Distribution of orexin receptor mRNA in the rat brain

Prashant Trivedi[1], Hong Yu, Douglas J. MacNeil, L.H.T. Van der Ploeg, Xiao-Ming Guan*

Department of Obesity Research, Merck Research Laboratories, P.O. Box 2000, R80M-213, Rahway, NJ 07065, USA

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Abstract The expression pattern of mRNA encoding two orexin receptors (OX_1R and OX_2R) in the rat brain was examined. OX_1R and OX_2R exhibited marked differential distribution. Within the hypothalamus, OX_1R mRNA is most abundant in the ventromedial hypothalamic nucleus whereas OX_2R is predominantly expressed in the paraventricular nucleus. High levels of OX_1R mRNA were also detected in tenia tecta, the hippocampal formation, dorsal raphe, and locus coeruleus. OX_2R mRNA is mainly expressed in cerebral cortex, nucleus accumbens, subthalamic and paraventricular thalamic nuclei, anterior pretectal nucleus. The presence of orexin receptor mRNA in the hypothalamus is in support of its proposed role in feeding regulation. Broad central distribution of orexin receptors may indicate additional functions for orexins.

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Key words: Orexin; G-protein coupled receptor; Feeding; In situ hybridization

1. Introduction

Feeding behavior is controlled by a complex feedback mechanism involving both central and peripheral systems. In the brain, a key site for regulating appetite and satiety is the hypothalamus, in which numerous neuropeptides have been demonstrated to stimulate or suppress food intake [1]. Recently, a novel hypothalamic peptide family was discovered, which, upon administration into the lateral ventricle of rats, elicited a strong stimulatory effect on food consumption, and was therefore termed orexins [2,3]. The orexins consist of orexin-A and orexin-B, which are proteolytically derived from the same 130 amino acid residue preproorexin precursor protein. In situ hybridization and immunohistochemical studies indicated that orexins are produced in neurons located in the dorsal, lateral and posterior hypothalamic areas, which project to multiple target fields both within and outside the hypothalamus [2,3].

To date, two orexin receptors (OX_1R and OX_2R) have been described, which belong to the G-protein coupled receptor superfamily with a proposed seven transmembrane topology [2]. The two receptors share 64% identity in their amino acid sequence, and display a distinct pattern of interaction with orexins: orexin-A has a high affinity for both receptors whereas orexin-B shows a 10-fold higher affinity at OX_2R than that at OX_1R [2]. In adult rats, the mRNA encoding orexin receptors were detected exclusively in the brain by Northern blot analysis [2], however, a detailed expression pattern of the receptors within the brain remained unknown. The present study was undertaken to compare the distribution of OX_1R and OX_2R mRNA throughout the rat brain in order to better understand the potential functions of orexin pathways.

2. Materials and methods

2.1. Tissue preparation

Male Sprague-Dawley rats (2–3 months of age) were purchased from Harlan Sprague Dawley (Indianapolis, IN). Animals were killed by decapitation and brains were quickly removed and frozen in −40°C isopentane and stored at −80°C until use. Coronal brain sections (14 μm) were cut at −17°C on a cryostat microtome and thaw-mounted on baked microslides. The sections were post-fixed in ice-cold 4% phosphate-buffered paraformaldehyde and stored in 95% ethanol at 4°C until use.

2.2. In situ hybridization

In order to enhance the signal intensity, an equal molar mixture of three antisense oligonucleotide probes was used for detecting each of the orexin receptors. The probes are specific to rat OX_1R or OX_2R, and do not cause cross-hybridization with each other. The nucleotide sequences of these probes are as follows: for OX_1R, 5′-TCCTCATAGTCTGGAGGCAGGTGGAAGGGTTCCCCACTGCTAGTG-3′, 5′-AAGGCTATGAGAAACACGGCCACGTAGGCCGCGATGAGAACCCAC-3′ and 5′-TGCTGAGCTTCCAGTTGCTCTGAGGGTCGCTTCCAGTTCCGCACC-3′; for OX_2R, 5′-AGTTGCGACGAGGGAGGGAATCCTCCAATTTGGTGCTGGACATCA-3′, 5′-AGAGCCACGACGAACACGATGATATACCCTGCGATCAGGACCCAC-3′ and 5′- CAAAGTTGCTGATCTGAGTAGTCAGGGACTTCCTGCTCTCTGTAC-3′. Various probes were 3′-end labeled with [α-^{33}P]dATP and terminal deoxynucleotidyl transferase and purified over Sephadex G-50 columns. The hybridization and washing conditions were as described previously [4]. To facilitate comparison between the two receptors, adjacent coronal sections from the same brain were used for detecting OX_1R or OX_2R. The signals are regarded as specific only if they can be displaced by the addition of 100-fold molar excess of the corresponding non-labeled oligonucleotides. Additional evidence for specific hybridization came from the detection of an identical pattern of distribution for a given receptor when using distinct, non-overlapping oligonucleotide probes individually. Following air-drying, the slides were exposed to Kodak BioMax X-ray film for about 3 weeks prior to development according to standard procedures. Autoradiographic images of the film were captured and analyzed with the MCID/M2 image analysis system (Imaging Research Inc., Ontario, Canada). Anatomical localization was verified according to the rat brain atlas [5].

3. Results and discussion

The expression patterns of OX_1R and OX_2R mRNA in the rat brains were examined by using in situ hybridization with receptor subtype-specific oligonucleotide probes. Fig. 1 shows the representative autoradiograms of different brain areas expressing specific signals (marked with arrowheads) for mRNA of OX_1R and OX_2R. The specificity of hybridization was determined by the criteria that the signals were displaceable by the presence of 100-fold molar excess of the corresponding unlabelled probes, and that the same distribution pattern of the receptors was detected with distinct, non-overlapping oligonucleotide probes (data not shown).

*Corresponding author. Fax: (1) (732) 594-3337.
E-mail: xiaoming_guan@merck.com

[1]These authors contributed equally to this study.

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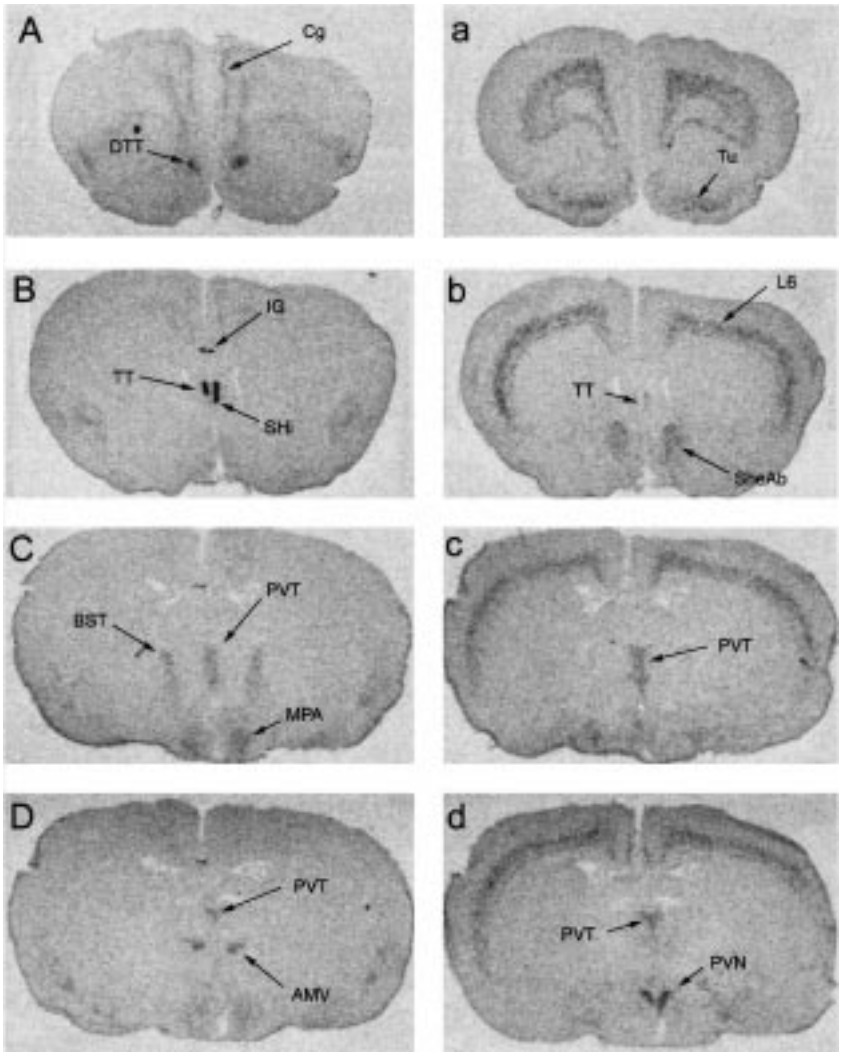

Fig. 1. Distribution of OX_1R and OX_2R mRNA in the rat brain. Adjacent coronal sections (14 μm) from the same brain were used for hybridization with specific probes against either OX_1R (panels A–H) or OX_2R (panels a–h) mRNA. Data are representative autoradiograms of three independent experiments. Cg: cingulate cortex; DTT, dorsal tenia tecta; L6, layer VI of cerebral cortex; Tu, olfactory tubercle; IG, indusium griseum; TT: tenia tecta; Shi: septohippocampal nucleus (nu); SheAb: shell of nucleus accumbens; BST: bed nucleus of the stria terminalis; PVT, paraventricular thalamic nu; MPA: medial preoptic area; AVM: ventral anteromedial thalamic nu; PVN: paraventricular hypothalamic nu; VMH: ventromedial hypothalamic nu; DMH: dorsomedial hypothalamic nu; CM: central medial thalamic nu; CA1, CA2, CA3: hippocampus; DG: dentate gyrus; AhiPM: amygdalohippocampal area; APTD: anterior pretectal nu; Sth: subthalamic nu; DR: dorsal raphe; LC: locus coeruleus.

Overall, the mRNA for both orexin receptors is widely distributed in the rat brain. However, the expression patterns for OX_1R and OX_2R are strikingly different (Fig. 1 and Table 1). The hypothalamus is generally regarded as the primary brain center for the regulation of feeding behavior and energy metabolism. Within this region, OX_1R mRNA is most abundant in the ventromedial hypothalamic nucleus (VMH). Moderate levels of hybridization were also detected in the medial preoptic area, lateroanterior and dorsomedial hypothalamic nuclei, lateral mamillary nucleus and posterior hypothalamic area. In contrast, OX_2R mRNA is predominantly expressed in the paraventricular nucleus (PVN). Weak to moderate signals were detected in the VMH and dorsomedial hypothalamic nuclei, and posterior and lateral hypothalamic areas. Previous reports have shown that both preproorexin mRNA and immunoreactive orexins are localized primarily in neurons around the lateral and posterior hypothalamic areas [2,3]. It has been demonstrated that the expression of preproorexin mRNA is up-regulated during fasting, and that orexin peptides, when administered intracerebroventricularly, stimulate food intake in rats [2]. These results suggest that orexins play a role in controlling food intake. Our data reveal a high level of orexin receptor expression in specific hypothalamic nuclei in support of the above notion. The present results also indicate that even though orexin signals could not be detected previously in neurons of certain hypothalamic nuclei known to control feeding, such as the PVN and VMH [2], the latter may be important targets receiving orexinergic inputs from the posterior and lateral part of the hypothalamus. It is of interest to note the sharp contrast in the distribution pattern for OX_1R and OX_2R mRNA in the PVN and VMH. Since both nuclei have been implicated to serve key roles in the neuronal circuitry of feeding regulation [6–8], it is difficult to predict which orexin receptor is the primary mediator for orexigenic action of the peptides.

Besides feeding behavior, the hypothalamus is the main

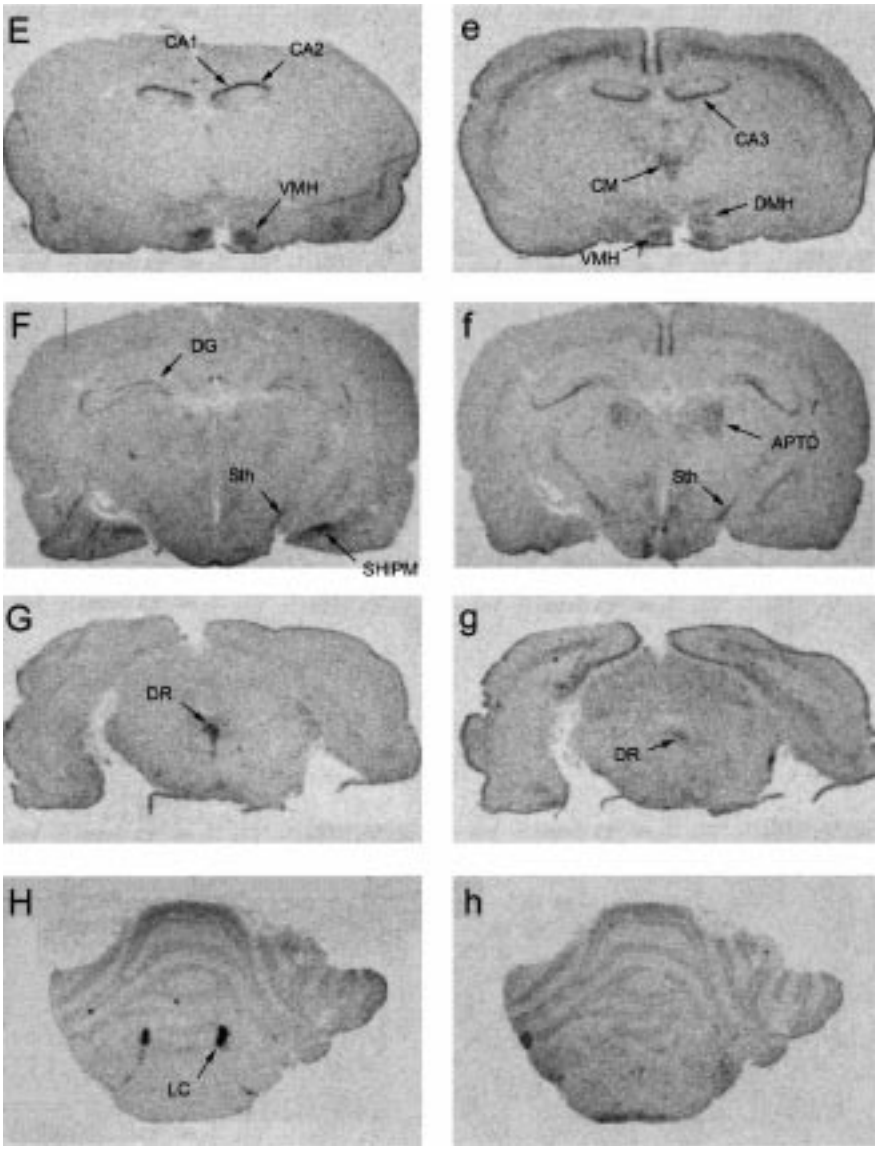

Fig. 1. (*continued*)

integrative center for a number of other neuroendocrine functions. For example, the medial preoptic area has been associated with maternal, sexual behaviors and sexual differentiation [9,10]. The PVN, which is enriched with a vast number of neuroendocrine modulators, has been implicated in multiple functions including stress response, control of pituitary function, body fluid homeostasis, analgesia, cardiovascular and gastrointestinal functions [6]. Whether orexins are involved in these hypothalamic activities will require further investigation.

In contrast to orexins, the expression of orexin receptors is not restricted to the hypothalamus (Fig. 1 and Table 1). Other brain regions that display high levels of expression of OX_1R include tenia tecta, indusium griseum, septohippocampal nucleus, bed nucleus of the stria terminalis, paraventricular thalamic nucleus, CA1, CA2 regions of the hippocampus, amygdalohippocampal area, dorsal and median raphe nuclei and locus coeruleus. On the other hand, OX_2R mRNA is mainly expressed in the olfactory tubercle, layer VI of the cerebral cortex, shell of the nucleus accumbens, paraventricular and central medial thalamic nuclei, subthalamic nucleus, anterior pretectal nucleus, CA3 of the hippocampus. The relative abundance of orexin receptor mRNA in various brain areas is summarized in Table 1.

The wide CNS distribution of mRNA encoding OX_1R and OX_2R suggests that orexins may be involved in multiple functional pathways. Among various regions mentioned above, several nuclei such as the locus coeruleus, the paraventricular thalamic nucleus, the preoptic area and septal nuclei have been previously shown to be the major terminal fields for orexin-containing fiber projections [3]. In the absence of functional data, however, the potential physiological significance of orexin innervation in these and other brain areas remains unclear and speculative. The locus coeruleus and dorsal/median raphe nuclei are major centers for the noradrenergic and serotonergic neurons, respectively, which project extensively to various parts of the brain and participate in a number of diverse neuronal functions [11,12]. High levels of OX_1R expression in these nuclei may suggest a regulatory role of orexins on the monoaminergic systems. In addition, the OX_1R

Table 1
Relative abundance of OX_1R and OX_2R mRNA in the rat brain

	OX_1R	OX_2R
Cingulate cortex	+	−
Cortex layer VI	−	+++
Olfactory tubercle	−	++
Tenia tecta	+++	+
Indusium griseum	+++	−
Septohippocampal nucleus	+++	−
Shell of nucleus accumbens	−	++
Bed nucleus of the stria terminalis	++	−
Medial septal nucleus	−	+
Vertical limb of diagonal band nucleus	−	+
Lambdoid septal zone	−	+
Paraventricular thalamic nucleus	++	++
Ventral anteromedial thalamic nucleus	++	−
Central medial thalamic nucleus	−	++
Subthalamic nucleus	+	++
Anterior pretectal nucleus	−	++
Medial preoptic area	++	−
Lateroanterior hypothalamic nucleus	++	−
Paraventricular hypothalamic nucleus	−	+++
Ventromedial hypothalamic nucleus	+++	++
Dorsomedial hypothalamic nucleus	+	+
Lateral mamillary nucleus	+	−
Lateral hypothalamic area	−	+
Posterior hypothalamic area	+	+
CA1	++	−
CA2	++	−
CA3	−	++
Dentate gyrus	+	+
Medial amygdaloid nucleus	+	−
Amygdalohippocampal area, posteromedial part	++	−
Dorsal raphe	++	+
Median raphe	+	−
Locus coeruleus	+++	−

Nomenclature was based on Paxinos and Watson [5]. The relative densities were estimated using a four-point scale: +++ highest density; ++ moderate density; + low, but above the background; − indistinguishable from the background intensity.

mRNA is also present in basal forebrain structures (i.e. the amygdala and bed nucleus of the stria terminalis), linked to such functions as memory storage, emotion and attention [13–15]. OX_2R mRNA is abundant in several areas related to basal ganglia, including the ventral striatum (nucleus accumbens and part of olfactory tubercle) and subthalamic nucleus. The accumbens is the major recipient of the mesolimbic dopaminergic projection from the ventral tegmental area, and serves a key role in the brain reward mechanism, which may mediate the positive reinforcing effect of food, thereby providing an indirect control of feeding behavior [1]. The anterior pretectal nucleus, another site specifically expressing OX_2R, has been proposed to be involved in nociceptive sensory processing [16]. Finally, both orexin receptors are heavily expressed in the hippocampus and associated structures (i.e. OX_1R: CA1, CA2, indusium griseum, septohippocampal nucleus; OX_2R: CA3), and the paraventricular thalamic nucleus (PVT). The hippocampus is presumably an important brain center for learning and memory [17,18], whereas the PVT is believed to be a part of the circadian timing system and relay information to the regions involved in motivational components of behaviors and visceral functions [19,20]. Obviously, more direct functional tests will be required before any definitive physiological role can be assigned to orexins.

In conclusion, the present data revealing hypothalamic expression of orexin receptors are in support of their proposed role in feeding regulation. However, high level expression of these receptors in multiple brain sites may indicate additional as yet undefined physiological functions for the novel peptide family. Finally, the strikingly divergent distribution of OX_1R and OX_2R suggests that these receptors may mediate distinct functions for orexins.

References

[1] Smith, G.P. (1997) in: The Encyclopedia of Neuroscience (Adelman, G. and Smith, B.H., Eds.), 2nd edn., Elsevier Science, Amsterdam.
[2] Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R.S., Buckingham, R.E., Haynes, A.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.-S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J. and Yanagisawa, M. (1998) Cell 92, 573–585.
[3] De Lecea, L., Kilduff, T.S., Peyron, C., Gao, X.-B., Foye, P.E., Danielson, P.E., Fukuhara, C., Battenberg, E.L.F., Gautvik, V.T., Bartlett II, F.S., Frankel, W.N., Van den Pol, A.N., Bloom, F.E., Gautvik, K.M. and Sutcliffe, J.G. (1998) Proc. Natl. Acad. Sci. USA 95, 322–327.
[4] Guan, X.-M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J.S., Smith, R.G., Van der Ploeg, L.H.T. and Howard, A.D. (1997) Mol. Brain Res. 48, 23–29.
[5] Paxinos, G. and Watson, C. (1997) The Rat Brain in Stereotaxic Coordinates, 3rd edn., Academic Press, New York.
[6] Kiss, J.Z. (1988) Brain Res. Bull. 20, 699–708.
[7] Kalra, S.P., Dube, M.G., Sahu, A., Phelps, C.P. and Kalra, P.S. (1991) Proc. Natl. Acad. Sci. USA 88, 10931–10935.
[8] Stoynev, A.G. and Ikonomov, O.C. (1987) Appetite 9, 217–229.
[9] Numan, M. (1986) Ann. NY Acad. Sci. 474, 226–233.
[10] Gorski, R.A. (1984) Prog. Brain Res. 61, 129–146.

[11] Foote, S.L. (1997) in: The Encyclopedia of Neuroscience (Adelman, G. and Smith, B.H., Eds.), 2nd edn., Elsevier Science, Amsterdam.
[12] Halliday, G., Harding, A. and Paxinos, G. (1995) in: The Rat Nervous System (Paxinos, G., Ed.), pp. 929–974, Academic Press, New York.
[13] McGaugh, J.L., Cahill, L. and Roozendaal, B. (1996) Proc. Natl. Acad. Sci. USA 93, 13508–13514.
[14] Davis, M., Walker, D.L. and Lee, Y. (1997) Ann. NY Acad. Sci. 821, 305–331.
[15] Pitkanen, A., Savander, V. and LeDoux, J.E. (1997) Trends Neurosci. 20, 517–523.
[16] Rees, H. and Roberts, M.H. (1993) Pain 53, 121–135.
[17] Eichenbaum, H., Otto, T. and Cohen, N.J. (1992) Behav. Neural Biol. 57, 2–36.
[18] Jarrard, L.E. (1993) Behav. Neural Biol. 60, 9–26.
[19] Moga, M.M., Weis, R.P. and Moore, R.Y. (1995) J. Comp. Neurol. 359, 221–238.
[20] Bubser, M. and Deutch, A.Y. (1998) Brain Res. 787, 304–310.