

Orexins and appetite regulation

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Summary Initial research on the functional significance of two novel hypothalamic neuropeptides, orexin-A and orexin-B, suggested an important role in appetite regulation. Since then, however, these peptides have also been shown to influence a wide range of other physiological and behavioural processes. In this paper, we review the now quite extensive literature on orexins and appetite control, and consider their additional effects within this context. Although the evidence for orexin (particularly orexin-A and the orexin-1 receptor) involvement in many aspects of ingestive physiology and behaviour is incontrovertible, central administration of orexins is also associated with increased EEG arousal and wakefulness, locomotor activity and grooming, sympathetic and HPA activity, and pain thresholds. Since the orexin system is selectively activated by signals indicating severe nutritional depletion, it would be highly adaptive for a hungry animal not only to seek sustenance but also to remain fully alert to dangers in the environment. Crucial evidence indicates that orexin-A increases food intake by delaying the onset of a behaviourally normal satiety sequence. In contrast, a selective orexin-1 receptor antagonist (SB-334867) suppresses food intake and advances the onset of a normal satiety sequence. These data suggest that orexin-1 receptors mediate the episodic signalling of satiety and appear to bridge the transition from eating to resting in the rats' feeding-sleep cycle. The argument is developed that the diverse physiological and behavioural effects of orexins can best be understood in terms of an integrated set of reactions which function to rectify nutritional status without compromising personal survival. Indeed, many of the non-ingestive effects of orexin administration are identical to the cluster of active defences mediated via the lateral and dorsolateral columns of the midbrain periaqueductal gray matter, i.e., somatomotor activation, vigilance, tachycardia, hypertension and non-opioid analgesia. In our view, therefore, the LH orexin system is very well placed to orchestrate the diverse subsystems involved in foraging under potentially dangerous circumstances, i.e., finding and ingesting food without oneself becoming a meal for someone else. © 2002 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Obesity, defined as a body mass index (BMI) of ≥ 30 kg/m², arises from a chronic net excess of energy intake over energy expenditure and is currently recognised as the largest and fastest growing public health problem in the developed world (WHO, 1998). More than 100 million people worldwide are considered obese, with recent UK statistics indicating that prevalence in both men and women has almost tripled (from c7% to c20%) in the past 20 years (Prescott-Clarke and Primatesta, 1998). The medical

significance of this alarming trend is that obesity not only impairs general quality of life but also increases morbidity (e.g., Type II diabetes, ischaemic heart disease, hypertension, stroke, osteoarthritis and cancer) and leads to a 2-fold increase in the risk of premature mortality (Kopelman, 2000; Macdonald, 2000; McIntyre, 1998). Furthermore, economic surveys estimate that the direct costs of obesity account for up to 6% of annual health budgets in developed countries (Wolf and Colditz, 1998) which, in the UK, amounts to around £350 million p.a. As a 10 kg weight loss markedly reduces the adverse effects of obesity (WHO, 1998), there is an urgent need for the development of effective treatments for those who are already obese or who will become obese in the future (Finer, 1999).

The current prevalence of obesity reflects a dynamic interaction between societal change (i.e., low energy

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expenditure associated with an increasingly sedentary existence) and the ready availability of palatable foods (i.e., high energy intake associated with energy-dense foods). It is therefore unsurprising that the majority of treatment programmes have emphasised the primacy of behaviour modification through exercise and dieting. However, while such lifestyle changes can lead to successful weight loss, this is often only modest in degree and temporary in duration (Chiesi et al., 2001; Clapham et al., 2001; Macdonald, 2000). For these reasons, it has been argued that drug treatment should be an active option for those patients unable to benefit from behaviour modification programmes alone (Carpino, 2000). Nevertheless, it is pertinent to note that, largely as a result of the unsympathetic public view that sufferers are responsible for their own misfortune, scientific interest in the precise mechanisms of obesity has traditionally been relatively low on the list of research priorities. Indeed, following the withdrawal of fenfluramine/phenteramine in 1997, only three drugs are currently marketed in Europe for the treatment of obesity, mazindol (an adrenergic agonist), sibutramine (a serotonin/noradrenaline reuptake inhibitor) and orlistat (a lipase inhibitor). Unfortunately, none of these agents achieves a weight loss greater than 10–15% and serious doubts have been expressed about the sustainability of even this very modest response (Clapham et al., 2001). However, at least three factors have contributed to a significant change in attitude towards obesity among scientists, the public, regulatory authorities and the pharmaceutical industry: (i) the realisation that this disorder is in the same league as the world's most serious diseases, (ii) the rapidly growing prevalence of obesity in the developed/developing world, and (iii) the discovery of novel brain mechanisms that regulate appetite and which present exciting opportunities for the development of novel treatment strategies (Dourish, 1999).

HYPOTHALAMIC NEUROPEPTIDES

Contemporary biological approaches to the problem of obesity involve multiple therapeutic targets (for recent

reviews: Ahima and Osei, 2001; Carpino, 2000; Chiesi et al., 2001; Clapham et al., 2001; Collins and Williams, 2001; Proietto et al., 2000). Among these diverse avenues of research, the potential therapeutic significance of hypothalamic neuropeptides is currently attracting much research attention (for recent reviews: Arch et al., 1999; Beck, 2001; Dhillon and Bloom, 2001; Inui, 1999; Inui, 2000; Kalra et al., 1999; Lawrence et al., 1999; Mercer and Speakman, 2001; Salton et al., 2000; Schwartz et al., 2000). The crucial role of the basal hypothalamus in appetite regulation and energy balance has been established since early demonstrations that damage to the lateral hypothalamus (LH) produces profound aphagia and weight loss whereas damage to the neighbouring ventromedial nucleus (VMH) results in marked hyperphagia and obesity (reviews: Bernardis and Bellinger, 1996; Oomura, 1980). On the basis of these findings, Stellar's (1954) classical 'dual centre theory' proposed that activity in the LH 'hunger centre' is controlled by inhibitory pathways from the VMH 'satiety centre': damage to the VMH itself, or to pathways from the VMH to the LH, prevented implementation of the VMH satiety signal, leading to over-eating and obesity. Although this elegantly simple theory has undergone many changes over the past half-century, including incorporation of several vitally important nuclei (e.g., paraventricular (PVN), arcuate (ARC), dorsomedial (DMN) and suprachiasmatic (SCN) nuclei), as well as the crucial role of peripheral feedback regulation, it is only within the last 10 years that significant advances have been made in elucidating the key biological signals and pathways involved (Beck, 2000; Kalra et al., 1999; Mercer and Speakman, 2001). This work has resulted not only in the identification of a multitude of orexigenic (appetite-stimulating) and anorexigenic (appetite-inhibiting) neurochemicals, but has also strengthened the concept that a distinct neuronal circuitry operates locally within the hypothalamus to regulate food intake and energy homeostasis (Kalra et al., 1999). Foremost among these appetite-related neurochemicals is a large group of neuropeptides which, when centrally administered, either enhance or reduce food intake (Table 1).

Table 1 Orexigenic and anorexigenic neuropeptides (adapted from Ahima and Osei, 2001; Inui, 1999; Kalra et al., 1999)

Orexigenic peptides ↑ food intake	Anorexigenic peptides ↓ food intake
Agouti-related protein (AgRP)	α -Melanocyte stimulating hormone (α -MSH)
Endogenous opioids	Cholecystokinin (CCK)
Galanin	Cocaine and amphetamine-regulated transcript (CART)
Ghrelin	Corticotropin releasing hormone (CRH)
Growth-hormone releasing factor	Glucagon-like peptide-1 (GLP-1)
Melanin-concentrating hormone (MCH)	Melanocortin
Neuropeptide Y (NPY)	Neurotensin
Orexin-A	Thyrotropin releasing hormone (TRH)
	Urocortin

Hypothalamic neuropeptides are believed to be the principal biological mechanism through which the adiposity (negative) feedback signal, leptin, influences brain circuitry involved in appetite and bodyweight regulation (Blundell et al., 2001; Kalra et al., 1999; Schwartz et al., 2000). A product of the *ob* gene, leptin is a major peripheral signal to the brain linked to the size of (predominantly white) adipose tissue stores (Friedman and Halaas, 1998). Consistent with this physiological role, malfunction of either the leptin gene (as in the *ob/ob* mouse) or the leptin receptor gene (as in the *db/db* mouse and *fa/fa* rat) results in an obese phenotype (Beck, 2001; Inui, 2000; Salton et al., 2000), as can genetic mutations in the coding region for leptin or leptin receptor genes in humans (Mantzoros, 1999; Trayhurn, 1999). Although initially conceptualised as a 'satiety' or 'starvation' signal which may yield therapeutic innovation, leptin is now known to have additional physiological roles (e.g., in the reproductive and immune systems) which may undermine its direct use as a treatment for obesity (Chiesi et al., 2001). However, as leptin-induced inhibition of food intake is thought to be mediated through a combined upregulation of hypothalamic anorexigenic peptides and a down-regulation of hypothalamic orexigenic peptides (Kalra et al., 1999), current research endeavours are strongly focussed on the neuropeptide signalling pathways downstream of leptin.

In addition to the traditional focus on the LH and VMH in hypothalamic regulation of energy balance, more recent research has emphasised the integrative role played by the ARC nucleus. This region of the mediobasal hypothalamus contains a high density of neurons that not only produce a large number of orexigenic (e.g., neuropeptide Y, NPY) and anorexigenic (e.g., α -melanocyte stimulating hormone, α -MSH) peptides but which also express leptin receptors. Furthermore, these ARC neurons send projections to adjacent hypothalamic sites (e.g., VMN, DMN, PVN) where local peptide infusions have been shown to significantly alter food intake and/or energy homeostasis (Inui, 1999; Kalra et al., 1999; Schwartz et al., 2000). At present, the NPY system is thought to be a (if not the) final common pathway for this signalling cascade (Gehlert, 1999; Kalra et al., 1999). Unsurprisingly, the recognition that expression of appetite is chemically encoded in the hypothalamus has generated the corollary position that dysfunction of this system may have a causal role in eating disorders such as obesity and anorexia (Kalra, 1997). As such, novel anti-obesity drugs are being designed to promote weight loss/prevent weight gain by modifying the function of key neuropeptides (Ahima and Osei, 2001; Carpino, 2000; Chiesi et al., 2001; Clapham et al., 2001; Collins and Williams, 2001; Proietto et al., 2000), including several NPY receptor antagonists (Chamorro et al., 2002; Gehlert, 1999)

and melanocortin-4 receptor agonists (Vergoni et al., 2000). Against this background, the recent discovery of yet two more hypothalamic neuropeptides (the orexins) should not only lead to further refinement in our understanding of the central mechanisms of appetite regulation but may also provide novel molecular targets for therapeutic drug development.

THE OREXINS: DISCOVERY

In early 1998, two research groups independently (and virtually simultaneously) reported the discovery of two novel hypothalamic neuropeptides. Employing subtractive cDNA cloning techniques, de Lecea et al. (1998) described a hypothalamic-specific mRNA encoding preprohypocretin, the putative precursor of two peptides (hypocretin-1 and hypocretin-2) sharing substantial amino acid identities both with each other and with the gut hormone, secretin. Hypocretin protein products were found to be restricted to neuronal perikarya of the dorsal and lateral hypothalamus, with extensive fibre projections throughout the neuraxis. Very shortly after this report appeared, Sakurai et al. (1998) published the discovery of two closely related peptide ligands (orexin-A and orexin-B) for a previously identified G protein-coupled orphan receptor. In this very detailed study, the authors not only delineated the structures of the two novel peptides but also those of their prohormone (prepro-orexin) and receptors (orexin-1 and orexin-2; OX₁R and OX₂R). Furthermore, they outlined the tissue distribution of the peptides and their receptors in rat brain and, crucially, provided the first insights into their likely functional (i.e., behavioural) significance.

In view of the hypothalamic expression of prepro-orexin mRNA, Sakurai et al. (1998) proposed that the orexins may be involved in the regulation of ingestive behaviour. Consistent with this hypothesis, they found that central administration of either peptide resulted in a dose-dependent stimulation of food intake in rats. They also demonstrated that orexin production and release is affected by the nutritional state of the animal, with 48 h fasting resulting in a 2.4-fold up-regulation of prepro-orexin mRNA. It was on the basis of these initial behavioural and physiological observations that the authors specifically chose the name 'orexins' for the novel peptides (after the Greek 'orexis', meaning appetite). Furthermore, in the conclusion to their seminal paper, they suggested that 'pharmacological intervention directed at the orexin receptors may prove to be an attractive avenue toward the discovery of novel therapeutics for diseases involving dysregulation of energy homeostasis such as obesity and diabetes mellitus'. The principal aim of the present paper is to review relevant evidence that has accumulated over the past four years.

Note on terminology

We have deliberately adopted the term 'orexins' rather than 'hypocretins' for the current review because of the specific focus on mechanisms appetite and the more common use of orexin-A and orexin-B in feeding experiments. The latter point is not insignificant in view of the fact that, despite the frequent interchangeable use of the terms orexin and hypocretin in the literature, the amino acid sequences of hypocretin-1 and orexin-A do not totally correspond (de Lecea et al., 1998; Sakurai et al., 1998), while at least some commercially available (deamidated) forms of hypocretin-1 and hypocretin-2 are largely inactive at orexin receptors (Smart et al., 2000).

OREXINS AND THEIR RECEPTORS

In humans, the prepro-orexin gene is located on chromosome 17q21, spans 1432 bp and consists of 2 exons and 1 intron (Sakurai et al., 1999). The cDNA sequence of prepro-orexin indicates that orexin-A and orexin-B are produced by proteolytic processing from the same 130-residue (rodent) or 131-residue (human) polypeptide. Orexin-A comprises a 33-amino acid peptide of 3562 Da (the structure of which is completely conserved in several mammalian and amphibian species) while orexin-B is a 28-amino acid peptide of 2937 Da that is 46% identical in sequence to orexin-A (Dyer et al., 1999; Sakurai et al., 1998; Shibahara et al., 1999). In rat brain, prepro-orexin mRNA and immunoreactive orexin are confined to a distinct population of neurons in the perifornical, lateral and dorsal hypothalami, with projections to several neighbouring hypothalamic nuclei as well as diverse forebrain, midbrain and brainstem loci (Briski and Sylvester, 2001; Cutler et al., 1999; Date et al., 1999; de Lecea et al., 1998; Fadel and Deutch, 2002; Peyron et al., 1998; Sakurai et al., 1998; Nambu et al., 1999; Taheri et al., 1999). The extensive extra-hypothalamic orexin projections in rat brain include the cerebral cortex, hippocampus, amygdala, septum, medial thalamic nuclei, periaqueductal gray, raphe nuclei, ventral tegmental area, locus coeruleus, area postrema, nucleus of the solitary tract, and spinal cord. Very similar CNS orexin distribution patterns have also been reported for non-human primates and humans (Horvath et al., 1999; Moore et al., 1998). Although not detectable in most peripheral tissues (e.g., heart, liver, kidney, lung), orexin-immunoreactive neurons have been identified in the testes (Sakurai et al., 1998), pituitary gland (Date et al., 2000) and, significantly, the enteric nervous system as well as endocrine cells of the gut and pancreas (Kirchgessner, 2002; Kirchgessner and Liu, 1999; Nowak et al., 2000; Takahashi et al., 1999).

The orexins activate two closely related G-protein-coupled receptors, termed the orexin-1 and orexin-2 receptor (OX₁R and OX₂R), which are also highly con-

served across mammalian species (94% and 95% homology rat v human, respectively). While orexin-A has equal affinity at OX₁R and OX₂R, orexin-B has an appreciably greater (approximately 10-fold) affinity at OX₂R (Sakurai et al., 1998; Smart et al., 1999; Smart et al., 2000). Early mapping of orexin receptor mRNA (Lu et al., 2000; Trivedi et al., 1998) and more recent immunohistochemical/*in situ* hybridization studies (Greco and Shiromani, 2001; Hervieu et al., 2001; Marcus et al., 2001) have revealed that both receptors have a widespread, though differential, distribution in rat brain. For example, the expression of OX₁R is high throughout the hypothalamus (including ARC, VMH and SCN), whereas hypothalamic expression of OX₂R is highest in the PVN. Outside the hypothalamus, the highest density of OX₁R is found in the locus coeruleus with appreciable levels in the olfactory nuclei, pyriform cortex, thalamus, hippocampus, dorsal tegmental nucleus, ventral tegmental area, dorsal raphe nucleus and spinal cord. In contrast, OX₂R is most strongly expressed in layer VI of the cortex, the amygdala, mesencephalic trigeminal nucleus, pontine reticular nuclei and ventral cochlear nucleus. Consistent with studies on peptide distribution, mRNA for both orexin receptors has also been detected in the gut (Kirchgessner and Liu, 1999) and pituitary gland (Date et al., 2000), with additional evidence for the presence of OX₁R in brown adipose tissue (Haynes et al., 2002) and OX₂R in the adrenal medulla (Lopez et al., 1999).

In the most detailed examination of hypothalamic OX₁R distribution thus far reported, Backberg et al. (2002) have employed indirect immunofluorescence histochemistry, combined with direct/indirect double-labelling and confocal laser microscopy, to characterise OX₁R immunoreactivity in chemically defined neurons within the hypothalamus. Confirming previous reports, neurons displaying OX₁R immunoreactivity were found to be very widely distributed within the hypothalamus, including cell bodies in the SCN, PVN, ARC, VMN, DMN and the LH. Of particular relevance in the present context are the observations of OX₁R immunoreactivity in over 90% of NPY- and POMC-expressing neurons of the ARC nucleus, and in both MCH- and orexin-expressing neurons of the LH. From a functional perspective, the latter finding indicates that the OX₁R may serve both as a self-regulatory inhibitory somatodendritic autoreceptor as well as a postsynaptic receptor. Clearly, these data indicate that, via the OX₁R, orexins are capable of influencing a constellation of hypothalamic neuropeptide signalling pathways implicated in appetite regulation.

The extensive anatomical distribution of orexin fibres and orexin receptors suggests a diversity of (perhaps unrelated) autonomic, neuroendocrine and behavioural functions (Sakurai, 1999). However, it must be appreciated at the outset that feeding does not occur in a vacuum but,

rather, is part of a complex, integrated and adaptive system designed to maintain energy balance and homeostasis (Blundell, 1986). It may therefore be the case that the orexin system plays a vitally important role in orchestrating the multiple physiological and behavioural subsystems involved in appetite regulation.

OREXIN RECEPTOR ANTAGONISTS

To date, most research on the behavioural significance of the orexins has involved the central (i.e., intracerebroventricular (ICV) or intracerebral (IC)) administration of the peptides themselves. While an invaluable research strategy, this approach alone suffers some drawbacks including concerns about the physiological relevance of the doses found to produce significant behavioural change as well as problems in establishing the receptor specificity of such effects (Arch, 2000). As reviewed below, several additional strategies have recently been developed including prepro-orexin gene knockout and the use of specific antibodies directed at either the peptides or their receptors. However, by far the most important technical advance in this area has been the development of selective orexin receptor antagonists, the most potent and selective of which is the OX₁R antagonist, SB-334867 (1-(2-Methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl-urea HCl). This compound has nanomolar affinity at the OX₁R, possesses at least 50-fold selectivity over the OX₂R and a wide range of other G-protein-coupled receptors, and is brain penetrant following systemic administration (Duxon et al., 2001; Porter et al., 2001; Smart et al., 2002; Smart et al., 2001). Understandably, therefore, SB-334867 is increasingly being used as a tool to study not only the receptor mediation of responses to exogenously administered peptides but also (when administered alone) to assess the physiological and behavioural significance of endogenous orexins.

OREXINS AND APPETITE

Since the initial demonstration of orexin-induced hyperphagia in rats (Sakurai et al., 1998), a large body of evidence has accumulated in support of the involvement of these peptides (particularly, orexin-A) in mechanisms of appetite regulation.

Neuroanatomy, neurophysiology and biochemistry

As reviewed above, a large number of orexigenic (e.g., NPY) and anorexigenic neuropeptides (e.g., α -MSH) are localised to leptin-sensitive neurons of the ARC nucleus (Arch et al., 1999; Sakurai, 1999). A convincing neuro-anatomical argument for orexin involvement in signalling pathways associated with feeding is suggested by the

strong reciprocal connections between LH orexin neurons and these ARC cells (Broberger et al., 1998; Elias et al., 1998; Guan et al., 2001; Horvath et al., 1999), the high levels of OX₁R immunoreactivity in the majority of NPY- and POMC- expressing ARC neurons (Backberg et al., 2002), and the large proportion of LH orexin neurons that express leptin receptors (Funahashi et al., 2000; Moriguchi et al., 1999; Niimi et al., 2001). Consistent with this hypothalamic circuitry, and with the extensive CNS projections of LH orexin fibres (Briski and Sylvester, 2001; Cutler et al., 1999; Date et al., 1999; de Lecea et al., 1998; Nambu et al., 1999; Peyron et al., 1998; Sakurai et al., 1998; Taheri et al., 1999), central infusions of orexin-A not only increase *cFos* immunoreactivity in ARC, PVN and VMH neurons but also in a range of extra-hypothalamic structures involved in appetite regulation, e.g. the amygdala and the nucleus of the solitary tract (NTS) (Date et al., 1999; Edwards et al., 1999; Mullett et al., 2000). Furthermore, the direct application of orexins has not only been found to activate neurons in many of these structures, including the PVN (Shirasaka et al., 1999, 2001) and ARC (Rauch et al., 2000) nuclei, but also to bidirectionally influence glucose-sensitive (activated) and glucose-responsive (inhibited) neurons of the LH and VMH, respectively (Shiraishi et al., 2000).

From a systems perspective, it is equally important to note that manipulations of nutritional state induce pronounced changes in orexin neurons. Thus, prolonged fasting (48–72 h) and/or insulin-induced hypoglycaemia have been found to increase hypothalamic prepro-orexin mRNA expression (Cai et al., 1999; Griffond et al., 1999; Mondal et al., 1999; Sakurai et al., 1998; Yamamoto et al., 2000), hypothalamic OX₁R densities (Lopez et al., 2000), and the activity of LH orexin neurons (Moriguchi et al., 1999). In contrast, leptin administration not only decreases LH concentrations of orexin-A (Beck et al., 1999) but also inhibits fasting-induced increases in hypothalamic prepro-orexin mRNA (Lopez et al., 2000). Intriguingly, however, if insulin-treated animals are given access to food, no changes are detected in hypothalamic prepro-orexin mRNA levels despite comparable levels of hypoglycaemia to similarly treated animals not given food (Cai et al., 1999). Together, these findings indicate that orexin neurons are highly sensitive to nutritional status and food availability, and that feedback-induced changes in orexin function can modulate activity in the elaborate neuronal circuitry involved in the expression of appetite.

Cephalic phase reflexes, mastication, and insulin secretion

Well over a century ago, Pavlov found that sensory stimulation associated with sham feeding evokes gastric acid secretion (Pavlov and Schumowa-Simanowskaja,

1895). It has since been firmly established that gastric acid secretion occurs as part of a series of cephalic phase reflexes, initiated by the thought, sight, smell and taste of food, and which serve to prepare the gut for the arrival of food. In this context, LH orexin neurons are known to innervate the dorsal motor nucleus of the vagus (DMV) (e.g., Peyron et al., 1998), a key area involved in the control not only of cephalic phase responses (such as gastric acid secretion) but also of gut motility. A role for orexins in these preparatory responses is strongly suggested by the observations that hypoglycaemia-induced activation of orexin neurons induces *c-fos* immunoreactivity in the DMV (Cai et al., 2001), and that centrally administered orexin-A dose-dependently increases both gastric acid secretion (Takahashi et al., 1999) and gut motility (Zbigniew et al., 2001).

LH orexin neurons also project to the brainstem trigeminal mesencephalic nucleus (Vme) and the trigeminal motor nucleus (Vmo) (Date et al., 1999; Nambu et al., 1999), areas that have also been shown to express mRNA for OX₁R and OX₂R (Greco and Shiromani, 2001). These important nuclei contain sensory and motor neurons innervating jaw elevator muscles and form a crucial component of the neural circuitry involved in the control of mastication (Luo et al., 2001). Recently, Zhang and Luo (2002) have not only found orexin-B-like terminals in both the Vme and Vmo but have also shown that these terminals closely contact neurons that have been retrogradely labelled with HRP injected into the jaw elevator muscles. LH orexin neurons would therefore appear capable of exerting direct synaptic control on the sensory and motor neurons involved in mastication, an essential executive episode of feeding behaviour. It is therefore of interest that rats display repetitive mouth/jaw movements shortly after ICV infusion of orexin-A (Rodgers et al., unpublished observations).

In the hindbrain, orexin-immunoreactive fibres are found in the NTS, which is known to be intimately involved in relaying vagally transmitted afferent signals from the gut (e.g., Bray, 2000). As ICV injection of orexins induces *c-fos* reactivity in the NTS (Edwards et al., 1999; Mullett et al., 2000), it seems likely that orexins are capable of modulating the responsiveness of NTS neurons to gastrointestinal stimuli. Consistent with this proposal, Cai et al. (2001) have recently reported that insulin-induced hypoglycaemia produces marked *c-fos* activation not only in LH orexin neurons but also in NTS neurons. As the NTS projects back to the LH (Horst et al., 1989), this pathway may be implicated in the regulation of LH orexin neurons by satiety signals.

In the periphery, endocrine cells of the pancreas also display orexin-like immunoreactivity, with orexin-A and OX₁R mRNA present in insulin-immunoreactive islet cells (Kirchgessner and Liu, 1999). Consistent with the possi-

bility that orexins may modulate insulin release, Nowak et al. (2000) found that orexin-A dose-dependently stimulates insulin secretion both in vivo and in vitro. In a recent review of these findings, Kirchgessner (2002) has suggested that the resultant drop in circulating glucose levels could itself contribute to orexin-induced hyperphagia. Alternatively, as insulin secretion occurs in preparation for food ingestion, orexin-stimulated insulin secretion could be yet another component of the cephalic phase response.

Together, the above findings indicate that the LH orexin system is involved in many physiological functions relevant to appetite control – from cephalic phase reflexes, through mastication, to insulin secretion. These neurons also appear to be regulated by input from the NTS, providing crucial feedback on circulating glucose levels and the present state of the gut. On this view, a precipitous fall in plasma glucose may be the stimulus for orexin activation ('on' switch) while gastric distension may serve to inhibit these neurons ('off' switch) (Cai et al., 2002).

Studies on food intake

Peptide administration

Over the past 4 years, many laboratories have confirmed the initial report by Sakurai et al. (1998) that centrally administered orexins stimulate food intake (Table 2). Although most work in this area to date has been done using ICV peptide administration in rats, it is important to note that similar effects have also been observed in mice and goldfish. While the hyperphagic response to orexins in mice appears to be less robust than in rats, only two studies have thus far been conducted in this species (Lubkin and Stricker-Krongrad, 1998; Marsh et al., 1999), and it may be relevant that both involved peptide administration via the third (rather than lateral) ventricle. Furthermore, the observation that orexins induce hyperphagia in fish (e.g., Volkoff et al., 1999) as well as mammals is consistent with very recent evidence for the existence of the hypocretin sequence in puffer fish and the likelihood that the hypocretin gene evolved as long ago as 650 million years (Alvarez and Sutcliffe, 2002).

Acute ICV administration of orexin-A in rats has been found to dose-dependently elevate food consumption in several different strains (Wistar, Sprague-Dawley, Lister hooded) and in both male and female subjects (e.g., Haynes et al., 2000). Furthermore, central mediation of this effect has been confirmed by the absence of a hyperphagic response following intravenous administration of orexin-A (0.3–5.0 mg/kg) (Haynes et al., 1999). Although orexin-B has also been reported to enhance food

intake, this effect is much less reliably obtained than the response to orexin-A and it has been argued that this pattern may either reflect the greater importance of the OX₁R in orexin-induced hyperphagia or the more rapid *in vivo* clearance of orexin-B (Smart et al., 2001). To avoid potential 'ceiling effects' (arising from high basal food intake), the vast majority of studies have administered the peptide during the early mid light phase of the operating light/dark cycle. However, the finding that orexin-A significantly increases the consumption of highly palatable wet mash indicates that orexin-A-induced hyperphagia is not limited to low basal intake (Rodgers et al., 2000, 2001).

As shown in Table 2, the typically effective hyperphagic dose range for orexin-A has been 2–10 nmol ICV, although increased intake has also been reported for doses as low as 0.07 nmol (Smart et al., 2002) and as astonishingly high as 300 nmol–3 μ M (Shiraishi et al., 2000). However, it is relevant to note that, as for the unreliable effects of orexins on food intake in mice, the latter study involved administration via the third ventricle. Furthermore, several observations indicate that, while still capable of stimulating food intake, orexin-A doses \geq 2.4 nmol ICV (lateral) can induce initial behavioural suppression (Rodgers et al., 2000), possibly linked to epileptogenic-like activity (Ida et al., 1999). Nevertheless, the potency with which orexin-A stimulates food intake (i.e., minimum effective dose reported = 0.07 nmol ICV) is not dissimilar to that of other orexigenic peptides (Smart et al., 2002). However, it should be emphasised that, while the maximum effect of orexins on intake is similar to that produced by galanin and MCH, it is substantially less than that seen in response to NPY (Edwards et al., 1999; Haynes et al., 1999; Ida et al., 1999; Sahu, 2002; Sakurai et al., 1998; Yamanaka et al., 2000).

In addition to acute effects, several studies have assessed the impact of chronically administered orexin-A on food intake in rats. Haynes et al. (1999) used an 8-day ICV infusion regimen of 18 nmol/day (i.e., 0.75 nmol/h), while Yamanaka et al. (1999) employed a 7-day ICV infusion regimen of 0.5 nmol/h (i.e., 12 nmol/day). Both studies reported an increase in food intake during the light phase and a compensatory (counter-regulatory) reduction in intake during the dark phase. This circadian pattern would be consistent with the additional observations in these experiments that chronically administered orexin-A does not alter 24 h food intake, bodyweight gain, fat pad weights, brown adipose tissue (BAT) temperature, or plasma levels of glucose, insulin, leptin, triglycerides, cholesterol, corticosterone or aldosterone. These data combine to suggest that orexin-A is more likely to be involved in the short-term rather than long-term regulation of energy balance. Although Lin et al. (2002) have recently failed to find any effects of

chronically administered orexin-A or orexin-B (50 pmol/h ICV for 14 days) on food intake in rats, it is important to emphasise that this study involved: (i) substantially lower orexin doses and, perhaps even more significantly in view of the counter-regulation described above, and (ii) the measurement of food intake at 24 h intervals only.

Although not extensively researched thus far, the neural sites supporting orexin-A-induced hyperphagia appear to be restricted to several discrete nuclei within the hypothalamus. Significant increases in food intake have consistently been observed following local application of orexin-A (0.03–1 nmol) in the LH, PFH and DMN. However, somewhat more variable responses have been obtained from the PVN and ARC nucleus, and only negative findings have been reported for infusions in the preoptic area, VMH, central nucleus of the amygdala, ventral tegmental area and NTS (Dube et al., 1999; Edwards et al., 1999; Kotz et al., 2001; Sakurai, 2002; Sweet et al., 1999). It is also worth noting that, where examined in these studies, intracerebral administration of orexin-B has failed to elicit a statistically reliable feeding response.

Antibody, gene knockout and antagonist effects

In view of the consistently observed hyperphagic response to acute orexin-A infusion, it would be predicted that disruption of orexin signalling should result in hypophagia. In accord with this prediction, fasting-induced food intake and/or dark phase food intake in rats is dramatically reduced by central, but not peripheral, administration of anti-orexin-A antibodies (Ida et al., 2000; Yamada et al., 2000; Niimi et al., 2001). Furthermore, intake is also significantly inhibited by antibodies directed at the OX₁R (Smith et al., 2000). In addition to these observations, knockout of the prepro-orexin gene (Willie et al., 2001) and genetic ablation of orexin neurons (Hara et al., 2001) have both been reported to result in a hypophagic phenotype. Although consistent with the hyperphagic profile observed in mice overexpressing the prepro-orexin gene (Inui, 2000), there are some puzzling anomalies in the phenotypes of these genetically modified animals. For example, despite undereating, prepro-orexin knockout mice apparently grow quite normally; despite overeating, mice overexpressing prepro-orexin do not become obese; and, perhaps most curious of all, despite producing a 30% reduction in food intake, genetic ablation of orexin neurons is actually associated with late-onset obesity. Although these unusual patterns may well be explained by concomitant alterations in metabolism and/or overall energy expenditure (e.g., Sakurai, 2002; Willie et al., 2001), the limitations of gene targeting research (such as developmental compensation, background gene effects) should not be underestimated (e.g., Crawley, 2000). Indeed, in a recent review, Salton et al.

Table 2 Summary of acute effects of orexin-A and orexin-B on food intake in rats, mice and goldfish

Authors	Species	Doses	Route	Test diet	Test time	Effects on food intake
Sakurai et al. (1998)	R, W, m	3 and 30 nmol	ICV(L)	Powder chow	Light phase	D/D increase with both peptides, orexin-A effect longer-lasting. Weaker effect than NPY.
Lubkin and Stricker-Krongrad (1998)	M, C57, m	1, 3, and 10 nmol	ICV(3)	Chow pellets	Light phase	No effects orexin-B; increase with orexin-A at 1 nmol only. Weaker effect than NPY
Samson et al. (1999)	R, SD, m	1 and 5 nmol	ICV(L)	Chow pellets	Light phase	Non D/D increase with hypocretin-1 and 2
Marsh et al. (1999)	M, WT, m	1.7–2.4 nmol	ICV(3)	Chow pellets	Light phase	No effects either peptide
Ida et al. (1999)	R, W, m	3 nmol	ICV(L)	Chow pellets	Light phase	Non-significant increase with orexin-A, but not orexin-B
Sweet et al. (1999)	R, SD, m	15 nmol	ICV(L)	Chow pellets	Light phase	Increase with orexin-B
Edwards et al. (1999)	R, W, m	3–30 nmol	ICV(L)	Chow pellets	Light phase	Increase with orexin-A and B; effects of orexin-A longer-lasting. Similar effect to galanin and MCH but weaker than NPY
Volkoff et al. (1999)	Goldfish	1–100 ng/g	ICV	Trout pellets	N/a	Increase with orexin-A and B (1–10 ng/g): more reliable response to orexin-A. No effect at highest dose
Kunii et al. (1999)	R, W, m	3–30 nmol	ICV(L)	Chow pellets	Light phase	Increase with both peptides
Haynes et al. (1999)	R, SD/W, m	2.3–23.4 nmol	ICV(L)	Chow pellets	Light phase	D/D increase with orexin-A but not orexin-B (both strains). No effect systemic administration
Rodgers et al. (2000)	R, H, m	0.78–7.0 nmol	ICV(L)	Wet mash	Light phase	Non D/D increase with orexin-A. Highest dose, behaviourally disruptive
Yamanaka et al. (2000)	R, W, m	10 nmol	ICV(L)	Chow pellets	Light phase	Increase with orexin-A
Shiraishi et al. (2000)	R, W, m	300 nmol–3 μ M	ICV(3)	Chow pellets	Light phase and dark phase	Non D/D increase with orexin-A in both phases
Volkoff and Peter (2000)	Goldfish	10 ng/g	ICV	Trout pellets	N/a	Increase with orexin-A
Ida et al. (2000)	R, SD, m	3 pmol–30 nmol	ICV(L)	Chow pellets	Dark phase	Non-significant increase with orexin-A and B
Dube et al. (2000)	R, SD, m	15 nmol	ICV(L)	Chow pellets	Light phase	Increase with orexin-A
Jain et al. (2000)	R, SD, m	3–30 nmol	ICV(L)	Chow pellets	Light phase	D/D increase with both peptides
Haynes et al. (2000)	R, SD, m/f	2.3–7.0 nmol	ICV(L)	Chow pellets	Light phase	Dose-dependent increase with orexin-A in both sexes
Rodgers et al. (2001)	R, H, m	2.4 nmol	ICV(L)	Wet mash	Light phase	Significant increase with orexin-A
Sunter et al. (2001)	R, W, m	1.0–3.0 nmol	ICV(L)	Chow pellets	Light phase	No effects of orexin-A or B
Smart et al. (2002)	R, SD, m	0.07–23 nmol	ICV(L)	Chow pellets	Light phase	D/D increase with orexin-A, MED = 0.07 nmol
Espana et al. (2002)	R, SD, m	3 nmol	ICV(L)	Chow pellets	Light phase and dark phase	Increase with hypocretin-1 in both phases (significant only in light phase)
Lopez et al. (2002)	R, SD, m	3 nmol	ICV(L)	Chow pellets	Light phase	Increase with orexin-A
Sahu (2002)	R, SD, m	1.0–3.0 nmol	ICV(3)	Chow pellets	Light phase	D/D increase with hypocretin-1; max response similar to MCH but less than NPY

Key: R: rat; M: mouse; W: Wistar, SD: Sprague–Dawley; H: Lister hooded; m: male; f: female; C57: C57BL/6J. WT: wild-type; ICV(L): lateral ventricle; ICV(3): third ventricle; D/D: dose-dependent; MED: minimum effective dose.

(2000) emphasise the *lack* of coherence in findings emerging from genetic and pharmacological/physiological research on the regulation of energy homeostasis.

Much more revealing are the results of recent experiments using the novel and selective OX₁R receptor antagonist, SB-334867. Acute systemic pretreatment with this antagonist (3.0–30.0 mg/kg, i.p.) completely blocks the hyperphagic response to ICV orexin-A (2.4–7.0 nmol), thereby implicating OX₁R-mediation of the peptide's effect on appetite regulation (Haynes et al., 2000; Rodgers et al., 2001; Fig. 1). Furthermore, when administered alone, acute SB-334867 (30.0 mg/kg, i.p.) not only significantly suppresses food intake in fasted rats and in rats tested at the start of the dark phase (Haynes et al., 2000) but also in non-deprived rats presented with highly palatable food during the light phase (Rodgers et al., 2001; Fig. 1).

Although SB-334867 is only detectable in brain up to 2 h after dosing (10 mg/kg, i.p.), acute treatment with 30 mg/kg has been reported to significantly suppress cumulative food consumption measured in male and female rats over 24 h following treatment (Haynes et al., 2000). Although most suppression appeared to occur during the initial 4 h period after dosing, the data reported suggest that food intake was also lower than in controls during the subsequent 20 h period. This finding is made all the more intriguing in view of the observation that acute treatment with 30 mg/kg SB-334867 also significantly suppresses 3-day post-treatment bodyweight gain in male rats (Rodgers et al., 2001). We have since replicated this finding on two occasions and further demonstrated that: (i) a significant decrease in bodyweight is evident 24 h following antagonist treatment (see also Fig. 4 legend in Haynes et al., 2000), with the growth curve subsequently paralleling that of vehicle

controls, and (ii) food consumption is significantly suppressed by SB-334867 (30 mg/kg) when animals are not actually tested for intake until 24 h following treatment (Ishii et al., unpublished observations). Together, these observations in rats strongly suggest that acute antagonism of the OX₁R initiates a sequence of downstream events that extend far beyond the period of receptor blockade.

Consistent with the acute anorectic effects of SB-334867 in rats, chronic (14 day) administration of the OX₁R antagonist has very recently been found to significantly reduce both cumulative daily food intake and 14-day bodyweight gain in a genetic model of obesity, the *ob/ob* mouse (Haynes et al., 2002). These striking effects were associated with a reduction in total fat mass and intracapsular BAT weight as well as increases in metabolic rate and OX₁R mRNA expression in BAT. Fasting (5 h) blood glucose levels were also markedly reduced at the end of the treatment period which, together with a trend towards lowered plasma insulin levels, suggests that chronic SB-334867 may improve insulin sensitivity in *ob/ob* mice.

Together, these findings with SB-334867 in rats and mice clearly indicate a potential role for OX₁R antagonists in the treatment of obesity with the additional effects on blood glucose and insulin sensitivity perhaps also indicative of a therapeutic role in diabetes (Haynes et al., 2002).

Interactions with other hypothalamic neuropeptide signalling pathways

A number of studies have explored the extent to which orexin-induced hyperphagia may involve other neuropeptide signalling systems implicated in hypothalamic regulation of appetite. We have already commented upon

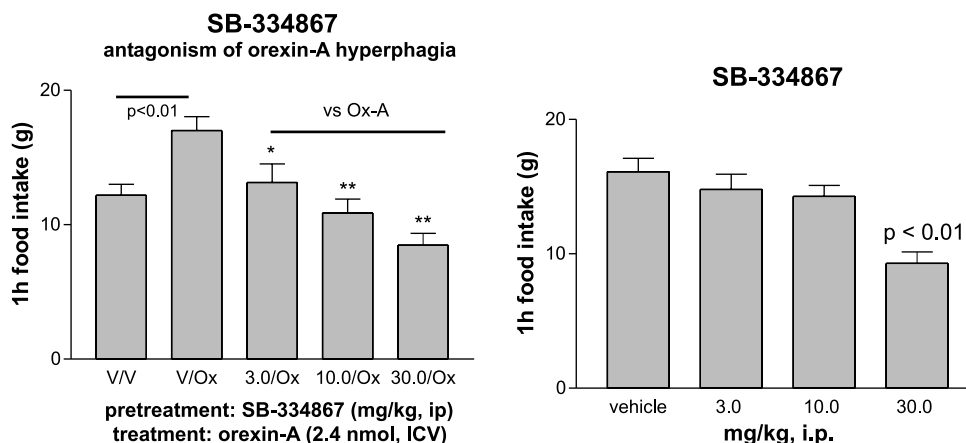


Fig. 1 Effects of the selective OX₁R antagonist, SB-334867 (3–30 mg/kg, i.p.), on basal food intake (right panel), and orexin-A-induced (2.4 nmol ICV) hyperphagia (left panel) in rats presented with palatable wet mash for 1 h. V: vehicle. Data redrawn from Rodgers et al. (2001).

the extensive projections of LH orexin neurons to NPY-expressing (and leptin-sensitive) neurons of the ARC nucleus, as well as the very high proportion (>90%) of ARC neurons that display OX₁R immunoreactivity (Backberg et al., 2002). An elegant study by Lopez et al. (2002) has provided evidence for the functionality of this pathway, in that a hyperphagic dose of orexin-A (3 nmol ICV) was found to selectively increase NPY expression in the rat ARC nucleus. Two additional aspects of this study deserve comment: (i) the timecourse for the effect of orexin-A on NPY expression closely paralleled the timecourse for its effects on food intake (both observed up to but not beyond 2–3 h post-infusion), and (ii) orexin-A did not alter the expression of MCH in the LH or, intriguingly, the expression of AgRP in ARC neurons co-expressing this peptide and NPY. These findings agree well with the recent observation of increased food intake in rats following combined ICV(3) treatment with intrinsically inactive doses of NPY (0.023 nmol) and hypocretin-1 (0.25 nmol), but not combined treatment with either hypocretin-1 and MCH or NPY and MCH (Sahu, 2002).

Additional evidence for the involvement of NPY signalling in orexin-stimulated feeding has been provided by pharmacological studies. Of the six NPY receptor subtypes thus far identified, Y1 and Y5 receptor populations have most strongly been implicated in NPY-induced feeding (Chamorro et al., 2002; Gehlert, 1999), i.e., antagonists selective for these receptor subtypes (e.g., 1229U91 and BIBO3304 for Y1, and CGP71683A for Y5) have been shown to inhibit both NPY-induced and spontaneous feeding in rats. In this context, three experiments have shown that, at intrinsically non-anorectic doses, 1229U91 (Jain et al., 2000), BIBO3304 (Yamanaka et al., 2000) and CGP71683A (Dube et al., 2000) inhibit the hyperphagic response to orexin-A and/or orexin-B. Although these data suggest that orexin-induced hyperphagia is at least partly mediated through downstream NPY activation of Y1 and Y5 receptors, Niimi et al. (2001) have provided evidence that, reciprocally, NPY-induced hyperphagia may in part be mediated through activation of orexin mechanisms. Firstly, they found that a significant proportion of LH orexin neurons display *Fos*-like immunoreactivity in response to NPY administration, and that this response of LH orexin neurons is substantially reduced by leptin. And, secondly, they demonstrated that pretreatment with an antiserum to orexin-A significantly attenuates the feeding response to NPY (1 nmol ICV). Although the effective dose of the orexin-A antiserum had significant anorectic effects when administered alone, these findings are consistent with the reciprocal connections between LH orexin neurons and NPY-expressing ARC neurons (Horvath et al., 1999). It would therefore appear

that, despite probable involvement with different aspects of energy balance (long-term versus short-term, respectively), NPY- and orexin-signalling systems are to some extent implicated in one another's acute effects on food intake.

An interesting study by Ida et al. (2000) has provided one possible explanation for the few reported failures to obtain orexin-A-induced hyperphagia (Table 2). Although replicating an earlier failure to find a significant increase in food intake with either orexin-A or orexin-B (Ida et al., 1999), they found that pretreatment with either a corticotropin releasing factor (CRF) antagonist or anti-CRF antiserum (themselves without significant effect on intake) resulted in a significant hyperphagic response to orexin-A (3 nmol ICV). Furthermore, mirroring the results of Jain et al. (2000) on basic orexin hyperphagia, the NPY Y1 receptor antagonist, 1229U91, blocked the feeding response to orexin-A in animals pretreated with either the CRF antagonist or antiserum. These findings not only confirm the involvement of NPY-signalling in orexin-induced hyperphagia but, intriguingly, also suggest that the ability of orexins to enhance food intake may be partly dependent upon the extent to which they stimulate CRF release. CRF is synthesised in the PVN, a nucleus that receives orexin innervation and which expresses OX₁R (Backberg et al., 2002), orexins are known to activate the HPA axis (Kuru et al., 2000), and CRF per se decreases food intake (e.g., Inui, 1999; Kalra et al., 1999). As such, between-laboratory variation in the stress reactivity of experimental animals, arising from genetic and/or environmental factors, may be a crucial determinant of orexin hyperphagia. If stress reactivity is high, or if animals are tested under non-habituated conditions, the anorectic response to endogenously released CRF may serve to counteract any hyperphagic response to exogenous administration of orexins.

As shown in Table 1, CART (cocaine- and amphetamine-regulated transcript) is a novel anorexigenic hypothalamic neuropeptide (e.g., Lambert et al., 1998) with recent evidence suggesting possible interactions with the LH orexin system. Thus, significant OX₁R immunoreactivity has been found in CART neurons of the ventrolateral division of ARC (Backberg et al., 2002), and it is probable that these CART neurons in turn project back to orexin cells in the LH (Horvath et al., 1999). The possible functional relevance of these anatomical connections has been suggested by the observation that administration of CART (55–102) inhibits both normal food intake and orexin-A-stimulated food intake in goldfish (Volkoff and Peter, 2000). Although interpreted by the authors as evidence of an inhibitory interaction between CART and orexin-A in the regulation of food intake, the strength of this conclusion is at least partially

undermined by the use of intrinsically anorectic doses of the CART fragment.

Orexins and serotonin

As serotonin (5-HT) has long been implicated in satiety (e.g., Blundell, 1977), it is of considerable interest that LH orexin neurons project strongly to the dorsal raphe nucleus (DRN) (Briski and Sylvester, 2001; de Lecea et al., 1998; Cutler et al., 1999; Dube et al., 1999; Nambu et al., 1999; Peyron et al., 1998; Sakurai et al., 1998; Taheri et al., 1999). Furthermore, the DRN displays a high density of OX₁R (Greco and Shiromani, 2001; Hervieu et al., 2001; Lu et al., 2000; Marcus et al., 2001; Trivedi et al., 1998), orexin-A excites 5-HT cells in the DRN (Brown et al., 2001), and both orexin-A and orexin-B have been found to inhibit depolarisation-stimulated 5-HT release in the hypothalamus (Orlando et al., 2001). Although, as reviewed below, there is some evidence for 5-HT involvement in orexin-induced stimulation of grooming (Duxon et al., 2001; Matsuzaki et al., 2001), no studies have yet been published on the effects of 5-HT manipulations on orexin-induced hyperphagia. However, as 5-HT exerts an inhibitory effect on food intake, most probably via 5-HT^{1A} (e.g., Simansky, 1996) and 5-HT^{2C} (e.g., Hewitt et al., 2002) receptor mechanisms, orexin projections to DRN may form part of an inhibitory feedback loop back to the hypothalamus (Brown et al., 2001). In this context, 5-HT^{1A} receptor immunoreactivity has very recently been identified in NPY-, AgRP-, POMC- and CART-containing neurons of the ARC nucleus as well as in MCH- and orexin-containing neurons of the LH (Collin et al., 2002). Undoubtedly, studies specifically addressing possible orexin-5-HT interactions in appetite regulation will appear in the near future.

OREXINS AND FEEDING BEHAVIOUR

Although many substances influence food consumption, changes in intake per se actually reveal comparatively little about how and why such changes occur. For example, is orexin-induced hyperphagia mediated directly through systems involved in appetite regulation or is it merely an indirect consequence of some other effect of the peptide? Even if directly mediated, are such effects produced through an increase in the motivation to eat or a decrease in satiety signalling? Although answers to these sorts of questions can be obtained through detailed behavioural analysis, it is somewhat surprising that relatively few studies to date have specifically focused on the behavioural effects of orexins. Nevertheless, these reports have produced some intriguing findings which, for reasons that should become apparent, will be considered as a function of whether food was present or absent during testing.

Behavioural effects of orexins: food present

Several studies have reported on the general behavioural effects of orexins within a feeding context, while others have more specifically examined peptide and antagonist effects on the natural structure of feeding behaviour.

Orexin-induced behavioural changes

In the first study to report in detail on the behavioural effects of orexins, Ida et al. (1999) found that 3 nmol ICV orexin-A (but not orexin-B) in rats significantly increased time spent feeding during a 3 h test. This enhancement of feeding behaviour was largely restricted to the first hour, an observation that may explain the absence of a significant treatment effect on 3 h food consumption. However, very much more pronounced stimulatory effects of orexin treatment (orexin-A generally > orexin-B) were observed on face-washing, grooming, searching and burrowing. These behavioural changes had a fast onset and, like feeding, were generally most evident during the first half of the test session. Interestingly, while orexin-A-induced searching was most evident after the main bout of feeding (70–80 min), animals treated with orexin-B showed regular bouts of searching throughout the entire 3 h period. Although the authors were somewhat cautious in their interpretation of the overall behavioural patterns observed, it is notable that all orexin-elicited behaviours form part of the normal appetitive-consummatory sequence associated with the pre-ingestive (searching), ingestive (feeding) and post-ingestive (grooming) phases. Interestingly, very similar findings have more recently been reported by Sunter et al. (2001) who failed to find a significant effect of ICV orexin-A (1 and 3 nmol) or orexin-B (3 nmol) on food intake or feeding behaviour in rats measured 4 h after peptide administration. While the negative result on ingestion may be similarly explained by the measurement interval employed (i.e., masking of an initial hyperphagic response), the authors nevertheless observed significant stimulatory effects of peptide treatment on 4 h measures of grooming, rearing, sniffing, locomotion (orexin-A only) and burrowing (orexin-B only). Furthermore, somewhat reminiscent of the counter-regulatory mechanisms proposed by Haynes et al. (1999), total food intake as well as the frequency of feeding episodes were significantly reduced in the period 4–24 h following treatment with orexin-A (3 nmol).

In addition to this work on rats, detailed behavioural studies on goldfish (Volkoff et al., 1999; Volkoff and Peter, 2000) have found that ICV orexin-A or orexin-B (1–10 ng/g) stimulates 1 h food intake and also markedly increases the total number of feeding acts, including those defined as incomplete (i.e., approach without consumption and/or food rejection). However, of relevance

to later discussion, the authors also reported a significant orexin-A-induced increase in the total number of behavioural acts (including non-feeding behaviours, such as gravel-spitting and interactions with other fish), suggesting the possibility of a general arousing effect of peptide treatment rather than a specific effect on mechanisms of appetite.

Most research on orexin-induced hyperphagia in rats has been conducted during the light phase of the LD cycle, a procedure normally adopted to control for possible ceiling effects arising from high basal intake during the dark phase. However, in a very recent study, Espana et al. (2002) examined in detail the behavioural effects of hypocretin-1 (orexin-A, 3 nmol ICV) administered prior to 90 min observation sessions in either the light or dark periods. Irrespective of time of administration, hypocretin-1 increased time spent awake, locomotor activity and chewing inedible objects. In contrast, while peptide administration in the light period also significantly increased feeding, drinking and grooming, only minimal effects on these behaviours were recorded following administration during the dark period. Although the data suggest that hypocretin-1-induced hyperphagia may largely be accounted for by the increase in waking time, there are a number of problems with this conclusion. Firstly, when administered in the dark period, hypocretin-1 *did* increase food intake, as well as time spent feeding and grooming; however, in view of high control baseline scores (as would be expected during the dark phase), these effects were not statistically reliable. Secondly, as the authors themselves admit, there was a significant increase in feeding during the first 30 min of the light period, a time when both the vehicle- and hypocretin-1-treated animals displayed comparable levels of waking. Thirdly, if ingestive responses to hypocretin-1 are simply an indirect consequence of time awake (arousal), it is odd that the locomotor stimulant response to the peptide did not display the circadian-dependency claimed for ingestive responses. To their credit, however, the authors conclude that only some of the ability of hypocretin-1 to increase feeding, drinking and grooming may reflect a more general behavioural activation.

The issue of a mediating role for general arousal in the hyperphagic response to orexin-A has also been investigated following direct peptide administration into the LH (Kotz et al., 2001). Using a novel behavioural approach, these authors examined the effects of intra-LH orexin-A (1 nmol unilateral) on food intake and locomotor activity in rats previously habituated to a running wheel. Behavioural measures were taken over a 2 h period in either the light or dark phase of the cycle. The authors argued that if orexin-induced hyperphagia were simply a consequence of enhanced arousal, then the presence of a

familiar running wheel should diminish or abolish the feeding response. Furthermore, if arousal explains hyperphagia, then the peptide should always induce feeding in situations where it enhances arousal. The results showed that, when administered during the light phase, orexin-A stimulated feeding to a similar extent in the presence *or* absence of the running wheel, and increased the number of wheel rotations to a similar extent in the presence *or* absence of food. During the dark phase, orexin-A stimulated locomotor activity but as in previous studies (reviewed above), did not significantly increase food intake. Importantly, these findings show that orexin-A-induced stimulation of feeding is not always coincident with increased activity, thereby demonstrating that the hyperphagic response is not merely an artifact of enhanced arousal. A similar conclusion regarding the behavioural specificity of orexin-A-induced hyperphagia has also been reached in studies on the effects of peptide administration directly into the rat ARC nucleus (unpublished data, cited in Sakurai, 2002) and, as reviewed below, in experiments that have examined the effects of the peptide (and an OX₁R antagonist) on the natural structure of feeding behaviour in rats.

Orexin effects on the natural structure of feeding behaviour

It has been recognised for some time that important insights into treatment-induced changes in appetite can be obtained through detailed analysis of the microstructure of feeding behaviour (e.g., Blundell and Latham, 1979; Clifton, 2000; Smith, 2000). In rodent research, one of the most informative microstructural approaches involves the behavioural satiety sequence (BSS), a concept that describes the natural progression from eating ⇒ grooming ⇒ resting (Fig. 2). First documented some 40 years ago (Bindra and Blond, 1958; Bolles, 1960), and subsequently elaborated in the mid-1970s (Antin et al., 1975; Blundell and Latham, 1979), the BSS has since been extensively used to characterise the influence of pharmacological and non-pharmacological manipulations on the normal pattern of behaviour associated with feeding in rats (for recent review: Halford et al., 1998). This work has confirmed that the preservation of the structure of this stochastic sequence can be taken to signify changes in food intake by a post-ingestive mechanism of satiety. In contrast, disruption of the sequence demonstrates that a treatment modifies food intake by mechanisms other than satiety i.e., via induction of nausea, pain, sedation or hyperactivity.

We have recently employed BSS methodology to characterise the dose-response effects of orexin-A and SB-334867 (a selective OX₁R antagonist), alone and in combination, on the behaviour of non-deprived male rats during a 1 h test with palatable mash (Rodgers et al.,

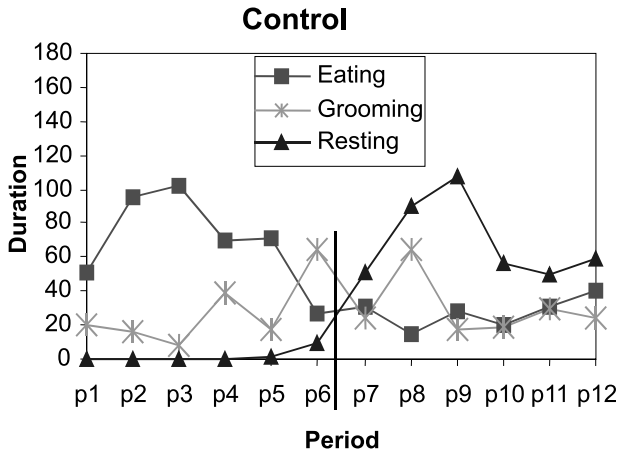


Fig. 2 Typical behavioural satiety sequence (BSS) in a group of non-deprived male Lister hooded rats ($n = 10$) presented with palatable wet mash for 1 h (unpublished control group data). Eating is the predominant behaviour during the first half of the test session and resting the predominant behaviour over the final 30 min. Regular bouts of grooming are also apparent and, typically, follow significant bouts of feeding. Duration scores are in seconds, period (p1–p12) refers to 5-min timebins during the 1-h test, and the vertical line denotes the transition point between eating and resting.

2000; Rodgers et al., 2001). In addition to recording 1 h food intake, videotaped sessions were scored for eat latency as well as the frequency and duration of feeding, drinking, grooming, resting, locomotion, rearing and sniffing. Close attention was paid to the natural structure of feeding behaviour and, in particular, to the timing of the transition between eating and resting (a marker of behavioural satiety).

When administered alone, orexin-A (0.78–7.0 nmol ICV) stimulated food intake without significantly influencing total session scores for any behavioural category (Rodgers et al., 2000). The only exception to this pattern was a small (though significant) increase in drinking observed at the highest dose tested (see also Espana et al., 2002; Kunii et al., 1999). Despite the general absence of orexin-A effects on total behavioural scores, microstructural analysis (i.e., timecourse of behavioural change within session) revealed significant effects of peptide treatment that were both dose- and time-dependent. Although significantly enhancing food intake, the highest dose tested (7 nmol) totally disrupted the BSS. Very atypically, these animals engaged in high levels of resting over the first 15–20 min of the test session while displaying a concomitant suppression of eating and other active behaviours. This sedative-like effect dissipated by 20 min post-infusion and was followed by somewhat higher than control (rebound) levels of most behaviours (including feeding) over the second half of the test session.

In contrast to the clearly disruptive effects of the high dose, behavioural structure was fully preserved at the two lower doses (0.78–2.4 nmol) of orexin-A, with the

principal effect of treatment being to delay the onset of behavioural satiety (i.e., transition from eat to rest) by approximately 12–15 min. Very importantly, these effects were seen in the absence of a general stimulation of behaviour, i.e., no increases were apparent in locomotor activity, rearing or sniffing. Furthermore, as neither eat latency nor initial feeding (frequency or duration) was significantly altered at these dose levels, it may be concluded that orexin-A does not affect initial motivation to eat. Rather, treatment with this peptide extends the time devoted to feeding prior to the onset of grooming and resting. The observations that enhanced grooming and suppressed resting were evident only in the latter part of the test (i.e., after the main bout of feeding) are fully consistent with this interpretation. In view of the relatively short test duration employed in these studies, these findings suggest that the delay in behavioural satiety under orexin-A treatment may occur through modulation of vagally mediated feedback to the NTS and beyond.

In a follow-up study (identical test conditions), we examined the dose-response effects of SB-334867 (3–30 mg/kg, i.p.) prior to determining the ability of this OX_1R antagonist to block the hyperphagic effects of 2.4 nmol ICV orexin-A (Rodgers et al., 2001). Administered alone, SB-334867 (30 mg/kg but not lower doses) significantly reduced food intake and most active behaviours except resting (which was increased). Although suggestive of a behaviourally non-selective (i.e., sedative-like) action, the structure of feeding behaviour was fully preserved at this dose level with the reduction in overall behavioural output clearly attributable to a much earlier onset of behavioural satiety. Thus, relative to the vehicle control condition, the transition between eating and resting occurred approximately 15 min earlier than normal, with general activity measures (such as rearing and sniffing) only showing significant suppression *beyond* this timepoint. Equally importantly, neither eat latency nor the initial bout of feeding behaviour were affected by antagonist treatment, thereby paralleling (though opposite in direction to) effects observed with orexin-A and further suggesting that, via the OX_1R , orexin-A influences satiety signalling rather than initial hunger motivation. In the second part of the study, we replicated earlier observations that orexin-A (2.4 nmol ICV) stimulates food intake, reduces resting, increases grooming and delays the onset of behavioural satiety. Pretreatment with SB-334867 was found to dose-dependently block all these effects of orexin-A and, most importantly, to do so even at dose levels below those required to induce intrinsic behavioural effects under identical test conditions (i.e., <30 mg/kg).

These BSS analyses clearly confirm the behavioural selectivity of orexin-A (2.4–7.0 nmol)-induced hyperphagic

gia as well as SB-334867 (30 mg/kg)-induced aphagia. When administered alone, both treatments fully preserved the normal structure of feeding behaviour yet induced meaningful and diametrically opposite effects on food intake, feeding behaviour and behavioural satiety. In addition, pretreatment with intrinsically non-anorectic doses (3–10 mg/kg) of SB-334867 dose-dependently blocked the hyperphagic and behavioural effects of orexin-A. As neither the peptide nor the OX₁R antagonist influenced initial motivation to eat, it seems reasonable to propose that, via the OX₁R, orexin-A specifically influences appetite through modulating the efficacy of satiety signalling from gut to brain. This conclusion would not be inconsistent with recently expressed views on the role of orexins in the short-term regulation of energy balance (e.g., Arch, 2000; Cai et al., 2002; Smart et al., 2002).

Behavioural effects of orexins: food absent

In addition to its classical role in the neural mediation of hunger, the LH has long been implicated in the regulation of drinking behaviour and fluid homeostasis. LH lesions not only produce aphagia but also adipsia (e.g., Oltmans and Harvey, 1976), while LH stimulation increases water intake as well as feeding (e.g., Mogenson and Stevenson, 1967). In this context, Kunii et al. (1999) have provided convincing evidence that LH orexins may also act as physiological regulators of fluid homeostasis and water intake. They found that orexin-immunoreactive fibres are present in two circumventricular organs (the subfornical organ and area postrema) associated with fluid intake, and that 48 h water deprivation upregulates hypothalamic prepro-orexin mRNA. Furthermore, in behavioural studies, they observed that ICV administration of orexin-A (3–10 nmol) and orexin-B (3–30 nmol) dose-dependently stimulates home-cage drinking behaviour in rats. These effects occurred within a few minutes of peptide infusion and, while less intense, were longer-lasting than the hyperdipsic response to 0.3 nmol angiotensin II. Paralleling the hyperphagic effect of orexins (reviewed above), orexin-A produced a more potent effect on drinking than orexin-B, i.e., the effect observed with 10 nmol orexin-A was greater than that produced by 30 nmol orexin-B. Furthermore, as no food was present during these experiments, the increases in drinking reported in several feeding studies (e.g., Espana et al., 2002; Rodgers et al., 2000) would not appear to be an indirect consequence of enhanced food intake.

The locus coeruleus (LC) receives a particularly dense projection from LH orexin neurons (Briski and Sylvester, 2001; Cutler et al., 1999; Date et al., 1999; de Lecea et al., 1998; Nambu et al., 1999; Peyron et al., 1998; Shirasaka et al., 1999; Taheri et al., 1999), and displays a high

density of OX₁R (Hervieu et al., 2001; Greco and Shiromani, 2001; Lu et al., 2000; Marcus et al., 2001; Trivedi et al., 1998). In this context, Hagan et al. (1999) have shown that orexin-A (30 nM to 3 μM) increases the firing of noradrenergic LC neurons *in vitro* and, when administered *in vivo* (2.4 nmol ICV), enhances EEG arousal at the expense of paradoxical sleep (see also Piper et al., 2000). Consistent with these observations, the authors also found that orexin-A (0.24–7.2 nmol ICV) potently stimulates both locomotor activity and grooming, with very similar effects having recently been reported for orexin-B (Jones et al., 2001). As these behavioural changes were recorded in a neutral test arena in the absence of food, it would appear that (as for drinking) the increases in locomotion and grooming sometimes observed with orexins in a feeding context are independent of their hyperphagic effects. Although it might be argued that these EEG and general behavioural arousal effects of orexins can account for their hyperphagic effects, several studies reviewed above render such a facile interpretation untenable (e.g., Espana et al., 2002; Kotz et al., 2001; Rodgers et al., 2000, 2001). Furthermore, while both orexin peptides stimulate locomotor activity and grooming to a similar degree, hyperphagia is much less reliably seen in response to orexin-B compared with orexin-A (see earlier).

In a further exploration of the effects of orexin-A on grooming, Duxon et al. (2001) observed that orexin-A-evoked grooming in rats is not only totally blocked by pretreatment with the OX₁R antagonist, SB-334867, but also by the selective serotonin (5-HT)_{2C} receptor antagonist, SB-242084. These findings suggest that orexin-A indirectly activates 5-HT_{2C} receptors downstream of OX₁R to elicit grooming. This result is not only important in itself but also because of (a) established LH orexin projections to the DRN (Briski and Sylvester, 2001; Cutler et al., 1999; de Lecea et al., 1998; Dube et al., 1999; Nambu et al., 1999; Peyron et al., 1998; Sakurai et al., 1998; Taheri et al., 1999), the high density of OX₁R in the DRN (Greco and Shiromani, 2001; Hervieu et al., 2001; Lu et al., 2000; Marcus et al., 2001; Trivedi et al., 1998), and reports that orexin-A excites 5-HT cells in the DRN (Brown et al., 2001), (b) other evidence pointing to the involvement of 5-HT mechanisms in orexin-induced grooming (Matsuzaki et al., 2001), and (c) the well-established inhibitory role of 5-HT, and especially 5-HT_{2C} receptors, in feeding behaviour (e.g., Hewitt et al., 2002).

As already noted, orexin-A and orexin-B stimulate locomotor activity in rats, and recent studies with SB-334867 have again pointed to OX₁R mediation of this effect (Jones et al., 2001). In this context, the dopaminergic ventral tegmental area (VTA) receives projections from LH orexin neurons (e.g., Fadel and Deutch, 2002; Nakamura et al., 2000), OX₁R and OX₂R are present in high densities in the VTA (Lu et al., 2000), and orexin-A

dose-dependently increases Ca^{2+} influx in isolated VTA dopamine neurons (Nakamura et al., 2000). Two recent behavioural studies have therefore explored the extent to which orexin-stimulated locomotion may depend upon the activation of dopaminergic receptors downstream of the OX_1R . Confirming the greater variability in behavioural effects observed with orexin-B, Matsuzaki et al. (2001) found similar locomotor and grooming responses to both peptides, whereas Nakamura et al. (2000) found such effects only with orexin-A. Nevertheless, orexin-A-induced (3–10 nmol ICV) hyperlocomotion was abolished by pretreatment with dopamine D_2 receptor antagonists (haloperidol and sulpiride) but not by 5-HT receptor antagonists (ritanserin and metergoline) (Matsuzaki et al., 2001; Nakamura et al., 2000). Interestingly, orexin-induced grooming was blocked not only by the 5-HT receptor antagonists but also by haloperidol and SCH23390 (a D_1 receptor antagonist). Although these findings suggest dopaminergic involvement in orexin-A-induced locomotor stimulation and grooming, it is somewhat unusual that neither study found any intrinsic locomotor effects with the dopamine receptor antagonists employed (haloperidol – 0.1 and 1.0 mg/kg; sulpiride – 20 and 200 mg/kg; SCH23390 – 0.1 and 1.0 mg/kg).

In addition to physiological and behavioural effects directly associated with appetite control, central administration of orexin-A and/or orexin-B has been found to induce a wide range of other physiological actions, including substantial sympathetic activation. Widely reported increases in arterial blood pressure and heart rate in response to orexin administration appear to be mediated via LH orexin inputs to the PVN and rostral ventrolateral medulla, both of which project directly to sympathetic preganglionic neurons of the spinal cord (Dun et al., 2000; Samson et al., 1999; Shirasaka et al., 2001). Orexins also enhance metabolic rate (Lubkin and Stricker-Krongrad, 1998), body temperature (Yoshimichi et al., 2001), and activity in the hypothalamic–pituitary–adrenal (HPA) axis (Hagan et al., 1999; Jones et al., 2001; Kuru et al., 2000). Of yet further significance is recent evidence that orexin-A, administered either intravenously (10–30 mg/kg) or ICV (0.7–7.2 nmol), elicits an analgesic reaction in rats and mice equivalent in efficacy to 10 mg/kg morphine (Bingham et al., 2001). Although this analgesic reaction is blocked by the OX_1R antagonist, SB-334867, it is insensitive to naloxone and would therefore be classed as a non-opioid form of pain inhibition.

FORAGING, VIGILANCE, AND DEFENCE

The apparent diversity of orexin effects on physiology and behaviour is of course consistent with the extensive distribution of orexin fibres and receptors throughout the

neuraxis. On the one hand, it is theoretically possible that effects on appetite regulation, CNS arousal, locomotor activity, grooming, sympathetic activation, HPA function and pain perception are entirely independent of each other. On the other hand, however, it is conceivable that the small group of LH orexin neurons coordinates a constellation of behavioural, physiological and endocrine changes designed to meet the demands of acute nutritional depletion. More specifically, Cai et al. (2001) have noted that the only manipulations to consistently produce activation of LH orexin neurons are low blood glucose levels and an empty stomach, both of which signify an urgent need for sustenance. This context-dependency may help to explain both major classes of effect associated with orexin activation – namely, enhanced food intake/delayed satiety and increased wakefulness/vigilance/behavioural activity. As recently suggested by several authors, the latter actions would help to ensure that foraging took precedence over sleeping, with increased wakefulness providing more feeding opportunities (e.g., Arch, 2000; Kirchgessner, 2002; Mieda and Yanagisawa, 2002; Sakurai, 2002; Willie et al., 2001). However, they would also ensure that, while foraging, animals remained alert to potential dangers in the environment, with increased vigilance serving to defend against predation (Kirchgessner, 2002). These coordinated effects of LH orexin activation would undoubtedly have adaptive value in aiding survival under conditions of severe nutritional depletion.

In the wild, rats are opportunistic foragers (Barnett, 1963) and, in order to feed, must leave the relative security of the home burrow. However, doing so immediately introduces the competing demands of finding food and avoiding danger. Indeed, it is widely recognised that predation risk shapes animal vigilance, and that the conflict between feeding and vigilance is the classic behavioural trade-off (Treves, 2000). In an excellent review of antipredator defense, Kavaliers and Choleris (2001) discuss two concepts widely used in behavioural ecology research. ‘Apprehension’ refers to any reduction in attention to foraging that occurs as a result of increasing the allocation of attention to detecting and/or responding to potential predators. Vigilance on the other hand is the behavioural state of alertness and scanning for predators. These opposing demands result in an ‘apprehension gradient’, ranging from the extremes of having no interest in the risk of predation to total preoccupation with such risk. As such, the baseline level of apprehension determines the prey’s level of vigilance in the absence of any tangible evidence of a predator’s presence. Obviously, if this is set too high, animals may miss valuable feeding opportunities but, if too low, they may well themselves be killed and eaten. Indeed, the results of many empirical studies and theoretical analyses

have established that foragers always behave as if the risk of predation is high, i.e., a 'better safe than sorry' strategy (Lima, 1998).

The relevance of these issues to the functional significance of orexins can perhaps best be illustrated by a question posed by Kavaliers and Choleris (2001) – namely, what should an animal do when it is simultaneously very hungry yet under the risk of predation? Although predation obviously carries a much higher penalty than any temporary loss of food, extreme hunger can also be life-threatening. According to the apprehension gradient, the animal in question should search for food while maintaining a high level of vigilance. As already discussed, the LH orexin system appears to be activated only under physiological conditions signifying severe nutritional depletion, i.e., low glucose levels (insulin-induced hypoglycaemia) and/or an empty stomach (prolonged fasting). Through its extensive hypothalamic and extrahypothalamic projections, the orexin system may therefore simultaneously initiate not only active foraging (searching and feeding) but also the requisite state of alertness to danger (vigilance) (Fig. 3).

In this context, detailed studies on the defensive behaviour of animals have revealed two basic coping strategies (active and passive defence), each involving

hypothalamic projections to effector mechanisms running rostro-caudally throughout the midbrain periaqueductal gray matter (PAG) (Bandler and Shipley, 1994). The active defensive strategy, in particular, has considerable significance for our understanding of the constellation of physiological and behavioural changes that accompany activation of the LH orexin system. Mediated by the dorsolateral and lateral columns of the PAG, this strategy is associated with increased somatomotor activity, increased vigilance, tachycardia, hypertension and non-opioid analgesia (for recent review: Keay and Bandler, 2001). It is surely no coincidence that all of these physiological changes have been described in response to central administration of orexins. Furthermore, as vigilance is associated with high levels of risk assessment (Blanchard et al., 1993; Kavaliers and Choleris, 2001), it is pertinent to note that orexin-A enhances corticosterone release (e.g., Hagan et al., 1999) and that a high positive correlation exists between risk assessment and plasma corticosterone titres (Rodgers et al., 1999).

In contrast to active defence, the passive defensive strategy (mediated via the ventrolateral PAG column) is most often seen in response to dire circumstances, such as capture and handling by a predator, and is associated with behavioural quiescence (tonic immobility), brady-

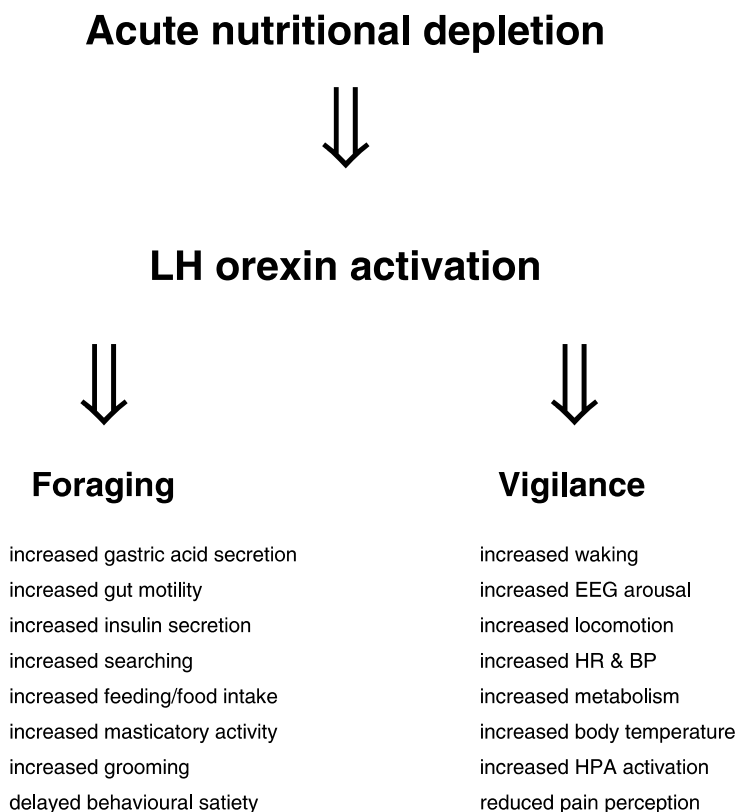


Fig. 3 Activation of LH orexin system by severe nutritional depletion (low blood glucose and/or empty stomach) simultaneously induces foraging and enhances vigilance. HR: heart rate, BP: blood pressure. See text for further details.

cardia, hypotension and opioid analgesia (Keay and Bandler, 2001). Clearly, the response requirements of passive defence are incompatible with those of active defence, implying the existence of mutual inhibitory interactions. In this context, considerable interest has recently been expressed in the potential involvement of orexins (and OX_2R mechanisms in particular) in the sleep/arousal disorder, narcolepsy. Although a detailed review of the relevant literature is beyond the scope of the present paper, such interest has stemmed not only from the effects of orexins on behavioural and EEG arousal (as reviewed above) but also from the effects of naturally occurring and/or engineered mutations of genes encoding prepro-orexin and/or orexin receptors. For example, in mice, knockout of the prepro-orexin gene or combined knockout of the genes encoding OX_1R and OX_2R has been found not only to result in hypophagia but also in behavioural changes consistent with bouts of cataplexy (for recent reviews: Hara et al., 2001; Hungs and Mignot, 2001; Sutcliffe and de Lecea, 2002; Willie et al., 2001). Cataplexy, defined as a sudden and complete loss of muscle tone, is a hallmark of narcolepsy and has traditionally been interpreted as rapid eye movement (REM)-sleep atonia. However, in humans, cataplexy is known to be triggered by strong emotions and the question therefore arises as to how and why cataplexy should result from inactivation of the orexin system. This issue has recently been explored in a thought-provoking paper by Overeem et al. (2002) who argue that, rather than REM-sleep atonia, cataplexy represents an 'atavism' (i.e., the recurrence of an ancestral pattern) and, more specifically, the recurrence of tonic immobility as the ultimate defensive response to extreme danger. From this perspective, the authors argue that LH orexin neurons may normally suppress brain areas involved in the initiation of tonic immobility. This suggestion raises the possibility that activation of the LH orexin system under conditions of severe nutritional depletion may simultaneously initiate foraging/active defense while inhibiting passive defense. Logically, therefore, complete inactivation of the orexin system (requiring severe loss of orexin neurons or loss of both types of orexin receptor) would not only disrupt the former but also disinhibit the latter.

Behavioural effects of orexins revisited

In virtually all published reports, hyperphagia is the predominant behavioural response to orexins (especially, orexin-A) when food is present in the test environment. However, it is clear that other behavioural changes can also occur in this context depending, amongst other things (e.g., dose), upon the nature of the food source, the reactivity of the subjects, familiarity with the test environment, and the presence of water and bedding in

that environment. These additional behavioural changes include searching, burrowing, drinking, increased locomotor activity and grooming. As many of these changes have also been observed outside a feeding context (e.g., an activity chamber), some authors have queried the behavioural selectivity of orexin hyperphagia (e.g., Espana et al., 2002) while others have suggested that, at best, orexins play only a minor role in appetite regulation (Sutcliffe and de Lecea, 2002). However, if (as suggested above) orexin activation signals nutrient depletion, thereby simultaneously engaging mechanisms associated with foraging and vigilance, there is no reason why the absence of food should prevent the occurrence of orexin-A-induced non-ingestive responses. Consistent with this line of thinking, it is known that orexins are capable of producing sympathetic activation even in anaesthetised animals (e.g., Shirasaka et al., 2001). Indeed, it might even be argued that the absence of food for an animal in 'foraging mode' might further intensify its state of arousal, and this may explain why orexin-induced locomotor activation and grooming are, if anything, even more readily observed in the absence of food. Furthermore, under these specific circumstances, the excessive grooming seen in response to orexins may represent a displacement, or stress-related, response arising from the failure to locate food. Clearly, such arguments lead to a number of testable hypotheses, e.g., contrasting the behavioural effects of orexins in novel versus familiar test situations, in situations where food is present, absent, or hidden in the test environment, and even in situations containing unconditioned or conditioned danger cues.

CONCLUSIONS

The empirical evidence for orexin involvement in the regulation of appetite is diverse and appears to us to be incontrovertible (Table 3). In particular, the hyperphagic effects of these peptides (especially orexin-A) have been found in several species and, in rats, are associated with a delay in the onset of behavioural satiety. Studies with SB-334867 strongly suggest these effects of orexin-A are mediated via the OX_1R while the observation that, when given alone, this antagonist inhibits feeding and phase advances behavioural satiety implicates OX_1R mechanisms in the regulation of normal feeding behaviour. Furthermore, the pattern of effects on feeding induced both by the peptide and the antagonist suggests that the primary role of orexin-A in appetite involves satiety signalling with orexins functioning to increase intake by prolonging feeding behaviour. This would be consistent with the view that the LH orexin system is activated in response to acute energy depletion and thus subserve short-term appetite regulation (Arch, 2000; Cai et al., 2002; Smart et al., 2002). The additional effects of

Table 3 Orexin involvement in appetite regulation: summary of evidence

Neuroanatomy	<ul style="list-style-type: none"> • LH orexin neurons project to NPY- and POMC- expressing cells in ARC nucleus • LH orexin neurons project to extrahypothalamic sites involved in appetite regulation
System activation	<ul style="list-style-type: none"> • Orexin receptors (OX1R and OX2R) are present in all of these brain areas
Ingestive physiology	<ul style="list-style-type: none"> • LH orexin neurons are activated by insulin-induced hypoglycaemia and by prolonged fasting • Orexins increase gastric acid secretion, gut motility, and insulin secretion • Orexins influence neural mechanisms involved in control of mastication • Orexins modulate activity in NTS
Food intake, feeding behaviour and bodyweight	<ul style="list-style-type: none"> • Orexins enhance food intake in several species, with neural sites of action in LH, PFN and DMN • Orexins increase feeding behaviour and delay behavioural satiety; effects blocked by OX1R antagonist, SB-334867 • Food intake/feeding behaviour inhibited by anti-orexin-A antibodies and OX1R antagonist • OX1R antagonist phase advances behavioural satiety and suppresses bodyweight gain in rats • OX1R antagonist reduces food intake and decreases bodyweight gain in genetically obese mice
System interactions	<ul style="list-style-type: none"> • Orexin-A selectively increases NPY expression in cells of the ARC nucleus • Orexin-A hyperphagia is blocked by NPY Y1 and Y5 receptor antagonists • Orexin-A antibodies block NPY-induced hyperphagia

ARC: arcuate nucleus; DMN: dorsomedial nucleus; LH: lateral hypothalamus; NPY: neuropeptide Y; NTS: nucleus of the solitary tract; OX1R and OX2R: orexin-1 receptor and orexin-2 receptor; PFN: perifornical nucleus; POMC: proopiomelanocortin.

orexins on CNS arousal, sympathetic activation, neuroendocrine function and pain perception can be understood not as independent effects of these peptides but rather as part of an adaptive constellation of changes in the physiology and behaviour of animals that must find food by foraging in potentially dangerous environments. The alternate perspective that 'the sleep-wake aspects of orexins are dominant and that a secondary involvement in metabolic concerns will therefore be subservient to the demands of the arousal cycle' (Sutcliffe and de Lecea, 2002) seems to miss the point that arousal cannot be an end in itself but, rather, is the means whereby animals engage in certain adaptive behaviours – such as foraging while simultaneously maintaining the level of vigilance necessary to avoid becoming a meal for someone else. In other words, the 'why?' question in neuroscience should be just as important as the 'how?'.

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REFERENCES

- Ahima RS, Osei SY (2001) Molecular regulation of eating behavior: new insights and prospects for therapeutic strategies. *Trends Molec Med* 7: 205–213.
- Alvarez CE, Sutcliffe JG (2002) Hypocretin is an early member of the incretin family. *Neurosci Lett* 324: 169–172.
- Antin J, Gibbs J, Holt J, Young RC, Smith GP (1975) Cholecystokinin elicits the complete behavioral sequence of satiety in rats. *J Comp Physiol Psychol* 89: 748–760.
- Arch JRS (2000) Orexins, feeding and the big picture. *Br J Nutr* 84: 1–4.
- Arch JRS, Williams G, Wilson S (1999) Orexins and obesity. *Neurosci News* 2: 37–42.
- Backberg M, Hervieu G, Wilson S, Meister B (2002) Orexin-receptor-1 (OX-R1) immunoreactivity in chemically identified neurons of the hypothalamus: focus on orexin targets involved in control of food and water intake. *Eur J Neurosci* 15: 315–328.
- Bandler R, Shipley MT (1994) Columnar organization in midbrain periaqueductal gray: modules for emotional expression? *Trends Neurosci* 17: 379–389.
- Barnett SA (1963) *The Rat. A study in behaviour*. Methuen, London.
- Beck B (2000) Neuropeptides and obesity. *Nutrition* 16: 916–923.
- Beck B (2001) KO's and organisation of peptidergic feeding behavior mechanisms. *Neurosci Biobehav Rev* 25: 143–158.
- Beck B, Richy S, Stricker-Krongrad A (1999) Regulation of hypocretins/orexins and neuropeptide Y during leptin treatment, energy restriction and hyperphagia in rodents. *Soc Neurosci Abstr* 25: 214.9.
- Bernardis LL, Bellinger LL (1996) The lateral hypothalamic area revisited: ingestive behavior. *Neurosci Biobehav Rev* 20: 189–287.
- Bindra D, Blond J (1958) A time sample method for measuring general activity and its components. *Can J Psychol* 12: 74–76.
- Bingham S, Davey PT, Babbs AJ, Irving EA, Sammons MJ, Wyles M, Jeffrey P, Cutler L, Riba I, Johns A, Porter RA, Upton N, Hunter AJ, Parsons AA (2001) Orexin-A, an hypothalamic peptide with analgesic properties. *Pain* 92: 81–90.
- Blanchard RJ, Yudko EB, Rodgers RJ, Blanchard DC (1993) Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behav Brain Res* 58: 155–165.
- Blundell JE (1977) Is there a role for 5-HT (5-hydroxytryptamine) in feeding? *Int J Obesity* 1: 15–42.
- Blundell JE (1986) Serotonin manipulations and the structure of feeding behaviour. *Appetite* 7: 39–56.
- Blundell JE, Goodson S, Halford JCG (2001) Regulation of appetite: role of leptin in signalling systems for drive and satiety. *Int J Obesity* 25 (Suppl 1): S29–S34.

- Blundell JE, Latham CJ (1979) Serotonergic influences on food intake: effect of 5-HT on parameters of feeding behaviour in deprived and free-feeding rats. *Pharmacol Biochem Behav* 11: 431–437.
- Bolles RC (1960) Grooming behavior in the rat. *J Comp Physiol Psychol* 53: 306–310.
- Bray GA (2000) Afferent signals regulating food intake. *Proc Nutr* 59: 373–384.
- Briski KP, Sylvester PW (2001) Hypothalamic orexin-A-immunopositive neurons express Fos in response to central glucopenia. *Neuroendocrinology* 12: 531–534.
- Broberger C, de Lecea L, Sutcliffe JG, Hokfelt T (1998) Hypocretin/orexin- and melanocortin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* 402: 460–474.
- Brown RE, Sergeeva O, Eriksson KS, Haas HL (2001) Orexin-A excites serotonergic neurons in the dorsal raphe nucleus of the rat. *Neuropharmacology* 40: 457–459.
- Cai XJ, Evans ML, Lister CA, Leslie RA, Arch JRS, Wilson S, Williams G (2001) Hypoglycaemia activates orexin neurons and selectively increases hypothalamic orexin-B levels. Responses inhibited by feeding and possibly mediated by the nucleus of the solitary tract. *Diabetes* 50: 105–112.
- Cai XJ, Liu XH, Evans M, Clapham JC, Wilson S, Arch JRS, Morris R, Williams G (2002) Orexins and feeding: special occasions or everyday occurrence? *Regul Pept* 104: 1–9.
- Cai XJ, Widdowson PS, Harrold J, Wilson S, Buckingham RE, Arch JRS, Tadayyon M, Clapham JC, Wilding J, Williams G (1999) Hypothalamic orexin expression: modulation by blood glucose and feeding. *Diabetes* 48: 2132–2137.
- Carpino PA (2000) Patent focus on new anti-obesity agents: September 1999 – February 2000. *Exp Opin Ther Patents* 10: 819–831.
- Chamorro S, Della-Zuana O, Fauchere J-L, Feletou M, Galizzi J-P, Levels N (2002) Appetite suppression based on selective inhibition of NPY receptors. *Int J Obesity* 26: 281–298.
- Chiesi M, Huppertz C, Hofbauer KG (2001) Pharmacotherapy of obesity: targets and perspectives. *Trends Pharmacol Sci* 22: 247–254.
- Clapham JC, Arch JRS, Tadayyon M (2001) Anti-obesity drugs: a critical review of current therapies and future opportunities. *Pharm Ther* 89: 81–121.
- Clifton PG (2000) Meal patterning in rodents: psychopharmacological and neuroanatomical studies. *Neurosci Biobehav Revs* 24: 213–222.
- Collin M, Backberg M, Onnestam K, Meister B (2002) 5-HT^{1A} receptor immunoreactivity in hypothalamic neurons involved in body weight control. *NeuroReport* 13: 945–951.
- Collins P, Williams G (2001) Drug treatment of obesity: from past failures to future successes? *Brit J Clin Pharmacol* 51: 13–25.
- Crawley JN (2000) *What's Wrong with My Mouse?* Wiley-Liss, New York.
- Cutler DJ, Morris R, Sheridhar V, Wattam TAK, Holmes S, Patel S, Arch JRS, Wilson S, Buckingham RE, Evans ML, Leslie RA, Williams G (1999) Differential distribution of orexin-A and orexin-B immunoreactivity in the rat brain and spinal cord. *Peptides* 20: 1455–1470.
- Date Y, Mondal MA, Matsuhara S, Ueta Y, Yamashita H, Kaiya H, Kangawa K, Nakazato M (2000) Distribution of orexin/hypocretin in the rat median eminence and pituitary. *Mol Brain Res* 76: 1–6.
- Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura S, Kangawa K, Sakurai T, Yanagisawa M, Nakazato M (1999) Orexins, orexigenic hypothalamic peptides interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Soc USA* 96: 748–753.
- de Lecea L, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE, Fukuhara C, Battenberg ELF, Gautvik VT, Bartlett FS, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA* 95: 322–327.
- Dhillon WS, Bloom SR (2001) Hypothalamic peptides as drug targets for obesity. *Curr Opin Pharmacol* 1: 651–655.
- Dourish CT (1999) New frontiers in obesity research. *Neurosci News* 2: 4.
- Dube MG, Horvath TL, Kalra PS, Kalra SP (2000) Evidence of NPY Y5 receptor involvement in food intake elicited by orexin-A in sated rats. *Peptides* 21: 1557–1560.
- Dube MG, Kalra SP, Kalra PS (1999) Food intake elicited by central administration of orexins/hypocretins: identification of hypothalamic sites of action. *Brain Res* 842: 473–477.
- Dun NJ, Dun SL, Chen C-T, Hwang LL, Kwok EH, Chang J-K (2000) Orexins: a role in medullary sympathetic outflow. *Regul Pept* 96: 65–70.
- Duxon MS, Stretton J, Starr K, Jones DNC, Holland V, Riley G, Jerman J, Brough S, Smart D, Johns A, Chan W, Porter RA, Upton N (2001) Evidence that orexin-A-evoked grooming in the rat is mediated by orexin-1 (OX₁) receptors, with downstream 5-HT^{2C} receptor involvement. *Psychopharmacology* 153: 203–209.
- Dyer CJ, Touchette KJ, Carroll JA, Allee GL, Matteri RJ (1999) Cloning of porcine prepro-orexin cDNA and effects of an intramuscular injection of synthetic porcine orexin-B on feed intake in young pigs. *Domest Anim Endocrinol* 16: 145–148.
- Edwards CMB, Abusnana S, Sunter D, Murphy KG, Ghatel MA, Bloom SR (1999) The effect of orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *J Endocrinol* 160: R7–R12.
- Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, Tatro JB, Hoffmann GE, Ollmann MM, Barsh GS, Sakurai T, Yanagisawa M, Elmquist JK (1998) Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamus. *J Comp Neurol* 402: 442–459.
- Espana RA, Plahn S, Berridge CW (2002). Circadian-dependent and circadian-independent behavioral actions of hypocretin/orexin. *Brain Res* 943: 224–236.
- Fadel J, Deutch AY (2002) Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience* 111: 379–387.
- Finer N (1999) Obesity: the need for new therapies. *Neurosci News* 2: 5–11.
- Friedman JM, Halaas JL (1998) Leptin and the regulation of bodyweight. *Nature* 372: 425–432.
- Funahashi H, Hori T, Shimoda Y, Mizushima H, Ryushi T, Katoh S, Shioda S (2000) Morphological evidence for neural interactions between leptin and orexin in the hypothalamus. *Regul Pept* 92: 31–35.
- Gehlert DR (1999) Role of hypothalamic neuropeptide Y in feeding and obesity. *Neuropeptides* 33: 329–338.
- Greco MA, Shiromani PJ (2001) Hypocretin receptor protein and mRNA expression in the dorsolateral pons of rats. *Molec Brain Res* 88: 176–182.

- Griffond B, Risold PY, Jacquemard C, Colard C, Fellman D (1999) Insulin-induced hypoglycemia increases prehypocretin (orexin) mRNA in the rat lateral hypothalamic area. *Neurosci Lett* 262: 77–80.
- Guan J-L, Saotome T, Wang Q-P, Funahashi H, Hori T, Tanaka S, Shioda S (2001) Orexinergic innervation of POMC-containing neurons in the rat arcuate nucleus. *NeuroReport* 12: 547–551.
- Hagan JJ, Leslie RA, Patel S, Evans ML, Wattam TA, Holmes S, Benham CD, Taylor SG, Routledge C, Hemmati P, Muntton RP, Ashmeade TE, Shah AS, Hatcher JP, Hatcher PD, Jones DNC, Smith MI, Piper DC, Hunter AJ, Porter RA, Upton N (1999) Orexin A activates locus coeruleus cell firing and increases arousal in the rat. *Proc Natl Acad Sci USA* 96: 10911–10916.
- Halford JCG, Wanninayake SCD, Blundell JE (1998) Behavioral satiety sequence (BSS) for the diagnosis of drug action on food intake. *Pharmacol Biochem Behav* 61: 159–168.
- Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM, Sinton CM, Sugiyama F, Yagami K-I, Goto K, Yanagisawa M, Sakurai T (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. *Neuron* 30: 345–354.
- Haynes AC, Chapman H, Taylor C, Moore GBT, Cawthorne MA, Clapham JC, Arch JRS (2002) Anorectic, thermogenic and anti-obesity activity of a selective orexin-1 receptor antagonist in ob/ob mice. *Regul Pept* 104: 153–159.
- Haynes AC, Jackson B, Chapman H, Tadayyon M, Johns A, Porter RA, Arch JRS (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul Pept* 96: 45–51.
- Haynes AC, Jackson B, Overend P, Buckingham RE, Wilson S, Tadayyon M, Arch JRS (1999) Effects of single and chronic intracerebroventricular administration of the orexins on feeding in the rat. *Peptides* 20: 1099–1105.
- Hervieu GJ, Cluderay JE, Harrison DC, Roberts JC, Leslie RA (2001) Gene expression and protein distribution of the orexin-1 receptor in the rat brain and spinal cord. *Neuroscience* 103: 777–797.
- Hewitt KN, Lee MD, Dourish CT, Clifton PG (2002) Serotonin 2C receptor agonists and the behavioural satiety sequence in mice. *Pharmacol Biochem Behav* 71: 691–700.
- Horst GJT, de Boer P, Luiten PGM, van Willigen JD (1989) Ascending projections from the solitary tract nucleus to the hypothalamus: a phaseolus vulgaris lectin tracing study in the rat. *Neuroscience* 31: 785–797.
- Horvath TL, Diano S, van den Pol AN (1999) Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* 19: 1072–1087.
- Hungs M, Mignot E (2001) Hypocretin/orexin, sleep and narcolepsy. *BioEssays* 23: 397–408.
- Ida T, Nakahara K, Katayama T, Murakami N, Nakazato M (1999) Effect of lateral cerebroventricular injection of the appetite-stimulating neuropeptides, orexin and neuropeptide Y, on the various behavioral activities of rats. *Brain Res* 821: 526–529.
- Ida T, Nakahara K, Kuroiwa T, Fukui K, Nakazato M, Murakami T, Murakami N (2000) Both corticotropin releasing factor and neuropeptide Y are involved in the effect of orexin (hypocretin) on the food intake in rats. *Neurosci Lett* 293: 119–122.
- Inui A (1999) Feeding and body-weight regulation by hypothalamic neuropeptides – mediation of the actions of leptin. *Trends Neurosci* 22: 62–67.
- Inui A (2000) Transgenic approach to the study of body weight regulation. *Pharmacol Rev* 52: 35–62.
- Jain MR, Horvath TL, Kalra PS, Kalra SP (2000) Evidence that NPY Y1 receptors are involved in stimulation of feeding by orexins (hypocretins) in sated rats. *Regul Pept* 87: 19–24.
- Jones DNC, Garlton J, Parker F, Taylor SG, Routledge C, Hemmati P, Muntton RP, Ashmeade TE, Hatcher JP, Johns A, Porter RA, Hagan JJ, Hunter AJ, Upton N (2001) Effects of centrally administered orexin-B and orexin-A: a role for orexin-1 receptors in orexin-B-induced hyperactivity. *Psychopharmacology* 153: 210–218.
- Kalra SP (1997) Appetite and body weight regulation: is it all in the brain? *Neuron* 19: 227–230.
- Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra P (1999) Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocr Rev* 20: 68–100.
- Kavaliers M, Choleris E (2001) Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neurosci Biobehav Rev* 25: 577–586.
- Keay KA, Bandler R (2001) Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neurosci Biobehav Rev* 25: 669–678.
- Kopelman PG (2000) Obesity as a medical problem. *Nature* 404: 635–643.
- Kotz CM, Teske JA, Levine JA, Wang CF (2001) Feeding and activity induced by orexin-A in the lateral hypothalamus in rats. *Regul Pept* 104: 27–32.
- Kirchgessner AL (2002) Orexins in the brain-gut axis. *Endocr Rev* 23: 1–15.
- Kirchgessner AL, Liu M-T (1999) Orexin synthesis and response in the gut. *Neuron* 24: 41–951.
- Kunii K, Yamanaka A, Nambu T, Matsuzaki I, Goto K, Sakurai T (1999) Orexins/hypocretins regulate drinking behaviour. *Brain Res* 842: 256–261.
- Kuru M, Ueta Y, Serino R, Nakazato M, Yamamoto Y, Shibuya I, Yamashita H (2000) Centrally administered orexin/hypocretin activates HPA axis in rats. *NeuroReport* 11: 1977–1980.
- Lambert PD, Couceyro PR, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ (1998) CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse* 29: 293–298.
- Lawrence CB, Turnbull AV, Rothwell NJ (1999) Hypothalamic control of feeding. *Curr Opin Neurobiol* 9: 778–783.
- Lima SL (1998) Stress and decision-making under the risk of predation: recent developments from behavioural, reproductive and ecological perspectives. *Adv Study Behav* 27: 215–290.
- Lin Y, Matsumura K, Tsuchihashi T, Lida M (2002) Chronic central infusion of orexin-A increases arterial pressure in rats. *Brain Res Bull* 57: 619–622.
- Lopez M, Senaris R, Gallego R, Garcia-Caballero T, Lago F, Seoane L, Casaneuva F, Dieguez C (1999) Orexin receptors are expressed in the adrenal medulla of the rat. *Endocrinology* 140: 5991–5994.
- Lopez M, Seoane L, del Carmen Garcia M, Dieguez C, Senaris R (2002) Neuropeptide-Y, but not agouti-related peptide or melanin-concentrating hormone, is a target peptide for

- orexin-A feeding actions in the rat hypothalamus. *Neuroendocrinology* 75: 34–44.
- Lopez M, Seoane L, del Carmen Garcia M, Lago F, Casanueva FF, Senaris R, Dieguez C (2000) Leptin regulation of prepro-orexin and orexin receptor mRNA in the hypothalamus. *Biochem Biophys Res Commun* 269: 41–45.
- Lu X-Y, Bagnol D, Burke S, Akil H, Watson SJ (2000) Differential distribution and regulation of orexin-1 and orexin2/hypocretin receptor messenger RNA in the brain upon fasting. *Horm Behav* 37: 335–344.
- Lubkin M, Stricker-Krongrad A (1998) Independent feeding and metabolic actions of orexins in mice. *Biochem Biophys Res Commun* 253: 241–245.
- Luo P, Moritani M, Dessem D (2001) Jaw-muscle spindle afferent pathways to the trigeminal motor nucleus in the rat. *J Comp Neurol* 435: 341–353.
- Macdonald IA (2000) Obesity: are we any closer to identifying causes and effective treatments? *Trends Pharmacol Sci* 21: 334–336.
- Mantzoros CS (1999) The role of leptin in human obesity and disease: a review of current evidence. *Ann Intern Med* 130: 671–680.
- Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper C B, Yanagisawa M, Elmquist JK (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* 435: 6–25.
- Marsh DJ, Hollopeter G, Huszar D, Laufer R, Yagaloff KA, Fisher SL, Burn P, Palmiter RD (1999) Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat Genet* 21: 119–122.
- Matsuzaki I, Sakurai T, Kunii K, Nakamura T, Yanagisawa M, Goto K (2001) Involvement of the serotonergic system in orexin-induced behavioral alterations in rats. *Regul Pept* 104: 119–123.
- McIntyre AM (1998) Burden of illness review of obesity: are the true costs realised? *J R Soc Health* 118: 76–84.
- Mercer JG, Speakman JR (2001) Hypothalamic neuropeptide mechanisms for regulating energy balance: from rodent models to human obesity. *Neurosci Biobehav Rev* 25: 101–116.
- Mieda M, Yanagisawa M (2002) Sleep, feeding, and neuropeptides: roles of orexins and orexin receptors. *Curr Opin Neurobiol* 12: 339–345.
- Mogenson GJ, Stevenson JA (1967) Drinking induced by electrical stimulation of the lateral hypothalamus. *Exp Neurol* 17: 119–127.
- Mondal MS, Nakazato M, Date Y, Murakami N, Yanagisawa M, Matsukura S (1999) Widespread distribution of orexin in rat brain and its regulation upon fasting. *Biochem Biophys Res Commun* 256: 495–499.
- Moore RY, Speh JC, van den Pol AN (1998) Hypocretin localization in the human hypothalamus. *Soc Neurosci Abstr* 24: 762.1.
- Moriguchi T, Sakurai T, Nambu T, Yanagisawa M, Goto K (1999) Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced hypoglycemia. *Neurosci Lett* 264: 101–104.
- Mullett MA, Billington CJ, Levine AS, Kotz CM (2000) Hypocretin 1 in the lateral hypothalamus activates key feeding-regulatory brain sites. *NeuroReport* 11: 103–108.
- Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, Sakurai T (2000) Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res* 873: 181–187.
- Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K (1999) Distribution of orexin neurons in the adult rat brain. *Brain Res* 827: 243–260.
- Niimi M, Sato M, Taminato T (2001) Neuropeptide Y in central control of feeding and interactions with orexin and leptin. *Endocrine* 14: 269–273.
- Nowak KW, Mackowiak P, Switonska MM, Fabis M, Malendowicz LK (2000) Acute orexin effects on insulin secretion in the rat: in vivo and in vitro studies. *Life Sci* 66: 449–454.
- Oltmans GA, Harvey JA (1976) Lateral hypothalamic syndrome in rats: a comparison of the behavioral and neurochemical effects of lesions placed in the lateral hypothalamus and nigrostriatal bundle. *J Comp Physiol Psychol* 90: 1051–1062.
- Oomura Y (1980) Input-output organisation in the hypothalamus relating to food intake behavior. In: *Physiology of the Hypothalamus*. Morgane PJ, Panksepp J, (eds). Handbook of the Hypothalamus, vol. 2. Marcel Dekker, New York pp. 557–620.
- Orlando G, Brunetti L, Di Nisio C, Michelotto B, Recinella L, Ciabattini G, Vacca M (2001) Effects of cocaine- and amphetamine-regulated transcript peptide, leptin and orexins on hypothalamic serotonin release. *Eur J Pharmacol* 430: 269–272.
- Overeem S, Lammers GJ, van Dijk JG (2002) Cataplexy: ‘tonic immobility’ rather than ‘REM-sleep atonia?’ *Sleep Med*, in press.
- Pavlov IP, Schumowa-Simanowskaja EO (1895) Beitrage zur Physiologie der Absonderungen Die innervation der Magenrusen beim Hunde. *Arch Anat Physiol (Lpz) Physiol Abt*: 53–69.
- Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG, Kilduff TS (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 18: 9996–10015.
- Piper DC, Upton N, Smith MI, Hunter AJ (2000) The novel brain neuropeptide, orexin-A, modulates the sleep-wake cycle of rats. *Eur J Neurosci* 12: 726–730.
- Porter RA, Chan WN, Coulton S, Johns A, Hadley MS, Widdowson K, Jerman JC, Brough SJ, Coldwell M, Smart D, Jewitt F, Jeffrey P, Austin N (2001) 1,3-Biarylyureas as selective non-peptide antagonists of the orexin-1 receptor. *Bioorg Med Chem Lett* 11: 1907–1910.
- Prescott-Clarke P, Primatesta P (1998) *Health Survey for England 1996*. HMSO, London.
- Proietto J, Fam BC, Ainslie DA, Thorburn AW (2000) Novel anti-obesity drugs. *Exp Opin Invest Drugs* 9: 1317–1326.
- Rauch M, Riediger T, Schmid HA, Simon E (2000) Orexin-A activates leptin-responsive neurons in the arcuate nucleus. *Pflugers Arch-Eur J Physiol* 440: 699–703.
- Rodgers RJ, Halford JCG, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JRS, Blundell JE (2000) Dose-response effects of orexin-A on food intake and the behavioural satiety sequence in rats. *Regul Pept* 96: 71–84.
- Rodgers RJ, Halford JCG, Nunes de Souza RL, Canto de Souza AL, Piper DC, Arch JRS, Upton N, Porter RA, Johns A, Blundell JE (2001) SB-334867, a selective orexin-1 receptor antagonist, enhances behavioural satiety and blocks the hyperphagic effect of orexin-A in rats. *Eur J Neurosci* 13: 1444–1452.

- Rodgers RJ, Haller J, Holmes A, Halasz J, Walton TJ, Brain PF (1999) Corticosterone response to the plus-maze: high correlation with risk assessment in rats and mice. *Physiol Behav* 68: 47–53.
- Sahu A (2002) Interactions of neuropeptide, Y., hypocretin-I orexin-A and melanin-concentrating hormone on feeding in rats. *Brain Res* 944: 232–238.
- Sakurai T (1999) Orexins and orexin receptors: implication in feeding behavior. *Regul Pept* 85: 25–30.
- Sakurai T (2002) Roles of orexins in regulation of feeding and wakefulness. *NeuroReport* 13: 987–995.
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JRS, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu W-S, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding. *Cell* 92: 573–585.
- Sakurai T, Moriguchi T, Furuya K, Kajiwara N, Nakamura T, Yanagisawa M, Goto K (1999) Structure and function of human prepro-orexin gene. *J Biol Chem* 274: 17771–17776.
- Salton SRJ, Hahm S, Mizuno TM (2000) Of mice and men: what transgenic models tell us about hypothalamic control of energy balance. *Neuron* 25: 265–268.
- Samson WK, Gosnell B, Chang J-K, Resch ZT, Murphy TC (1999) Cardiovascular regulatory actions of the hypocretins in brain. *Brain Res* 831: 248–253.
- Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. *Nature* 404: 661–671.
- Shibahara M, Sakurai T, Nambu T, Takenouchi T, Iwaasa H, Egashira S, Ihara M, Goto K (1999) Structure, tissue distribution, and pharmacological characterisation of *Xenopus* orexins. *Peptides* 20: 1169–1176.
- Shiraishi T, Oomura Y, Sasaki K, Wayner MJ (2000) Effects of leptin and orexin-A on food intake and feeding related hypothalamic neurons. *Physiol Behav* 71: 251–261.
- Shirasaka T, Miyahara S, Kunitake T, Jin Q-H, Kato K, Takasaki M, Kannan H (2001) Orexin depolarizes rat hypothalamic paraventricular nucleus neurons. *Am J Physiol Regulatory Integrative Comp Physiol* 281: R1114–R1118.
- Shirasaka T, Nakazato M, Matsukura S, Takasaki M, Kannan H (1999) Sympathetic and cardiovascular actions of orexins in conscious rats. *Am J Physiol* 277: R1780–R1785.
- Simansky KJ (1996) Serotonergic control of the organisation of feeding and satiety. *Behav Brain Res* 73: 37–42.
- Smart D, Haynes AC, Williams G, Arch JRS (2002) Orexins and the treatment of obesity. *Eur J Pharmacol* 440: 199–212.
- Smart D, Jerman JC, Brough SJ, Neville WA, Jewitt F, Porter RA (2000) The hypocretins are weak agonists at recombinant human orexin-1 and orexin-2 receptors. *Br J Pharmacol* 129: 1289–1291.
- Smart D, Jerman JC, Brough SJ, Rushton SL, Murdock PR, Jewitt F, Elshourbagy NA, Ellis CE, Middlemiss DN, Brown F (1999) Characterisation of recombinant human orexin receptor pharmacology in a Chinese hamster ovary cell line using FLIPR. *Br J Pharmacol* 128: 1–3.
- Smart D, Sabido-David C, Brough SJ, Jewitt F, Johns A, Porter RA, Jerman JC (2001) SB-334867-A: the first selective orexin-1 receptor antagonist. *Br J Pharmacol* 132: 1179–1182.
- Smith JC (2000) Microstructure of the rat's intake of food, sucrose and saccharin in 24-hour tests. *Neurosci Biobehav Rev* 24: 199–212.
- Smith SL, Fraser DC, Needham AM, Jackson HC, Cheetham SC, Heal DJ (2000) Effect of central injection of anti-orexin1 receptor antibody on food intake in rats. *Int J Obesity* 24 (Suppl. 1): S37, 102.
- Stellar E (1954) The physiology of motivation. *Psychol Rec* 61: 5–22.
- Sunter D, Morgan I, Edwards CMB, Dakin CL, Murphy KG, Gariner J, Taheri S, Rayes E, Bloom SR (2001) Orexins: effects on behavior and localisation of orexin receptor 2 messenger ribonucleic acid in the rat brainstem. *Brain Res* 907: 27–34.
- Sutcliffe JG, de Lecea L (2002) The hypocretins: setting the arousal threshold. *Nat Rev Neurosci* 3: 339–349.
- Sweet DC, Levine AS, Billington CJ, Kotz CM (1999) Feeding response to central orexins. *Brain Res* 821: 535–538.
- Taheri S, Mahmoodi M, Opacka-Juffry J, Ghattei MA, Bloom SR (1999) Distribution and quantification of orexin A in rat tissues. *FEBS Lett* 457: 157–161.
- Takahashi N, Okumura T, Yamada H, Kohgo Y (1999) Stimulation of gastric acid secretion by centrally administered orexin-A in conscious rats. *Biochem Biophys Res Commun* 254: 623–627.
- Trayhurn P (1999) Leptin: much more than an anti-obesity hormone. *Neurosci News* 2: 29–32.
- Treves A (2000) Theory and method in studies of vigilance and aggregation. *Anim Behav* 60: 711–722.
- Trivedi P, Yu H, MacNeil DJ, van der Ploeg LHT, Guan X-M (1998) Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett* 438: 71–75.
- Vergoni AV, Schioth HB, Bertolini A (2000) Melanocortins and feeding behavior. *Biomed Pharmacother* 54: 129–134.
- Volkoff H, Bjorklund JM, Peter RE (1999) Stimulation of feeding behavior and food consumption in the goldfish, *Carassius auratus*, by orexin-A and orexin-B. *Brain Res* 846: 204–209.
- Volkoff H, Peter RE (2000) Effects of CART peptides on food consumption, feeding and associated behaviors in the goldfish, *Carassius auratus*; actions on neuropeptide Y- and orexin-induced feeding. *Brain Res* 887: 125–133.
- WHO (world Health Organization) (1998) Obesity – preventing and managing the global epidemic. Report of a WHO consultation on obesity. Geneva 3–5 June 1997.
- Willie JT, Chemelli RM, Sinton CM, Yanagisawa M (2001) To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci* 24: 429–458.
- Wolf AM, Colditz GA (1998) Current estimates of the economic costs of obesity in the United States. *Obesity Res* 6: 97–106.
- Yamada H, Okumura T, Motomura W, Kobayashi Y, Kohgo Y (2000) Inhibition of food intake by central injection of anti-orexin antibody in rats. *Biochem Biophys Res Commun* 267: 527–531.
- Yamamoto Y, Ueta Y, Serino R, Nomura M, Shibuya I, Yamashita H (2000) Effects of food restriction on the hypothalamic

- prepro-orexin gene expression in genetically obese mice. *Brain Res Bull* 51: 515–521.
- Yamanaka A, Kunii K, Nambu T, Tsujino N, Sakai A, Matsuzaki I, Miwa Y, Goto K, Sakurai T (2000) Orexin-induced food intake involves neuropeptide Y pathway. *Brain Res* 859: 404–409.
- Yamanaka A, Sakurai T, Katsumoto T, Yanagisawa M, Goto K (1999) Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. *Brain Res* 849: 248–252.
- Yoshimichi G, Yoshimatsu H, Masaki T, Sakata T (2001) Orexin-A regulates body temperature in coordination with arousal status. *Exp Biol Med* 226: 468–476.
- Zbigniew KK, Berthoud HR, Burmeister MA, Hornby PJ (2001) Orexin-A in the dorsal motor nucleus of the vagus potently increases gastric motor function via orexin-1 receptor in rats. *Gastroenterology* 120 (Suppl 1): A113.
- Zhang J, Luo P (2002) Orexin B immunoreactive fibers and terminals innervate the sensory and motor neurons of jaw-elevator muscles in the rat. *Synapse* 44: 106–110.