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Intracisternal injection of orexin-A prevents ethanol-induced gastric mucosal damage in rats

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Background. Accumulating evidence indicates that orexin-A in the brain stimulates vagal flow projecting to the stomach. Since the vagal system plays an important role in gastric mucosal integrity, we hypothesized that orexin-A in the brain might have a gastroprotective action. ***Methods.*** We examined the effect of centrally administered orexin-A on the development of gastric mucosal damage evoked by ethanol and its possible mechanism of action in rats. ***Results.*** Intracisternal but not intraperitoneal injection of orexin-A significantly inhibited the severity of gastric mucosal damage by 70% ethanol in a dose-dependent manner, suggesting that orexin-A acts in the brain to prevent ethanol-induced gastric mucosal damage. The antiulcer action was observed in rats administered with orexin-A centrally but not orexin-B, indicating that the action is mediated through orexin 1 receptors. The gastroprotective action of centrally administered orexin-A was blocked by pretreatment with atropine, N^{ω}-nitro-L-arginine methylester, or indomethacin. ***Conclusions.*** These results suggest that orexin-A acts on orexin 1 receptors in the brain to exert a gastroprotective action against ethanol. The vagal muscarinic system, nitric oxide, and prostaglandins may mediate the cytoprotective action of centrally administered orexin-A.

Key words: orexin-A, brain, gastroprotection, prostaglandins, nitric oxide

Introduction

Orexins/hypocretins are novel neuropeptides that are localized in neurons in the lateral hypothalamus.[1,2] On the other hand, orexin-immunoreactive fibers and terminals, and specific orexin receptors, are distributed in a wide variety of nuclei in the central nervous system.[3,4] Based upon this neuroanatomical evidence, orexinergic projection should be involved in a number of biological functions. To date, it has been demonstrated that orexins may be implicated in a wide variety of physiological functions, including feeding,[1,5,6] behavioral activity,[7] the sleep/awake cycle,[8–10] anxiety,[11] energy balance,[12] neuroendocrinological response,[13] and cardiovascular functions.[14,15] In addition to these functions, we have demonstrated that orexin-A is involved in central regulation of gastric acid secretion.[16–18] Central but not peripheral injection of orexin-A dose-dependently stimulates gastric acid secretion in conscious rats.[16] Acid stimulation by centrally administered orexin-A was completely blocked by atropine or surgical vagotomy, suggesting that orexin-A acts in the brain to stimulate gastric acid secretion through the vagal system. Considering the potent orexigenic action of orexin-A, orexin-A may be an important candidate as a mediator of cephalic-phase secretion, as proposed by Pavlov.[19] The vagal-dependent stimulation of gastric acid secretion by orexin-A furthermore supports the hypothesis that orexin-A plays a vital role in cephalic-phase gastric secretion, because the importance of the vagus in conveying the neural impulses that mediate cephalic-phase gastric secretion has been recognized.[20]

The vagal system is involved in not only the regulation of gastric acid secretion but also maintaining gastric mucosal integrity. For instance, vagal stimulation induced by 2-deoxy-D-glucose prevents ethanol-induced lesions in intact but not vagotomized rats.[21] Recent accumulating evidence[22,23] that orexin-A acts centrally in the brain to influence vagal tone led us to speculate that brain orexin-A might possess gastroprotective action through modulation of vagal tone. The aims of the present study were (1) to examine whether orexin-A in the brain exerts gastroprotective action against ethanol-induced gastric mucosal damage and (2) to investigate

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the mechanisms underlying the protective effect of orexin-A.

Materials and methods

Animals

Male Sprague-Dawley rats weighing approximately 120 g were housed under controlled light/dark conditions (lights on, 07:00–19:00) with the room temperature regulated to 23°–25°C. Rats were allowed free access to standard rat chow (Solid rat chow, Oriental Yeast, Tokyo, Japan) and tap water. All experiments were performed in conscious animals deprived of food for 24 h but with free access to water up to the initiation of the experiments.

Chemicals

Synthetic orexin-A (human/bovine/rat/mouse) and orexin-B (human/rat) were purchased from the Peptide Institute, Osaka, Japan, and were dissolved in normal saline just before the experiments. Atropine sulfate and N^{ω}-nitro-L-arginine methylester (L-NAME), an inhibitor of nitric oxide (NO) synthase, obtained from Sigma (St Louis, MO, USA) were dissolved in saline and injected subcutaneously at 1 ml/kg. Indomethacin was purchased from Sigma, dissolved in 7% sodium bicarbonate solution, and injected intraperitoneally at 1 ml/kg.

Treatments

Gastric mucosal damage was induced by 1 ml of 70% ethanol through an oroesophageal tube. Rats were killed 60 min after ethanol administration, and their stomachs were removed and examined for mucosal lesions. We initially examined the dose-related effects of an intracisternal injection of orexin-A on the severity of gastric mucosal lesions by ethanol. As a control, we examined whether intracisternal injection of orexin-A by itself, without administration of ethanol, was capable of inducing gastric mucosal damage. All animals received intracisternal injection (10 µl) of several doses of orexin-A. Intracisternal injection was performed under brief ether anesthesia with a 10-µl Hamilton microsyringe after rats were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) as previously described.[24] Following the intracisternal injection, rats were returned to their cages. One hour after the treatment, rats were administered 70% ethanol, and 1 h later the stomach was removed. Effects of intracisternal injection of orexin-B or intraperitoneal administration of orexin-A on the severity of gastric mucosal damage induced by 70% ethanol were similarly examined.

Effects of atropine, L-NAME, or indomethacin on the gastroprotective action of orexin-A were examined to clarify possible mechanisms by which orexin-A exerts cytoprotective action against ethanol. Rats received each chemical subcutaneously or intraperitoneally, and 60 min after the administration, 70% ethanol was given to the stomach. One hour after ethanol was given by intubation, the stomach was removed. The doses of atropine (2 mg/kg), L-NAME (70 mg/kg), or indomethacin (5 mg/kg) were selected on the basis of previous studies.[25–27]

Assessment of gastric mucosal lesions

Gastric lesions were assessed macroscopically. Each stomach was opened along the greater curvature, gently rinsed in saline, opened to expose the mucosa, and photographed with a digital camera. The lesion area out of the total area of corpus was calculated, and the percentage of the total area occupied by the lesion was determined as the severity of the gastric lesions.

Statistical analysis

The results are expressed as means ± SEM. Statistical analysis comprised analysis of variance and subsequent Fisher's LSD test. $P < 0.05$ was considered statistically significant.

Ethical considerations

Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the Public Health Service. The approval of the Research and Development and Animal Care committee at the Asahikawa Medical College was obtained for all studies.

Results

First, we examined whether intracisternal injection of orexin-A by itself induces gastric mucosal damage. Intracisternal injection of a 10-µg dose of orexin-A did not induce any gastric mucosal lesions in ten rats. Next, the effect of intracisternal administration of orexin-A on the severity of gastric mucosal damage caused by 70% ethanol was assessed macroscopically. Figure 1 shows the macroscopic appearance of a representative gastric mucosa in a rat treated with an intracisternal injection of saline or orexin-A at a dose of 10 µg. A 10 µg dose of orexin-A completely protected against ethanol-induced gastric mucosal lesions when compared with the saline control. As shown in the Fig. 2, the gastroprotective action of centrally administered orexin-A

Fig. 1. Representative macroscopic appearance of ethanol-induced gastric mucosal damage in rats injected intracisternally with either saline control (10 µl) or orexin-A (10 µg/10 µl). One hour after the injection, the animals were given 1 ml of 70% ethanol through an oroesophageal tube. One hour after ethanol, the animals were killed and the stomachs were removed. Each stomach was opened along the greater curvature

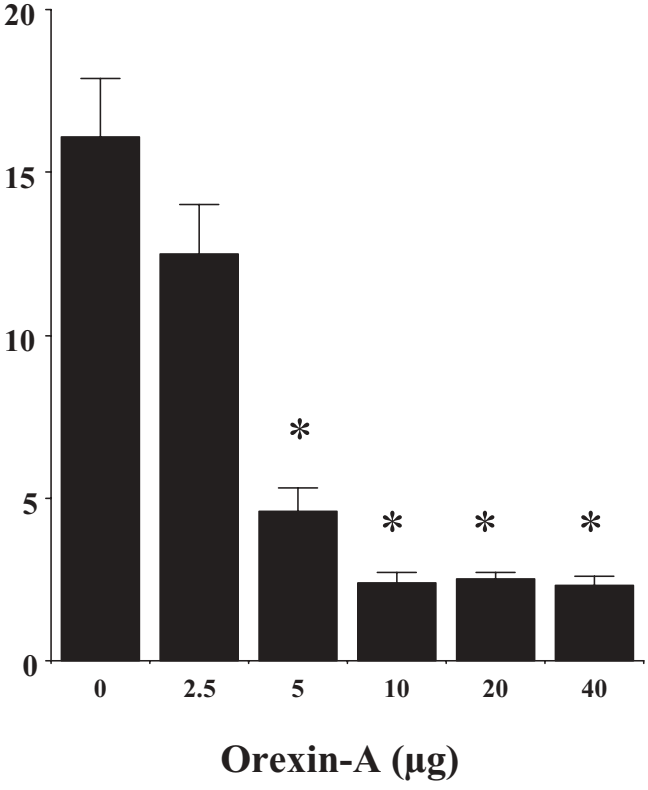

Fig. 2. Dose–response effect of intracisternal injection of orexin-A on the severity of ethanol-induced gastric mucosal damage. Each bar represents the mean ± SEM of 5–12 animals. *$P < 0.01$ compared with the control (orexin-A, 0)

was dose-dependent. To clarify whether orexin-A acts centrally to exert its gastroprotective action, we examined the effect of peripherally administered orexin-A on the development of gastric mucosal damage by ethanol. Intracisternal injection of orexin-A at a dose of 10 µg potently inhibited the severity of gastric mucosal lesions, while intraperitoneal injection of orexin-A at the same dose failed to protect against ethanol-induced gastric lesions (Table 1), suggesting that the gastroprotective action of orexin-A is mediated via the central nervous system.

We next compared the effects of orexin-A and orexin-B on the severity of ethanol-induced gastric mucosal damage. Intracisternal injection of orexin-A (10 µg) but not orexin-B (10 µg) suppressed the development of gastric mucosal lesions by ethanol (Fig. 3), indicating that orexin-A specifically exerts gastroprotective action against ethanol.

In the next step, we tried to clarify the mechanisms by which centrally administered orexin-A exerts gastroprotective action against ethanol. The role of the vagal–muscarinic system, nitric oxide, or prostaglandins in the gastroprotective action by orexin-A was evaluated. Rats were pretreated with atropine (2 mg/kg), L-NAME (70 mg/kg), or indomethacin (5 mg/kg), and the effect of orexin-A on the severity of gastric mucosal lesions by ethanol was assessed. As illustrated in Figs. 4 and 5 and Table 2, atropine, L-NAME, or indomethacin blocked the cytoprotective action by orexin-A.

Atropine, L-NAME, or indomethacin alone did not modify gastric lesions induced by ethanol, in agreement with previous reports.[25–28]

Discussion

The present study demonstrated for the first time that centrally but not peripherally administered orexin-A exerts a dose-dependent gastroprotective effect on ethanol-induced gastric mucosal damage, indicating that the site of action of orexin-A must be in the brain. It has been shown that a number of chemicals act centrally in the brain to exert gastroprotective action.[27,29–32] On the basis of the present evidence, we suggest that orexin-A should be listed as one of neuropeptides in the brain that have gastroprotective action against ethanol.

Orexin-A and orexin-B were initially identified as endogenous peptide ligands for two orphan G protein-coupled receptors.[1] In the present study, the effect of intracisternal injection of orexin-A or -B on the severity of gastric mucosal damage by ethanol was examined, and it was clearly demonstrated that the gastroprotective action was induced by orexin-A but not orexin-B. It has been previously shown that orexins bind to two specific receptors, named OX1R and OX2R. According to in vitro binding and functional assays, OX1R is selective for orexin-A and OX2R is nonselective, for orexin-A and orexin-B.[1] Based upon this finding, the lack of

Table 1. Effect of intraperitoneal injection of orexin-A on the severity of gastric mucosal damage induced by ethanol

	Number of animals	Gastric lesions (%)
Saline	5	12.3 ± 1.7
Orexin-A	5	13.2 ± 2.6

Table 2. Effect of intraperitoneal injection of indomethacin on the severity of gastric mucosal damage induced by ethanol

	Number of animals	Gastric lesions (%)
Vehicle (ip) + saline (ic)	5	14.4 ± 2.1
Indomethacin (ip) + saline (ic)	5	13.8 ± 1.9
Vehicle (ip) + orexin (ic)	7	3.4 ± 0.5*
Indomethacin (ip) + orexin (ic)	8	14.1 ± 1.6

ip, intraperitoneal; ic, intracisternal
* $P < 0.01$ compared with vehicle (ip) + saline (ic)

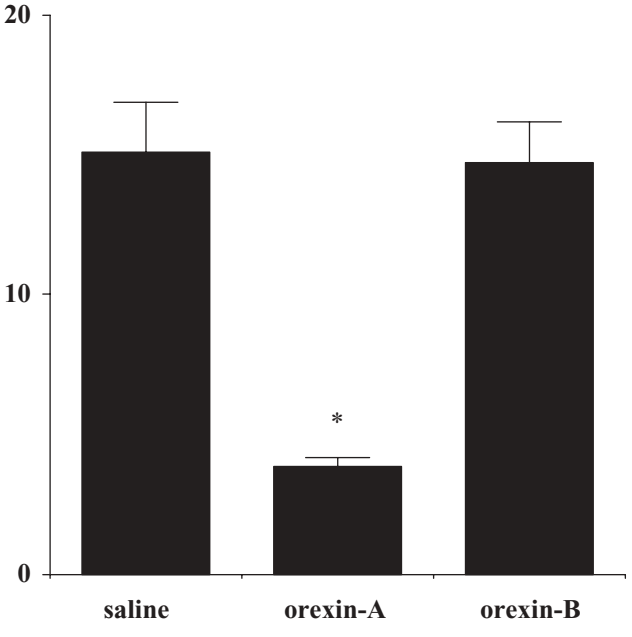

Fig. 3. Effect of intracisternal injection of orexin-A or orexin-B on the severity of ethanol-induced gastric mucosal damage. Each bar represents the mean ± SEM of 6 animals. *$P < 0.01$ compared with the saline control

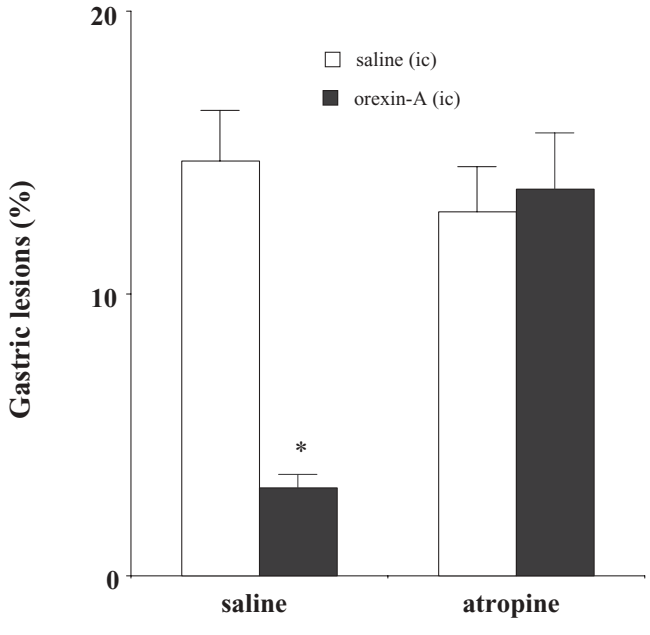

Fig. 4. Effect of atropine on the gastroprotection by centrally administered orexin-A. Each bar represents the mean ± SEM of 7 animals. *ic*, intracisternal. *$P < 0.01$ compared with the saline control

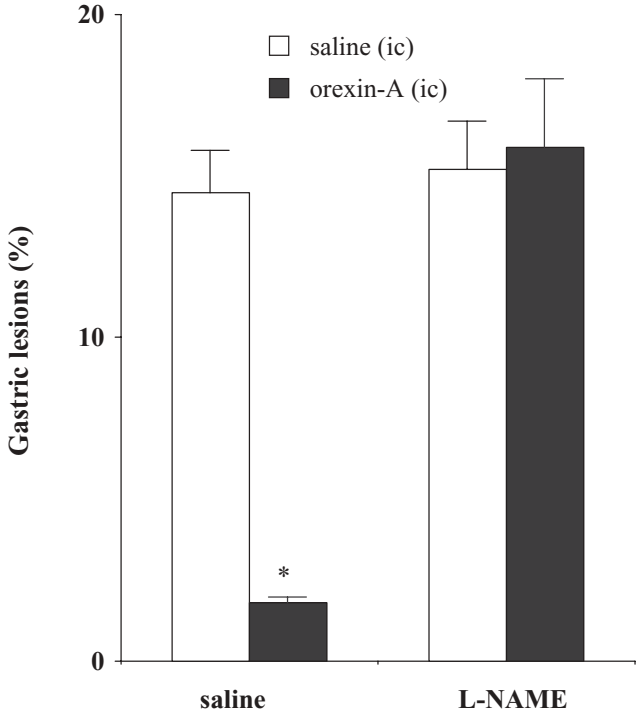

Fig. 5. Effect of N^{ω}-nitro-L-arginine methylester (*L-NAME*) on gastroprotection by centrally administered orexin-A. Each bar represents the mean ± SEM of 7 animals. $^{*}P < 0.01$ compared with the saline control

gastroprotective action of orexin-B suggests that the orexin-A-induced antiulcer action against ethanol may be mediated by OX1R.

The vagal system is involved in maintaining gastric mucosal integrity. For instance, vagal activation can exert gastroprotective action against ulcerogenic stress, including ethanol.[21,27] Since accumulating evidence indicates that orexin-A administered into the cerebrospinal fluid acts in the dorsal motor nucleus (DMN) of the vagus neurons in the medulla oblongata, cells of origin innervating the stomach through the vagus nerve, to activate vagal flow,[22,33,34] the gastroprotective action by centrally administered orexin-A may be mediated by the vagal system. In fact, the present study demonstrated that atropine completely blocked the gastroprotective action by centrally administered orexin-A, supporting the above speculation that the vagal system plays an important role in the gastroprotective action by centrally administered orexin-A.

It has been demonstrated that nitric oxide plays an important role in maintaining gastric mucosal integrity.[35] Kiraly et al.[36] have demonstrated that NO is involved in gastroprotection by central vagal stimulation, suggesting that gastric NO synthesis and release might be under vagal control. The gastroprotective action of centrally administered orexin-A appears to be mediated by the NO pathway because inhibition of NO synthesis by L-NAME completely abolished the gastroprotective effect of orexin-A, as shown in the present study. Interestingly, Farr et al.[37] have very recently demonstrated that subcutaneous injection of L-NAME-blocked orexin-A-induces an increase in food intake in rats, and orexin-A fails to increase food intake in NO synthase knockout mice. They further demonstrated that L-NAME drastically inhibits NO synthase activity in the hypothalamus. These results suggest that NO in the brain plays a vital role in the orexin-A-induced food consumption. The present data that L-NAME also blocked the gastroprotective effect by orexin-A raises the possibility that NO in the brain might also contribute to the antiulcer action by centrally administered orexin-A. According to the observation by Zheng et al.,[38] as many as 20% of hypothalamic orexin neurons project to the dorsal vagal complex, including the DMN neurons in the medulla, and some are in close anatomical apposition with nitric oxide synthase-immunoreactive neurons. These observations further support that NO is implicated in signal transduction from the vagal preganglionic neurons in the brain to the stomach to exert the gastroprotective action by centrally administered orexin-A.

Endogenous prostaglandins in the gastric mucosa are thought of as mediators of cytoprotection.[28] In fact, exogenously administered or endogenously released prostaglandins are well established to protect gastric mucosa against ethanol.[28] Yoneda and Tache[27,39] have demonstrated that endogenous prostaglandins are involved in gastroprotection by vagal stimulation by centrally administered thyrotropin-releasing hormone (TRH), suggesting that the vagus nerve plays a vital role in gastric prostaglandin synthesis and release. The gastroprotective action of centrally administered orexin-A appears to be mediated by endogenous prostaglandins, similarly to TRH, because inhibition of prostaglandin synthesis by indomethacin completely blocked the gastroprotective effect of orexin-A, as shown in the present study. In addition to endogenous prostaglandins, heat-shock protein, known to be a gastroprotective molecule, expressed in the gastric mucosa[40] might be related to the mechanism by which centrally administered orexin-A protects gastric mucosa against ethanol. Further studies are needed to clarify the above speculation.

In conclusion, the present study demonstrated for the first time that orexin-A acts in the brain to prevent ethanol-induced gastric mucosal damage. It is also suggested that OX1R in the brain, the vagal pathway, and endogenous NO and prostaglandins are implicated in gastroprotection by centrally administered orexin-A.

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