ELSEVIER

European Journal of Pharmacology 452 (2002) 105–109

www.elsevier.com/locate/ejphar

Involvement of cholinergic neurons in orexin-induced contraction of guinea pig ileum

Katsuhisa Matsuo [a,b], Muneshige Kaibara [b], Yasuhito Uezono [b], Hideki Hayashi [b], Kohtaro Taniyama [b,*], Yoshibumi Nakane [a]

[a] *Department of Psychiatry, Nagasaki University School of Medicine, Nagasaki 852-8523, Japan*
[b] *Department of Pharmacology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*

Received 24 April 2002; received in revised form 9 August 2002; accepted 16 August 2002

Abstract

The mechanism underlying orexin-induced contraction was examined in isolated preparations of guinea pig ileum, in relation to cholinergic transmission. Orexin-A caused contraction of ileal strips in a concentration-dependent manner. 1-(2-Methylbenzoxazol-6-yl)-3-[1,5]napthyridin-4-yl-urea hydrochloride (SB-334867-A) antagonized the orexin-A-induced contraction, with no effects on the acetylcholine-induced contraction and twitch contractions. The orexin-A-induced contraction was inhibited by tetrodotoxin and atropine, but not by hexamethonium, an antagonist of vasoactive intestinal peptide and a mixture of 5-hydroxytryptamine receptor antagonists. Orexin-A evoked an outflow of [^{3}H]acetylcholine from the ileal strips preincubated with [^{3}H]choline, in a concentration-dependent manner, and the orexin-A-evoked outflow was inhibited by tetrodotoxin, indicating that the outflow of [^{3}H]acetylcholine originates from the nerve terminals. The orexin-A-evoked outflow of [^{3}H]acetylcholine was antagonized by SB-334867-A. Thus, orexin-A evokes the release of acetylcholine from the enteric cholinergic neurons due to stimulation of the orexin-1 receptors and then causes contractions of guinea pig ileum.

Keywords: Orexin-A; Orexin-1 receptor; SB-334867-A; Acetylcholine release; Tetrodotoxin

1. Introduction

The recently discovered orexins consist of orexins A and B, and their receptors are classified into two types, orexin-1 and orexin-2 receptors (Sakurai et al., 1998). Orexin-containing neurons are localized in the dorsal and lateral hypothalamic area and perifornical hypothalamus, and the nerve fibers are widely distributed throughout the brain (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999; Willie et al., 2001). Orexins have been described as playing a role in the control of feeding (Sakurai et al., 1998; Edwards et al., 1999; Ida et al., 1999; Sweet et al., 1999), sleep–wakefulness (Chemelli et al., 1999; Lin et al., 1999), neuroendocrine homeostasis (Date et al., 1999; Horvath et al., 1999), and autonomic regulation (van den Pol, 1999) in the central nervous system (Willie et al., 2001). Recent studies have shown that orexin-containing neurons and orexin receptors (Kirchgessner and Liu, 1999; López et al., 1999) are present outside the central nervous system. Orexin-containing neurons and orexin receptors were found to be present in the guts of rat, mouse, guinea pig, and human, and the peptide was found to cause an increase in the rate of propulsion, and further varicosities of orexin-immunoreactive nerve fibers were found to enter a myenteric ganglion from a connective ganglion and encircle neuronal somata, and orexin-immunoreactive neurons were found to coexpress choline acetyltransferase immunoreactivity (Kirchgessner and Liu, 1999). These findings led to the idea of possible interaction of orexin-containing neurons and enteric cholinergic neurons. Thus, the present study attempted to determine the mechanism underlying orexin-induced contraction of intestine, in relation to enteric cholinergic neurons, using longitudinally oriented, whole-thickness strips from guinea pig ileum. Orexin-A has equal affinity for both orexin-1 and orexin-2 receptors, while orexin-B displays 10-fold greater selectivity for orexin-2 receptors (Sakurai et al., 1998). Orexin-A was, therefore, used as an agonist for orexin receptors in the present study.

* Corresponding author. Tel.: +81-95-849-7047; fax: +81-95-849-7048.
E-mail address: taniyama@net.nagasaki-u.ac.jp (K. Taniyama).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02300-2

2. Materials and methods

The study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of Nagasaki University, as adopted and promulgated by the notification of the Director General of the Science and International Affairs Bureau of the Japanese Ministry of Education, Science, Sports and Culture, Japan. Adult guinea pigs of either sex (300–350 g) were killed by cervical dislocation. Strips of ileum were excised 10 cm proximal to the ileocaecal sphincter, and approximately 1.5-cm preparations were dissected.

2.1. Measurements of the mechanical activity

The mechanical activity of longitudinally oriented muscle was measured in strips prepared by cutting parallel to the oral–anal axis. The ileal strips were placed in a 5-ml organ bath in the presence of Krebs–Ringer solution of the following composition (in mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, KH_2PO_4 1.18, and glucose 11, which was continuously gassed with 95% O_2 and 5% CO_2 and maintained at 34–36 °C and pH 7.4. Approximately 5 mN of resting tension was applied and was kept constant by readjustment during the equilibration period. Mechanical responses to single concentrations of agonists were recorded by means of an isometric transducer (Nihon Kohden, Japan, SD-1T). Experiments were started after stabilization of the contraction induced by acetylcholine (10^{-7} M). An interval of 30 min (with intervening washings) between single concentrations of agonists (acetylcholine and orexin-A) completely prevented any desensitization, and the amplitudes of contractions induced by agonists produced a reliable response. To evaluate effects of antagonists, the strips were exposed to the antagonists 15 min before and during applications of agonists. The effects of agonists in the absence and presence of antagonists were obtained using the same strips and represented as a percent of amplitude of acetylcholine (10^{-7} M)-induced contraction. The twitch contractions were obtained by electrical transmural stimulation (1 ms pulse duration, 15 V intensity, at a frequency of 0.1 Hz) of longitudinally oriented, whole-thickness strips positioned between two parallel platinum electrodes.

2.2. Measurement of the outflow of [^{3}H]acetylcholine

The methods used have been described previously (Kan et al., 1994; Akehira et al., 1995). Briefly, the whole-thickness strips of ileum were incubated with 2×10^{-7} M [^{3}H]choline for 60 min in Krebs–Ringer solution. After washing in fresh Krebs–Ringer solution for 30 min, the preparations were mounted in the apparatus and perfused at 2 ml/min with the same solution gassed with 95% O_2 and 5% CO_2, maintained at 34–36 °C and pH 7.4. Krebs solution, containing 10^{-5} M hemicholinium-3 to prevent the uptake of choline formed from acetylcholine, was used for perfusion. Furthermore, to exclude the effect of mechanical distortion accompanied by orexin-A-induced contraction on the [^{3}H]acetylcholine outflow, the perfusion solution contained 10^{-7} M atropine. Experiments were started 60 min after the spontaneous outflow of tritium had reached steady state. The perfusates were collected every 30 s, and the radioactivity was counted in a liquid scintillation spectrometer. At the end of release experiments, the radioactivity of the tissue dissolved in Soluene was counted in a liquid scintillation spectrometer.

The validity of assuming total tritium as a measure of [^{3}H]acetylcholine outflow under the present experimental conditions had been documented in our previous studies (Kan et al., 1994; Akehira et al., 1995). The outflow of tritium was represented as the fractional rate obtained by dividing the amount of tritium in the perfusate by the respective amount of tritium in the tissue. The tritium content of the tissue at each period was calculated by adding cumulatively the amount of each fractional tritium outflow to the tritium content of the tissue at the end of the experiment. From each of the outflow curves obtained by plotting the fractional outflow of tritium against time, the peak outflow of tritium evoked by stimulation with orexin-A in each condition was calculated from the difference between the peak tritium outflow and the basal outflow. When the preparations were stimulated with orexin-A three times successively (S1, S2, and S3) at 30-min intervals, the stimulation-evoked outflow of tritium markedly decreased or increased from the first (S1) to second stimulation period (S2). Therefore, the outflow of tritium evoked by S2 and S3 was used. The ratio of S3/S2 calculated from the peak outflow of tritium in the S2 and S3 with orexin-A was used as control, and the effects of substances on the orexin-A-evoked outflow were evaluated from the ratio of S3/S2 calculated from S3 in the presence of substances. The data were analyzed using Dunnett's *t*-test and a P value of 0.05 or less was considered statistically significant.

2.3. Drugs and chemicals

Substances used were as follows: [^{3}H]choline (60 Ci/mmol) (New England Nuclear, Boston, MA), orexin-A (Peninsula, Belmont, CF), hemicholinium-3 (Sigma, St. Louis, MO), acetylcholine chloride, atropine sulphate, hexamethonium bromide, and tetrodotoxin (Wako, Osaka, Japan), ketanserin tartrate (Research Biochemicals Int., Natick, MA, USA), [Ac-Tyr^1, D-Phe^2]growth hormone-releasing factor 1–29, amide ([Ac-Tyr^1, D-Phe^2]GRF-(1–29); Tocris Cookson, Ballwin, MO, USA) and Soluene® (Packard, Downers Groves, IL, USA). Other chemicals used were of reagent grade. 1-(2-methylbenzoxazol-6-yl)-3-[1,5]napthyridin-4-yl-urea hydrochloride (SB-334867-A), (1-*n*-butyl-4-piperidinyl) methyl-8-amino-7-chloro-1,4-benzodioxane-5-carboxylate (SB204070) and granisetron were generously provided by Smith Kline Beecham, UK.

3. Results

3.1. Mechanical response to orexin-A

Orexin-A, 10^{-8}–10^{-6} M, caused contractions of ileal strips, in a concentration-dependent manner, and the maximum contraction was obtained at 3×10^{-7} M (Fig. 1A). Pretreatment with SB-334867-A at 3×10^{-7} M for 15 min shifted rightward the concentration-response curve of orexin-A-induced contractions, and pretreatment with SB-334867-A at 10^{-6} M prevented the maximum contraction induced by orexin, 10^{-7} M (Fig. 1A). SB-334867-A, 3×10^{-7} and 10^{-6} M, did not affect the acetylcholine (10^{-7} M)-induced contraction (Fig. 1B) and twitch contractions which were atropine-sensitive and tetrodotoxin-sensitive ($n = 5$) (data not shown).

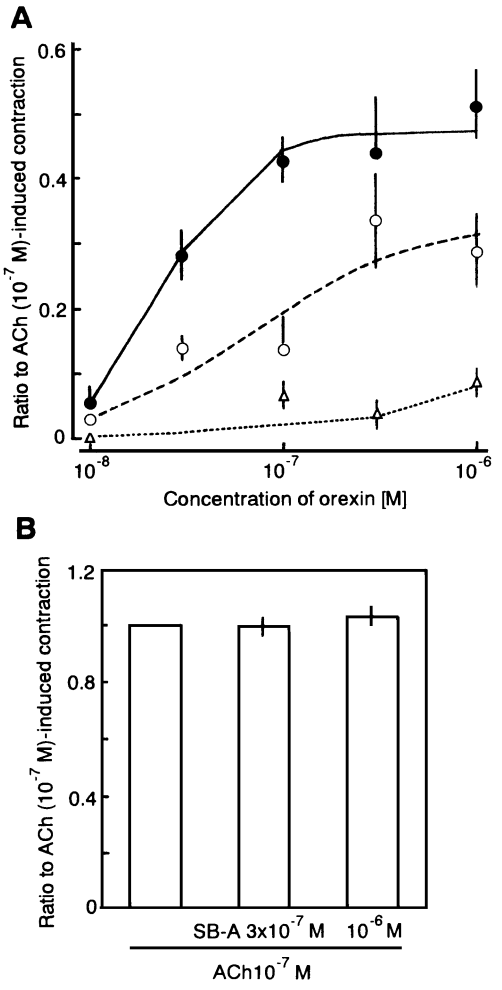

Fig. 1. Effect of SB-334867-A on the orexin-A-induced contraction (A) and acetylcholine-induced contraction (B) of the isolated guinea pig ileum. (A) Concentration–response curves of orexin-A-induced contraction, in the absence (●) and presence of SB-334867-A at 3×10^{-7} (○) and 10^{-6} M (△). The contraction induced by orexin-A is presented as relative to that induced by 10^{-7} M acetylcholine (ACh). Each point represents the mean ± S.E.M. for five animals. (B) Acetylcholine-induced contraction in the absence and the presence of SB-334867-A (SB-A). Each column represents the mean ± S.E.M. for five animals. SB-334867-A, 3×10^{-7} and 10^{-6} M, was present 15 min before and during application of various concentrations of orexin-A or 10^{-7} M acetylcholine (ACh).

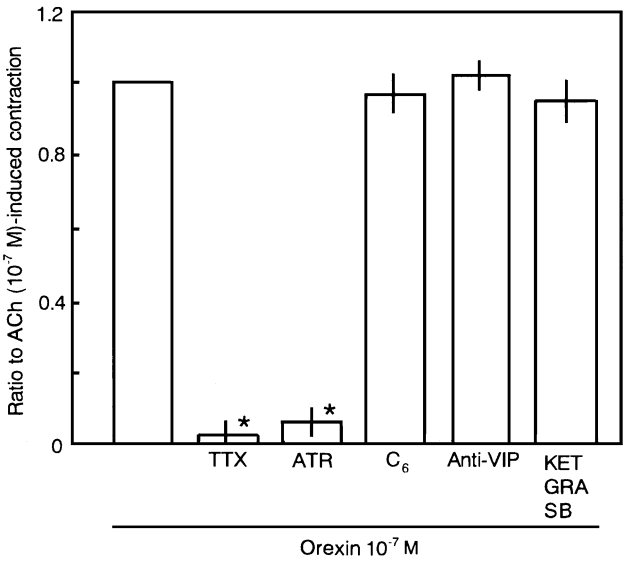

Fig. 2. Effects of tetrodotoxin, atropine, hexamethonium, VIP receptor antagonist and serotonin receptor antagonists on the orexin-A-induced contractions of the isolated guinea pig ileum. Tetrodotoxin (TTX), 3×10^{-7} M, atropine (ATR), 10^{-8} M, hexamethonium (C_6), 10^{-4} M, [Ac-Tyr^1, D-Phe^2]GRF-(1–29) (Anti-VIP), 10^{-6} M, and a mixture of ketanserin (KET), 10^{-7} M, granisetron (GRA), 10^{-7} M, and SB204070 (SB), 10^{-7} M, were present 15 min before and during addition of orexin-A, 10^{-7} M. Each column represents the mean ± S.E.M. for five animals. * Significantly different from value for the nontreated preparations ($P < 0.05$).

The orexin-A (10^{-7} M)-induced contraction was prevented by pretreatment with either tetrodotoxin, 3×10^{-7} M, for 15 min or atropine, 10^{-8} M, for 15 min, but not by pretreatment with hexamethonium, 10^{-4} M (Fig. 2).

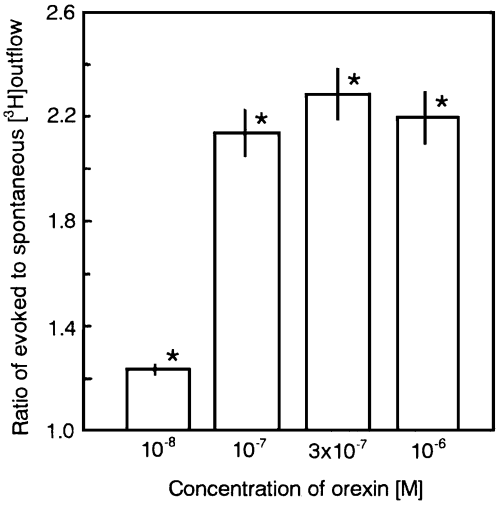

Fig. 3. Orexin-A-evoked outflow of [^{3}H]acetylcholine from ileal strips preloaded with [^{3}H]choline. Orexin-A at various concentrations was perfused for 2 min. Each column represents the mean ± S.E.M. of the ratio of [^{3}H]acetylcholine (ACh) outflow in the second stimulation with orexin-A (S2) to spontaneous outflow from five animals. * Significantly different from value for spontaneous outflow ($P < 0.05$).

Table 1
Effects of tetrodotoxin and SB-334867-A on the orexin-A-evoked outflow of [^{3}H]acetylcholine from ileal strips

Agents	Ratio of S3/S2
Orexin-A 10^{-7} M	0.868 ± 0.170
Orexin-A 10^{-7} M + TTX 3×10^{-7} M	0.087 ± 0.056[a]
Orexin-A 10^{-7} M + SB 3×10^{-7} M	0.277 ± 0.082[a]
Orexin-A 10^{-7} M + SB 10^{-6} M	0.014 ± 0.040[a]

The S3/S2 ratio was calculated by dividing the [^{3}H]acetylcholine outflow in the third stimulation with orexin-A, 10^{-7} M (S3), by that in the second stimulation with orexin-A, 10^{-7} M (S2). Tetrodotoxin (TTX), 3×10^{-7} M, and SB-334867-A, 3×10^{-7} and 10^{-6} M, were present 15 min before and during the S3. Each number is presented as the mean ± S.E.M. of the ratio of S3/S2 in the orexin-A-evoked outflow of [^{3}H]acetylcholine from five animals.

[a] Significantly different from value for the nontreated preparations ($P < 0.05$).

The effects of an antagonist for vasoactive intestinal polypeptide (VIP) receptor and antagonists for serotonin receptors on the orexin-A (10^{-7} M)-induced contraction were examined, using [Ac-Tyr^1, D-Phe^2]GRF-(1–29), 10^{-6} M, and a mixture of antagonists of serotonin receptors, ketanserin, 10^{-7} M (5-HT$_2$ receptor antagonist), granisetron, 10^{-7} M (5-HT$_3$ receptor antagonist), and SB204070, 10^{-7} M (5-HT$_4$ receptor antagonist). The VIP (10^{-6} M)-induced contraction was inhibited by [Ac-Tyr^1, D-Phe^2]GRF-(1–29) at 10^{-6} M to approximately 20% ($n = 5$), and the 5-HT (10^{-6} M)-induced contraction was almost completely inhibited by a mixture of antagonists of serotonin receptors ($n = 5$). The orexin-A (10^{-7} M)-induced contraction, however, was not affected by these antagonists for VIP receptors and serotonin receptors (Fig. 2).

3.2. Effect of orexin-A on the [^{3}H]acetylcholine outflow

The spontaneous outflow of [^{3}H]acetylcholine reached steady state, and a single exponential curve was obtained with a fractional rate of 0.00660 ± 0.00062/min ($n = 6$) 60 min after perfusion. Orexin-A, 10^{-8}–10^{-6} M, evoked the outflow of [^{3}H]acetylcholine in a concentration-dependent manner (Fig. 3). The [^{3}H]acetylcholine outflow evoked by orexin-A, 10^{-7} M, was prevented by 15-min pretreatment with 3×10^{-7} M tetrodotoxin (Table 1). Pretreatment with SB-334867-A, 3×10^{-7} and 10^{-6} M, for 15 min inhibited, by approximately 70% (3×10^{-7} M) and completely (10^{-6} M), the [^{3}H]acetylcholine outflow evoked by orexin-A, 10^{-7} M, without affecting the spontaneous outflow of [^{3}H]acetylcholine (Table 1).

4. Discussion

The orexin-A-induced contractions of isolated preparations of guinea pig ileum were antagonized by SB-334867-A. It has been shown that SB-334867-A is a selective antagonist of the orexin-1 receptor in the central nervous system (Smart et al., 2001; Soffin et al., 2002). SB-334867-A did not affect the acetylcholine-induced contraction and cholinergic nerve-mediated twitch contractions; therefore, the response to orexin-A may be mediated by stimulation of the orexin-1 receptor, also in intestinal tissue. The orexin-A-induced contraction was tetrodotoxin-sensitive and atropine-sensitive, while it is insensitive to hexamethonium. These results indicate that orexin-A acts at orexin-1 receptors, but not at nicotinic acetylcholine receptors, and causes contraction mediated by stimulation of the excitatory neurons, especially cholinergic neurons.

Orexin-A evoked the outflow of [^{3}H]acetylcholine from ileal strips preloaded with [^{3}H]choline. The outflow of radiolabels from the preparations preloaded with radiolabelled choline has been shown to represent the outflow of radiolabelled acetylcholine (Vizi et al., 1984). As the orexin-A-evoked outflow was also antagonized by SB-334867-A, the evoked outflow is due to stimulation of orexin-1 receptors, as noted in the contraction. The orexin-A-evoked outflow of acetylcholine was tetrodotoxin-sensitive. Tetrodotoxin is known to block nerve conduction and not to affect the release of transmitter substances induced from nerve terminals by local depolarizing stimulation (Vizi et al., 1973; Gonella et al., 1980; Starke, 1981; Sawa et al., 1995); therefore, the orexin-A-evoked outflow of [^{3}H]acetylcholine may be induced by the impulse generated in the soma-dendritic regions of neurons. Thus, orexin-A may act at the orexin-1 receptors located on the soma-dendritic regions of enteric cholinergic neurons and evoke the release of acetylcholine, then cause contraction of ileal smooth muscle cells. The nerve cell bodies with orexin-1 receptors within myenteric ganglia shown in an immunohistochemical study (Kirchgessner and Liu, 1999) may correspond to enteric cholinergic nerve cells. Both orexin receptors, orexin-1 and orexin-2 receptors, have been shown to be present in the gastrointestinal tract, including myenteric ganglia (Kirchgessner and Liu, 1999). The orexin-2 receptor within the myenteric ganglia may be located on nerve cells other than cholinergic neurons.

It has been proposed that orexin-containing neurons play a role in intestinal secretion, based on the immunohistochemical findings that submucosal VIP-containing neurons costore orexin and the electrophysiological findings that orexin evokes spike activity in VIP secretomotor neurons (Kirchgessner and Liu, 1999). VIP is known to be an inhibitory neurotransmitter in the gastrointestinal tract, while the peptide has been shown to produce a contractile response mediated by stimulation of cholinergic neurons only in the guinea pig ileum (Cohen and Landry, 1980; Kusunoki et al., 1986). Furthermore, orexin-containing enterochromaffin cells contain serotonin, and orexin nerve fibers innervate the orexin/serotonin-containing enterochromaffin cells (Kirchgessner and Liu, 1999). Orexin-A has been shown to excite serotonergic neurons in the rat dorsal raphe nucleus (Brown et al., 2001). Serotonin is well known to control the motility of the gastrointestinal tract through

stimulation and inhibition of various enteric neuronal activities (Taniyama et al., 2000). In the present study, the orexin-A-induced contractions were not affected by an antagonist of VIP and a mixture of antagonists of serotonin receptors; thus, orexin-A may express the contractile effect mediated by stimulation of the cholinergic neurons in the guinea pig small intestine, not through VIP and serotonin. As orexin has been shown to decrease the amplitude of evoked slow IPSPs which are mediated by stimulation of α_2 adrenoceptor (Kirchgessner and Liu, 1999), there is a possibility that disinhibition by orexin of adrenergic regulation of cholinergic nerve activity adds to the orexin-evoked acetylcholine release and contraction. There is a circuit, with orexin as focus, within the hypothalamus related to energy homeostasis and endocrine regulation (Horvath et al., 1999). The interaction of orexin and the enteric nervous system is the subject of ongoing study.

Acknowledgements

This work was supported by grants from the Ministry of Education, Science, Sports, and Culture, Japan.

References

Akehira, K., Nakane, Y., Hioki, K., Taniyama, K., 1995. Site of action of galanin in the cholinergic transmission of guinea pig small intestine. Eur. J. Pharmacol. 284, 149–155.

Brown, R.E., Sergeeva, O., Eriksson, K.S., Haas, H.L., 2001. Orexin A excites serotonergic neurons in the dorsal raphe nucleus of the rat. Neuropharmacology 40, 457–459.

Chemelli, R., Willie, J., Sinton, C., Elmquist, J., Scammell, T., Lee, C., Richardson, J., Williams, S., Xiong, Y., Kisanuki, Y., Fitch, T., Nakazato, M., Hammer, R., Saper, C., Yanagisawa, M., 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98, 437–451.

Cohen, M.L., Landry, A.S., 1980. Vasoactive intestinal polypeptide: increased tone, enhancement of acetylcholine release, and stimulation of adenylate cyclase in intestinal smooth muscle. Life Sci. 26, 811–822.

Date, Y., Ueta, Y., Yamashita, H., Yamaguchi, H., Matsukura, S., Kangawa, K., Sakurai, T., Yanaagisawa, M., Nakazato, M., 1999. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proc. Natl. Acad. Sci. U. S. A. 96, 748–753.

Edwards, C.M., Abusnana, S., Sunter, D., Mutphy, K.G., Ghatei, M.A., Bloom, S.R., 1999. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J. Endocrinol. 160, R7–R12.

Gonella, J., Niel, J.P., Roman, C., 1980. Mechanism of the noradrenergic motor control on the lower oesophageal sphincter in the cat. J. Physiol. (Lond.) 306, 251–260.

Horvath, T., Diano, S., van den Pol, A., 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.

Ida, T., Nakahara, K., Nakahara, K., Katayama, T., Murakami, N., Nakazato, M., 1999. Effect of lateral cerebroventricular injection of the appetite-stimulating neuropeptide, orexin and neuropeptide Y, on the various behavioral activities of rats. Brain Res. 821, 526–529.

Kan, S., Niwa, M., Taniyama, K., 1994. Specific receptor for vasoactive intestinal contractor in myenteric cholinergic neurons. Eur. J. Pharmacol. 258, 139–143.

Kirchgessner, A.L., Liu, M.-T., 1999. Orexin synthesis and response in the gut. Neuron 24, 941–951.

Kusunoki, M., Tsai, L.H., Taniyama, K., Tanaka, C., 1986. Vasoactive intestinal polypeptide provokes acetylcholine release from the myenteric plexus. Am. J. Physiol. 251, G51–G55 (Gastrointest. Liver Physiol. 14).

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qin, X., de Jong, P.J., Nishino, S., Mignot, E., 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 98, 365–376.

López, M., Senaris, R., Gallego, R., Garcia-Caballero, T., Lago, F., Seoane, L., Casanueva, F., Dieguez, C., 1999. Orexin receptors are expressed in the adrenal medulla of the rat. Endocrinology 140, 5991–5994.

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.

Peyron, C., Tighe, D., van den Pol, A., de Lecea, L., Heller, H., Sutcliffe, J., Kilduff, T., 1998. Neurons hypocretin (orexin) project to multiple neuronal systems. J. Neurosci. 18, 9996–10015.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R., Tanaka, H., Williams, S., Richardson, J., Kozlowski, G., Wilson, S., Arch, J., Buckingham, R.E., Haynes, A., Carr, S., Annan, R., MacNutty, D., Li, W., Terrett, J., Elshourbagy, N., Bergsma, D., Yanagisawa, M., 1998. Orexins and orexins receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. Cell 92, 573–585.

Sawa, T., Mameya, S., Yoshimura, M., Itsuno, M., Makiyama, K., Niwa, M., Taniyama, K., 1995. Differential mechanism of peptide YY and neuropeptide Y in inhibiting motility of guinea-pig colon. Eur. J. Pharmacol., 223–230.

Smart, D., Sabido-David, C., Brough, S.J., Jewitt, F., Johns, A., Porter, R.A., Jerman, J.C., 2001. SB-334867-A: the first selective orexin-1 receptor antagonist. Br. J. Pharmacol. 132, 1179–1182.

Soffin, E.M., Evans, M.L., Gill, C.H., Harries, M.H., Benham, C.D., Davies, C.H., 2002. SB-334867-A antagonizes orexin mediated excitation in the locus coeruleus. Neuropharmacology 42, 127–133.

Starke, K., 1981. Presynaptic receptors. Annu. Rev. Pharmacol. Toxicol. 21, 7–30.

Sweet, D.C., Levine, A.S., Billington, C.J., Kotz, C.M., 1999. Feeding response to central orexins. Brain Res. 821, 535–538.

Taniyama, K., Makimoto, N., Furuichi, A., Sakurai-Yamashita, Y., Nagase, Y., Kaibara, M., Kanematsu, T., 2000. Functions of peripheral 5-hydroxytryptamine receptors, especially 5-hydroxytryptamine4 receptor, in gastrointestinal motility. J. Gastroenterol. 35, 575–582.

van den Pol, A., 1999. Hypothalamic hypocretin (orexin): robust innervation of the spinal cord. J. Neurosci. 19, 3171–3182.

Vizi, E.S., Bertaccini, G., Impicciatore, M., Knoll, J., 1973. Evidence that acetylcholine release by gastrin and related polypeptides contributes to their effects on gastrointestinal motility. Gastroenterology 64, 268–277.

Vizi, E.S., Ono, K., Adam-Vizi, V., Duncalf, D., Foldes, F., 1984. Presynaptic inhibitory effect of Met-enkephalin on [14C]acetylcholine release from the myenteric plexus and its interaction with muscarinic negative feedback inhibition. J. Pharmacol. Exp. Ther. 230, 493–499.

Willie, J.T., Chemelli, R.M., Sinton, C.M., Yanagisawa, M., 2001. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu. Rev. Neurosci. 24, 429–458.