REVIEW

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Role of orexins in regulation of gastrointestinal motility

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Abstract: Orexins (hypocretins) – hypothalamic peptides, appear to play an important role in the regulation of energy balance, feeding behaviour and sleep-wakefulness state. The presence of orexins and their receptors in the gastrointestinal tract argues also for a local action of these neuropeptides. The structure of orexin A and orexin B molecules is similar and these peptides act via two orexin receptors OX1R and OX2R. Orexin actions on gastrointestinal motor activity are complex and can involve central and peripheral mechanisms. The reported data suggest that orexins acting centrally within the dorsomedial nucleus exert a potent increase of gastric motility. Orexins also modulate small bowel motility through peripheral actions. These neuropeptides significantly increase the intestinal motility in isolated segments of the small bowel and colon. Furthermore, orexin A and orexin B increase the length of the MMC cycle. Orexins might therefore be included into the group of regulatory peptides controlling digestive tract motility.

Key words: orexin (hypocretin), orexin receptors, gastrointestinal motility, migrating motor complex

INTRODUCTION

Orexins represent the group of neuropeptides which are synthesized in the neurons of the lateral hypothalamus, and are therefore also defined as hypocretins. In 1998, two independent groups of researchers discovered two new peptides and their receptors utilizing different methodology [1, 2]. These peptides were called orexins and hypocretins. Precise genetic analysis, however, showed that the molecular structure of these peptides was identical [1, 2]. Orexin release was proved to occur within the suprarenal medulla, pancreatic islets and testes [1-5]. These neuropeptides are engaged especially in sleep regulation and the maintenance of energy balance [1, 4]. Furthermore, orexins interact with other endogenous substances to contribute in maintaining body homeostasis [4-7]. The concentration of orexins in the blood is dependent on total body metabolism [4]. Orexins, their precursors and orexin receptors are also located within the wall of the digestive tract [8]. Orexins are synthesized in the endocrine cells of the stomach and intestine [8-9]. Orexin-containing neurons and orexin receptors were found in the gut of mice, rats, guinea pigs, and also in man. Some studies exhibited possible interaction of orexin-containing neurons and enteric cholinergic neurons [10, 11]. Orexin-containing neurons have been found in submucosal and myenteric ganglia. Two types of orexins – orexin A (OXA) and orexin B (OXB) – were found. The half-life of OXA in the blood and cerebrospinal fluid is longer than OXB [10]. OXA was identified in submucosal neurons and the myenteric plexus of the ileum, where choline acetyltransferase (ChAT) is also present [13]. Some of submucosal neurons contain orexin and vasoactive intestinal peptide (VIP) [8, 10]. Thus it is possible that orexins participate in the control of gastrointestinal motility. Plasma orexin level is relatively high during the fasting state in man and rats [8, 12, 13]. Orexins are therefore a new group of gastrointestinal peptides, and can also be included among the peptides of the "brain-gut axis" [14]. The aim of this article is to present the current data summarizing the role of orexins in the regulation of gastrointestinal motor activity.

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CHARACTERISTICS OF OREXIN MOLECULES AND OREXIN RECEPTORS

Although first studies on orexins were performed in rats [1, 2], it is clear now that this group of newer neuropeptides occurs in man and various animal species. Orexins, like other hormonal peptides, are synthesized as inactive molecules, i.e. as pre-pro-orexins. Pre-pro-orexins are activated in proteolytic chain reactions. Secretion of orexin OXA and orexin OXB is thus performed *via* activation of pre-pro-orexins. OXA is a 33 amino acid peptide, while OXB is a 28 amino acid peptide [5]. Molecular structures of OXA and OXB reveal 46% of amino acid homology. Neurotensin, vasopressin, oxytocin, and glucagon-like peptide (GLP-1) were found to induce orexin synthesis [15-17]. Furthermore, the orexin release might be affected by the cholecystokinin and corticotropin-releasing factor [16, 17]. Serotonin, adrenaline, and probably leptin, belong to the inhibitors of orexin synthesis [6, 15-17]. OXA and OXB have different affinity to orexin receptors [2, 10]. Two subtypes of orexin receptors, such as OX1R and OX2R, were isolated in human and animal gastrointestinal systems [18-20]. OX1R and OX2R are G-protein coupled receptors and these two receptors have 65% structural homology [3]. Both OX1R and OX2R are present in the central nervous system and also in peripheral tissues [5, 21]. Mucosal endocrine cells contain OX2R receptors [22]. OXB can act mainly *via* OX2R, while OXA can act *via* both OX1R and OX2R receptors. OXB exerts a stronger effect in physiological conditions than OXA [10]. Orexin increased the cytosolic Ca^{2+} concentration in the

majority (80%) of ghrelin- responsive neuropeptide Y (NPY) neurons [6]. It is also possible that ghrelin and orexin can regulate the activity of NPY neurons in the hypothalamus [6]. NPY neurons in the hypothalamic arcuate nucleus (ARC) play an important role in the stimulation of food intake [6]. Ghrelin activates orexin neurons in the central nervous system [23]. Stimulation of food intake by ghrelin and orexin, together with their effect on gastrointestinal motility, may explain their combined effect on body mass gain [6, 23].

ROLE OF OREXINS IN REGULATION OF GASTRIC MOTILITY

Role of orexins in gastric contractility. In the course of some experiments the influence of orexins on gastric antral and fundic motor activity was shown in rats and mice [21, 24]. Exogenous OXA reduced the frequency of the gastric fundus contraction (relaxation of the proximal part of stomach) and increased the contractility of the distal part of the stomach after intracisternal administration [24]. Central action of OXA in the regulation of gastric motility *via* the vagus nerve was demonstrated [24]. The study of Kobashi et al. also showed that OXA induced relaxation of the proximal stomach lasting more than 30 min in rats [24]. Phasic contractions in the distal stomach were facilitated after orexin administration [24]. As reported by Krowicki et al., intravenous injection of OXA and OXB increased motility of the gastric antrum *via* activation of dorsal motor nucleus (DMN) of the vagal neurons after an overnight fast in rats [25]. When OXA and OXB were administered to the DMN, rostrally to the obex (at the dose 10 pmol/animal), antral motility increased. These authors also showed that OXA and OXB, at the same dose, increased the intragastric pressure. The highest dose of OXA, i.e. 100 pmol/animal, did not evoke significant changes in gastric contractility. When OXA was administered into the DMN, caudally to the obex, gastric motor function did not alter significantly. Excitation of gastric motor function evoked by OXA injected into the DMN was abolished by vagotomy [25]. OXA in mouse gastric fundus exerts a modulatory action on the nitrergic neurotransmission of postganglionic neurons [26]. Relaxation of the proximal stomach was observed after vagotomy [26]. Baccari et al. [26] have shown that nitric oxide is an important factor in the influence of orexins on gastric motility in the mouse [26]. The data suggests that the action of orexins in the regulation of gastric motility is control *via* the central nervous system.

Role of orexins in gastric emptying. The influence of orexins on gastric emptying was dependent upon the site of the injection of exogenous orexins into the DMN. When OXA was injected rostrally to the obex, gastric emptying was accelerated; however, injection of OXA ventrally to the obex altered the gastric emptying insignificantly [25]. Grabauskas et al. showed that the excitatory effects of OXA and OXB is accompanied by a prolonged neuronal membrane depolarization in rats [12]. OXA and OXB at doses oif 30-300 nM induced a slow depolarization, and increased firing in DMN neurons: 70% of neurons which projected to the gastric corpus and fundus and 3/13 of neurons which supplied the antrum/pylorus in rats [2, 10]. The study of Kirchgessner et al. showed that gastric emptying was not altered after intravenous injection of exogenous OXA at the dose 500 pmol/kg/min in fasting rats [14]. Ehrström et al., however, showed that peripheral OXA significantly decreased the rate of gastric emptying in man [27, 28]. These studies were performed after an overnight fast in both man and rats. Acceleration of gastric emptying was observed after intravenous injection of orexin-receptor antagonist, SB-334867-A, at the dose of 10 mg/kg in rats [10]. These results suggest that endogenous OXA influences gastric emptying in these animals [10]. In man, gastric emptying was not altered after intravenous injection of OXA at the dose of 10 mg/kg/min [10]. The lag-phase and half-emptying time were not affected by exogenous OXA [10, 27]. Only the transient inhibitory effect on the rate of gastric emptying was observed [10]. Gastric emptying is faster after orexins injections in the DMN [27]. In another study, Ehrström et al. also showed that endogenous OXA stimulates gastric emptying in non-fasted rats [28]. Gastric emptying was studied with a liquid non-nutrient or nutrient meal, using ^{51}Cr as radioactive marker. Plasma OXA levels decreased after intake of the nutrient meal and after injection of OX1R antagonist [28]. The influence of orexins on gastric motility, especially on gastric emptying, is regulated *via* the central nervous system [16]. Orexins are involved in the development of depression and gastrointestinal disorders, which are frequently accompanied by inhibition of the gastric and intestinal function because the lack of orexin activity might evoke inhibition of gastric function [29, 30-32].

ROLE OF OREXINS IN REGULATION OF INTESTINAL MOTILITY

Role of orexins in the regulation of small bowel motility. The experiments searching for the influence of orexins on small-intestinal motor activity were carried out both *in vivo* and *in vitro*. Korczyński et al. showed that OXA and OXB induce dose-dependent contractions of intestinal segments isolated from fasted and non-fasted rats [29]. In this protocol, the selective antagonist of OX1R, i.e. SB-334867-A, atropine, and tetrodotoxin (TTX) were also applied. SB-334867-A reduced contractions in the intestinal segment evoked by OXA injection in fasted and non-fasted rats. Orexin-induced contractions were not fully inhibited by TTX and atropine [29]. These results suggest that OXA and OXB stimulate motility *via* both myogenic and neural mechanisms. Korczyński et al. also showed that only partial inhibition of OXA-induced contractions was observed when SB-334867-A was used; however, OXB-induced contractions were not affected [29]. These authors reported that motility of the gastrointestinal tract is regulated by orexins with a predominant role of OXB [29]. It was recently reported by Matsuo et al. that OXA induced contractions of isolated preparations of guinea pig ileum [11]. They showed that OXA-induced contraction was TTX- and atropine-sensitive, while hexamethonium did not change OXA-induced contractions. This study suggests that OXA can act *via* OXR1 receptors located on the soma-dendritic regions of the enteric cholinergic neurons, and OXA can cause contractions of ileal smooth muscle cells. OXA may exert a contractile effect mediated by cholinergic neurons in guinea pig small bowel [11]. Immunohistochemical findings showed that submucosal VIP-containing neurons co-store orexin [11]. Orexin-containing enterochromaffin cells contain serotonin, and orexin nerve fibres innervate the orexin/serotonin enterochromaffin cells. The authors suggested that OXA-induced contractions were not affected by

an antagonist of VIP and serotonin receptors. Thus, Matsuo et al. showed that OXA cannot be mediated by VIP or serotonin [11]. VIP is a neuromodulator of inhibitory motor neurons in the myenteric plexus, and mediates relaxation of the circular muscle. OXA appears to mediate in part non-adrenergic non-cholinergic relaxation *via* the activation of nitric oxide synthase-containing myenteric neurons [33]. The authors showed co-localization of OXA and neuronal nitric oxide synthase in myenteric neurons of the duodenum and nerve fibres in circular muscle [33]. OXA could modulate motility by binding to the receptors located at neural synapses within the enteric plexus, enteric mucosa, and gut muscles [11]. Yazdani et al. showed the complex effect of orexins on gastrointestinal motility [34]. The study *in vitro* showed that OXA at the doses of 10 μM and 10 nM evoked a dose dependent increase in ileal muscle contraction in fed rats. Fasting for 24h led to a 60% reduction in OXA response. The authors showed also that TTX partially inhibited OXA-induced contractility. Yazdani et al. suggested that OXA induces motility both *via* neural and myogenic pathway [34].

Experiments *in vivo* revealed that OXA and OXB can affect small bowel motility *via* modification of the migrating motor complex (MMC) cycle length in rats [22]. The MMC is a specific motor pattern travelling along the gut. In man, dog, and rat, the MMC cycle is present only during fasting intestinal motility. The MMC cycle is characterized by three or four phases. In man and dog phase 3 of the MMC occurs every 90-150 min. In rats, phase 3 of the MMC occurs about every 15 min [10]. The MMC cycle length decreased after OXR1 antagonist SB-334867-A- administration at the dose 10 mg/kg [35]. Intravenous injection of orexins during the interdigestive period lengthened the time between two consecutive phases of the MMC cycle in rats, thus prolonging the whole cycle (Fig. 1).

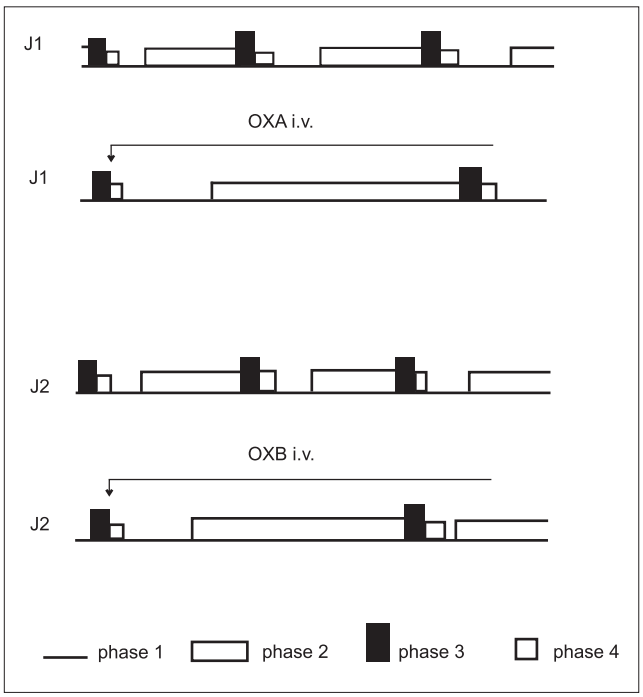

Figure 1 Schematic presentation of influence of orexin A and orexin B on migrating motoric complex (MMC) cycle length, and influence of these two orexins on each phase of the MMC cycle in rat ileum.
J1 – proximal segment of ileum; J2 – distal segment of ileum;
OXA – orexin A; OXB – orexin B;
i.v. – intravenously.
Time of recording – 40 min (adapted from 10,22,29,30,34).

This effect was dose dependent [10]. Higher doses of OXA – 50-5000 pmol/kg/min – evoked considerable alterations in the interdigestive motility since the MMC cycle was replaced by irregular spiking activity for more than 1h [10]. Vagotomy was not able to inhibit this effect.

Therefore, the peripheral action of orexins on small bowel motility was suggested [22].

Intravenous infusion of OXA and OXB produced an inhibitory effect similar to VIP on the MMC [22, 36]. Orexin nerve fibres in the circular muscle of the duodenum appear to contain VIP. Thus, the effect of OXA on the MMC might be mediated by VIP or, at least, involve the same mechanism as VIP [14, 22, 37]. However, the study *in vitro* showed that OXA cannot be mediated by VIP [11]. The effect of orexins on MMC cycle length can also be mediated by nitric oxide [10]. As reported by Satoh et al., OXA evoked non-adrenergic, non-cholinergic (NANC) inhibitory response in mouse small bowel [34]. This effect is evoked due to the activation of nitrergic neurons [33, 36]; however, the study did not explain how OXA releases nitric oxide from the nitrergic neurons. OXA probably activates nitrergic neurons directly or indirectly *via* activation of the interneuron in the gut. OXA modulate NANC-dependent relaxation of the longitudinal muscle in the mouse duodenum, jejunum and ileum [33]. Relaxation of the longitudinal muscle in the mouse small bowel was inhibited by TTX [33]. In another study, it was shown that NO contributes to the inhibitory effect of OXA on the MMC, and the effect of OXA on fasting motility is OX1R-specific [35]. The duration of the activity front and its propagation velocity was unchanged after OXA injected at various doses, 50-1,000 pmol/kg/min, in rats [35]. Näslund et al. also showed that there was no effect of OXA or OXB infused at the doses of 100 and 500 pmol/kg/min in rats on the duration and propagation velocity of the activity front [22]. Direct influence *via* orexin receptors is possible on smooth muscles; thus, orexins can influence peripherally on gastrointestinal motility [22,35]. Figure 2 presents two possible pathways of the influence of orexins on gastrointestinal motility [13,38,39].

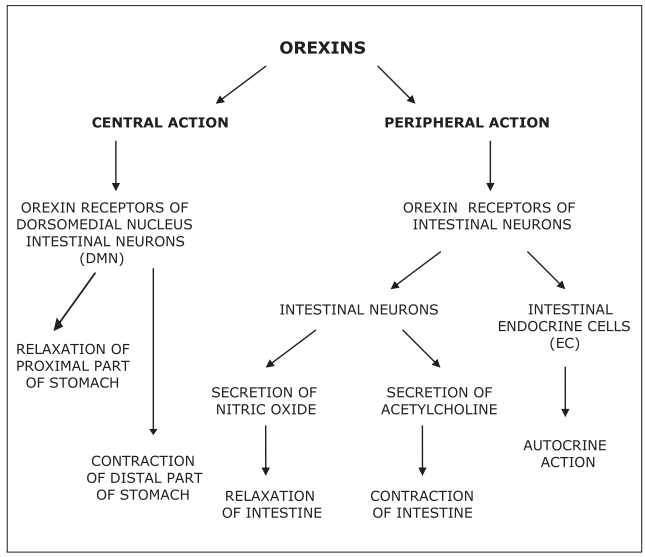

Figure 2 Possible mechanisms of action of orexins on gastrointestinal motility. (5,10,11,21,25,26,30,34,39).

Role of orexins in regulation of colonic motility. The studies *in vitro* showed the influence of orexins on the propulsion of the guinea pig distal colonic segments, possibly *via* the direct action on the myenteric plexus [8, 10]. These studies showed that this effect was dose-dependent. Incubation of the colonic segments for 10 min with OXA at the dose 0.3-30 nM caused a concentration-dependent-increase in the rate of propulsion. OXA stimulates colonic motility directly [8], an observation that suggested that enteric orexin receptors are present. This effect of orexin was also shown in the human colon [40]. Sanger et al. analysed the *in vitro* effect of cumulative concentration of OXA on isometric muscle tension and TTX-sensitive responses evoked by electrical field stimulation. OXA at the concentrations of 0.1, 1 and 10 μM increased muscle tone or initiated a slow contraction followed by prolonged facilitation of spontaneous contractile activity [40].

CONCLUSION

The reported influences of orexins on gastrointestinal motility suggest a role of these neuropeptides in this complex function. Orexins and orexin receptors are present throughout the human and animal gut. In majority of studies OXA was used in the experiments. Based on the localization of OXA and OXR and effects on gastric emptying rate or intestinal motility, it seems that endogenous OXA may have a role in modulating food intake and gastrointestinal function. However, some studies showed a predominant role of OXB in the regulation of gastrointestinal motility. Intravenous infusion of OXA and OXB produced a similar inhibitory effect to VIP on the MMC. In conclusion, orexins may influence the gastrointestinal motility as neuromodulators in the central nervous system. OXA could modulate motility also by acting on receptors located at nerve synapses within the enteric plexus, enteric mucosa and gut muscles. The site of the orexin action has not yet been precisely located. However, central and peripheral action of orexins on gastrointestinal motility revealed rather a modulatory than initiating role of these peptides. Detection of the mechanisms of action of many neuropeptides on gastrointestinal tract is helpful for understanding the control of gastrointestinal motility in the digestive and interdigestive periods in man and various animal species. Increasing knowledge concerning new isolated peptides provides more possibilities for looking for the tools useful for the treatment of various gastrointestinal diseases.

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