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Activation of sensory nerves participates in stress-induced histamine release from mast cells in rats

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Abstract

To elucidate the mechanism by which stress induces rapid histamine release from mast cells, Wistar rats, pretreated as neonates with capsaicin, were subjected to immobilization stress for 2 h, and histamine release was measured in paws of anesthetized rats by using in vivo microdialysis after activation of sensory nerves by electrical or chemical stimulation. Immobilization stress studies indicated that in control rats stress induced a 2.7-fold increase in the level of plasma histamine compared to that in freely moving rats. Whereas pretreatment with capsaicin significantly decreased stress-induced elevation of plasma histamine. Microdialysis studies showed that electrical stimulation of the sciatic nerve resulted in a 4-fold increase of histamine release in rat paws. However, this increase was significantly inhibited in rats pretreated with capsaicin. Furthermore, injection of capsaicin into rat paw significantly increased histamine release in a dose-dependent manner. These results suggest that activation of sensory nerves participates in stress-induced histamine release from mast cells.

Keywords: Stress; Histamine release; Sensory nerve; Mast cells; Capsaicin; Neuropeptides; Electrical stimulation; Microdialysis

Previous studies from our laboratory demonstrated that exposure of rats to stress induces mast cells to release histamine [9,10] which has been implicated in neurogenic inflammation [19] and development of gastric ulcer [9]. However, the mechanism by which stress induces histamine release from mast cells is still unknown. Several non-immunologic secretagogues such as substance P (SP) [7], neurokinin A [11] and corticotropin-releasing hormone [19], have been reported to cause the degranulation of mast cells. In the peripheral system, SP is mainly present in peripheral sensory neurons, particularly in small unmyelinated fibers and has been identified in nerve endings throughout the body, including the skin, joints, and vascular, gastrointestinal, and mucosal tissues [26], where mast cells are predominantly localized [12]. Mast cells are found in close proximity to [4], or in direct contact with [18,23], peripheral nerve endings, especially neuropeptide-containing afferent nerves [1,21]. Furthermore, increasing evidence suggests that the secretory function of mast cells is regulated by the nervous system [7]. Taken together, these findings suggest that activation of sensory nerves might be involved in the stress-induced histamine release from mast cells. In this study, we observed the effect of sensory nerve degeneration on stress-induced histamine release in the rats pretreated with capsaicin as neonates, and measured histamine release in rat paw after activation of sensory nerves by in vivo microdialysis.

Normal, capsaicin and vehicle pretreated, male Wistar rats, weighing 240–250 g, were used in the study. Rats were housed under conditions of controlled temperature, humidity and illumination (lights on from 07:00 to 19:00 h), and allowed free access to food and water. The rats were deprived of food 18 h before experiments, but were permitted intake of water ad libitum. Neonatal rats from four litters were injected subcutaneously at 24–36 h of age with 50 mg/kg capsaicin in vehicle (10% ethanol, 10% Tween 80, 80% sterile saline) under ether anesthetic. Age-matched pups from different litters were injected with an equal volume of vehicle only. Two months later, capsaicin-pretreated rats showing at least 40% increase in latency in response to noxious thermal stimulation (52°C) compared

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with vehicle-pretreated rats, were used as described by Yonehara et al. [25].

Experiment 1: the effects of immobilization stress on plasma histamine levels were investigated in capsaicin and vehicle pretreated rats. Rats were subjected to immobilization stress at around 10:00 h. Each rat was restrained firmly with a stainless steel mesh, and the immobilized rats were kept at 24°C for 2 h. Blood samples at a volume of 0.15 ml each, were drawn from an indwelling jugular catheter, inserted 3 days before according to a previously described method [9], at times 0, 5, 15, 30, 45, 60, 90 and 120 min during the stress. The samples were immediately centrifuged ($800 \times g$) for 15 min at 4°C. An aliquot (50 μl) of plasma from each blood sample was stored at −84°C until histamine determination. Blood samples were obtained from control rats under freely moving conditions [9].

Experiment 2: the effect of electrical stimulation of the sciatic nerve on histamine release was examined by microdialysis in rat paw as previously described [3]. After insertion of the microdialysis probe (4 mm, BAS, Japan) into the right hind paw of urethane anesthetized rats (1.2 g/kg, i.p.), the right sciatic nerve was exposed and impaled with a thread. Two hours later, three baseline dialysates (40 μl each) were collected at 20-min intervals prior to stimulation. At the start of collection of the 4th sample, the nerves were placed on bipolar, enamel-insulated, stimulating electrodes under liquid paraffin, and stimulated for 10 min with a 10 V square wave (frequency 5 Hz, duration 1 ms). Dialysates were collected every 20 min up to 2 h and kept at −20°C until histamine assay. In control animals, only a sham operation without electrical stimulation was performed. This experiment was carried out in both capsaicin and vehicle pretreated rats.

Experiment 3: the effect of capsaicin injection on histamine release was investigated by microdialysis as previously described in the paws of normal rats anesthetized with urethane. Baseline samples were collected 2 h after insertion of the microdialysis probe, and then either capsaicin, dissolved in 50 μl vehicle (as above) at concentrations of 10^{-9} M, 10^{-7} M, or the same volume of vehicle was injected into the subcutaneous space of right rat paw about 2 mm from the probe. The dialysate was collected every 20 min for up to 3 h after injection. The concentrations of histamine in plasma and the dialysates were determined by high performance liquid chromatography-fluorometry [3,9,24].

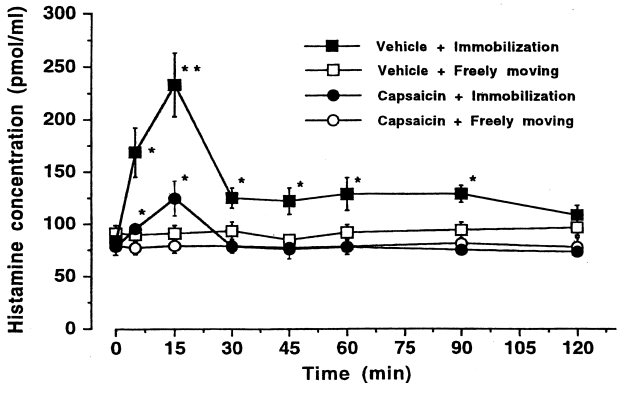

Fig. 1. Plasma concentrations of histamine in freely moving rats and rats exposed to immobilization stress for 2 h. Freely moving rats served as the control group. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with the respective value in the control group.

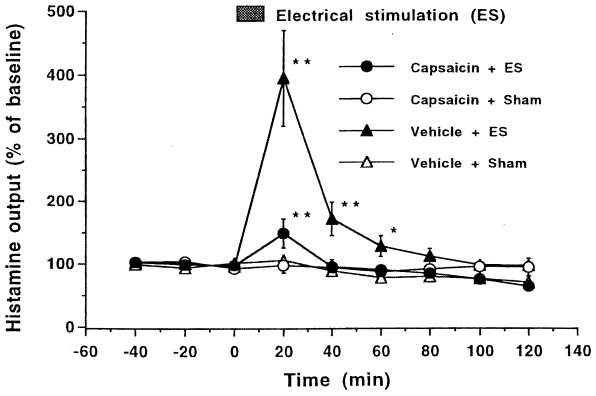

Fig. 2. Changes in the release of histamine into microdialysis perfusates in capsaicin and vehicle pretreated rats after electrical stimulation (10 V, 5 Hz, 1 ms for 10 min) or sham operation of the sciatic nerve. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with the respective value in the sham operated group.

In the microdialysis study, histamine output was observed to be stable 2 h after implantation of the probe. Thus, the mean value of histamine output observed during the next 1 h was defined as the basal output and subsequent fractions were expressed as percentages of this value. For plasma histamine data, the concentration of plasma histamine, was expressed as pmol/ml. All data were expressed as means ± SEM ($n = 5$ in microdialysis study, $n = 4$ in plasma study). Two-way analysis of variance, followed by the Fisher's protected least significant differences test were used to determine if differences between groups were statistically significant.

Fig. 1 illustrates the effect of immobilization stress on the level of plasma histamine in rats pretreated with capsaicin. In the vehicle group, the maximal elevation of plasma histamine, reached 15 min after immobilization, was 2.7-fold higher than that in the freely moving rats. Thereafter, the level of histamine declined but was sustained for up to at least 90 min in the range 110–130 pmol/ml, which was significantly higher than in controls. In contrast, the mean basal level of plasma histamine in freely moving control rats was 91.5 ± 2.2 pmol/ml. In the capsaicin pretreated group, the stress-induced plasma histamine increase was significantly inhibited. Significant increases were found only at 5 and 15 min after onset of immobilization; plasma histamine levels then declined and returned to basal levels after 30 min. At 15 min the peak level increased by 1.6-fold, but was significantly lower than in the vehicle treated group ($P < 0.01$). There was no difference in the average values

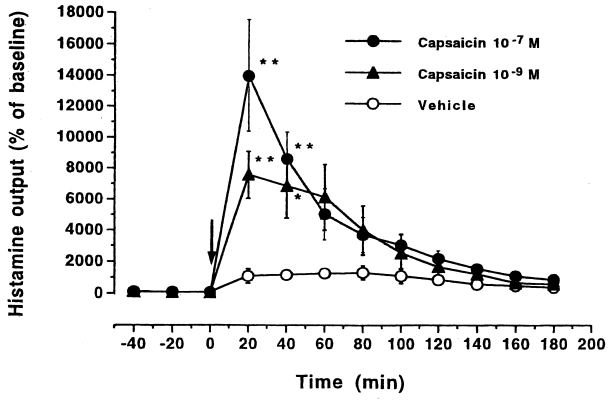

Fig. 3. Changes in the release of histamine into microdialysis perfusates in rats after subplantar injection of capsaicin. Capsaicin, at concentrations of 10^{-7} M, 10^{-9} M, or 50 μl vehicle, was injected after collection of three baseline fractions as indicated by an arrow. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with the vehicle treated group.

of plasma histamine between freely moving, capsaicin and vehicle pretreated rats.

Fig. 2 shows the effect of electrical stimulation of the paws of capsaicin treated rats on histamine output. In the vehicle treated group, 10 min electrical stimulation of the sciatic nerve, induced a 4-fold increase in histamine release in the first sample compared with the basal level. The histamine release then declined, but levels were still significantly higher for 60 min, returning to basal levels in next 20 min. In the capsaicin pretreated rats, the increase in histamine release induced by electrical stimulation, was only found in the first sample and was significantly lower than in the vehicle group ($P < 0.01$).

Fig. 3 shows the effect of subplantar injection of capsaicin on histamine release in rats. Capsaicin, at concentrations of 10^{-7} M and 10^{-9} M, induced significant 142- and 75-fold increases in histamine release, compared to basal levels, respectively, in the first sample which then declined to the level of vehicle group within 1 h. The injection of vehicle alone induced only an 11- fold increase in histamine release.

In the present study, pretreatment with capsaicin, which depletes SP in sensory nerve endings [14,26] but does not decrease the content of histamine in tissues [6], significantly decreased the stress-induced elevation of plasma histamine (Fig. 1), suggesting that stress might activate sensory nerves to release neuropeptides which stimulate mast cell degranulation.

We next examined whether activation of sensory nerves in anesthetized rat paws by in vivo microdialysis induced histamine release. Acute electrical or chemical stimulation of sensory nerve fibers is a well established pharmacological tool used to selectively activate sensory nerves [2]. In our experiment, electrical stimulation of the sciatic nerve significantly increased histamine release in the rat hind paw, whereas pretreatment with capsaicin, which depletes neuropeptides in sensory nerve endings, significantly inhibited this increase (Fig. 2). Furthermore, injection of capsaicin was shown to induce histamine release in a dose-dependent manner (Fig. 3). It is interesting that the injection of vehicle also resulted in an 11-fold increase in histamine, in agreement with our previous report that insertion of injection needles and also infusion of saline increased subcutaneous levels of histamine [3]. These results clearly indicate that activation of sensory nerves results in histamine release from mast cells in the paws of anesthetized rats. However, Petersen et al. [20] reported that intradermal administration of capsaicin, in concentrations which caused intense pain and flare reactions, did not release histamine or SP in intact human skin.

Previous reports support the notion of interaction between mast cells and sensory nerves. Mast cells are in direct anatomical contact with neuropeptide-containing sensory nerves [18,23], and electrical stimulation of the sciatic nerve [5,25] or injection of capsaicin [8,13,15] elicited release of neuropeptides in rat paw, in particular SP. In addition, neuropeptides such as SP, released from sensory nerve endings, have been suggested to stimulate histamine release from mast cells [7,16], showing that neuropeptides are involved in histamine release induced by the activation of sensory nerves. The depletion of neuropeptides by capsaicin pretreatment of rat neonates was shown to decrease histamine release from mast cells induced by stress or activation of the sensory nerve (Figs. 1 and 2), indicating that stress-induced histamine release is mediated by neuropeptides released from sensory nerves. Neuropeptide release from sensory nerve endings has been reported to be induced by stress [22,26], while pretreatment with capsaicin decreased stress-induced SP release [26]. It is revealed that stress activates sensory nerves to release neuropeptides which then stimulate mast cells to release histamine, resulting in elevated levels of plasma histamine. Some observations suggest that direct activation of G proteins is the physiological mechanism by which SP triggers mast cells to release histamine [17].

In conclusion, we found that activation of sensory nerves elicited histamine release in anesthetized rats. Stress resulted in histamine release from mast cells, whereas the degeneration of sensory nerves significantly inhibited this response, suggesting that activation of sensory nerves participates in stress-induced histamine release from mast cells.

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