Orexins: a new family of neuropeptides

Orexin-A and -B are, respectively, 33- and 28-residue peptides, isolated recently from the rat hypothalamus. Both peptides are derived by proteolytic processing from a 130-amino acid precursor, prepro-orexin, which is encoded by a gene localized to chromosome 17q21 in humans. The prepro-peptide has also been identified in human brain, and in other primates and mice.

Prepro-orexin has been shown by northern blot analysis to be abundant in the brain, and detectable concentrations have also been found in the testes, but not in a variety of other peripheral tissues. In situ hybridization maps confirm prepro-orexin mRNA expression in the hypothalamus, particularly the ventral hypothalamus. Immunocytochemical mapping of orexin-A has identified a population of medium sized neurones within the hypothalamus, medium eminence and ventral thalamic nuclei of the rat brain. This distribution has been confirmed recently in both humans and non-human primates, indicating that this particular family of peptides is well conserved. More importantly, there is emerging evidence suggesting the existence of extensive extra-hypothalamic projections of orexin-immunoreactive neurones. In addition to confirming the presence of immunoreactive cells within the hypothalamus, Peyron and colleagues reported immunolabelled fibres throughout extra-hypothalamic regions, including the septal nuclei, substantia nigra and the raphe nuclei, with a particularly dense innervation of the locus coeruleus. This was confirmed using an antibody which was selective for orexin-A.

The orexins activate two related G-protein coupled receptors (OX1 and OX2) which are 64% homologous and were identified originally as orphans from a human cDNA library. They are most closely (26%) related to the neuropeptide Y receptor. In radioligand binding studies, orexin-A has equal affinity at OX1 and OX2 receptors, while orexin-B has a higher affinity for the OX2 receptor. Activation of either receptor causes an increase in intracellular Ca2+ concentration (Table 1), possibly by stimulating Ca2+ influx via voltage-sensitive calcium channels via a protein kinase C-mediated mechanism. Orexin-B has also been shown to activate Ca2+-sensitive K+ channels.

Northern blot analysis has shown that mRNA for the orexin receptors is found predominantly in the brain. Subsequent in situ hybridization studies have mapped the distribution of the receptors in more detail. OX1 receptor mRNA has been identified in significant quantities in the locus coeruleus, hippocampal formation, dorsal raphe and, to a lesser extent, other brain areas, including the cortex. OX2 receptor mRNA has been identified in the cortex, nucleus accumbens, paraventricular nuclei, the thalamic nuclei, the CA3 layer of the hippocampus and the hypothalamus.

This distribution of the orexins and their receptors suggests that these peptides may play an important role in several key physiological functions. These proposed functions and the evidence for them are discussed below.

The dense innervation of the locus coeruleus (LC) and lateral hypothalamic regions, most notably the tuberomammillary nucleus, by orexin-containing fibres indicates that this family of peptides may play an important role in arousal and maintenance of the waking state. More recent studies using selective antibodies have shown that most of the fibres in these regions express orexin-A rather than orexin-B. In addition, the OX1 receptor has been localized to the monoaminergic neurones in these regions. In vitro electrophysiological studies have shown that orexin-A depolarizes cells from the LC and so increases their rate of firing, indicative of increased arousal in vivo. Indeed, intracerebroventricular (i.c.v.) administration of orexin-A increased the level of arousal in rats, as measured by EEG and EMG (D. C. Piper, personal communication). These studies showed that orexin-A i.c.v. increased the proportion of time awake at the expense of a reduction in paradoxical sleep, and indeed at higher doses virtually abolished paradoxical sleep while reducing deep sleep (SWS2) (D. C. Piper, personal communication). Taken collectively, these data clearly demonstrate that orexin-A acts at multiple sites to play an integratory role in the sleep–wake cycle.

A high density of orexin-immunoreactive neurones are contained in the perifornical nucleus, which is intimately involved in the neural control of food intake. Orexin-containing fibres are also found in the ventromedial hypothalamus (VMH) and the paraventricular nucleus (PVN), as are both orexin receptors. Moreover, recent immunostaining studies in rat and primate tissues have shown that some orexin-containing neurones in these regions express leptin receptors and synapse onto neuropeptide Y containing neurones. Furthermore, both the prepro-peptide and receptors have been shown to be upregulated in fasted animals.

Additional evidence for a role in feeding has been provided by in vitro electrophysiological studies which showed that orexin-A stimulates glutamate release from the arcuate nucleus (ACN), a region associated with the neuroendocrine control of energy balance. In addition, i.c.v. administration of orexin-A increased food intake in rats both by
prolonging feeding and inhibiting satiety.11 Orexin-B has also been reported to stimulate feeding,1 2 although this remains controversial.11 However, neither peptide affected body weight,11 fat distribution11 or terminal concentrations of blood glucose, leptin or insulin (G. Williams, personal communication). It has also been reported that prepro-orexin mRNA is increased by low blood glucose concentrations but inhibited by the presence of food in the gut (G. Williams, personal communication). Taken collectively, these data clearly demonstrate a role for orexins in the regulation of feeding and energy homeostasis.

The hypothalamic distribution of both the peptides and their receptors,5 7 9 especially in the ACN and PVN, suggests strongly that these peptides play a regulatory role in neuroendocrine function, especially energy metabolism and reproduction.5 6 Furthermore, orexin-A and orexin-B stimulate the neuroendocrine neurones of the ACN in vitro.6 I.c.v. administration of orexin has been shown to modify several key hormones, including luteinizing hormone,15 growth hormone, leptin, prolactin and corticosterone (G. Williams and J. J. Hagan, personal communication).

The dense innervation of the perifornical nucleus with orexin-immunoreactive fibres,1 5 in addition to other regions, including the LC and the rostral ventrolateral medulla,5 suggest that orexins may play a role in the regulation of arterial pressure and other cardiovascular functions.5 Indeed, the dense localization of OX2 receptors in the PVN further strengthens this case.9 Moreover, in vivo, orexin has been shown to modulate secretion of prolactin (J. J. Hagan, personal communication) which plays a major role in water balance,14 increases EMG activity (J. J. Hagan, personal communication) and increases arterial pressure in rats.15

The distribution of the orexin peptides and their receptors suggests that these peptides may be involved in a range of other physiological functions, including thermoregulation and nociceptive processing.5 7 9 However, orexin-A has been shown to have no effect on body temperature in rats.11

Finally, the distribution of orexin receptors, especially localization of OX2 to layer VI of the cortex,9 suggests that the orexins may be involved in neurodegeneration and the pathophysiology of head injury. This case is supported by demonstration that orexin-B stimulates glutamate and GABA release in vitro.6

In summary, the orexins are a new family of neuropeptides which are of particular interest to the anaesthetist as they play a pivotal role in the regulation of the sleep–wake cycle, energy metabolism and neuroendocrine function, in addition to potentially influencing arterial pressure and other cardiovascular variables. Moreover, the orexins may also be involved in neurodegeneration, nociceptive processing and the pathophysiology of head injury.

D. Smart
Neuroscience Department
SmithKline Beecham Pharmaceuticals
New Frontiers Science Park
Third Avenue, Harlow
Essex CM19 5AW, UK

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