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Abstract

Accumulating evidence have indicated that orexin-A in the brain stimulates vagal flow projecting to the stomach. Since the vagal system palys an important role in gastric mucosal integrity, we examined in the present study the effect of central orexin-A on the development of gastric mucosal damage evoked by ethanol in rats. 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 anti-ulcer action was observed in rats received with central administration of orexin-A but not orexin-B, indicating that the action is mediated through orexin 1 receptors. The gastroprotective action of central orexin-A was blocked by pretreatment with atropine, N^{w}-nitro-L-arginine methylester or indomethacin, respectively All these results suggest that orexin-A acts in orexin 1 receptors in the brain to exert a gastroprotective action against ethanol. Vagal muscarinic system, nitric

oxide and prostaglandins may mediate the cytoprotective action by central orexin-A.

Introduction

Orexins/hypocretins are novel neuropeptides that are localized in neurons in the lateral hypothalamus[1, 2,]. On the other hands, 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 these neuroanatomical evidence, orexinergic projection should be involved in a number of biological functions. It has been so far demonstrated that orexins may be implicated in a wide variety of physiological functions. These include feeding[1, 5, 6], behavioral activity [7], sleep/awake [8-10], anxiety[11], energy balance [12], neuroendocrinological response [13] and cardiovascular functions [14, 15]. In addition to these functions, we have demonstrated for the first time that orexin-A is involved in central regulation of gastric acid secretion [16-18]. Central but not peripheral injection of orexin-A dose-despondently stimulated gastric acid secretion in conscious rats [16]. The acid stimulation by central 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 the cephalic phase secretion as proposed by Pavlov [19]. The vagal dependent stimulation of gastric acid secretion of orexin-A furthermore supports the hypothesis that orexin-A may play a vital role in cephalic phase gastric secretion because it has been recognized the importance of the vagus in conveying the neural impulses that mediate cephalic phase gastric secretion [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 prevented ethanol-induced lesions in intact but not in 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 modulating 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 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^{o}C. Rats were allowed free access to standard rat chow (Solid rat chow, Oriental Yeast Co., 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 Peptide Institute Inc., Osaka, Japan and were dissolved in normal saline just before experiments. Atropine sulfate, and N^{w}-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 in 1 ml/kg. Indomethacin was purchased from Sigma, dissolved in 7 %sodium bicarbonate solution and injected intraperitoneally in 1 ml/kg.

Treatments

Gastric mucosal damage was induced by 1 ml of 70 % ethanol through an oroesophageal tube. Rats were sacrificed 60 min after ethanol administration. The stomachs were removed and examined for mucosal lesions. We initially examined the dose-related effects of intracisternal injection of orexin-A on the severity of gastric mucosal lesions by ethanol.

As a control, whether intracisternal injection of orexin-A by itself is capable of inducing gastric mucosal damage was examined. Rats in this group did not receive the administration of ethanol. 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, Tijunga, CA, USA) as previously described [24]. Following the intracisternal injection, rats were returned to their cages. One h after the treatment, rats received administration of 70 % ethanol, and one 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 also examined similarly.

Effects of atropine, L-NAME or indomethacin on the gastroprotective action by orexin-A were examined to clarify possible mechanisms by which orexin-A exerts cytoprotecetive action against

ethanol. Rats received each chemical subcutaneously or intraperitoneally and 60 min after the administration, 70 % ethanol was given to the stomach. One h after intubation of ethanol, the stomach was removed. The doses of atropine (2 mg/kg), L-NAME (70 mg/kg) or indomethacin (5 mg/kg) were selected according to 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 using a digital camera. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions.

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was performed by 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 committees 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 orexin-A at 10 μg dose did not induce any gastric mucosal lesions in 10 rats. Next, the effect of intracisternal administration of orexin-A on the severity of gastric mucosal damage evoked by 70 % ethanol was assessed macroscopically. Figure 1 shows the representative macroscopic appearance of gastric mucosa in rats that had been treated with

intracisternal injection of saline or orexin-A in a dose of 10 μg. Ten μg dose of orexin-A completely protected against ethanol-induced gastric mucosal lesions when compared with saline control. As shown in the Figure 2, the gastropeotective action of central orexin-A 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. Intrascisternal injection of orexin-A in a dose of 10 μg potently inhibited the severity of gastric mucosal lesions while intraperitoneal injection of orexin-A in a 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 have 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 (Figure 3), indicating that orexin-A specifically exerts its 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. Role of the vagal-muscarinic system, nitric oxide and 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 Figure 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, being 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 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]. Based upon the present evidence, we would 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 so far 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 non-selective for orexin-A and orexin-B[1]. Based upon the finding, the

lack of gastroprotective action of orexin-B may suggest that orexin-A-induced anti-ulcer action against ethanol may be mediated by OX1R.

The vagal system is involved in maintaining gastric mucosal integrity. For instance, vagal activation could exert gastroprotective action against ulcerogenic stress including ethanol [21, 27]. Since accumulating evidence have indicated that orexin-A administered into the cerebrospinal fluid acts in the dorsal motor nucleus of the vagus (DMN) 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 central orexin-A may be mediated by the vagal system. In fact, the present study demonstrated that atropine completely blocked the gastroprotective action by central 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. have demonstrated that NO is involved in the gastroprotection by central vagal stimulation [36], 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. have very recently demonstrated that subcutaneous injection of L-NAME blocked orexin-A-induced increase in food intake in rats and orexin-A failed to increase food intake in the NO synthase knockout mice [37]. They further demonstrated that L-NAME drastically inhibited 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 gastroproteective effect by orexin-A may raise a possibility that NO in the brain might also contribute to the anti-ulcer action by central orexin-A. According to the observation by

Zheng et al., as many as 20 % of hypothalamic orexin neurons project to the dorsal vagal complex including the DMN neurons in the medulla and some of which are in close anatomical apposition with nitric oxide synthase-immunoreactive neurons [38]. These observation furthermore support that NO is implicated in the signal transduction from the vagal preganglionic neurons in the brain to the stomach to exert the gastroprotective action by central 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 et al., have demonstrated that endogenous prostaglandins are involved in the gastroprotection by vagal stimulatio by central TRH, suggesting that vagus nerve plays a vital role in gastric prostaglandin synthesis and release [27, 39]. The gastroprotective action of centrally administered orexin-A appears to be mediated by endogenous prostaglandins similarly as 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 as a gastroprotective molecule expressed in the gastric mucosa [40] might be related to the mechanisms by which central orexin-A protects gastric mucosa against ethanol. Further studies shhoud be 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, vagal pathway, and endogenous NO and prostaglandins are implicated in the gastroprotection by central orexin-A.

Acknowledgments

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Table 1

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

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

Rats received intraperitoneal injection of either saline or orexin-A (10 μg). One h after the injection, the animals were given 1 ml of 70 % ethanol through an oroesophageal tube. One h after ethanol, the animals were sacrificed and the stomachs were removed. Each stomach was opened along the greater curvature and photographed using a digital camera. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions. Each result is expressed as mean ± SEM.

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

Rats received intraperitoneal injection of either vehicle or indomethacin (5 mg/kg) and intracisternal injection of saline or orexin-A (10 μg/10 μl) 30 min after the intraperitoneal injection. One h after the injection, the animals were given 1 ml of 70 % ethanol through an oroesophageal tube. One h after ethanol, the animals were sacrificed and the stomachs were removed. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions. Each result is expressed as mean ± SEM. * $p < 0.01$ when compared with vehcle (ip) + saline (ic).

Figure legends

Figure 1

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

Figure 2

Dose response effect of intracisternal injection of orexin-A on the severity of ethanol-induced gastric mucosal damage. Rats received intracisternal injection of orexin-A (0, 2.5, 5, 10, 20 or 40 µg/10 µl). One h after the injection, the animals were given 1 ml of 70 % ethanol through an oroesophageal tube. One h after ethanol, the animals were sacrificed

and the stomachs were removed. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions. Each bar represents the mean ± SEM of 5-12 animals. * $p < 0.01$ when compared with control (orexin-A, 0).

Figure 3

Effect of intracisternal injection of orexin-A or orexin-B on the severity of ethanol-induced gastric mucosal damage. Rats received intracisternal injection of saline, orexin-A (10 μg/10 μl) or orexin-B (10 μg/10 μl). One h after the injection, the animals were given 1 ml of 70 % ethanol through an oroesophageal tube. One h after ethanol, the animals were sacrificed and the stomachs were removed. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions. Each bar represents the mean ± SEM of 6 animals. * $p < 0.01$ when compared with saline control.

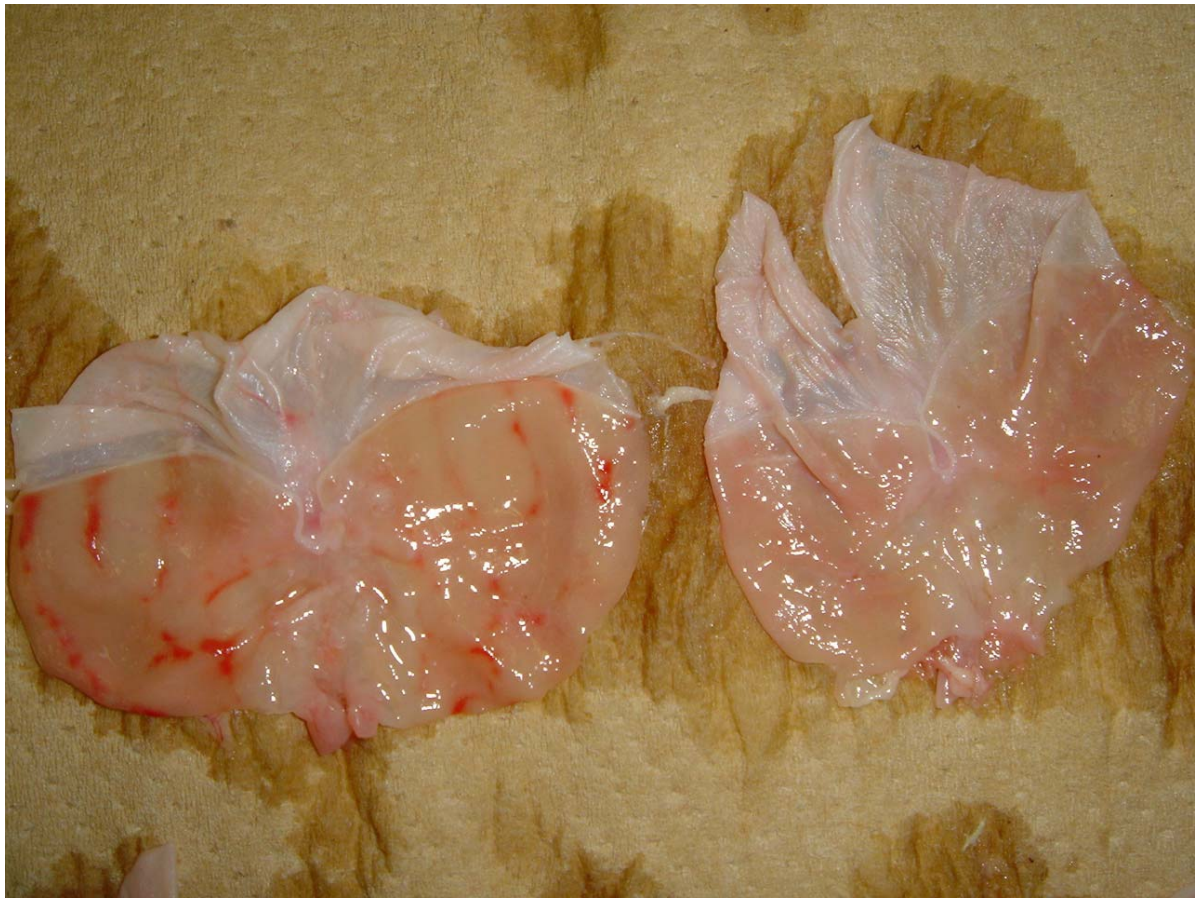
Figure 4

Effect of atropine on the gastroprotection by central orexin-A. Rats received subcutaneous administration of saline or atropine (2 mg/kg) and intracisternal injection of saline or orexin-A (10 µg/10 µl) 30 min after the subcutaneous injection. One h after the intracisternal injection, the animals were given 1 ml of 70 % ethanol through an oroesophageal tube. One h after ethanol, the animals were sacrificed and the stomachs were removed. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions. Each bar represents the mean ± SEM of 7 animals. * $p < 0.01$ when compared with saline control.

Figure 5

Effect of L-NAME on the gastroprotection by central orexin-A. Rats received subcutaneous administration of vehicle or L-NAME (75 mg/kg) and intracisternal injection of saline or orexin-A (10 µg/10 µl) 30

min after the subcutaneous injection. One h after the intracisternal injection, the animals were given 1 ml of 70 % ethanol through an oroesophageal tube. One h after ethanol, the animals were sacrificed and the stomachs were removed. The lesion area out of the total area of corpus was calculated and the percentage was determined as the severity of gastric lesions. Each bar represents the mean ± SEM of 7 animals. * $p < 0.01$ when compared with saline control.



Control Orexin-A

Figure 1

Figure 2

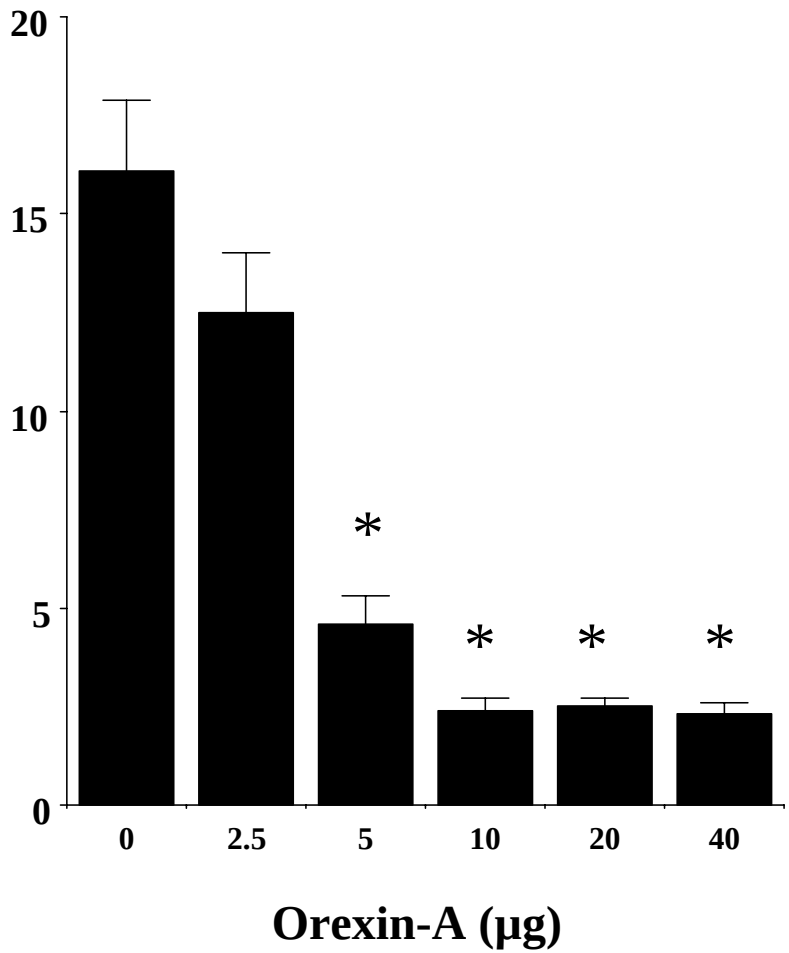


Figure 3

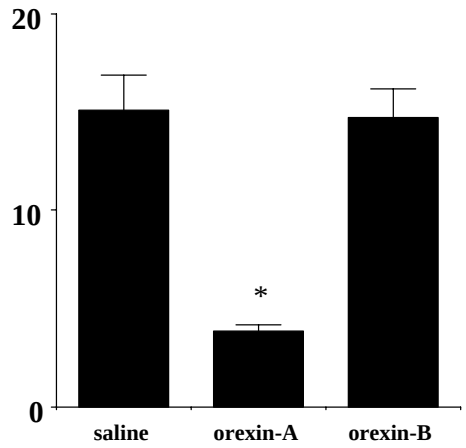


Figure 4

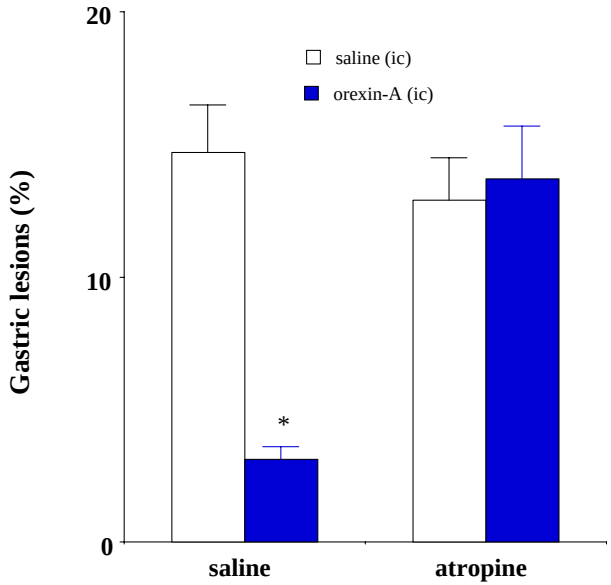

20
10
0
Gastric lesions (%)
saline (ic)
orexin-A (ic)
*
saline
atropine

Figure 5