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Effects of the vasoactive intestinal peptide on stress-induced mucosal ulcers and modulation of methylation of histamine in gastric tissue of the rats

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Abstract

Vasoactive intestinal peptide (VIP) prevents stress-induced gastric ulcers, inhibits mast cell degranulation and protects gastric tissue from lipid peroxidation. Histamine has an important role in the development of gastric ulcers and mast cell derived histamine might be essential in this process.
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Keywords: Vasoactive intestinal peptide; Stress; Stress-induced gastric ulcers; histamine

1. Introduction

In our previous reports, it was concluded that vasoactive intestinal peptide (VIP) administration in experimental stress-induced ulcer models inhibits mast cell degranulation and prevents mucosal ulcers and protects gastric tissue from lipid peroxidation [1]. Tunçel et al. [2,3] showed that VIP decreases histamine content of testicular tissue of stress rats and also inhibits degranulation and proliferation of mast cells in testicular interstitium.

It has been proposed that histamine has an important role in the development of gastric ulcers, and mast cell derived histamine might be essential in this process [4–7]. Despite the fact that histamine is the main mediator of stress-induced ulceration, reports related to the levels of gastric tissue histamine during stress are limited in number and are controversial [8,9].

In the present study, we have planned to determine the levels of histamine in gastric tissue of rats either stress-induced gastric ulcer development or healing of lesions by the administration of VIP. Additionally, the inactivation of histamine was also determined. The main metabolic pathways for the degradation of histamine are ring methylation and oxidative deamination [10,11]. In gastric tissue, methylation appears to be the main path of inactivation in many species. Accordingly, in the present study, to evaluate the inactivation of histamine in gastric tissue, in addition to histamine, N^{τ}-methylhistamine was measured in tissue extract of stressed and VIP treated rats. Quantification of histamine and N^{τ}-methylhistamine was performed by HPLC.

2. Materials and methods

2.1. Methods

Histamine and N^{τ}-methylhistamine were measured in gastric tissue of stressed and VIP treated rats. Quantification of histamine and N^{τ}-methylhistamine were performed by HPLC. Sprague–Dawley rats of either sex were used and divided into eight groups. The animals were exposed to cold-restraint stress for 3 h, then 25 ng kg^{-1} VIP was given intraperitoneally. VIP

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was administered, once daily, for 1, 2 and 3 days. The rats were decapitated 24 h after the last administration of VIP.

Three hours of stress induced hemorrhagic ulcers in the glandular mucosa that appeared for 3 days. VIP significantly suppressed gastric lesions formation. The levels of histamine in the tissue was not changed significantly by three hour of stress. The tissue histamine levels of stressed rats significantly decreased below that of controls 24 h after stress exposure. The concentration of tissue histamine increased to the control level in the 2nd day (48 h) and decreased again below that of controls in the 3rd day (72 h). Histamine levels of tissue remained below that of controls throughout 3 days by VIP administration. N^{τ}-methylhistamine concentrations of gastric tissue was increased significantly by 3 h of stress. The levels of N^{τ}-methylhistamine in the tissue of stressed rats decreased to the control levels for 3 successive days. VIP treatment significantly decreased N^{τ}-methylhistamine concentration of the tissue in the 1st and 2nd day, and enhanced the levels of N^{τ}-methylhistamine above the values those of controls and stressed groups on the 3rd day.

The regulation of histamine inactivation in gastric tissue seems to be important as well as the levels of histamine during either progression or healing of mucosal ulcers. VIP, regulating both synthesis and inactivation, possibly equilibrates beneficial and harmful effects of histamine in the gastric tissue of stressed rats and effectively heals mucosal ulcers.

2.2. Experimental procedure

In this experiment, Sprague–Dawley rats (200–220 g) of either sex were used. The study was approved by local animal use and care ethic committee (Medical Faculty of Osmangazi University, Eskisehir). Rats were divided into eight groups. The animals were fasted for 24 h with water ad libitum and kept at room temperature. The rats were exposed to restraint stress in cold for 3 h (from 08:30 to 11:30 h) as described previously [1,8].

Group I were the controls; group II was exposed to cold-restraint stress and saline was given intraperitoneally. The rats were decapitated at the end of the stress exposure and their stomachs were removed. In groups III, IV and V the animals were exposed to stress for 3 h, then 25 ng kg^{-1} VIP was given intraperiotoneally. VIP was administered, once daily, for 1, 2 and 3 days for groups III, IV and V, respectively. The rats were decapitated 24 h after the last administration of VIP. The stomach of each rat was removed.

Groups VI, VII and VIII were the controls of groups III, IV and V. The rats were exposed to stress for 3 h, then saline was administered for 1, 2 and 3 days for groups, VI, VII and VIII, respectively.

2.3. Ulcers index

The stomach was opened along the lesser curvature, washed with saline and pinned out flat in a standard position for macroscopic examination and scoring of ulcer with the help of a magnifying glass. Lesion size (mm) was determined by measuring each lesion along its greatest diameter; in the case of petechial lesions, five such lesions were considered the equivalent of a 1-mm ulcer. The sum of the total severity scores in each group of rats divided by the number of animals was expressed as the mean ulcer index (UI).

2.4. Measurement of gastric tissue histamine and $N^{\prime}$-methylhistamine concentration

N^{τ}-methylhistamine (NMH) (1-methyl-4-[β-aminoethyl] imidazole) and histamine were purchased from Sigma (St. Louis, MO). Internal standard: Ro–methylhistamine (RMH) (3-methyl-5 [β-aminoethyl] imidazole) was purchased from Calbiochem (Lucerne, Switzerland). All chemicals were analytical grade and they were provided from Merck Co. (Darmstadt, Germany).

Histamine and N^{τ}-methylhistamine were extracted from the tissue by the *n*-butanol extraction method [12]. Two milliliters aliquots containing histamine and N^{τ}-methylhistamine were transferred to the polyethylene test tubes. The eluates were evaporated to dryness by lyophilization in a Leybold–Heraeus freeze-dryer and stored at $-70\,^{\circ}C$ until further use [13]. Lyophilized samples were redissolved in 250 μl double-distilled water, added 1×10^{-5} M 250 μl Ro–methylhistamine as an internal standard and mixed with 200 μl reaction buffer [14]. Thirty microliters of OPA (*o*-phytaldehyde) solution (0.1% in methanol) was added to the mixture. After 3 min of incubation time at room temperature, the reaction was stopped by addition of 20 μl 1 N HCl and 10 μl of the sample were immediately injected to the HPLC column. Ro–methylhistamine was the internal standard. Recovery of histamine and N^{τ}-methylhistamine from the tissue was calculated with the extraction of 1×10^{-6} M histamine and 5×10^{-7} M N^{τ}-methylhistamine under the same condition.

2.5. Chromatographic conditions

An HPLC gradient system was consisting of models of LC-10 AT pump and RF-10 AXL fluorescence detector and CBM-10 A communication bus module was processing the data using the software of LC Workstation CLASS-LC 10 (Version 1) (Shimadzu, Tokyo, Japan), a 10 μl loop injection port (Rheodyne, Cotati, CA), Hyperisil 3 μm-C_{18} column (100 mm × 4.6 mm ID) (Phenomenex, CA). The mobile phase was prepared by measuring 52 volume of methanol and 48

volume of 0.07 M Na_2HPO_4 at pH 9.45 and it was filtered from a glass filter, degassed by a model of B-220 ultrasonic bath (Branson, CA).

Fluorometric detection was performed at λ_{ex}: 335 nm and λ_{em}: 445 nm. The flow-rate was 1 ml/min and analysis was made at ambient temperature.

2.6. Statistics

The data was expressed as the means ± SE. Statistical analyses were performed using one-way ANOVA and Duncan's multiple range test.

3. Results

3.1. Ulcer index

Fig. 1 shows the results of ulcer indices. Exposure of rats to cold-restrain stress for 3 h induced hemorrhagic ulcers in the glandular mucosa. Mucosal ulcers appeared for three days and ulcer index slightly increased in the second day. When VIP was administered for 3 consecutive days after the application of 3 h stress, gastric lesions formation was significantly suppressed as compared to saline treatment.

3.2. Histamine and N^t-methylhistamine levels of gastric tissue

Fig. 2 shows the histamine levels of gastric tissue of the rats. The levels of histamine in the tissue did not change significantly by the three hour of cold-restraint stress. The tissue histamine levels of saline-treated rats were significantly decreased below that of controls 24 h after stress exposure. However, the concentration of tissue histamine increased to the control level in the 2nd day (48 h), in which ulcer index slightly increased, while it decreased again below that of controls in the 3rd day (72 h). When VIP was administrated after the application of 3 h stress, histamine levels of tissue remained below that of controls throughout 3 days. Histamine levels of gastric tissue of VIP-treated rats was significantly less than that of the saline-treated rats only on the 2nd day.

N^t-methylhistamine concentrations of gastric tissue were presented in Fig. 3. N^t-methylhistamine concentrations of gastric tissue were increased significantly by 3 h of cold-restraint stress as compared to control. The levels of N^t-methylhistamine in the tissue of saline-treated rats decreased to the control levels for three successive days after the application of 3 h stress. VIP treatment significantly decreased N^t-methylhistamine concentration of the tissue below those of controls and saline-treated groups on the 1st day. On the 2nd day, VIP caused a decrease in the tissue N^t-methylhistamine concentration below that of the controls while increased enhanced the levels of N^t-methylhistamine above the values those of controls and saline-treated groups on the 3rd day.

4. Discussion

The experiments reported here include measurements of the levels of histamine, N^t-methylhistamine for the untreated stressed rats with lesion formation in gastric tissue and VIP treated rats. It was found that histamine content of gastric tissue is not increased after 3 h of cold-restraint stress. However, the levels of histamine decreased below that of control values for successive 1st and 3rd days. As to the histamine content of gastric tissue of rats exposed to cold-restraint stress, Bouclier et al. [9], using RIA technique for the measurement of

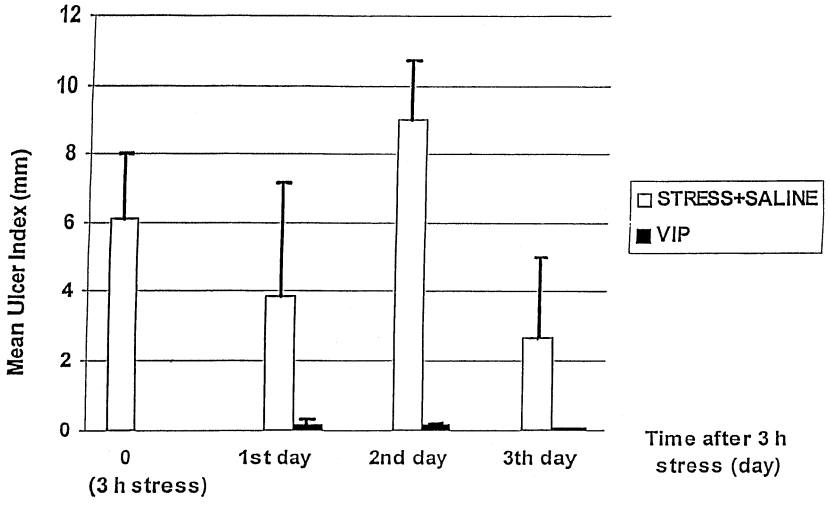

Fig. 1. Mean ulcer indices ($n = 6$ for each group). □, stress+saline; ■, stress+VIP.

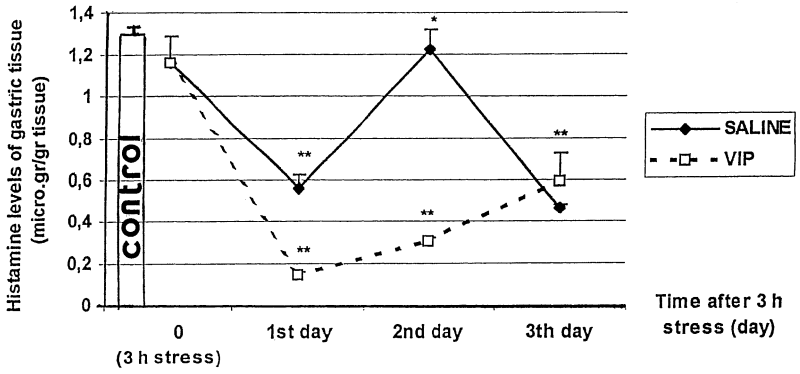

Fig. 2. Histamine levels of gastric tissue stressed and VIP-treated rats (saline-given, $n = 5$; VIP-given, $n = 6$). * $P < 0.01$: significantly different from VIP-given; ** $P < 0.01$: significantly different from the control. –◆–, stress+saline; –□–, stress+VIP.

histamine levels, reported similar findings to ours that histamine concentration is not changed significantly after 4 h cold-restrain stress. On the contrary, Das and Banerjee [8], determining histamine by a fluorometric method, reported a controversial finding that 2 h cold-restraint stress causes time-dependent increases in histamine but decreases in acid content of gastric fluid of rats. Their contrary results were probably due to a significant difference in technique, since their method, which does not differentiate between histamine and its metabolite histamine, is less sensitive than RIA and HPLC. It is highly likely that both histamine and N^{τ}-methylhistamine could be measured together. Cold-restraint-induced gastric ulcer formation has been one of the most studied models of experimental gastric ulcer formation has been one of the most studied models of experimental gastric mucosal injury [4,6–9,15]. The relationship between acid secretion and lesion formation is not clear. There is usually no evidence of acid hyper secretion during the production of the stress-induced acute mucosal ulcer [15,16]. Many investigators have documented either no change or decline in acid output during cold-restraint stress [8,16,17]. Since histamine is a well-documented pharmacological stimulant of gastric acid secretion, our findings suggest that suppression of acid secretion may be associated by reduction of tissue histamine level [18].

In the present study, VIP significantly suppressed gastric lesions and caused a significant decease in the histamine content of the tissue. The mechanisms of the action of VIP on gastric tissue histamine levels can be explained by its inhibitory effect on the release of gastrin hormone, mast cells and enterochromaffin-like (ECL) cells. Mast cells and ECL cells are histamine storing compartment in the gastric tissue [19–21]. It was shown that VIP inhibits degranulation and reduces the number of mast cells in gastric tissue of rats exposed to cold-restraint stress [1]. It was also reported that VIP diminishes the number of mast cells and ECL cells in the cecum and left colon of rats [2,22]. Moreover, VIP is an inhibitory peptide on release of gastrin hormone which activates synthesis and release of histamine from ECL cells [23]. Thus, in the present study, gastric tissue histamine levels could be decreased by VIP according to its actions mentioned above. Our results related to methylated histamine indicate that inactivation of histamine enhances in the gastric tissue during the progression of gastric ulcers, N^{τ}-methylhistamine content of the tissue increased after 3 h of cold-restraint stress and remained in control values throughout three days despite the low levels of tissue histamine for the 1st and 3rd days. VIP lowered N^{τ}-methylhistamine levels of gastric tissue except for the 3rd day, and reduced mucosal lesion. On the other hand, gastric ulcers were

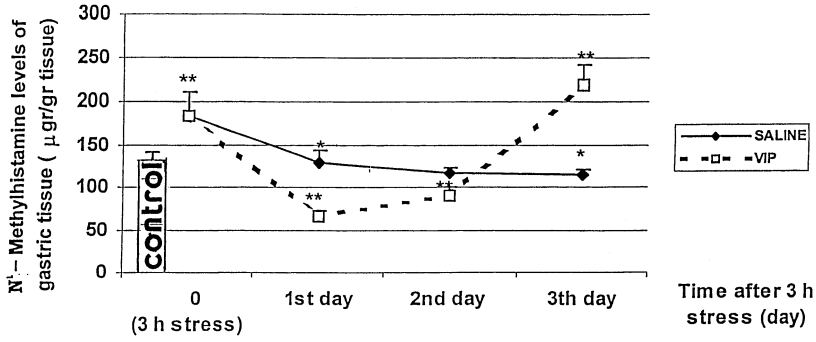

Fig. 3. N^{τ}-methylhistamine levels of gastric tissue of stressed and VIP-treated rats (saline-given, $n = 5$; VIP-given, $n = 6$). * $P < 0.01$: significantly different from VIP-given; ** $P < 0.01$: significantly different from control. –◆–, stress+saline; –□–, stress+VIP.

completely removed when inactivation of histamine significantly increased by VIP on the 3rd day. Because lesions did not disappear from mucosa of saline-treated rats even though histamine content of the tissue is low, inactiviation rate of histamine seems to be important for healing of mucosal ulcers. Our results show that VIP decreases histamine levels of gastric tissue and at the same time, modulates inactivation of histamine by methylation, and it effectively heals mucosal ulcers. Thus, our results indicate that VIP, regulating both synthesis and inactivation, possibly equilibrates of the beneficial and harmful effects of histamine. It is proposed that histamine is an important mediator for tissue repair and wound healing [24,27]. The beneficial effect of histamine on the repair of intestinal mucosa after ischemia reperfusion has been well documented [25–27] However, it is suggested that if synthesis and inactivation of histamine is not regulated precisely, beneficial actions of histamine are outweighed by its undesirable side effects. Our present results strongly suggest that VIP provides the beneficial actions of histamine which are not outweighed by its undesirable side effects.

In conclusion, VIP treatments of rats significantly inhibited development of gastric mucosal ulcers induced by cold-restraint stress. As we reported previously, VIP has combined actions that counteract gastric ulcer development induced by cold-restraint stress [1,23]. VIP, acting as an antioxidant and anti-inflammatory and modulating the activity of mast cells, prevents gastric mucosa from the damages of cold-restraint stress. The present study demonstrated additional favorable actions of VIP that counteract gastric ulcer development induced by cold-restraint stress. It can be suggested that as VIP is curing the gastric lesions, while it regulates synthesis and inactivation of histamine in the tissue.

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