, AY mice (in which MC4-R and MC3-R are inhibited by agouti) become obese despite dramatically elevated plasma leptin levels [116]. However, the view that MC4-R lie downstream of the leptin was challenged by the production of a genetic cross between AY and ob/ob mice. Obesity in AY mice was originally thought to be caused wholly by POMC signalling, thus blocking the leptin signal. Therefore, introduction of the AY mutation into the leptin-deficient ob/ob mouse should cause no additional weight gain. However, the effects of detective POMC signalling and the absence of the leptin appeared, at least in part, to be independent and additive, as the presence of AY increased weight gain to a similar extent in both the wild-type and ob/ob backgrounds [117].

4.1.2. AGRP

A unique feature of the melanocortin system is the presence of two endogenous antagonists. The peptide agouti has already been mentioned. Recently, a novel hypothalamic peptide, AGRP, was isolated and cloned based on its sequence homology to agouti [99]. However, unlike agouti, AGRP is normally expressed in the CNS although restricted to the NPY neurones of the ARC. In some hypothalamic nuclei, e.g., PVN and DMH, AGRP is thought to be released from NPY endings in the same synaptic complex as α-MSH. AGRP expression is increased in both ob/ob and db/db mice [99,118]. Moreover, overexpression of AGRP in transgenic mice results in obesity, as occurs in AY and MC4-R knockout mice [99]. Therefore, AGRP appears to also function as an MC4-R and MC3-R antagonist, pointing to a role for this peptide in the normal control of body weight. Consistent with this is evidence that central administration of AGRP significantly increases food intake and can reverse leptin-induced inhibition of feeding [119].

Interestingly, AGRP may regulated more robustly by changes in metabolic state than is POMC [120]. We have reported alterations in AGRP in dietary-obese and food-restricted rats but no changes in α-MSH and POMC [121]. AGRP release is also elevated in the hypothalamus of fasted rats [122]. This information suggest that AGRP may fine
tune the melanocortin axis and its tonic restraining effect on food intake and body weight. Curiously, AGRP is absent from other areas critical to the regulation of food intake, e.g., the VMH [2]. This may suggest that another ligand may be the principal controller of receptor activity in this nucleus.

Fig. 3 summarizes the proposed relationships between the melancortin system, leptin and AGRP and the way in which these interactions may determine susceptibility to dietary obesity in rats.

4.1.3. NPY

The melanocortin and NPY neuronal systems apparently interact with each other in a reciprocal fashion to regulate each other’s activity. Strangely, obese Ay mice have unaltered ARC-NPY mRNA, but expression occurs at high levels in an additional site, the DMH [123]. This finding was also observed in obese MC4-R knockout mice and suggests that NPY may partly mediate obesity in this model. Recently, this argument has been strengthened by the finding that the NPY-Y1 receptor antagonist 1229U91 significantly attenuates the feeding effects of the MC4-R selective antagonist HSO14 [124], while MC4-R knockout mice respond to the orexigenic actions of NPY [125]. Thus, the melanocortinergic neurones would appear to exert control over the NPY system. However, interplay between the two systems is not simply one of the hierarchical inhibition by the melanocortins, because of the coexpression of AGRP within the NPY neurones of the ARC [2].

Activation of these NPY/AGRP neurones could stimulate feeding through a dual effect, with both activation of NPY receptors and antagonism of MC4-R by AGRP. Additionally, MC4-R activity could also be antagonised by NPY itself: POMC neurones express the NPY-Y1 receptor [93,126] and receive input from NPY terminals (as discussed previously) [126,127]. Potentially, NPY released by the ARC neurones could therefore inhibit the ARC melanocortinergic system directly at the cell body [128] as well as postsynaptically through AGRP release (Fig. 4).

4.1.4. Mahogany

Mahogany was recently identified as another potential regulator of the melanocortin system, which, when mutated, suppresses the obesity resulting from ectopic expression of agouti in the brain [129]. Its mode of action remains controversial. It has been suggested that mahogany acts as an accessory receptor, gathering extracellular molecules presented to melanocortin receptors and reducing signalling by either presenting antagonist or sequester ligand [130]. Further work is required to understand these interactions and their significance in the control of feeding.

4.2. Summary

The melanocortin system clearly plays an important role in the control of feeding behaviour and body weight in rodents. Its relevance to human energy balance has also been confirmed recently, with the observation that rare cases of severe obesity are associated with specific mutations that affect the melanocortin system. Such mutations include...
truncation and frameshift mutation of the POMC gene [131] and a frameshift mutation in the MC4-R, the latter being associated with dominantly inherited morbid obesity [132].

5. Orexins/hypocretins

Orexin-A and -B were first identified as endogenous ligands for an orphan G protein-coupled receptor. Orexin-A and -B are 33- and 28-residue peptides derived from the same precursor prepro-orexin, encoded by the prepro-orexin gene on chromosome 10 in the rats and 17 in humans [133]. Prepro-orexin is synonymous with “preprohypocretin,” which was identified earlier and was predicted to be cleaved into two peptides, hypocretin-1 and-2, which include the orexin-A and -B sequence, respectively. Hypocretin-1 has the same sequence as orexin-A but with five extra residues at the N-terminus and one at the C-terminus, whereas hypocretin-2 is identical to orexin-B but for one extra amino acid at the C-terminus [134].

Orexins/hypocretins are expressed by neurones restricted to the perifornical nucleus and dorsal and lateral areas of the hypothalamus. These neurones are adjacent to but distinct from MCH neurones and project to many sites, including the PVN, ARC, the NTS and the dorsal motor nucleus of the vagus (DMNX) [133,135] Orexin neurones interact closely with other appetite-regulating neuronal systems. Orexin neurones have reciprocal connection with leptin receptor-expressing ARC neurones that express either NPY/AGRP or POMC/CART [14,136]. LHA orexin fibres are distributed extensively in the NTS, which contains receptors for numerous appetite-regulating peptides including NPY, leptin and orexin.

Orexin receptors (OX1-R and OX2-R) are widely but differentially distributed in the brain [134]. OX1-R is relatively selective for orexin-A, with an affinity 10 times higher than for orexin-B, and is expressed abundantly in the VMH. OX2-R (which has comparable affinities for both orexin-A and -B) is found predominantly in the PVN. OX1-R is coupled to the Gg subunit of G-protein, whereas OX2-R is linked to both Gi and Gq. Activation of both receptors result in Ca\(^{2+}\) influx and phospholipase C stimulation [137].

5.1. Relationship of orexin to feeding

A physiological role for the orexins in energy homeostasis was first suggested by the finding that prepro-orexin gene expression is increased by 48-h fasting and that central orexin administration increases feeding. It now appears that orexin-A stimulates feeding acutely, especially during daytime [133], although the effect is short lived and does not increase overall 24-h intake [138]. Neither does obesity result from chronic intracerebroventricular administration [139]. Blocking of either orexin-A (by an anti-orexin-A antibody) or OX-1R (using a selective antagonist) results in reduced food intake, further supporting the role of orexin-A in regulating feeding [140,141]. The effects of orexin-A on feeding may partly be mediated via NPY [142–145], perhaps through activation of both phospholipase C and Ca\(^{2+}\) influx [146].

Systematic studies indicate that orexin-B has a weaker effect (if any) in stimulating feeding [138,147]. Curiously, orexin-B levels may be selectively altered (independently of orexin-A) under conditions of increased hunger [148,149]. As discussed below, these changes may be related to changes in arousal rather than in hunger per se. Overall, the orexin neurones could seem more likely to be involved in short-term regulation of feeding episodes rather than the long-term control of body weight (see below).

5.2. Regulation of orexin neurones

Various circulating and neural factors are known to influence orexin neuronal activity. We and others [138,139] have investigated whether increased activity of the orexin neurones could contribute to enhanced hunger. We found increased orexin expression only under two circumstances, namely prolonged food deprivation for 48-h and hypoglycaemia if food was withheld [149]. Strikingly, no changes in prepro-orexin expression were observed in other conditions, which included food restriction for 6 days, diabetes induced by STZ injection, glucoseprivation with 2-deoxy-R-glucose (2-DG) that produced comparable hunger to hypoglycaemia or voluntary overeating of palatable food [149]. Common factors to 48-h fasting and acute hypoglycaemia during starvation, but not to the other circumstances of enhanced appetite, are falling plasma glucose levels and absence of food from the gut.

Most orexin neurones carry functional leptin receptors OB-Rb [14,150] and show leptin-induced activation through the JAK/STAT signalling pathway [150]. Moreover, central leptin administration has been reported to decrease orexin expression [151]. This suggests that orexin neurones may be at least partly regulated by the status of peripheral fat stores. By contrast, we found that orexin gene expression was not changed when plasma leptin levels were decreased through food restriction or diabetes or if leptin was replaced peripherally in food-restricted rats [149]. One striking difference between 48-h fasting and these other conditions with decreased plasma leptin is the absence of food from the upper gut. We have suggested that the presence of food in the gut may generate inhibitory signals that reach the brain and prevent any increase in orexin due, for example, to a fall in glucose (see below). Such inhibitory signals could include gastric distension and a rise in the portal glucose level. Both these are known to be detected by vagal sensory fibres and transmitted indirectly to the LHA via the NTS.

Glucose, another signal that is essential in initiating and terminating feeding [153], also appears to regulate orexin neuronal activity. The LHA is a well-known site to contain glucose-receptive neurones (see below) and is essential to
glucoprivic feeding. Orexin expression is stimulated by insulin-induced hypoglycaemia [149,153] if food was not available, but this increase in orexin mRNA was not seen either if euglycaemia was maintained through a peripheral glucose infusion [153] or if the hypoglycaemic rats were allowed to eat (hypoglycaemia normally induces striking hyperphagia) [149]. These findings again suggest that orexin neurones are stimulated by falling blood glucose levels but are promptly inhibited by feeding-related signals that might include satiety signals from the gut as well as a rise in blood glucose [149]. As discussed below, it is not clear whether overactivity of orexin neurones would result in increased hunger and food-seeking activity (consistent with the experimental effects of orexin-A but not orexin-B) or might affect other physiological functions thought to be influenced by the orexins, such as wakefulness.

Others have found LHA concentrations of orexin-A and -B peptide not to be significantly increased by prolonged fasting [154]. The lack of any increases in orexin levels despite the twofold increase in prepro-orexin gene expression [133,149] may imply that during fasting there is enhanced turnover and release of orexins in the LHA. A reported decrease in orexin-A levels in other brain areas under the same condition suggests that fasting might increase orexin-A release, which would be predicted to drive hyperphagia [154]. Dietary restriction for 7 days (93% of normal food intake) was found to reduce orexin-A levels in the LHA [151]. By contrast, we observed that hypothalamic orexin mRNA levels were not significantly altered by food restriction for 6 days, which induced comparable weight loss and metabolic changes to 48-h fasting [149]. Difference in the orexin responses to total fasting vs. food restriction may be related to inhibitory signals generated by the presence of food in the gut, as discussed above.

Underfeeding also differentially regulates orexin receptors and peptides. Fasting for 20 h stimulates OX2-R expression only in the ARC [155], concomitant with overactivity of the NPY/AGRP neurones during fasting. On the other hand, OX1-R, which is assumed to mediate orexin-induced feeding, is markedly increased in the VMH by 20-h fasting [155]. Surprisingly, administration of either orexin-A or -B into this nucleus failed to elicit any effects on food intake [156].

A further mystery is that selectively increased hypothalamic orexin-B concentrations have been found under two conditions of increased hunger, namely fasting superimposed on insulin-induced hypoglycaemia [148] and food restriction (2 days) during lactation [147]. These increases may not relate directly to hunger. Orexin-B may cause a relatively higher tonic stimulation of OX2-R, which is essential in promoting wakefulness [157]: central injection of orexin-B increases general alertness, together with burrowing and food-seeking activity [158]. Levels of arousal may therefore be the physiological function controlled by orexin-B under these conditions. In theory, an increase in local hypothalamic orexin-B could result from either reduced release and/or decreased degradation of orexin-B, but the precise mechanisms remain speculative. Food-deprived hypoglycaemic rats become extremely drowsy and may enter coma and die if hypoglycaemia persists [148]. We suggest that release of orexin-B may be reduced in these rats and that this may contribute to decreased wakefulness.

The differential regulation of orexin receptors and divergent responses of orexin-A and -B during starvation raise the possibility that orexin neurones may be organised into specific subsets that participate in separate aspects of feeding behaviour but that complement each other during starvation. Increased orexin synthesis with postulated increased release of orexin-A in prolonged fasting [149], together with up-regulated OX1-R [155], may stimulate feeding.

Interestingly, orexins can modulate the release of both GABA and glutamate via presynaptic axons [159]. The possible importance of this is that GABA and glutamate are known to regulate both feeding behaviours and wakefulness [160], and they could conceivably participate in the mechanisms by which acute starvation increases alertness. Enhanced alertness and an increase in food-seeking activity associated with severe energy deficit may be among the adaptive responses that counteract a fuel crisis [161]. Thus, enhanced orexin release may per se reinforce feeding and food seeking-related activities directly or indirectly through GABA and/or glutamate.

5.3. Relationship of orexin to obesity

At present, orexins are not convincingly implicated in obesity. Unlike NPY, which potently increases food intake and induces obesity if given repeatedly [162], central administration or orexin-A affects daytime feeding only, without increasing 24-h food intake, while repeated injections have no effect on body weight [139]. We did not find any disturbance in orexin mRNA in dietary-obese rats [149], which were 20% heavier than rats fed on normal chow diet. However, orexin mRNA is reportedly decreased in ob/ob and db/db mice [163], in contrast to the unchanged expression in nonobese Wistar rats [149]. The authors suggested that up-regulation of prepro-orexin expression in ob/ob and db/db mice was due to normalisation of glucose levels, implying that hyperglycaemia causing tonic suppression of orexin expression in obese ob/ob and db/db mice was due to normalisation of glucose levels, implying that hyperglycaemia causing tonic suppression of orexin expression in obese ob/ob and db/db mice [163]. In contrast to that explanation, we found that orexin gene expression was unchanged in diabetic ZDF rats (compared with nonobese controls) but was significantly decreased when ZDF rats were treated with the thiazolidinedione rosiglitazone, which normalised hyperglycaemia and other metabolic disturbances [164]. Furthermore, orexin-A and -B levels show no consistent changes in various obese rats [166,167]. These conflicting data are difficult to reconcile at present.

5.4. Orexin in the gastrointestinal tract

A large population of orexin-containing neurones has recently been identified in the gastrointestinal tract [168].
Orexin neurones are extensively located throughout the enteric nervous system (ENS), and orexin receptors are also present in proximity to orexin terminals of mucosal nerve fibres [169], suggesting that the ENS may respond to orexin released locally. Orexins increase gut motility and intestinal secretion when incubated with guinea pig colon in vitro [168]. More intriguingly still, orexins are also expressed in endocrine cells in the gastric and intestinal mucosa as well as the pancreas [168], hinting that orexins may function as hormones and/or as paracrine or autocrine transmitters. Central administration of either orexin-A or -B increases plasma insulin [169]. Orexin-A given centrally also stimulates gastric secretion and increases blood glucose, whereas orexin-B has no such effects [170].

Enteric orexin neurones, like their hypothalamic counterparts, express functional leptin receptors on their surface [168] and are activated by fasting, perhaps specifically by the absence of food from the gut. This suggests that orexin neurones may somehow regulate aspects of energy balance during food ingestion. It is not yet clear whether they can sense food or specific nutrients or are concerned with other autonomic gut activities (motility and secretion) and whether or not they communicate with the orexin neurones in the hypothalamus.

5.5. Possible functions of orexin

We have discussed above the evidence that orexin neurones are stimulated by falls in plasma glucose and apparently inhibited by prandial signals, which could include the presence of food in the gut and/or a rising glucose concentration in the portal circulation [148,149]. These neurones may constitute a system for initiating and terminating short-term feeding episodes and may be involved in an integrated network in the gastrointestinal tract, brainstem and hypothalamus. They could function to modulate glucose homeostasis [171], as illustrated in Fig. 5, and perhaps participate in the “on–off” regulation of short-term feeding behaviour [149]. Orexin neurones certainly occupy strategically important anatomical structures that would allow them to fulfill such a role. Peripherally, they may sense nutritional status in the gut, modulating gastrointestinal secretion and motility in order to prime the digestive tract in preparation for food ingestion and energy uptake [168]. Centrally, orexin neurones sense glucose availability to regulate their own activity and could interact with other neurotransmitters to control feeding [148,149,153,172].

At same time, the widespread distribution of orexin terminals and receptors indicate that orexins may have broader functions that energy homeostasis. The orexin system is now known to be essential in regulating sleep–wake functions: mutations in OX2-R result in narcolepsy in the dog [157] while orexin-knockout mice have disordered wakefulness [173]. The relevance of this to food-seeking behaviour has been discussed above. Moreover, orexin also is involved in cardiovascular regulation of blood pressure [109,174,175] neuroendocrine [176–178] and autonomic functions.

6. Glucose-sensing neurones in hypothalamus and other regions

Almost half a century ago, Mayer [179] and Anand et al. [180] formulated the “glucostat” hypothesis that glucose availability to specific glucose-sensing neurones is an important factor regulating feeding behaviour and ultimately body weight. Glucose is normally the main metabolic fuel of the brain, and falls in blood glucose or blockade of neuronal glucose utilisation (i.e., with the antimetabolic 2-DG) powerfully stimulate feeding. It has long been recognised that specific parts of the CNS contain neurones that can detect changes in ambient glucose level, but their place in the hierarchy of the CNS systems that regulate feeding is uncertain. It is only now becoming clear that glucose-sensing neurones may communicate extensively with other appetite-regulating neuronal systems, including those expressing NPY and orexins.

Electrophysiological techniques can be used, either in vitro (brain slices) or in vivo (stereotactic implantation into the CNS of anaesthetised animals), to demonstrate that certain neurones alter their electrophysiological behaviour...
(i.e., membrane potential and firing rate) in response to change in ambient glucose levels. Glucose-responsive neurones increase their firing rate as glucose levels rise, while glucose-sensitive neurones are stimulated when glucose falls and vice versa [181–184]. The behaviour of a typical glucose-sensing neurone is illustrate in Fig. 6.

Several hypothalamic nuclei contain glucose-sensing neurones, including the ARC, DMH, PVN, VMH and LHA [182,184–187], all of which are intimately involved in the neural control of energy homeostasis [188,189]. Extrahypothalamic areas containing glucose-sensing neurones include the substantia nigra, locus coeruleus, neocortex, hippocampus and especially the NTS. As mentioned above, the NTS relays visceral afferent signals (carried via the vagus) to the LHA [190–194].

The importance of the glucose-sensing neurones is highlighted by the recent demonstration that obesity develops in mice injected with gold thioglucose, which selectively destroys glucose-responsive neurones of the VMH [195]. This indicates that long-term body weight regulation, as well as acute feeding behaviour, is controlled by glucose-sensing neurones.

The various populations of glucose-sensing neurones appear to have distinct anatomical distributions. For example, 30–40% of LHA neurones respond to a rise in extracellular glucose level with a decrease in firing rate (i.e., inhibition of activity; glucose-sensitive neurones), while glucose-responsive neurones are rare (~5%) in this area. By contrast, 40–45% of VMH neurones are glucose responsive (being stimulated by increased glucose) and glucose-sensitive neurones are sparse here [184,186,196]. This suggests that the VMH and LHA have distinct functions in the recognition of altered glucose availability, but it is not clear how these differences might be related to overall integration of feeding signals in these regions or in the hypothalamus as a whole.

6.1. How are glucose-sensing neurones activated by changes in ambient glucose levels?

The first insight into cellular mechanism of glucose-sensing neurones came from studies of slices of NTS, which also contains neurones sensitive to changes in extracellular glucose concentration. NTS neurones that were stimulated by a rise in glucose level became depolarised by 3–10 mV while their membrane conductance increased by 50–80%. Conversely, decreasing glucose to 3 mM hyperpolarised these same cells and reduced their membrane conductance [183]. The authors postulated that such behaviour most likely was due to alterations in the membrane permeability to K+ ions. Similar changes in membrane voltage following either a rise or a fall in glucose level were subsequently demonstrated in VMH neurones in vitro [197]. Moreover, cell-attached recordings from these neurones revealed the presence of K+ channels, which were closed by a rise in extracellular levels of either ATP or glucose, suggesting that closure of the ATP-sensitive K+ channels is responsible for the depolarisation and increased firing rate under hyperglycaemic conditions [197]. The same mechanism triggers insulin secretion by the islet β-cells [198,199]. This conclusion was supported by a recent in vivo study of Ashford et al. [196]. On the other hand, the glucose-sensitive neurones that predominate in the LHA are activated through different mechanism, which is dependent on alterations in Na+/K+ ATPase [196].

6.2. Other factors modulating glucose-sensing neurones

Some glucose-sensitive neurones in the LHA also respond to various sensory stimuli including cold, heat, pinching the tail and increased inspired CO₂ concentrations. These stimuli modulate the neuronal responses to glucose [196,200] and may be involved in the suppression of feeding induced by these noxious stimuli [200].
In addition to detecting changes in ambient glucose levels, most of the glucose-sensitive neurones are also affected by other circulating metabolites, all of which are known to influence feeding behaviour in one way to another (Table 2) [181–184,201,202].

6.2.2. Orexins

As discussed above, orexin-A and -B are expressed exclusively in the LHA and stimulate feeding acutely [206]. Using Fos-like immunoreactivity as a marker of neuronal activation, orexin-containing neurones are activated by insulin-induced acute hypoglycaemia [148,173]. Increased hypothalamic levels of orexin mRNA have been reported in response to falls in blood glucose level [150,173]. This raises the possibility that the LHA glucose-sensitive neurones, whose neurochemical identity is unknown, might be the orexin neurones. However, using a combined electrophysiological and immunohistochemical technique, we have recently demonstrated that none of the identified glucose-sensing neurones contain orexin-A. However, the two populations of neurones are intimately related. Some processes of orexin cells are closely intertwined around glucose-sensitive cell bodies and processes, while glucose-sensitive neurones send branches to several orexin-A cells. Strikingly, the cell bodies of an orexin cell and a glucose-sensing cell may be closely applied each other [207]. This implies that glucose-sensing neurones and orexin neurones interact with each other, and we have found that orexin-A potently excited glucose-sensing neurones in the LHA while it had relatively small effects on glucose insensitive neurones [205]. Together, glucose-sensing and orexin neurones in the LHA may cooperate to play a key role in controlling short-term feeding, especially in response to glucoprivic stimuli. As spontaneous feeding episodes in the rat are preceded and apparently initiated by small falls in plasma glucose level [155], this mechanism may have wider relevance to the regulation of food intake.

7. Conclusions

Recent advances in molecular biology and neuroscience have greatly expanded our understanding of the physiology of energy balance and neuroendocrine regulation. Here, we have discussed several hypothalamic neurotransmitters that mediate metabolic and neuroendocrine responses to alterations in energy balance and revealed the complexity of these processes, which involve multiple neural circuits probably operating at different levels and different degrees to integrate and maintain energy homeostasis. With the increasing numbers of obese individuals in the Western world as well as in societies exposed to Western life style, and with obesity-related disorders such as diabetes and cardiovascular diseases, an improved understanding of brain body weight control is an important priority.


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