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Review

Orexins and their receptors: structural aspects and role in peripheral tissues

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Abstract. Orexins, also named hypocretins, were discovered in 1998 by subtractive cDNA cloning or orphan receptor technologies. Prepro-orexin is enzymatically matured into two peptides, orexin-A and orexin-B which are 33- and 28-amino-acid peptides, respectively. Two cloned orexin receptors OX_1R and OX_2R are serpentine G-protein-coupled receptors, both of which bind orexins and are coupled to Ca^{2+} mobilization. Orexins are neuropeptides present in hypothalamic neurons that project throughout the central nervous system to nuclei involved in the control of feeding, sleep-wakefulness, neuroendocrine homeostasis and autonomic regulation. The interest of investigators in orexins has focused on narcolepsy, since genetic or experimental alterations of the orexin system are associated with this sleep disorder. However, orexins are not restricted to the hypothalamus and together with their receptors they are expressed in peripheral tissues. This new multifaceted aspect of orexin biology is reviewed here in descriptions of (i) the proform, maturation and structure of orexins, (ii) the structure, signal transduction and pharmacology of orexin receptors and (iii) the expression of orexins and orexin receptors as well as their biological role in the hypothalamus-pituitary-adrenal axis, gastrointestinal tract, endocrine pancreas and other peripheral tissues.

Key words. Neuropeptide; food intake; narcolepsy; pharmacology; signal transduction; hypothalamus-pituitary-adrenal axis, gastrointestinal tract.

Orexin history

Hypocretins/orexins were discovered by two independent groups using subtractive cDNA cloning [1, 2] or orphan receptor technologies [3]. Analysis of the expression pattern of subtracted hypothalamus-enriched cDNAs revealed an original 569-nucleotide clone encoding a 130-residue putative secretory protein with an apparent signal sequence [2]. Potential proteolytic maturation of this protein indicated the possible generation of two homologous peptides sharing weak sequence homology with secretin [2]. These hypothalamic peptides were named hypocretin 1 and 2 [2]. By screening rat brain extracts for their ability to induce intracellular Ca^{2+} transients in a battery of orphan G-protein-coupled-receptor-expressing cells, two peptides were purified and named orexin-A and orexin-B due to their orexigenic effects [3]. The 28-amino-acid orexin-B and the 33-amino-acid orexin-A were identical to hypocretin 2 and 1, respectively[1]. When Sakurai and collegues [3] reported the discovery of orexins, they also described the cloning of two G-protein-coupled receptors for orexins referred to as OX_1R and OX_2R [3].

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[1] Both nomenclatures are still used by the scientific community, but for the sake of clarity, the orexin nomenclature has been chosen in this review.

Orexin neurons originate predominantly from the hypothalamus and project throughout the central nervous system (CNS) to nuclei involved in the control of feeding, sleep-wakefulness, neuroendocrine homeostasis and autonomic regulation [4–6]. In consonance with this distribution, orexins were early shown to stimulate food consumption [3]. The discovery of the involvement of orexins in narcolepsy was made at the same time in dogs [7] and mice [8]. Canine narcolepsy was found to be caused by a mutation in the orexin receptor OX_2R gene [7]. This observation shifted research interest from appetite control to sleep regulation [9]. Most interesting, this disabling sleep disorder characterized by daytime sleepiness, cataplexy and striking transitions from wakefulness into rapid-eye-movement (REM) sleep is associated in humans with orexin deficiency [10–12]. The link between the orexin system and narcolepsy was further substantiated in mice in which the orexin had been knocked out [8, 13] or in canine narcolepsy where orexin receptor OX_2R mutations [7, 14] have been reported.

Recent evidence indicates that (i) expression of orexins and their receptors is not restricted to the CNS and (ii) orexins control several physiologic responses in peripheral tissues. In this review, current knowledge regarding orexins and orexin receptors is discussed with special emphasis on their role in the periphery. Recent reviews on the neurobiology of orexins in the CNS are available [6, 15–36].

Orexins: pro-form, maturation and structure

A single mRNA transcript encodes for prepro-orexin containing both orexin-A and orexin-B [2, 3, 37]. The human prepro-orexin gene has been localized on chromosome 17q21 (table 1) [3]. The structure of prepro-orexin (fig.1) deduced from the full-length cDNA is well conserved (75% amino acid identity) within the vertebrate genomes (human, rat, mouse, dog, pig and *Xenopus laevis* (table 1). This prepro-hormone has been immunohistochemically characterized in the lateral hypothalamus and adjacent areas [3, 38, 39]. The first 33 amino acids of prepro-orexin exhibit characteristics of a secretory signal sequence (hydrophobic core followed by residues with small polar side chains). The Ala^{32}-Gln^{33} position has been predicted as the most likely site for a signal sequence cleavage. Mature orexin-A and orexin-B peptides issue from post-translational modifications of prepro-orexin involving specific proteolytic processing. The

Table 1. Chromosome localisation for prepro-orexin and orexin receptors.

	Species	Chromosome localization	Size (amino acids)	Accesssion number	Reference
Prepro-orexin (orexin precursor)	dog	ND	130	AF285110	12
	human	17		NM-001524	2
		17q21	131	AF041240	3
		17q21–q22		AF118885	3
	mouse		130	AF041242	3
		11		AF019566	2
		11 61.0 cM		NM-010410	2
	pig	ND	129	AF169352	
				AF075241	82
	rat	10		NM-013179	2
			130	AF041241	2
	sheep	ND	66*	AF425237	GenBank
	Xenopus laevis	ND	144		24
OX_1 receptor	human	1p33	425	AF041243	3
				NM-001525	2
				AH009945	10
	mouse		260*	AF394596	GenBank
	pig		86*	AF097995	GenBank
	rat	5		NM-013064	3
			416	AF041244	3
OX_2 receptor	dog	12	109*	AF164626	GenBank
	human	6p11–q11		NM-001526	2
		6cen (p11–q11)	444	AF041245	3
				AH009943	10
	mouse		212*	AF394597	GenBank
	pig		77*	AF059740	GenBank
	rat	8		NM-013074	3
			460	AF041246	3

* partially cloned sequence; ND, not determined.

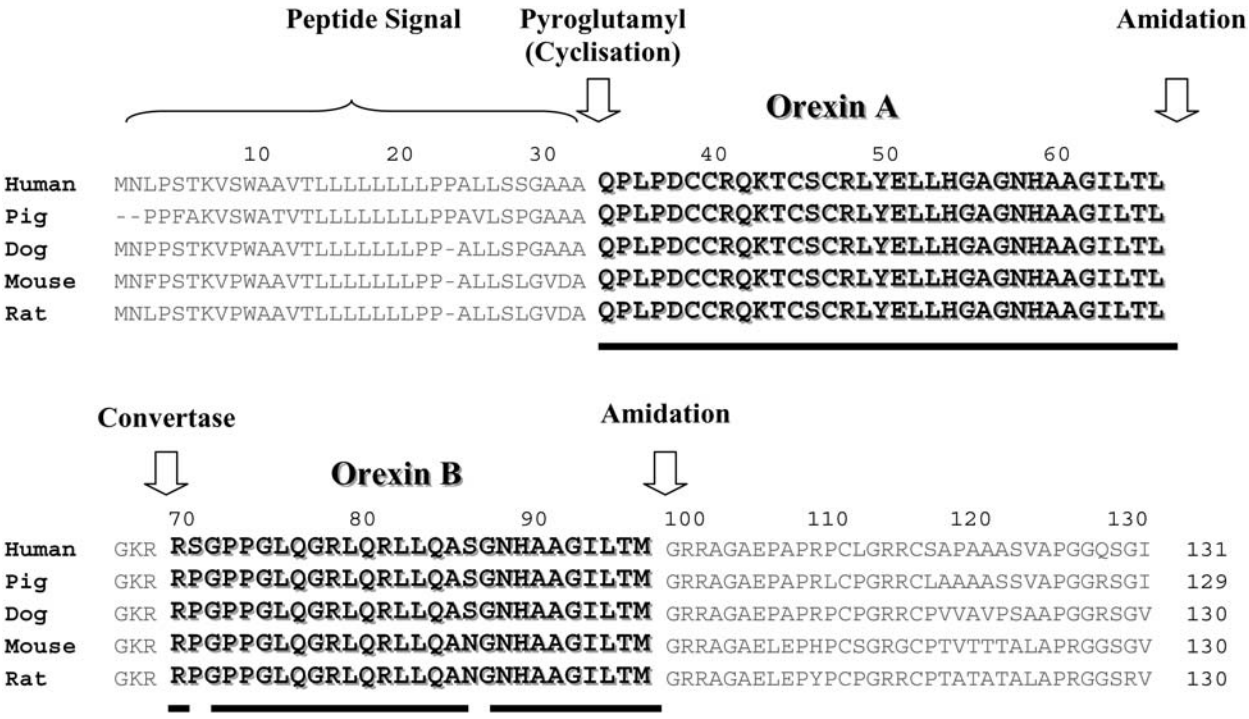

Figure 1. Multiple alignment of prepro-orexin in vertebrates. The deduced amino acid sequence of human prepro-orexin was used for all homology searches which were performed using the alignment tools (Blast) at the National Center for Biotechnology Information (NCBI). The scheme shows the structure of prepro-orexin and the location of orexin-A and orexin-B amino acid sequences (bold). Arrows indicate predictive enzymatic cleavage sites and chemical modifications. The N-terminal 32-amino-acid signal peptides present some amino acid substitutions in vertebrate species. The bold lines below orexin sequences indicate amino acid identity in human, pig, dog, mouse and rat sequences.

orexin-A sequence starts with Gln^{33}, which is cyclized into an N-terminal pyroglutamyl residue presumably by enzymatic transamidation [3, 37]. The last residue (Leu^{65}) of the mature orexin-A peptide is followed by a Gly residue which presumably serves as NH_2 donor for C-terminal amidation by sequential actions of peptidylglycine monooxygenase and peptidylamidoglycolate lyase [3, 37]. As expected, Gly^{66} is followed by a pair of basic amino acid residues, Lys^{67}-Arg^{68}, which constitute a recognition site for prohormone convertases [3, 37]. The next segment of the deduced prepro-orexin sequence, Arg^{69} to Met^{96}, is identical to the sequence of purified orexin-B. The Met^{96} residue is again followed by Gly-Arg-Arg, a C-terminal amidation signal [3, 37]. The orexin-B sequence is followed by a C-terminal part in which a majority of amino acid substitutions were found in all vertebrate cloned prepro-orexins (see fig.1).

The available amino acid sequences of vertebrate orexin-A reveal 100% interspecies identity (fig. 1). Orexin-A is a 33-amino-acid peptide (3562 Da) with an N-terminal pyroglutamyl and an amidated C terminus. It contains two intrachain disulfide bonds (Cys^6-Cys^{12} and Cys^7-Cys^{14}) (fig. 2, upper panel). The available amino acid sequences of vertebrate orexin-B reveal 93% interspecies identity (fig. 1). Orexin-B is a 28-amino-acid peptide (2937 Da) devoid of cysteine residues and thereby of intrachain disulfide bonds (fig. 2, upper panel). Human orexin-B shares 46% amino acid identity with orexin-A (fig. 2, upper panel) and also possesses an amidated C-terminal residue.

The three-dimensional structure of human orexin-B (fig. 2, right middle panel) has been determined by two-dimensional ^{1}H-NMR and dynamical simulated annealing calculations [40]. Human orexin-B consists of two alpha helices from Leu^7 to Gly^{19} and from Ala^{23} to Met^{28}, connected by a short linker [40]. The two helices are oriented about 60–80° relative to each other. Taking advantage of the high sequence homology between orexin-A and orexin-B, a three-dimensional model of orexin-A has been constructed using the Modeller 6.0 program and is shown in figure 2 (left middle panel) [authors' unpublished data]. The modeled structure for orexin-A is highly similar to the structure of orexin-B (fig. 2, lower panel). Like orexin-B, orexin-A exhibits two helices, a quite long alpha helix (residues 11–24) and a smaller one (residues 26–32) at the C terminus. At the N-terminal part of orexin-B, prior to the long alpha-helix, there are two proline residues forming a turn. In the corresponding segment of orexin-A, there is also a turn, even though proline residues are absent. The presence of two disulfide bridges

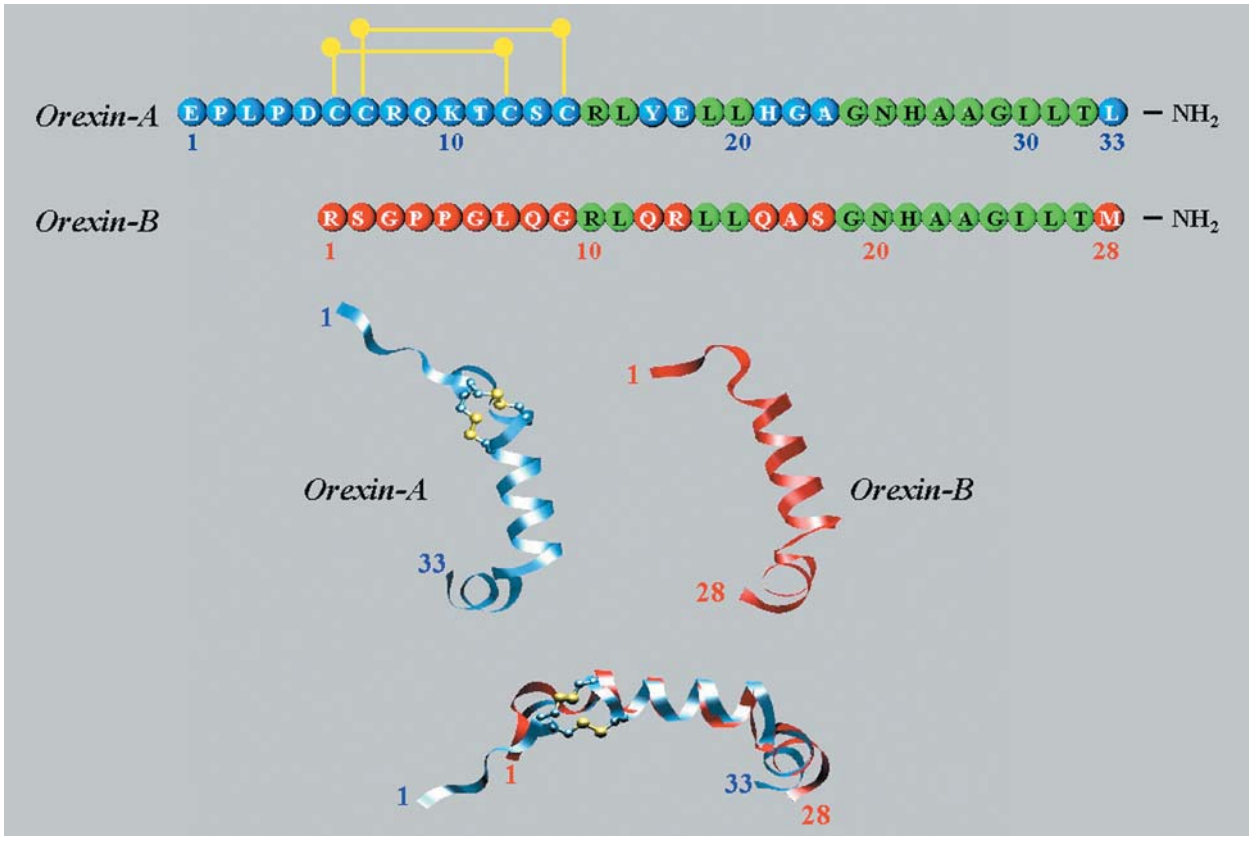

Figure 2. Comparison of human orexin-A and orexin-B structures. Upper panel: amino acid sequences of human orexin-A and orexin-B deduced from nucleic acid sequence (GenBank accession number AF041240). Green circles indicate amino acid identity. The two disulfide bonds in orexin-A are shown in yellow. Middle panel: schematic view of three-dimensional structures of human orexin-B, determined by two-dimensional 1H-NMR and dynamic simulated annealing calculations [40] and orexin-A, constructed by homology using the Modeller 6.0 program (Copyright 1989–2002 Andrej Šali). Lower panel: superposition of orexin-A and orexin-B three-dimensional structures.

[Cys^6–Cys^{12} and Cys^7–Cys^{14}] may ensure such a turn in the structure of orexin-A. These two bridges reduce the flexibility of the N-terminal part of orexin-A and make the region between Cys^6 and Lys^{10} structurally close to the corresponding region of orexin-B even if the sequences are completely different (fig. 2). Superposition of three-dimensional views of orexin-A and orexin-B is shown in figure 2 (lower panel). The high structural similarity between orexin-A and orexin-B may explain why orexin receptors discriminate poorly between the two peptides.

Orexin receptors

Structure

The orphan G-protein-coupled receptor HFGAN72 was identified as a human orexin receptor during the course of orexin discovery and was named OX_1R [3]. A BLAST search of the GenBank database with the OX_1R amino acid sequence resulted in the discovery of a second human orexin receptor (OX_2R) sharing 64% amino acid identity with OX_1R [3]. Both receptors belong to the class I subfamily within the superfamily of G-protein-coupled receptors. Full-length OX_1R and OX_2R cDNAs have also been cloned in rats (table 1).

The human OX_1R is a 425-amino-acid protein with seven putative transmembrane helices (fig. 3). A PROSITE search for consensus motifs of the SWISS-PROT database predicts the presence of an N-glycolysation site on N^{194} in extracellular loop II, protein kinase A phosphorylation sites on S^{262} and T^{296} in intracellular loop III, and multiple protein kinase C phosphorylation sites in intracellular loop II (T^{157}), intracellular loop III (T^{296}) and the C-terminal tail (S^{362}, S^{385}, S^{390} and S^{396}). An additional putative protein kinase C phosphorylation site is suggested in extracellular loop III on S^{331} (see fig. 3). Human OX_1R is encoded by seven exons [10] (fig. 3). The human OX_1R gene has been localized on chromosome 1p33 (table 1) [2, 3, 37]. Partial sequences of OX_1R have also been determined in mouse and pig (table 1).

Human OX_2R is a 444-amino-acid protein with seven putative transmembrane helices (fig. 4). A PROSITE search predicts the presence of three putative extracellular N-glycosylation sites in the N-terminal tail (N^{14} and N^{22})

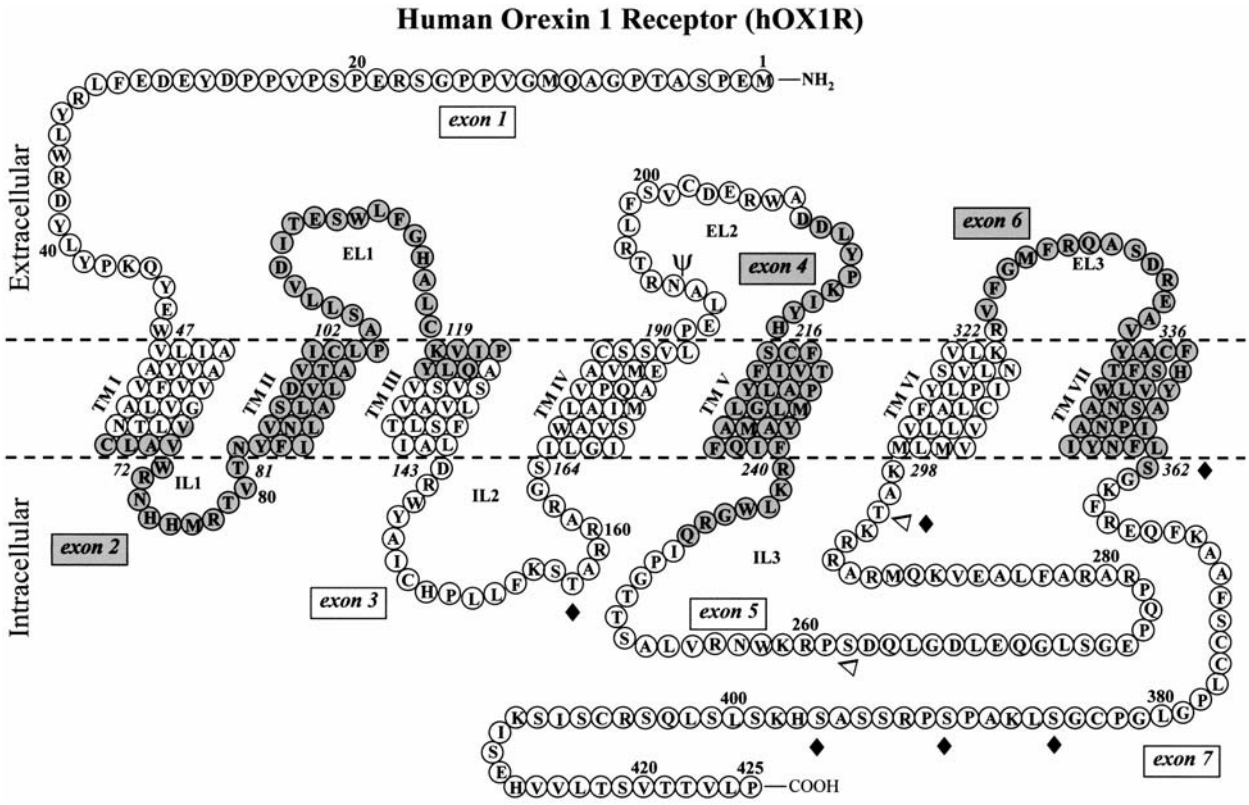

Figure 3. Schematic view of the secondary structure of the human orexin 1 receptor (hOX_1R). The positions of putative transmembrane segments have been assigned on the basis of the hydropathy plot of the cloned receptor cDNA (accession number AF041243). Amino acid residues are numbered starting from the N-terminal methionine and the one-letter code is used. Open circles indicate amino acid sequences encoded by exons 1, 3, 5 and 7; black circles indicate amino acid sequences encoded by exons 2, 4 and 6. ∇ and ♦ indicate consensus phosphorylation sites for protein kinase A and protein kinase C, respectively. Consensus N-glycosylation sites are indicated by ψ.

and loop II (N^{202}), a single protein kinase A phosphorylation site in intracellular loop III (T^{302}) and four protein kinase C phosphorylation sites in intracellular loop II (T^{165}), intracellular loop III (T^{302}) and the C-terminal tail (S^{368} and S^{403}). Human OX_2R is encoded by seven exons [10] (fig. 4). The human OX_2R gene has been localized on chromosome 6p11–q11 (table 1). Partial sequences of OX_2R have also been determined in mouse, pig and dog (table 1).

Comparison of human OX_1R and OX_2R amino acid sequences (fig. 5) shows an overall 64% identity and reveals interesting features: (i) all transmembrane segments, extracellular loops and intracellular loops I and II are highly conserved; (ii) the N-terminal extracellular domain, intracellular loop III and the C-terminal tail exhibit weak amino acid sequence identity. Moreover, these latter domains are different in length in OX_1R and OX_2R (fig. 5); (iii) both receptors share a common N-glycosylation site in extracellular loop II, a common protein kinase A phosphorylation site in intracellular loop III and three common protein kinase C phosphorylation sites in intracellular loop II and the C-terminal tail. OX_1R has several additional protein kinase C phosphorylation sites whereas OX_2R has two additional putative N-glycosylation sites in the N-terminal domain (figs. 3, 4).

Binding studies

To date, very few reports describe binding studies of orexins to their receptors. Using [^{125}I-Tyr^{17}]-orexin-A as a tracer, Sakurai and colleagues [3] showed that transfection of human OX_1R or OX_2R cDNA conferred tracer binding whereas mock-transfected CHO cells did not exhibit specific binding. Tracer binding to recombinant OX_1R was inhibited by orexin-A (IC_{50} of 20 nM) and by orexin-B (IC_{50} of 420 nM), suggesting that OX_1R discriminates between the two peptides and is selective for orexin-A. This binding was not inhibited by any of several unrelated peptides. In contrast, tracer binding to recombinant OX_2R was inhibited by similar concentrations of orexin-A and orexin-B (IC_{50} of 30–40 nM), suggesting that OX_2R is a nonselective receptor. These early binding studies were in good agreement with data obtained in a $[Ca^{2+}]_i$ transient assay. Similar data were reported by Shibahara et al. [38] using [^{125}I]-orexin-A as tracer and recombinant OX_1R or OX_2R expressed in CHO cells, and

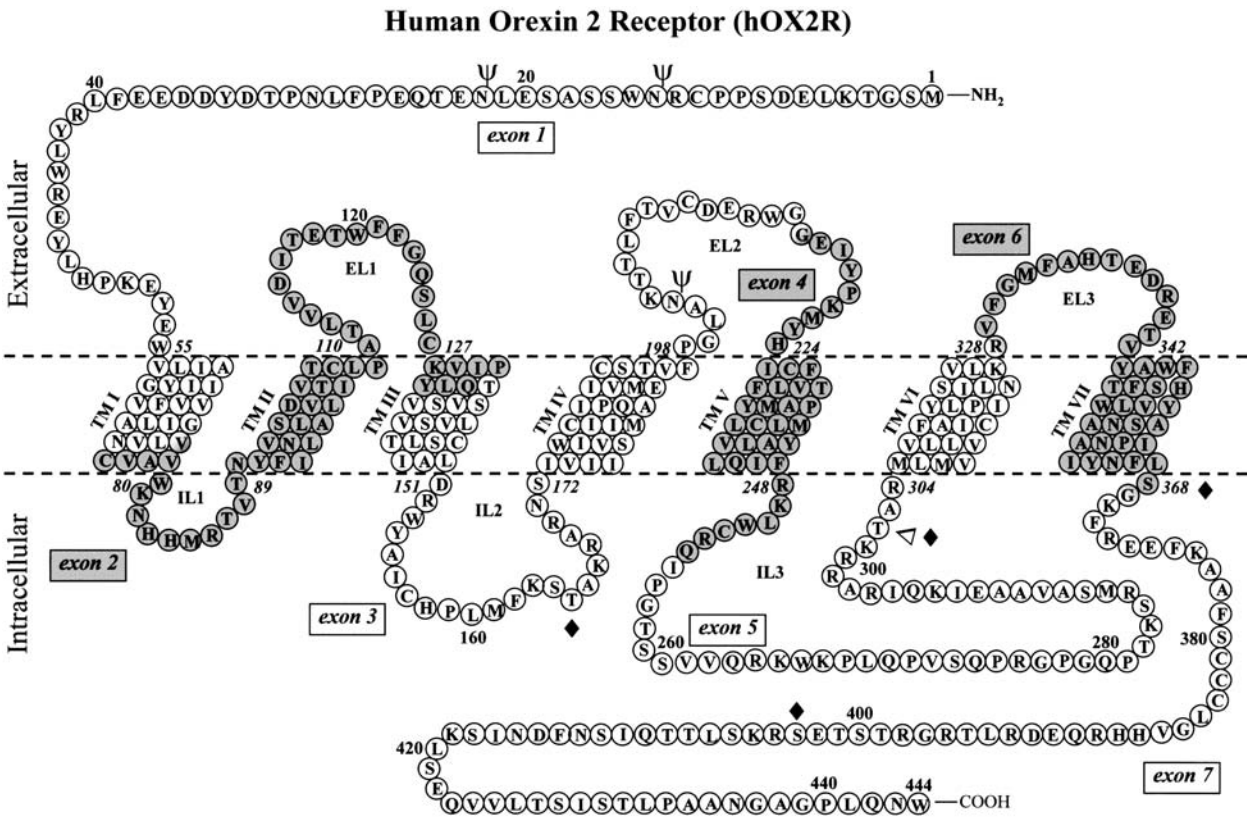

Figure 4. Schematic view of the secondary structure of the human orexin 2 receptor (hOX_2R). The positions of putative transmembrane segments have been assigned on the basis of the hydropathy plot of the cloned receptor cDNA (accession number AF041245). Amino acid residues are numbered starting from the N-terminal methionine and the one-letter code is used. Open circles indicate amino acid sequences encoded by exons 1, 3, 5 and 7; black circles indicate amino acid sequences encoded by exons 2, 4 and 6. ∇ and ♦ indicate consensus phosphorylation sites for protein kinase A and protein kinase C, respectively. Consensus N-glycosylation sites are indicated by ψ.

by Smart et al. [41] using rhodamine-green-tagged orexin-A as tracer and recombinant OX_1R expressed in CHO cells. Only one paper reported the binding of [^{125}I]-orexin-A to native receptors in rat anterior hypothalamus membranes [42]. Two binding sites for orexin-A were suggested by Scatchard analysis with K_ds of 34 and 671 nM. However, the specificity of tracer binding is highly questionable in this study since iodinated orexin binding is inhibitable by different unrelated peptides such as neuropeptide Y or PACAP [42]. In this context, two recent reports have clearly demonstrated that OX_1R and OX_2R neither bind nor are they functionally affected by secretin, PACAP, NPY variants or α-MSH [41, 43].

These rare binding studies suggest that the affinity of orexin-A or orexin-B is rather low with a $K_d > 20$ nM for both OX_1R and OX_2R. This is considerably lower than the K_d usually reported for most peptide receptors, i.e. in the 0.1 – 1 nM range [44]. Whether the low affinities reported for orexin receptors are a physiological reality or are related to problems with orexin tracers remains to be determined. In particular, the effect of iodinating Tyr^{17} the biological activity of orexin-A is still uncharacterized. On the other hand, orexins have been shown to trigger post-receptor events, for example calcium mobilization, at concentrations < 1 nM [45].

Signal transduction

Several observations indicate that orexins induce intracellular calcium transients in both receptor-transfected cells and native-receptor-expressing cells. Sakurai et al. [3] have shown that orexin-A and orexin-B induce detectable calcium transient in CHO cells expressing human orexin receptors. The EC_{50}s were 30 and 2500 nM, respectively, in CHO/OX_1R cells, and 34 and 60 nM, respectively, in CHO/OX_2R cells. These values are in agreement with the aforementioned binding data. Smart et al. [46] reported slightly lower EC_{50} values for human orexin receptors expressed in CHO cells. They were 10 and 100 nM for orexin-A and orexin-B, respectively, in CHO/OX_1R cells and 5 and 6 nM in CHO/OX_2R cells. More recent papers indicated that orexins may induce calcium mobilization at much lower concentrations [47, 48]. Indeed, the EC_{50} of orexin-A in CHO cells expressing human OX_1R is 1 or 2 nM in the studies by Lund et al. [47] and Darker et al. [48], respectively. Even more surpris-

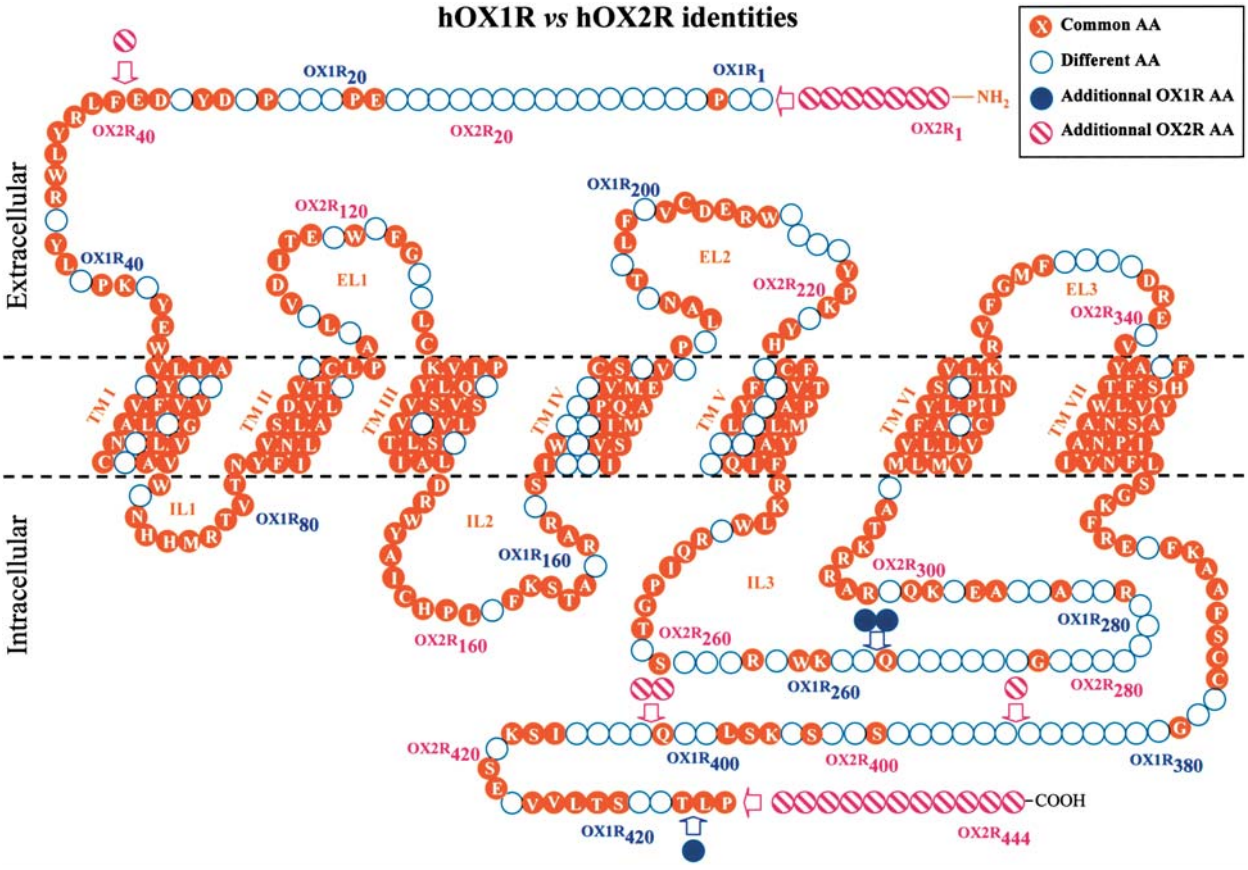

Figure 5. Superposition of schematic views of the human orexin receptors OX_1R and OX_2R. Orange circles correspond to identical amino acid residues; open green circles correspond to different amino acid residues. Comparison of amino acid sequences of OX_1R and OX_2R indicates the existence of the insertion of single residues or clusters of residues. Green and pink circles indicate residues which are specific for OX_1R and OX_2R, respectively.

ingly, Okumura et al. [45] reported EC_{50} values of 0.068 and 0.69 nM for orexin-A and orexin-B, respectively, in CHO/OX_1R cells and EC_{50} values of 0.057 nM for both peptides in CHO/OX_2R cells. The reason for such divergent data is not clear since the same receptors were transfected in the same cell line. Such huge differences in response to orexins could be due, at least in part, to different receptor densities in stably transfected cell lines. The orexin-induced calcium increase has also been observed in cultured rat hypothalamic neurons [49]. The origin of calcium upon orexin challenge has been documented in CHO cells expressing human OX_1R [50]. Orexin-A (3 nM) causes activation of two Ca^{2+} pathways in the cells: a receptor-operated Ca^{2+} influx and, subsequently, an IP3-mediated $[Ca^{2+}]_i$ transient from intracellular pools. Taken together, these observations suggest that orexin receptors are coupled to G_q heterotrimeric proteins.

A few reports suggest a link between orexin receptors and the cAMP pathway in isolated adrenal cells. Indeed, orexin-A and orexin-B stimulate cAMP production in dispersed rat zona fasciculata-reticularis cells, probably through interaction with OX_1R [51]. Similarly, orexin-A has been shown to increase basal cAMP release by dispersed human adrenocortical cells, probably through interaction with OX_1R [52]. In sharp contrast, orexin-A and orexin-B slightly inhibit the PACAP-induced increase in cAMP level in rat pheochromocytoma PC12 cells, probably through OX_2R [53]. These observations raise the possibility of orexin receptor coupling to G_s and/or G_i/G_o proteins [54]. The regulation by orexins of cAMP production may be tissue dependent, since no stimulatory or inhibitory effect of orexins was observed in cultured at hypothalamic neurons [49].

Structure-function relationship and pharmacology of orexins

Very few data are available regarding the structure-function relationship of orexins for interaction with their receptors. Deletion of the sequence 1–14 in the 1–33 orexin-A native peptide results in a 60- and 23-fold decrease of potency for inducing calcium responses in CHO/OX_1R and CHO/OX_2R cells, respectively [45]. Since the 1–14 sequence contains two disulfide bonds,

between Cys^6 and Cys^{12} and between Cys^7 and Cys^{14} (see fig. 2), their role has been investigated by introducing alanine residues [45]. Alanine-substituted analogues lacking either or both disulfide bonds display an ~tenfold decrease of potency for both OX_1R and OX_2R. The Cys^7-Cys^{14} bond seems to play a more important role than the Cys^6-Cys^{12} bond. These data indicated that disulfide bonds, though playing a role, are not essential for orexin-A activity. This is in accordance with the fact that orexin-B which is devoid of S-S bonds is active at both OX_1R and OX_2R (see above). Darker et al. [48] described the potencies of a series of N-terminally truncated orexin-A. They reported that orexin-A (15–33) is 180 times less potent than the native peptide for inducing calcium responses in CHO/OX_1R cells. Shorter orexin-A fragments have even lower potencies.

A breakthrough in orexin receptor pharmacology occured in 2001 when GlaxoSmithKline Pharmaceuticals reported the first orexin-1 receptor antagonist series of 1-aryl-3-quinolin-4-yl-3-naphthyridin-4-yl ureas [55]. In particular, the SB-334867 compound has excellent selectivity for OX_1R and blood-brain barrier permeability. In CHO/OX_1R cells, SB-334867 inhibits the orexin-A- (10 nM) and orexin-B- (100 nM) induced calcium responses with apparent pKb values of 7.27 and 7.23, respectively. SB-334867 is devoid of any agonist properties on either orexin receptors but displays a weak antagonist effect on OX_2R [41]. The activity of SB-334867 administrated intraperitoneally to rats (up to 30 mg/kg) on food intake and most active behaviors was also reported [56–58].

Expression of orexins and their receptors: relation to biological actions

Following the discovery of orexins, these peptides were initially considered as exclusively present in hypothalamic neurons that project throughout the brain [59, 60]. The origin of orexin neurons as well as their widespread central projections suggest that orexins play an important role in cognitive, autonomic, emotional and motivational aspects of feeding behavior. Indeed, intracerebroventricular (icv) injection of orexins increases food consumption in rats though they are not as potent as other orexigenic

Table 2. Orexin system expression in rat tissues.

Rat tissue		Prepro-orexin	OX_1 receptor	OX_2 receptor	References
Brain	PCR	+	+	+	
	ISH	+	+	+	2–4, 45
	IHC	+	+	+	
Spinal cord	IHC	ND	+	ND	83
Pituitary	PCR	–	+	+	45, 58, 66
Adrenal	PCR	–	+	+	45, 58, 66
	ISH	ND	+	+	45
Gonads	PCR	♂ +	+	–	45
	PCR	♀ –	+	–	45
	RIA	♂ +	ND	ND	84
Kidney	PCR	–	+	–	45
Small intestine	PCR	–	+	–	45
	PCR	+	+	+	50
	IHC	+	+	+	50
Stomach	PCR	–	–	–	45
Pancreas	PCR	–	+	+	50
	IHC	+	+	ND	50
Liver	PCR	–	–	–	45
Spleen	PCR	–	–	–	45
Heart	PCR	+	–	–	45
Lung	PCR	–	–	+	45
Thyroid	PCR	–	+	–	45
Skeletal muscle	PCR	–	–	–	45

PCR, polymerase chain reaction; ISH, in situ hybridization; IHC, immunohistochemistry; RIA, radioimmuno assay; +, detected; –, absent; ND, not done.

neuropeptides. Other central effects of icv administration of orexins include increased wakefulness and suppression of REM sleep, providing evidence that orexins play a causative role in sleep-wakefulness regulation. This was clearly substantiated by genetic and/or experimental alterations of the orexin/orexin receptor system in animal and human narcolepsy (see above). Centrally mediated actions of orexins on neuroendocrine and energy metabolism regulation have also been described. The orexin neuropeptide signaling system is now considered an important central pathway that promotes adaptative behavioral and physiological responses in response to metabolic and environmental signals. The distribution and actions of orexins in the CNS have been extensively documented in several excellent reviews [6, 15–36] and are listed in tables 2 and 3. It is now established that: (i) orexin as well as orexin receptors are not restricted to the hypothalamus, but are expressed in peripheral tissues, and (ii) orexins are involved in the regulation of an increasing number of peripheral functions either through a central control or direct interaction with peripheral target tissues. The presence of orexin-A in plasma has been detected in rats and humans [61–64]. This circulating plasma orexin and/or the local production of orexins in various tissues (see table 2) [54, 65, 66] may be essential for the peripheral actions of orexins. This new multifaceted aspect of orexin biology is reviewed below.

Hypothalamo-pituitary axis

Several lines of evidence suggest that orexins influence neuroendocrine homeostasis and modulate hormone secretions in the hypothalamo-pituitary-adrenal axis (HPA) (see table 3). Orexins control the secretion of several pituitary hormones acting on either hypothalamic neurons and/or directly on pituitary cells. This latter effect is supported by the identification of orexin receptors in rat hypophysis [61, 70, 71].

Luteinizing hormone and thyroid-stimulating hormone

Orexins administered in the third ventricule of ovariectomized rats suppress the pulsatile secretion of plasma luteinizing hormone (LH) [72]. This effect appears to be related to orexin action on gonadotropin-releasing hormone (GnRH) neurons at the hypothalamic level and no direct in vitro effect of orexins on basal LH release from the pituitary has yet been described. Moreover, orexins were shown to inhibit LH-releasing hormone (LHRH)-induced LH release from pituitary cells in proestrous female rats while no effect was seen in male or ramdomly cycled female rats [73]. Orexins modulate LH secretion in an ovarian-steroid-dependent manner [74]. Indeed, icv injection of orexins stimulates LH secretion in estradiol- and progesterone-pretreated ovariectomized rats. In contrast, orexins inhibit LH release in unprimed ovariectomized rats. This ovarian-steroid-dependent bimodal LH response is reminiscent of that of other orexigenic neuropeptides [74]. These observations reveal an important role for orexin-A in the hypothalamo-pituitary gonadal axis [73, 74].

Intravenous injection of orexin-A decreases the thyroid-stimulating hormone (TSH) plasma level though TSH release from the anterior pituitary in vitro is not affected by orexin-A [75].

Table 3. Biological effects of orexins.

Orexin-A and/or orexin-B	Experimental model	Administration route	Biological responses	References
Central effects after icv injection of orexins				
Orexins	rat (in vivo)	central	sleep/awake ↑ (narcolepsy)	1–11
Orexins	rat (in vivo)	central	food intake ↑ (obesity)	5, 10,12–22
Orexins	rat (in vivo)	central	drinking behaviour ↑	23
Orexins	rat (in vivo)	central	circadian process	24–28
Orexin-A	rat	central	thermoregulation (hypothermia without affecting metabolic rate)	36
Orexins	mouse	central (3rd ventricle)	energy metabolism control	42
Peripheral effects after icv injection of orexins				
Orexin-A	rat (in vivo)	icv	prolactin, GH ↓; corticosterone plasma level ↑	29
Orexins	ovariectomized rat	centrally 3rd ventricle	suppression of pulsatile LH secretion	30
Orexins	conscious rat	icv	mean arterial pressure ↑; heart rate ↑; renal sympathetic nerve activity ↑; plasma catecholamine ↑	31
Orexins	rat (in vivo)	icv	mean arterial pressure ↑	32
Orexin-A	rat (in vivo)	icv	plasma prolactin suppression	33

Table 3 (continued)

Orexin-A and/or orexin-B	Experimental model	Administration route	Biological responses	References
Orexins	decerebrated rat	central microinjection	muscle-tone ↓ noradrenergic tonus in CNS ↑	34
Orexin-A	conscious rat	central	plasma adrenocorticotropin ↑; corticosterone ↑; corticotropin-releasing factor ↑	35
Orexin-A	ovariectomized rat	icv	suppression of GnRH secretion (via β-endorphin)	37
Orexins	adult male rat	icv	activation of HPA system	38
Orexins	rat	icv	corticosterone release ↑ (NPY mediation)	39
Orexin-A	ovariectomized rat	icv	LH ↑ and prolactin ↑	40
Orexins	rat	icv	plasma corticosterone level ↑; stress-induced activation of HPA	41
Orexins	rat	central	hyperlocomotion↑ and emotional responses ↑ grooming behavior ↑ (via dopaminergic system)	43
Orexin-A	male rat (in vivo) hypothalamic explant (in vitro)	icv	plasma corticosterone and ACTH ↑ CRF and NPY release ↑	44
Orexins	urethan-anesthetized rat	intracisternal	mean arterial pressure ↑; heart rate ↑; positive feeding behaviour cardiovascular response	45;
Orexin-A	rat	intracisternal	acid secretion ↑ (vagal system)	46
C- Peripheral effects after peripheral injection of orexin or in vitro				
Orexin-A	rat (in vivo)	peripheral	corticosterone plasma level ↑	47
Orexin-A	rat (in vivo)	iv	epinephrine release and vascular tone heart rate and blood pressure ↑	48, 31, 32
Orexin-A	rat (in vivo)	peripheral	stress response	49
Orexin-A	rat (in vivo)	iv	gastric secretion ↑	46
Orexin-A Orexin-B	rat (in vivo)	subcutaneous	corticosterone plasma level ↑ proliferative activity ↑	51
Orexins	rat (in vivo)	chronic systemic administration	plasma aldosterone and corticosterone ↑ (chronic secretagogue action via OX_1R and OX_2R)	52
Orexin-B	pig (in vivo)	intramuscular	food intake ↑ ; appetite stimulation	53
Orexin-A	rat (in vivo)	Microdialysis perfusion	wakefulness ↑	54
Orexin-A	rat	ip	acid secretion →	46
Orexins	rat (in vitro)	cellular exposure	endocrine response stress	55
Orexin-A	rat (in vitro) hypothalamus and anterior pituitary slices	cellular exposure	hypothamic TRH ↑ plasma thyrotropin ↓ hypothalamic TRH release ↓ (in vitro)	56
Orexins	rat (in vivo)	subcutaneous	blood insulin ↑; blood glucose ↑	57
Orexin-A	rat (in vitro)	perfusion	insulin secretion ↑	
Orexins	adult male rat (in vivo) adrenal slices	intraperitoneal perfusion	ACTH basal secretion → corticosterone release →	38
Orexin-A	rat (in vivo)	subcutaneous	insulin and leptin blood levels ↑	38
Orexin-A	mouse (in vivo)	organ exposure	transient small intestinal muscle contraction ↑ (mediation of relaxation via activation of nitrergic neurons)	58
Orexins	rat (in vivo) rat (in vitro) (dispersed fasciculata-reticularis cells)	subcutaneous cellular exposure	glucocorticoid secretagogue action and plasma corticosterone ↑ corticosterone production ↑; cAMP ↑	59
Orexin-A	human (in vitro) adrenocortical cells	cellular exposure	cortisol response ↑; glucocorticoid secretion ↑	59
Orexin-B	mouse peritoneal macrophages	perforated patch-clamp method	immunocompetence (modulation of macrophage functions)	60
Orexins	rat PC-12 cell line (in vitro)	supplemented to medium	suppression of catecholamine release and synthesis	61

icv: intracerebroventricular; iv: intraveinous; ip: intraperitoneal; GH, growth hormone; LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; HPA, hypothalamo-pituitary adrenal axis; ACTH, adrenocorticotropic hormone; TRH, thyrotropin-releasing hormone; CRF, corticotropin-releasing factor; NPY, neuropeptide Y.

Growth hormone and prolactin

Icv injection of orexin-A in rats decreases prolactin and growth hormone (GH) plasma levels, possibly through a direct effect on hypothalamic neurons [76]. The inhibitory action of orexin-A on plasma prolactin occurs in part through an independent pathway of the dopaminergic system in rats [77]. An OX_1R has been identified by RT-PCR and immunohistochemistry in human pituitary GH-secreting cells [78].

HPA axis

Orexins may control adrenal secretions through the HPA axis or by direct actions on adrenal glands (fig. 6).

Icv injection of orexin-A [73, 79–84] or orexin-B [81–84] increases the plasma concentration of adrenocorticotropic homone (ACTH). This effect appears to be mediated, at least in part, through activation of a hypothalamic corticotropic-releasing factor (CRF) pathway. Indeed, orexin-A stimulates CRF release from hypothalamic explants in vitro [73, 80, 82]. Moreover, the effect of icv injection of orexin-A on the plasma ACTH level is blocked by a CRF antagonist [79] and central administration of orexin-A increases the CRF mRNA level in the paraventricular nucleus [85]. These observations suggest a potential role for endogenous orexins in the hypothalamic regulation of stress hormone secretion. Direct action of orexins on ACTH-secreting cells in the anterior pituitary has also been described [86]. Indeed, orexins inhibit CRF-stimulated ACTH secretion in cultured rat anterior pituitary cells, an effect probably mediated by OX_1R [86]. Though no functional data are available in humans, OX_2R has been detected by RT-PCR in the pituitary and located by immunohistochemistry in ACTH-secreting cells [78]. In consonance with the stimulation of ACTH secretion by orexins, several reports have found that icv administration of orexin-A or orexin-B increases the plasma corticosterone level [73, 76, 81, 83, 87–89]. Since Jaszberenyi et al. [83, 87–89] observed that intraperitoneal administration of orexins and incubation of adrenal slices with orexins did not alter corticosterone secretion in rats, these authors suggested that orexins activate the HPA system at a central level without direct interaction with adrenals. However, numerous observations by other groups highlight a direct action of orexins on adrenals. (i) Orexin receptors are expressed in adrenal medulla and cortex. In rats, both OX_1R and OX_2R mRNAs are present in adrenals [90], in medulla [91] and cortex [61, 92]. OX_1R is expressed in cultured porcine adrenal medulla and cortex [53]. OX_1R and/or OX_2R are also present in human adult [54, 93–96] and fetal [65] adrenals. (ii) Prepro-orexin and orexin-A have been detected by Western blot in human adult adrenals [54]. (iii) In vivo, prolonged systemic administration of orexins A and B in rats increases plasma corticosterone and aldosterone levels without affecting ACTH plasma concentration [92]. Similarly, subcutaneous administration of orexins stimulates corticosterone secretion in immature [90] and adult [51] rat adrenals. (iv) In vitro, orexins stimulate corticosterone secretion from dispersed rat zona fasciculata-reticularis cells, an effect which is not reversed by an ACTH receptor antagonist [51]. Similarly orexin-A stimulates cortisol release from cultured porcine adrenal cortex cells [97] and dispersed human adrenocortical cells [52].

In addition, orexins exert other direct secretagogue roles in adrenals. Indeed, orexin-A stimulates release of epinephrine and norepinephrine from cultured porcine adrenal medullary cells and of aldosterone from cultured porcine adrenal cortex cells [97]. A role of orexins in catecholamine biosynthesis is also suggested by the ability of orexin-A and orexin-B to reduce tyrosine hydroxylase mRNA in rat pheochromocytoma PC12 cells [53]. However, orexins may selectively stimulate glucocorticoid secretion without affecting aldosterone or catecholamine secretions in human adrenals [52]. In conclusion, orexins appear to control the HPA axis at central, pituitary and adrenal levels (see fig. 6).

Gastrointestinal tract

The presence of orexins in the lateral hypothalamus and the important role of this area in the control of gut function suggest that orexins may be involved in the central control of the gastrointestinal tract. Some evidence does support that central orexins can regulate some gut functions [98]. In addition, orexin-containing enteric neurons, orexin-immunoreactive endocrine cells (stomach and intestine) as well as orexin receptors have been characterized in the gastrointestinal tract [61, 66, 71, 99–102], suggesting that orexins exert local direct control on gut function.

Stomach

Intracisternal injection of orexin-A but not orexin-B stimulates gastric acid secretion in conscious rats [98]. Orexin-A likely acts in the brain to stimulate acid secretion by modulating the vagal system. Indeed intraperitoneal administration of orexin in rats is ineffective and vagotomy or atropine abolish the action of central orexin [98]. However, the stomach does expresses endogenous orexin at least in some species. In the guinea pig stomach, orexin-A immunoreactivity was found in endocrine cells of the pyloric glands and a subset of these cells contain gastrin [66]. Recent observations indicate that endocrine cells containing orexins are detected in mouse stomach at gestational day 14 [99].

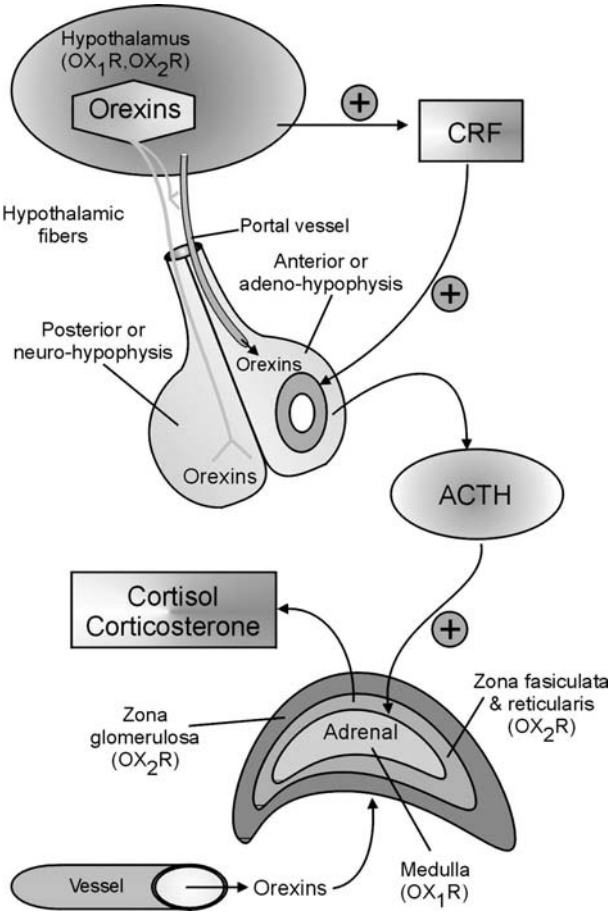

Figure 6. Orexin involvement in the hypothalamo-pituitary-adrenal (HPA) axis. The scheme represents possible roles of orexins in the HPA. The hypothalamus expresses both orexins (A and B) and their receptors (OX_1R and OX_2R). Hypothalamus orexin may reach pituitary cells by two routes: (i) hypothalamic orexin fibers mainly project to the neurohypophysis; the functional role of these projections is still unclear; or (ii) hypothalamic orexins are released into the hypothalamo-hypophyseal portal circulation (portal vessels). This may result in the direct control of adrenocorticotropic hormone (ACTH) cells by orexins. Another mechanism whereby orexins stimulate ACTH release is through a hypothalamic corticotropin-releasing factor (CRF) pathway. Then, ACTH induces release of corticosterone from adrenals. Direct action of orexins on adrenals has also been demonstrated. Orexin-A circulates in the blood, and adrenals express orexin receptors (OX_1R and OX_2R).

Intestine

Orexins are found throughout the small intestine of rodents (rats, mice and guinea pigs) and humans and in guinea pig distal colon [66]. Prepro-orexin mRNA was detected by PCR in longitudinal muscle of rat intestine in one study [66] whereas no PCR product was found in whole intestine of rats in another study [61] (see table 2). Orexin-containing neurons are immunodetected in both myenteric and submucosal plexuses [66]. Orexins are present in nerve terminals making synaptic contacts with other neurons, especially with vasoactive intestinal peptide (VIP)-containing submucosal neurons. Orexin-immunoreactive neurites are also observed in the circular muscle layer, surrounding submucosal blood vessels and mucosa [66]. The extensive network of orexin-containing nerve fibers in the mucosa encircles the crypts and travels within the lamina propria to the tips of the villi [66]. This large distribution of intestinal orexin fibers suggests the possibility of various biological effects of endogenous orexins, such as enteric neuronal excitation, gut motility, blood flow and processes of epithelial absorption and/or secretion. This hypothesis is in agreement with the presence of orexin receptors in the intestinal wall. Indeed, OX_1R and OX_2R immunoreactive neurons are found in submucosal and myenteric ganglia of the guinea pig and rat ileum [66]. Again, divergent data are reported regarding expression of OX_2R mRNA in rat intestine. RT-PCR experiments detected OX_2R mRNA in longitudinal intestinal muscle [66] but not in the whole intestine [61]. In addition, OX_1R immunoreactivity is detectable in the circular muscle and deep muscular plexus of the guinea pig duodenum. Finally, OX_1R mRNA is present in rat intestine [61, 66] in submucosa, circular muscle and longitudinal muscle myenteric plexus [66]. In this context, a few biological effects of orexins in the intestine have already been reported. Orexin-A excites secretomotor neurons in the guinea pig submocosal plexus and controls gut motility by increasing the velocity of propulsion in isolated guinea pig colon [100]. Orexin-A and orexin-B have also been shown to inhibit fasting motility in the rat duodenum [100]. Biological effects of orexins on intestinal blood vessels or epithelium have not yet been reported.

Intestinal orexins are not strictly neuronal since orexin-like immunoreactivity has also been found in endocrine cells [66, 99–102]. In rodents and humans, these cells are enterochromaphine cells and often co-store serotonin and substance P. Whether or not plasma orexins [62, 63, 103–105] originate mainly from gut endocrine cells remains to be determined.

Endocrine pancreas

Subcutaneous injection of orexin-A in rats results in an increase in blood insulin level [106–108]. A direct effect of orexins on endocrine pancreas is likely since orexin-A stimulates insulin secretion in perfused rat pancreas in vitro [106] and immunoreactivity from orexin-A and orexin receptor has been detected in rodent insulin-positive endocrine cells [66].

Miscellaneous organs

The expression of orexins and/or orexin receptors in several peripheral rat tissues (see tables 2 and 3) suggests a more widespread action of orexins at the periphery in

testis, kidney, heart, thyroid and lung [61, 75]. Very recent reports indicate that (i) orexin-containing neurons of the lateral hypothalamus project to brown adipose tissue [109] and (ii) orexins play an important role in the adipoinsular axis by stimulating insulin and leptin secretions [107] (see table 3).

Plasma orexin

Plasma orexin-A has been detected by radioimmunoassay in rats and humans. Jöhren et al. [45] reported plasma orexin levels of 3.1 and 3.9 pM in male and female rats, respectively. In healthy human subjects, the reported plasma orexin levels are highly variable: 1.9 pM [48], 5.7–9.4 pM [47], 50–250 pM [46]. These orexin plasma levels compare well with the reported concentrations of various peptide hormones in human plasma. For example, in the basal state in most mammals, the concentration of bioactive cholecystokinin is closed to 1 pM [78]. Gastrin concentrations in fasting plasma in humans are generally <25 pM [79]. Normal concentrations of gastric inhibitory polypeptide are ranged from 17 to 110 pM in humans [80]. Pancreatic polypeptide and peptide YY plasma levels in fasting normal human subjects are 15 and 10 pM, respectively [81]. Therefore, circulating concentrations of orexins seem to be compatible with an endocrine role. The endocrine cells storing orexins in the gastrointestinal tract [50] could be a source of plasma orexins. However, plasma orexin may also result from a local spillover from orexin-containing nerves. Whatever the origin of plasma orexins, the question is whether the concentration of circulating orexins is sufficient to trigger biological responses in target cells. In this respect, plasma orexin concentrations are much lower than the affinity reported for recombinant orexin receptors, e.g. >20 nM (see above). However, they compare more favorably with some reported EC_{50} values of orexin-A in triggering calcium mobilization through interaction with OX_1R or OX_2R, e.g. 60 pM [30]. In this context, further investigations are clearly needed to clarify the possible endocrine role of orexins.

Concluding remarks and perspectives

Orexins were discovered 4 years ago and several hundred papers already deal with these neuropeptides. The widespread central projections of hypothalamic orexin neurons to nuclei involved in feeding behavior, sleep-wakefulness control, neuroendocrine regulation and autonomic regulation early supported the idea that orexins have diverse and integrative actions in the CNS. However, little was known until recently regarding the physiological relevance of orexins in the periphery. Recent studies have clearly extended the multifaceted role of orexins to the regulation of peripheral functions either through a central control or direct interaction with peripheral target tissues such as hypophysis, adrenals, gastrointestinal tract or endocrine pancreas. Like many other neuropeptides, orexins belong to the so-called brain-gut axis since they are expressed in the CNS and gastrointestinal tract. The discovery of endocrine cells storing orexins in the stomach and intestine also indicates that orexins may play a dual role as neuropeptides and hormones in the periphery. This is in line with the presence of orexin-A in blood and variations in orexin circulating levels under some physiopathological circumstances such as narcolepsy or obesity. However, many questions remain unanswered regarding orexins as well as their receptors. Major tasks for the future will be the following: (i) to obtain reliable orexin tracers to document in more detail the specificity, affinity and structure-function relationship of orexin receptors which are still poorly characterized; (ii) to develop selective agonists and antagonists; (iii) to better understand the signal transduction pathways of orexin receptors; (iv) to evaluate the involvement of orexins in other peripheral functions; (v) to understand the physiological role, if any, of circulating orexin and its site(s) of origin, i.e. gastrointestinal tract or other sources, and (vi) to explore peripheral tissue functions in orexin or orexin receptor knock out mice. Following such studies, orexins will likely be promoted as major regulatory peptides.

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