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GASTRIC ACID SECRETION INDUCED BY PARAVENTRICULAR NUCLEUS MICROINJECTION OF OREXIN A IS MEDIATED THROUGH ACTIVATION OF NEUROPEPTIDE YERGIC SYSTEM

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Abstract—Very recently, we have reported that the modulatory effect of PVN on gastric acid secretion may be mediated through the orexin fibers and/or orexin-responsive neurons. In this study, we address the hypothesis which demonstrates the existence of a putative orexin A– neuropeptide Y Y1/Y5 receptors interaction to increase gastric acid secretion in pyloric-ligated conscious rats. Male Wistar rats were implanted with guide canula directed to the PVN and lateral ventricle. Intracerebroventricular (ICV) microinjections of GR-231118 (Y1 receptor antagonist) and CGP-71683 (Y5 receptor antagonist) on gastric acid secretion were considered. The effect of pretreatment with Y1 receptor antagonist, GR-231118, and Y5 receptor antagonist, CGP-71683, on PVN orexin A-induced acid secretion was assessed. Gastric acid secretion was measured using the pylorus-ligation method, and the amount of gastric acid was determined by titration with 0.01 N NaOH to a pH of 7.0. Key results: ICV microinjections of GR-231118 and CGP-71683 decreased acid secretion by 25 ± 0.05% and 67 ± 0.02%, respectively. ICV microinjections of GR-231118 and CGP-71683 inhibited effects of PVN-injected orexin-A on acid secretion. We suggest that Y1 and Y5 receptors stimulate gastric acid secretion and the stimulatory effect of PVN orexin receptors on gastric acid secretion may be mediated via interactions, at least in part, through activation of Y1 and Y5 receptors. These neural pathways may play key roles in the orexinergic action of orexins in the cephalic phase of gastric acid secretion.

Key words: orexins, NPY Y1 and Y5 receptors, hypothalamic paraventricular nucleus, gastric acid secretion, pylorus ligation.

INTRODUCTION

The cephalic phase of gastrointestinal secretion produces a coordinated secretory response that primes the gut to assist digestion of impending meal. The most important component of the cephalic phase response is gastric acid secretion. Little is known about the precise molecular mechanism of cephalic phase stimulation. Orexin A and neuropeptide Y (NPY) may be important candidates as mediators of the cephalic phase secretory response to feeding.

Matsuda et al. (1991) demonstrated that centrally administered NPY stimulated gastric acid and pepsin secretion in anesthetized rats (Matsuda et al., 1991). Furthermore, Geoghegan et al. (1993) indicated that intracerebroventricular (ICV) NPY increased sham feeding, gastric and pancreatic secretion, and insulin levels in dogs (Geoghegan et al., 1993). These data and others (Lee et al., 1994) support that central NPY functions as a modulator of cephalic phase acid secretion.

Central administration of orexin A has been shown to stimulate gastric acid secretion (Takahashi et al., 1999; Haynes et al., 2000; Okumura et al., 2001). Furthermore, we showed that orexin A acts, in part, on the ventromedial hypothalamus (VMH) (Eliassi et al., 2009) to stimulate acid secretion. In addition, it has been demonstrated that PVN is a site for modulation of the hypothalamic control of gastric acid secretion (Shiraishi, 1987, 1988). However, little is known about the mechanisms by which stimulation of PVN neurons increases gastric acid production in a molecular/neurotransmitter basis. Very recently, our results demonstrated that the modulatory effect of PVN on gastric acid secretion may be mediated, at least in part, through orexin fibers (orexin-sensitive neurons) and orexin-1 receptors (Chaleek et al., 2012). Morphological and functional interactions between orexinergic pathways have indeed been demonstrated between NPY and orexins (Horvath et al., 1999; Dube et al., 2000; Jain et al., 2000; Yamanaka et al., 2000) in rodents. Several evidences suggest that the action of NPY in the hypothalamus may involve orexin neurons (Niimi et al., 2001). In addition, the effects of orexin A are mediated, in part, by the NPY pathway (Dube et al., 2000; Ida et al., 2000; Jain et al., 2000). Furthermore, there are some evidences to show that the Y1 and Y5 receptors have been implicated in feeding (Gerald et al., 1996). These findings led us to a speculation that interaction between the PVN orexin fibers (and/or orexin-responsive neurons) and Y1/Y5 receptors may play a role in gastric secretion. Therefore, we examined in the present study the hypothesis that PVN-microinjected orexin A affects gastric secretory response, at least in part, through Y1/Y5 receptors in conscious rats.

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Abbreviations: DMSO, dimethyl sulfoxide, ICV, intracerebroventricular; NPY, neuropeptide Y; VMH, ventromedial hypothalamus.

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EXPERIMENTAL PROCEDURES

Ethical considerations

All experiments were executed in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences (1100/1-87/11/21; 2008).

Animals

Male Wistar rats (Pasteur Institute of Iran, Tehran, Iran), weighing between 180 and 200 g were housed with their dames and were exposed to a 12:12-h light/dark cycle at 22–24 °C and were fed laboratory standard chow and water *ad libitum*. They were deprived of food, but not water, for 20–24 h prior to experiments.

Drugs

Orexin-A (human/bovine/rat/mouse), GR-231118, CGP-71683, dimethyl sulfoxide (DMSO), [CPP^{1-7}, NPY^{19-23}, Ala^{31}, Aib^{32}, Gln^{34}] – pancreatic polypeptide and [Pro^{34}] – peptide YY were purchased from Sigma (St. Louis, MO, USA). Ketamine and xylazine were purchased from Rotex (Levallois-Perret, France) and Alfasan (Alfasan, Woerden, The Netherlands), respectively. Orexin A, GR-231118, [CPP^{1-7}, NPY^{19-23}, Ala^{31}, Aib^{32}, Gln^{34}] – pancreatic polypeptide and [Pro^{34}] – peptide YY were administered in 0.9% saline and CGP-71683 was dissolved in DMSO (Sigma, St. Louis, MO, USA).

Injection of compounds

Drugs or vehicle was injected in a volume of 0.5 and 5 μl into the PVN and ICV, respectively. The drug injections were performed under brief ether anesthesia using Hamilton 0.5 and 5 μl syringes through Silastic tubing.

Surgery

Rats were anesthetized with a mixture of ketamine/xylazine and fitted with a 23-gauge stainless steel canula placed just above the right PVN using the stereotaxic atlas of Paxinos and Watson (2007). Coordinates of the tip of the guide canula were as follows: lateral: 0.4 mm from midline; dorsoventral: 7 mm from skull surface; anteroposterior: −1.8 mm from the bregma. During the same surgical session, a 23-gauge stainless steel guide canula was inserted in the lateral cerebral ventricle (lateral: 1.6 mm from midline; dorsoventral: 3 mm from skull surface; anteroposterior: −0.8 mm from the bregma) and affixed with cranioplastic cement. The injector was extended 1 mm beyond the end of the guide canula. After surgery, the animals were allowed a 7-day recovery period before experimental trials. Drugs or vehicles were injected in a volume of 0.5 (PVN) or 5 μl (ICV) over 30 s via a microsyringe pump (Stoelting, Lane Dale, IL, USA). After injection, the needle was left in place for an additional 30 s. Gastric acid secretion was measured using the pylorus-ligation method as described previously (Eliassi et al., 2009).

Treatments

In a first and a second set of experiments, we considered the effects of ICV-microinjected GR-231118 (Y1 receptor antagonist) and CGP-71683 (Y5 receptor antagonist) on gastric acid secretion and juice volume. Rats received ICV injections of 5, 7.5, 15, and 30 μg of GR-231118 or 5, 15 and 30 μg of CGP-71683 and 5 μl of saline or DMSO as control groups, respectively. In a third set of experiments, in order to consider the interaction between orexin A receptor and Y1 receptor on gastric acid secretion, we examined the effect of Y1 receptor antagonist, GR-231118 on orexin A-induced acid secretion and juice volume. Five min after ICV injection of GR-231118 (5 μg; ineffective dose) or saline (control group), orexin-A (5 μg) was microinjected into the PVN. The dose of orexin A (5 μg) was based on that used in our previous study (Chaleek et al., 2012). In a fourth set of experiments, the effects of the selective Y5 receptor antagonist, CGP-71683, on stimulatory action of orexin A on gastric acid secretion and juice volume were evaluated. Finally, in a fifth set of experiments, the effects of Y1/Y5 receptor agonist, [Pro^{34}] – peptide YY, and the selective Y5 receptor agonist, [CPP^{1-7}, NPY^{19-23}, Ala^{31}, Aib^{32}, Gln^{34}] – pancreatic polypeptide, on gastric acid secretion were determined. Rats received PVN injections of 1.5 and 3 nmol of [Pro^{34}] – peptide YY (Corpa et al., 2001) and 6, 12 and 24 nmol of [CPP^{1-7}, NPY^{19-23}, Ala^{31}, Aib^{32}, Gln^{34}] – pancreatic polypeptide or 0.5 μl of saline as control groups. In all the aforementioned experiments, gastric contents were collected after one hour to determine gastric output and juice volume. All experiments were performed at 9:00 AM.

Measurements of gastric acid output

The stomach was removed and the gastric contents were collected and centrifuged. The volume of gastric secretion was measured and the amount of gastric acid was determined by titration with 0.01 N NaOH to a pH of 7.0 (Sartorius-professional meter PP-25, Germany).

Statistical analysis

Results are shown as mean ± SEM. The differences between groups were checked by a one-way ANOVA followed by Tukey's HSD test. Student's unpaired *t*-test was used to evaluate differences between two groups. $P < 0.05$ was considered statistically significant.

Histology

The brains were removed and fixed in formalin after each experiment. For histological examination of canula and injection placement in PVN, 100-μm thick sections were taken and canula and injection tracks were examined with light microscopy. The data reported here are only from animals in which the placements of canula were histologically verified (Fig. 1A). The total number of rats injected in the PVN was 54. Six rats were excluded due to canula misplacement (Fig. 1B). The injections outside of the PVN did not have significant effects on gastric acid secretion.

RESULTS

Effects of ICV-microinjected GR-231118 (Y1 receptor antagonist) on gastric acid secretion and juice volume

GR-231118 decreased gastric acid secretion and juice volume in male rats. Gastric acid output decreased in the presence of 7.5, 15 and 30 μg of GR-231118, but not 5 μg of GR-231118. In the presence of 15 μg of GR-231118, acid output reached 81.7 ± 6.3 μE/h, which was significantly different compared with acid output in the control (saline) group (104.8 ± 8.4 μEq/h) ($P < 0.001$). Fig. 2A shows the inhibitory effect of