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Role of Intracerebroventricular Vasopressin in the Development of Stress-Induced Gastric Lesions in Rats

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Summary

We investigated whether arginine vasopressin (AVP) has a central influence on the development of gastric mucosal lesions evoked by restraint-cold stress in rats. AVP and vasopressin V_1 receptor antagonist were injected intracerebroventricularly (icv) and the rats were exposed to restraint-cold stress for five hours. After decapitation the stomachs were examined for gastric mucosal lesions which were evaluated according to an ulcer score. Three different doses of AVP and V_1 receptor antagonist were administered in order to investigate the effects of exogenous and endogenous AVP on stress-induced gastric mucosal lesions. The intensity of gastric mucosal lesions was reduced when exogenous AVP was injected intracerebroventricularly. On the other hand, vasopressin V_1 receptor antagonist, which was administered by the same route, augmented gastric mucosal lesions. Our findings indicate that AVP, injected centrally, plays a role in preventing the formation of gastric mucosal lesions induced by stress *via* a central V_1 receptor.

Key words

Vasopressin • Restraint-cold stress • Gastric mucosal lesions • Vasopressin V_1 receptor antagonist

Introduction

Stress is a complex physiological phenomenon which activates the hypothalamo-hypophyseal-adrenal axis. The physiological response to stress includes increased activity of the hypothalamo-hypophyseal-adrenal axis as well as changes in gastrointestinal functions (Redei *et al.* 1994). The pathogenesis of stress-induced gastric ulcers is poorly understood. Several factors such as focal ischemia (Guth 1972), excessive hydrochloric acid secretion (Goldman and Rosoff 1968), back-diffusion of hydrogen ions through the gastric mucosal barrier (Gordon *et al.* 1973), and alterations of gastric mucus production (Shorrock and Rees 1988) have all been implicated in the formation of stress-induced gastric ulcers.

A large number of previous studies has clarified the role of enhanced activity of the hypothalamo-hypophyseal-adrenal axis or the autonomic nervous system as determinants of a variety of changes occurring in the endocrine, immune, gastrointestinal, cardiovascular or nervous systems in confrontation with some stressful situations. However, it is not known what role is played by arginine vasopressin (AVP) in the these changes. In addition to its direct vasopressor and hepatic glycogenolytic actions (V_1 receptor-mediated) and antidiuretic effect (V_2 receptor-mediated), AVP may act as a neurotransmitter or neuromodulator (Jard *et al.*

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1987). It has been demonstrated that AVP is involved in body temperature regulation in the central nervous system (CNS) (Pitman *et al.* 1988). Behavioral effects of vasopressin can be observed following systemic treatment and after intracerebroventricular (icv) or local administration in the brain (Koob *et al.* 1985).

Previous studies indicated that the production of AVP was stimulated under several stressful situations such as ether inhalation, electric stimulation or immobilization (Gibbs 1984, Williams *et al.* 1985, Onaka *et al.* 1986, Ivanyi *et al.* 1991). Similarly as many other neuropeptides, vasopressin is not confined to the hypothalamic-neurohypophyseal system, but it is widely distributed in the central nervous system of various species (Buijs 1978, Miakowski *et al.* 1988). Several hypothalamic nuclei play an essential role in the central regulation of the gastrointestinal function (Onaka *et al.* 1986, Miakowski *et al.* 1988). Controversial results have been published about the effects of AVP on gastric mucosal lesions (Honda *et al.* 1994, Laszlo *et al.* 1994).

It was established that AVP synergizes with the corticotropin-releasing factor (CRF) in stimulating the release of adrenocorticotropic hormone (ACTH) from the corticotrophs (Rivier and Vale 1985). The intracerebroventricular administration of AVP not only reduced gastric lesions but also elevated plasma ACTH levels after restraint and water immersion in rats (Honda *et al.* 1994).

Centrally administrated AVP also affected the activity of the autonomic nervous system. The administration of AVP significantly elevated plasma catecholamine concentrations and blood pressure (Tanaka *et al.* 1977, Martin *et al.* 1988). This evidence suggests that AVP in the CNS can stimulate the sympathetic nervous system directly and cause changes in various systems, such as the cardiovascular or the gastrointestinal system. Several reports dealt with the preventive role of the sympathoadrenergic system in stress-induced gastric ulcer development (Orlando *et al.* 1982, Hernandez *et al.* 1984). The exogenous administration of synthetic adrenal hormones potentiates the cytoprotective activity of endogenous adrenal hormones that are released during the exposure to restraint stress (Hernandez *et al.* 1984). In our study, we examined the effects of exogenous AVP or vasopressin V_1 receptor antagonist in an attempt to clarify the role of AVP in the development of stress-induced gastric lesions.

Methods

Animals

Fifty-nine female Sprague-Dawley rats, weighing 300-350 g (Experimental Animals Breeding and Research Center, Uludağ University Medical Faculty, Bursa) were used. In this study, rats were housed in a controlled environment animal facility and fed laboratory chow and water *ad libitum*. Rats were habituated to the animal facility for at least one week before the experiments. All rats were deprived of food, but not of water, for 24 h prior to the beginning of the experiments.

The surgical and experimental protocols were approved by the Animals Care and Use Committee of Uludağ University.

Surgical procedures

A cannula was implanted into one lateral cerebral ventricle under ether anesthesia and under aseptic conditions. A small hole was drilled through the skull 1.5 mm lateral to the midline and 1.0 mm posterior to the bregma. A 10 mm long 20-gauge stainless steel hypodermic needle was inserted through the hole into the lateral ventricle. The cannula was lowered 4.5-5.0 mm below the skull surface and was fixed to the skull with acrylic cement. Animals were housed individually and allowed to recover for 5 days.

Ulcer model and assessment of lesions

Individual rats were restrained under a wire mesh in the supine position in a cold room (7-9 °C) for 5 h. This regimen of cold-restraint stress has been reported previously to produce gastric ulcers reliably in food-deprived rats (Drago *et al.* 1993). After 5 h of stress, the animals were decapitated. The stomach was removed immediately, its content was washed out and the gastric mucosa was exposed by cutting along the lesser curvature. After the mucosa had been rinsed with normal physiological saline, it was pinned on a flat surface and examined with a dissecting microscope. The degree of gastric lesions was evaluated according to the following rating scale:

0: no lesion,
1: mucosal edema and petechiae,
2: 1-5 small lesions (1-2 mm),
3: more than 5 small lesions or 1 intermediate lesion (3-4 mm),
4: two or more intermediate lesions or gross lesions (longer than 4 mm).

The sum of scores was expressed as an ulceration score per stomach.

Effects of icv cannulation on gastric mucosal lesion development

An icv cannula was implanted in a group of rats in order to ascertain whether icv cannulation without stress exposure has an effect on gastric mucosal lesions (n=5). Another group served as controls without cannulation (n=5). Rats were decapitated after 5 days and their stomach was examined.

Effects of icv administration of AVP or V_1 receptor antagonist on the development of gastric mucosal lesion

To reveal the role of AVP in the CNS on the development of stress-induced gastric mucosal lesion, we injected AVP or V_1 receptor antagonist vasopressin (β-mercapto β , β-cyclopentamethylene-propionyl, O-Me-Tyr, Arg vasopressin) into the lateral cerebral ventricle of rats before the stress exposure.

An infusion pump was connected to the ventricular cannula, and three different doses of AVP (2, 2.5 and 3 ng/5 µl) or 5 µl saline were injected into the ventricle (n = 28). Three different doses of the V_1 receptor antagonist (4, 4.5 and 5 ng/5 µl) were injected intraventricularly (n = 21) using a Hamilton syringe. The drugs were injected slowly for 1 min into conscious and freely moving rats, because preceding stressful situations had already been shown to affect the responses of hormones to stress and the formation of gastric lesions induced by stress. Five minutes later, the rats were exposed to restraint-cold stress and the resulting gastric lesions were evaluated after decapitation 5 h later. At the end of the experiment, 5 µl of a methylene blue solution were injected into the cerebral ventricle through the cannula to verify the placement of the inner end of the cannula. After decapitation, the brain was removed and sections were observed macroscopically to ascertain whether the cannula had been correctly placed into the lateral cerebral ventricle.

Statistical analysis

The results were evaluated by using statistics programs (Systat 3.0 and Minitab). At first, the differences between groups were assessed by Kruskal-Wallis test. If there were differences among the groups ($P<0.001$), bilateral differences were examined by Mann-Whitney U test. The results were expressed as means ± standard deviations.

Results

In the present study, we investigated whether the surgical operations employed had any effect on stress-induced ulcer development. We compared the ulcer scores of rats in which icv cannula had only been implanted, with the scores of control animals without implantation. However, there were no differences were found in either of these groups (ulcer score 0).

Table 1. Effects of intracerebroventricular administration of arginine vasopressin (AVP) on the development of gastric mucosal lesions.

Groups	n	Ulcer score
Saline	7	3.6±0.2
AVP (2 ng/5 µl)	7	2.1±0.1*
AVP (2.5 ng/5 µl)	7	1.7±0.1**
AVP (3 ng/5 µl)	7	1.6±0.2**

*Difference from the saline group (*P<0.01, **P<0.001)*

The mean gastric mucosal lesion score of the saline-treated group was 3.57±0.20. Three different doses of AVP were applied in order to investigate the effect of icv AVP on stress-induced gastric mucosal lesions (Table 1). All three doses of AVP prevented the development of stress-induced gastric mucosal lesions as compared to the saline-treated group. In order to evaluate the relationship between the gastric mucosal lesions and the AVP doses, the Spearman order correlation coefficient test was calculated ($r=0.452$, $P<0.05$).

Table 2. Effects of intracerebroventricular administration of vasopressin V_1 receptor antagonist on development of gastric mucosal lesions.

Groups	n	Ulcer score
Saline	7	3.6±0.2
V_1 antagonist (4 ng/5 µl)	7	3.7±0.1
V_1 antagonist (4.5 ng/5 µl)	7	3.8±0.2*
V_1 antagonist (5 ng/5 µl)	7	4.5±0.1**

*Difference from the saline group (*P<0.01, **P<0.001)*

Three different doses of vasopressin V_1 receptor antagonist were applied in order to investigate the effect of icv administration of V_1 receptor antagonist on stress-induced gastric mucosal lesions. Higher doses of V_1 receptor antagonist enhanced the development of stress-induced gastric mucosal lesions as compared to the saline-treated group (Table 2).

Discussion

Several stress models have been designed and used in order to elucidate the pathogenesis of gastric mucosal lesions present in a large number of patients, being treated for severe trauma, burns, septic and cardiogenic shock or central nervous system damage (Laszlo *et al.* 1994). The models used for stress-induced gastric mucosal damage usually concern ethanol, reserpine, indomethacin, restraint-cold stress or hemorrhage (Drago *et al.* 1993, Laszlo *et al.* 1994).

Studies concerning the role of vasopressin in stress indicate that AVP is a significant stress hormone and may participate in stress-induced physiological mechanisms. While some researchers observed that the ulcer index in rats with induced diabetes insipidus (DI) or with a congenital defect of AVP synthesis was higher than in the control group (Honda *et al.* 1994). Some authors reported contrary results (Laszlo *et al.* 1994). It has been demonstrated that AVP action is synergic with CRF (Honda *et al.* 1994, Rivier and Vale 1985). Administration of AVP to rats with DI not only diminishes gastric lesions but also increases plasma ACTH levels. It was observed that a subcutaneous injection of 1-desamino-8-D-AVP, an antidiuretic analogue of AVP, to rats with DI did not cause significant changes in the ulcer index. However, icv administration of AVP decreased the ulcer index (Honda *et al.* 1994). In our experiments, icv administered AVP reduced gastric mucosal lesions. We propose that this AVP action is the result of sympathetic nerve activity because icv AVP can affect catecholamine metabolism in some parts of the brain and increase plasma catecholamine concentration and blood pressure (Tanaka *et al.* 1977, Martin *et al.* 1988). It is known that the sympathoadrenergic system plays a significant role in the protection of gastric mucosal integrity (Orlando *et al.* 1982, Hernandez *et al.* 1984). It is therefore suggested that the effect of AVP on the CNS may be mediated by the activation of the sympathoadrenergic system. Furthermore, it has been demonstrated that central administration of CRF has a preventive action on stress-induced ulcers (Gunion and Tache 1986, Shibasaki *et al.* 1990). Since AVP is synergic with CRF it will lead to an increase in blood ACTH and thus play a protective role against gastric mucosal lesions. Furthermore, icv CRF inhibits gastric acid secretion (Tache *et al.* 1984). Some pharmacological studies in which vasopressin receptor antagonists were used, indicated that gastric acid secretion was inhibited through prostaglandin E_2 stimulated by AVP secretion (Puurunen 1988). In our study, the icv administrations of vasopressin V_1 receptor antagonist augmented gastric mucosal lesions in rats. These results demonstrate that AVP plays a preventive role *via* the central V_1 receptor.

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