Invited Commentary

Orexins, feeding and the big picture

The number of hypothalamic neuropeptides that have been implicated in the regulation of feeding has grown steadily over recent years (Inui, 1999). Many of these reduce food intake but some increase it. These are neuropeptide Y, Agouti-related protein (a natural antagonist of the melanocortin-4 receptor), melanin-concentrating hormone, galanin and the orexins. Neuropeptide Y and Agouti-related protein are more powerful stimulants of feeding than melanin-concentrating hormone, galanin or the orexins, but this does not preclude a role for the weaker stimulants: strong arguments can be made for all of them (Arch *et al.* 1999; Kalra *et al.* 1999). None of these peptides is exclusively involved in the regulation of feeding, however. Now 2 years after the first description of the orexins (Sakurai *et al.* 1998), significant new information allows us to review the role of the orexins and their receptors in feeding and place this in the context of their wider role.

Orexins-A and -B were identified in extracts of rat brain and bovine hypothalamus by their ability to evoke transient elevations of intracellular Ca concentration in cells expressing an orphan 7-transmembrane, G-protein-coupled receptor, now known as the orexin-1 or OX_1-receptor. Subsequently, a second (OX_2) receptor was identified by its homology (64 % at the amino acid level) to the OX_1-receptor (Sakurai *et al.* 1998). Both functional and binding studies show that the OX_1-receptor has greater affinity (about 10-fold) for orexin-A than -B, whilst the OX_2-receptor has similar affinity for the two peptides (Smart *et al.* 1999, 2000). Orexin-A is thirty-three amino acids long and orexin-B twenty-eight amino acids long. They are produced by processing of the same prepro-orexin peptide. Hypocretins were discovered independently and are sometimes said to be synonymous with orexins, but hypocretin-1 was originally deduced to have five more N-terminal amino acids than orexin-A and both hypocretins were shown in a figure of the original paper (Sakurai *et al.* 1998) as having C-terminal glycines. These are removed (as predicted in the text) leaving C-terminal amides in the mature peptides (de Lecea *et al.* 1998). Researchers should be aware that commercial hypocretins corresponding to those shown in the figure of de Lecea *et al.* (1998) are far less potent than orexins (Smart *et al.* 2000).

The orexins were given their name because intracerebroventricular injection increased food intake in rats (Sakurai *et al*. 1998). A number of studies have reproduced these findings, but some find that orexin-B has little or no effect, or even that it may inhibit feeding (Haynes *et al.* 1999). The greater effect of orexin-A compared with -B appears to implicate the OX_1- rather than the OX_2-receptor in the regulation of feeding, but it is equally possible that orexin-B has less effect because it is more rapidly cleared from the area of injection: peripherally administered orexin-B is known to be metabolised faster than orexin-A (Kastin & Akerstrom, 1999). Moreover if the problem in eliciting a feeding effect with orexin-B is simply that it is 10-fold less potent at the OX_1-receptor, then it should be possible to obtain an effect by increasing the dose of the peptide. This is not what is found (Haynes *et al.* 1999). Orexin-A and -B are clearly inadequate tools with which to dissect the roles of OX_1- and OX_2-receptors *in vivo*.

Fortunately, there are now two tools which demonstrate a role for the OX_1-receptor in mediating the feeding effect of orexin-A. SB-334867-A is an OX_1-receptor antagonist that is about 30-fold selective relative to OX_2-receptor antagonism and has very little affinity for a wide range of other receptors (Arch, 2000); there is also an OX_1-receptor antibody (Smith *et al.* 2000). The antagonist has been shown to inhibit orexin-A-driven feeding when given intraperitoneally at doses of 3–30 mg/kg (Arch, 2000; Rodgers *et al.* 2000), suggesting that the OX_1-receptor is at least partly responsible for the orexigenic effect of orexin-A. It would, however, be unwise to exclude a role of the OX_2-receptor without having investigated the effects of an antagonist of this receptor. Suppression of feeding in response to leptin is blocked by antagonists of the melanocortin-4, glucagon-like peptide-1 and corticotrophin-releasing-hormone receptors (Cone, 1999): all three of these pathways must be operational for leptin to have any effect. Similarly, enhancement of a complex behaviour like feeding may depend upon stimulation of both orexin receptors.

It is one thing to demonstrate that a peptide can affect feeding when injected into the brain and quite another to show that it normally plays a role in the regulation of feeding. In support of orexins playing a physiological role, both the OX_1-receptor antagonist and the antibody, as well as an antibody to orexin-A (Yamada *et al.* 2000) have all been reported to inhibit natural feeding. The sceptic might argue that disruption of the orexin system is merely disrupting behaviour in general: the rat might be driven to indulge in other behaviours that preclude feeding. However, it is notable that the normal behavioural satiety sequence (eating, grooming, resting) is preserved in rats treated with the antagonist; what happens is that the transition points between behaviours are advanced. Conversely, low doses of the agonist delay the transition point so that more time is spent in feeding (Rodgers *et al.* 2000). It can still be argued that the effect of orexins on feeding is secondary to an effect on another behaviour. For example, the primary effect may be to stimulate activity and reduce

Table 1. Evidence that orexins stimulate feeding

Neuroanatomy
Cell bodies in perifornical nucleus, lateral and dorsal hypothalamus, eneteric nervous system
Prepro-orexin and receptor mRNA in intestinal enterochromaffin cells
Neurones project to NPY+AGRP and POMC neurones in arcuate nucleus
Physiology
Orexin-A (and in some reports orexin-B) stimulates feeding, gut motility, gastric acid secretion, insulin secretion
Antibodies to orexin-A and the OX_1-receptor and an OX_1-receptor antagonist inhibit feeding
OX_1-receptor mRNA is upregulated by fasting and hypoglycaemia

NPY, neuropeptide Y; AGRP, Agouti-related protein; POMC, proopiomelanocortin; OX_1, orexin-1.

rest, allowing more time for food seeking behaviour. This argument seems simplistic, however: many peptides and drugs that increase activity do not increase feeding.

Persuasive evidence that orexins directly regulate feeding has come from immunohistochemical studies. The original reason for investigating the effects of the orexins on feeding was that the cell bodies of the neurones that produce them were found exclusively in the lateral hypothalamus (the classical 'feeding centre') and nearby regions (perifornical nucleus, dorsal hypothalamus). Subsequently it has been shown (Elias *et al.* 1998; Horvath *et al.* 1999) that orexin neurones both send fibres to, and receive them from, neurones in the arcuate nucleus that express either neuropeptide Y and Agouti-related protein, or proopiomelanocortin mRNA (from which α-melanocyte-stimulating hormone, a neuropeptide that reduces food intake, is derived). These findings imply that orexins directly influence neurones that regulate feeding behaviour. Moreover, it has recently been reported that the orexins and their receptors are present in the enteric nervous system, and endocrine cells of the gut and pancreas. Orexin-A stimulates gut motility, insulin secretion and (when given centrally) gastric acid secretion (Kirchgessner & Liu, 1999; Takahashi *et al.* 1999; Nowak *et al.* 2000). Taken together, these findings are beginning to develop a strong case for a role for the orexins, at least orexin-A, in the regulation of feeding (Table 1).

This is far from being the whole story, however. Orexin neurones in the hypothalamus send fibres to many parts of the brain that have never been implicated in the regulation of feeding behaviour. Moreover, functional correlates have been discovered for some of these neuroanatomical findings. Orexin-A, and in some studies orexin-B, stimulates arousal, locomotor and sympathetic activities; corticosterone and growth hormone levels are raised, whilst prolactin and luteinising hormone levels are depressed (Hagan *et al.* 1999; Shirasaka *et al.* 1999; Tamura *et al.* 1999). Emerging evidence points to there being subpopulations of orexin neurones, but it is nevertheless probable that activation of orexin neurones often triggers more than one behavioural, autonomic or endocrine response. What do these responses have in common?

One way to approach this question is to investigate the physiological stimuli that activate orexin neurones. So far the only situations that have been reported to enhance the expression of hypothalamic or gut prepro-orexin mRNA are fasting for 48 h and insulin-induced hypoglycaemia: both are situations in which the demand for food is high (Cai *et al.* 1999; Kirchgessner & Liu, 1999). Intriguingly, if insulin-injected animals have access to food there is no change in the hypothalamic prepro-orexin mRNA level, although hypoglycaemia is almost as severe as when access to food is denied. It is as though a signal from the gut is telling the animal that the fuel crisis is almost over.

Could it be then that the orexins activate a range of responses needed to respond to a fuel crisis? After all, in a crisis the animal must be alert to seek out food, and activation of the sympathetic nervous system, whilst in itself leading to an increased fuel demand, may be a risk that has to be taken to support food-seeking behaviour. Furthermore, it is well known that reproductive activity is suppressed by poor nutritional status. More studies are needed to investigate the physiology of orexin neurone activation: what influence does sleep deprivation or manipulations that influence reproductive or autonomic function have, and if they do affect orexin neurones, how does this influence feeding behaviour? The interaction of leptin with the orexin system is a further puzzle that needs to be untangled. The current evidence is that orexins play no part in mediating the obesity of animals that have a defective leptin system, but reduced orexin synthesis may play a role in the hypophagic response to leptin (Arch *et al.* 1999; López *et al.* 2000).

A strong case can therefore be made for a role for at least orexin-A and the OX_1-receptor in the regulation of feeding, though this feeding role is only part of a bigger picture. The roles of orexin-B and the OX_2-receptor in feeding are less well established. It must be emphasised that this pairing of ligands and receptors regarding evidence for their role in feeding is in no way intended to suggest that the OX_1-receptor is the orexin-A receptor and the OX_2-receptor is the orexin-B receptor: orexin-A stimulates both receptors equally and orexin-B is only marginally more potent at the OX_2-receptor. Why there are two orexins, both derived from the same precursor, and two receptors is a question which this commentary cannot begin to address in our current state of knowledge.

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