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Central orexin-A changes the gastrointestinal motor pattern from interdigestive to postprandial in rats

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ABSTRACT

Orexin-A, also described as hypocretin-I, was discovered in the extracts of the rat brain. OXA is implicated in a wide variety of physiological functions, such as feeding, arousal, behavioral activity, energy homeostasis and gastrointestinal motility. Orexin receptor type-1 is highly expressed in the dorsal motor nucleus of vagus. Although peripherally administered OXA abolishes small intestinal interdigestive contractions in rats, it still remains unclear whether central OXA affects interdigestive GI motility in rats. Two strain gauge transducers were attached on the antrum and duodenum to record circular muscle contractions. Spontaneous gastroduodenal contractions were recorded in freely moving conscious rats. OXA (1-20 μg) was administered intracerebroventricularly (icv). Atropine pretreatment (1 mg/kg, ip) and truncal vagotomy were performed to elucidate the neural pathways of central OXA. OXA (1–20 μg) dose-dependently disrupted the interdigestive phase III-like contractions and induced an irregular postprandial-like motility pattern in the stomach and duodenum. The stimulatory effect of OXA on gastroduodenal postprandial-like motility pattern was abolished by atropine and truncal vagotomy. Central administration (icv) of selective OXA receptor antagonist, SB-334867 (16 μg), enhanced gastric spontaneous phase III-like contractions. It is suggested that central OXA changes GI motor pattern from interdigestive to postprandial via the vagal cholinergic pathways. Endogenous OXA may have an inhibitory role in interdigestive GI motility in rats.

1. Introduction

Orexins were initially isolated from a group of neurons, located in the lateral hypothalamic area, the region known as the feeding center (de Lecea et al., 1998; Sakurai et al., 1998). The orexin neuropeptide family consists of orexin-A (OXA) and orexin-B (OXB), which are coded from the same prepro-mRNA (Sakurai, 1999; Sakurai et al., 1999). The orexin receptors, OX1R and OX2R, have a G-protein-coupled receptor structure and display 64% homology in their amino acid sequences. OX1R preferentially binds OXA, while OX2R binds both OXA and OXB with similar affinity (Korczynski et al., 2006). The expression patterns for OX1R and OX2R are strikingly different in central nervous system (CNS). OX1R mRNA is most abundant in the ventromedial hypothalamic nucleus whereas OX2R mRNA is predominantly expressed in the paraventricular nucleus (Korczynski et al., 2006; Trivedi et al., 1998).

As orexin-producing neurons project in a wide range of brain regions, the orexinergic system is involved in not only feeding behavior but also in sleep/wakefulness and energy homeostasis (Peyron et al., 1998). Moreover, orexins regulate gastrointestinal (GI) functions through the brain–gut axis (Kirchgessner, 2002; Kukkonen et al., 2002).

The accumulating evidences suggest that central OXA acts on GI motor functions through the vagal pathways. Orexin receptor type 1 (OXR1) is highly expressed in the dorsal motor nucleus of vagus (DMV) (Paranjape et al., 2007). OXA excites the gastric-projecting vagal motor neurons (Grabauskas and Moises, 2003). Microinjection of OXA into the DMV increased intragastric pressure and antral motility in anesthetized rats (Krowicki et al., 2002). Intracisternal administration of OXA induces relaxation of the proximal stomach, while it facilitates the distal stomach contractions in anesthetized rats. Both of the relaxation and contractions induced by OXA were attenuated by vagotomy (Kobashi et al., 2002).

GI motility pattern in rats can be divided into the interdigestive and postprandial states. The interdigestive state is characterized by the migrating motor complex (MMC), while the postprandial stage is characterized by sustained and irregular contractions in the antrum and small intestine in rats (Ariga et al., 2008). The MMC pattern is disrupted by feeding. MMC occurs during the interdigestive state and consists of three distinct phases. Phase I (period of motor quiescence), phase II (period of irregular low-amplitude contractions) and phase III (period of regular high level contractions) (Szurszewski, 1969).

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The effect of OXA on interdigestive GI motility has not been fully studied. Previous studies have reported that peripheral administration of OXA-induced an inhibitory effect on small intestinal MMC in rats (Ehrstrom et al., 2003; Naslund et al., 2002). However, it is not clear whether centrally administered OXA modulates the interdigestive motility of the GI tract. To investigate the effect of OXA, we studied 1) whether central OXA administration affects gastroduodenal interdigestive motility, 2) whether the effect of OXA is mediated by the vagal pathways and 3) whether central endogenous OXA has a modulatory role in gastroduodenal interdigestive motility in conscious rats.

2. Methods

2.1. Animals

Male Sprague–Dawley (SD) rats weighing 250–300 g were kept in individual cages under conditions of controlled temperature (22–24 °C), humidity and light (12 h light cycle starting at 7:00 AM) with free access to laboratory chow and water. All experiments were started at 9:00 AM every day. All animals were weighed before and after the experimental period. Animal protocols were approved by the Institutional Animal Care and Use Committee of Zablocki VA Medical Center at Milwaukee and carried out in accordance with the National Institute of Health 'Guide for the Care and Use of Laboratory Animals'. All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

2.2. Surgery

2.2.1. Icv cannula placement

Following an overnight fasting, rats were anesthetized with isoflurane (2%) and placed in a stereotaxic apparatus. After the skin and muscles of the head were dissected, a 24-gauge plastic sterile cannula was implanted in the right lateral ventricle (1.5 mm caudal, 2 mm lateral from the Bregma; 6 mm ventral from the skull surface), as previously reported (Ishiguchi et al., 2001). The cannula was fixed with cement (Kyowa Electronic Instruments, Tokyo, Japan) and acrylic resin (Shofu, San Marcos, CA). At the end of the experiment, the rats were euthanized by an intraperitoneal injection of pentobarbital (200 mg/kg). The implantation site of icv cannula was confirmed by the presence of Evans blue (5%; 1 µl) after injection via the cannula.

2.2.2. Implantation of the transducers

Through a midline laparotomy, the stomach and duodenum were exposed and two strain gauge transducers (6×4 mm) (Kyowa Electronic Instruments, Tokyo, Japan) were implanted on the serosal surface of the gastric antrum and duodenum, as previously reported (Taniguchi et al., 2008). The wires from transducers were exteriorized through the abdominal wall and tunneled subcutaneously toward the back where they were secured using a protective jacket (Star Medical, Tokyo, Japan).

To evaluate the mediation of the vagal nerve, subdiaphragmatic vagotomy was performed before the implantation of the transducers. Under the dissecting microscope, the lower part of the esophagus was exposed and anterior and posterior branches of the vagal nerves were incised above the hepatic and celiac branches, as previously reported (Taniguchi et al., 2008). Sham-operated rats served as the control. After the surgery, rats were housed individually, with access to a standard diet and tap water and they were allowed to recover for 1 week before the experiments.

Phase III-like contractions of the stomach are not always observed even after 24 h of fasting in rats (Ariga et al., 2007; Ariga et al., 2008). To optimize the phase III-like contractions, we developed a fixed feeding regimen in rats (Ariga et al., 2008). We have previously shown that the interdigestive phase III-like contractions were augmented 1–2 weeks after a fixed feeding regimen in rats. During the recovery period, the rats were fed with a fixed feeding regimen. The rats were trained to the assigned meal feeding regimen, once daily at 02:00 to 06:00 PM, as previously reported (Ariga et al., 2008).

2.3. Motility recording

After a 24 h-fasting with free access to water, gastric and duodenal contractions were measured in conscious, freely moving rats. The wires from the transducers were connected to a recording system (Power Lab model 8SP; ADI instruments, Colorado Springs, CO) and GI contractions were monitored for 3–4 h to observe spontaneous phase III-like contractions. Phase III-like contractions were defined as clustered contractions with amplitude of more than 4 g, as previously reported (Ariga et al., 2008; Taniguchi et al., 2008).

Central (intracisternal) administration of OXA (2.8 nmol; 10 µg) has been shown to stimulate contractions in the distal stomach (Kobashi et al., 2002). To investigate the effects of central OXA administration on interdigestive motility, OXA (1–20 µg) was administered into the right lateral ventricle (icv). To study whether the cholinergic pathways are involved in mediating the OXA-dependent effects on MMC, atropine (1 mg kg^{-1}, ip) was administered 10 min before OXA injection.

A selective OX1R antagonist, SB-334867 (16 µg; icv), was used to block the effect of central endogenous OXA. The dose of SB-334867 was applied according to a previous report in rats (Kushikata et al., 2003).

2.4. Chemicals

All drugs were prepared on experiment days, immediately before administration. Atropine (Sigma, St. Louis, MO) was also dissolved in sterile saline; whereas, OXA (Sigma, St. Louis, MO) and SB-334867 (Tocris Cookson, Ellisville, MO) were dissolved in sterile artificial cerebrospinal fluid (Harvard Apparatus, Holliston, MA) and dimethyl sulfoxide (Sigma, St. Louis, MO), respectively. Icv injections were performed in a volume of 5 µl with a Hamilton syringe; whereas atropine was administered intraperitoneally in a volume of 300 µl with an insulin syringe.

2.5. Statistical analysis

The quantification of gastroduodenal motility was evaluated by calculating the motility index (area under the curve) with computer-based software (Power Lab; AD Instruments, Colorado Springs, CO), as previously reported (Ariga et al., 2007). The motility index change in response to OXA was compared to the motility index of phase I-like contractions during the baseline recording.

Results were expressed as mean ± standard error (SE). The non-parametric Mann–Whitney *U*-test was used to determine the differences among the experimental treatments. The repeated-measures ANOVA was also used in the dose–response study. A p-value <0.05 was considered to be statistically significant.

3. Results

3.1. Effect of feeding on gastroduodenal fasting motility pattern

Spontaneous gastric and duodenal phase III-like contractions were recorded in freely moving conscious rats. Immediately after feeding, GI motility pattern was changed from interdigestive to postprandial (Fig. 1).