ELSEVIER

European Journal of Pharmacology 360 (1998) 139–146

ejp

Biphasic elevation of plasma histamine induced by water immersion stress, and their sources in rats

Zhi-Li Huang [a], Takatoshi Mochizuki [a,1], Hirokazu Watanabe [a], Masatoyo Kagoshima [b], Kazutaka Maeyama [a,*]

[a] *Department of Pharmacology, Ehime University School of Medicine, Shigenobu-cho, Onsen-gun, Ehime 791-0295, Japan*
[b] *Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, Minato-ku, Tokyo 108-8641, Japan*

Received 18 June 1998; revised 9 September 1998; accepted 11 September 1998

Abstract

The effect of water immersion stress on the plasma concentration of histamine, in Wistar and mast cell-deficient (Ws/Ws) rats, was investigated. The histamine content of the plasma, skin and gastric mucosa, as well as the level of activity of histidine decarboxylase in the gastric mucosa, were determined by high performance liquid chromatography (HPLC)-fluorometry. In Wistar rats exposed to water immersion stress for a total of 6 h, an initial, acute, four-fold, transient increase in the plasma histamine level, followed by a sustained, though lower, elevation of the plasma histamine level, was observed. The initial acute increase in plasma histamine level was also seen in gastrectomized Wistar rats exposed to water immersion stress, but not in Ws/Ws rats exposed to stress. The sustained elevation of the plasma histamine level was observed in the Ws/Ws rats. However, in both the gastrectomized Wistar rats and gastrectomized Ws/Ws rats, the sustained elevation in plasma histamine level was not observed. The histamine content of the skin of Wistar rats after 15 min or more exposure to water immersion stress, was 20% lower than that of control rats. The mucosal histamine content of both Wistar rats and Ws/Ws rats, was 20% lower, whereas histidine decarboxylase activity in the gastric mucosa was enhanced by two-fold, during exposure to stress for 4 h. These findings indicate that water immersion stress causes a biphasic increase in plasma histamine concentration in Wistar rats; the initial acute increase in plasma histamine level originates from mast cells, and the second, sustained increase is attributed to enterochromaffin-like cells.

Keywords: Histamine; Enterochromaffin-like cell; Mast cell; Mast cell-deficient rat; Gastrectomy; Water immersion stress

1. Introduction

Histamine is synthesized and stored in several kinds of the cells throughout the mammalian body. Histamine is mainly stored in mast cells, basophils and enterochromaffin-like (ECL) cells in the periphery. An antigen binds with immunoglobulin E (IgE) on the surface of mast cells and basophils, thereby inducing these cells to release histamine. Once released, histamine induces anaphylactic responses including vasodilation, increased vascular permeability, and contraction of smooth muscle, all of which are mediated by histamine H_1-receptors (Pearce, 1989). Recent studies have revealed that non-immunologic secretagogues, such as substance P (Hua et al., 1996), neurokinin A (Joos et al., 1994) and corticotropin-releasing hormone (Theoharides et al., 1998), can induce the degranulation of mast cells. This suggests the relevance of mast cells not only to allergic disorders, but also to neuroinflammatory symptoms in which the nervous system interacts with the immune system (Sternberg et al., 1992; Ader et al., 1995).

Histamine is released from numerous ECL cells in the oxyntic mucosa of the stomach, under the control of both the vagus nerve and gastrin (Håkanson et al., 1984; Prinz et al., 1993; Sandor et al., 1996). Histamine plays a crucial role in regulating gastric acid secretion from parietal cells via histamine H_2-receptors (Waldum and Sandvik, 1989). Animal studies have shown that stress-induced gastric ulceration is frequently associated with acid secretion and

* Corresponding author. Tel.: +81-89-960-5258; Fax: +81-89-960-5263; E-mail: kazu@m.ehime-u.ac.jp

[1] Present address: Department of Molecular Behavioral Biology, Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565-0874, Japan.

0014-2999/98/$ - see front matter
PII: S0014-2999(98)00670-0

mucosal blood vessel engorgement, and these could probably be due to the effects of histamine (Cho et al., 1992).

These findings suggest that histamine released in the periphery, participates in the physiological and pathological responses to stress. However, no studies on the change in the amount of histamine released from peripheral histamine-storing cells under pathogenic conditions, have been reported. In the present study, we studied the plasma concentration of histamine in rats during exposure to water immersion stress. Water immersion stress is a useful model for examining stress-induced gastric ulcers (Honda et al., 1994). In an attempt to clarify the particular cells which release histamine during stress, water immersion stress was applied to Wistar rats, and to mutant rats that are homozygous at the white spotting (Ws) locus, that is, Ws/Ws rats, which are deficient in mast cells (Niwa et al., 1991). A total gastrectomy was then performed on these animals, and the gastrectomized rats were subjected to water immersion stress to determine if ECL cells contribute to the elevation in plasma histamine level.

2. Materials and methods

The animal experiments performed in this study, were conducted according to the guidelines of the Animal Care Committee of Ehime University School of Medicine. The experimental protocols were approved by the Animal Care Committee.

2.1. Animals

Male Wistar rats, weighing 240–250 g, were purchased from CLEA Japan (Osaka, Japan), and kept in our laboratory for 1 week before exposing them to stress. Male and female Ws/+ rats, both of the Donryu strain, were crossed to obtain male Ws/Ws rats, which are deficient in mast cells, and their wild type, +/+ rats, using the procedure described by Niwa et al. (1991). Each of the Ws/Ws rats and +/+ rats, had a weight of 230–250 g. All of the rats were housed at a constant temperature (24 ± 2°C), with a humidity of 55 ± 10%, on an automatically-controlled, 12:12 h light/dark cycle (lights on at 7:00 a.m.). The rats had free access to food and water. The rats were deprived of food 18 h before they were exposed to stress, but were permitted intake of water ad libitum.

2.2. Cannulation procedure

Cannulation was carried out as described by Kvetnansky et al. (1992). Briefly, the rats were first anesthetized with sodium pentobarbital (50 mg/kg i.p.). A catheter filled with a solution containing 600 IU/ml of sodium heparin, was implanted into the jugular vein; the outlet of the catheter was drawn out at the nape, and sealed. After the operation, the rats were kept in individual cages in a quiet room for an adaptation period of 3 days before water immersion stress was applied.

2.3. Water immersion stress procedure and blood sampling

The fasted rats were subjected to water immersion stress at 10:00 a.m., according to the method of Takagi et al. (1964). Each rat was removed from the home cage environment, and restrained firmly with a stainless steel mesh. The immobilized rat was vertically immersed in a water bath at 23°C to the level of the xiphoid process for 6 h. Blood samples of 0.2 ml each, were drawn from the jugular catheter at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min during the stress experiment. The collected blood samples were immediately centrifuged ($800 \times g$), at 4°C for 15 min. The precipitated blood cells after the third sampling, were resuspended in an equal volume of saline, and infused back into the animal by the catheter within 5 min after centrifugation (Knigge et al., 1990; Pacak et al., 1995). Fifty microliters each plasma sample was stored at −84°C, until the time of histamine determination.

The control rats were not subjected to stress. Blood samples were obtained from the rats under freely moving conditions during a 6 h time period. Each rat was placed in an acrylic cage, and the outlet of the catheter was connected to a liquid swivel attached to a balanced arm (Tsumura, Tokyo, Japan). This system enabled us to obtain plasma samples from freely moving rats without exposing them to any type of stress.

2.4. Total gastrectomy

Other Wistar and Ws/Ws rats were fasted, with only water available, the night before the operation. Under sodium pentobarbital anesthesia (50 mg/kg i.p.), a total gastrectomy was performed, followed by an end-to-end esophagoduodenostomy. All of the rats had an uneventful postoperative recovery. One week after the total gastrectomy was performed, the cannulae manipulations were carried out as described previously.

2.5. Preparation of the skin and gastric mucosa for analysis of histidine decarboxylase activity and histamine content

Other Wistar and Ws/Ws rats were subjected to water immersion stress without the implantation of catheters. They were decapitated after being exposed to water immersion stress for 15 min, 1 h, and 4 h. A piece of the circular dorsal skin, 10 mm in diameter, and the stomach, were removed from the animal. The stomach was opened along the major curvature and rinsed with ice-cold saline. The oxyntic mucosa was then scraped off the gastric wall. These preparations were stored at −84°C.

The control rats were quickly transferred from the home cage to an adjacent room, and decapitated immediately. The dorsal skin and oxyntic mucosa from the control rats, were obtained and prepared as described above.

2.6. Determination of the histamine content of the tissue preparations by high performance liquid chromatography (HPLC)-fluorometry

The concentration of histamine in the plasma, and in the tissue homogenates, was determined by an HPLC-fluorometry technique (Yamatodani et al., 1985; Guo et al., 1997). Fifty microliters of each plasma sample, was diluted with 350 μl of 0.46 M perchloric acid; this was centrifuged at $10\,000 \times g$ for 15 min at 4°C. The supernatant was obtained, and 50 μl of this was injected directly into a column packed with the TSKgel SP2SW Cation Exchanger (150 × 6 mm i.d.) (Tosoh, Tokyo, Japan). Histamine was eluted with 0.25 M potassium phosphate, at a flow rate of 0.6 ml/min. The histamine was post-labeled with *o*-phthalaldehyde in an alkaline condition, and detected fluorometrically in an F1080 Fluorometer (Hitachi, Tokyo, Japan), using excitation and emission wavelengths of 360 and 450 nm, respectively.

Each tissue preparation of dorsal skin and gastric mucosa, was weighed, and then promptly homogenized in 4 volumes of ice-cold potassium phosphate buffer (0.1 M, pH 6.8), which contained 0.01 mM pyridoxal 5′-phosphate, 0.2 mM dithiothreitol, 1% polyethylene glycol (average molecular weight, 300), and 100 μg/ml of phenylmethylsulfonyl fluoride, using a polytron homogenizer (Kinematica, Lucerne, Switzerland). One hundred microliters of the homogenate from each tissue preparation, was mixed with 900 μl of 0.46 M perchloric acid. This was centrifuged at $10\,000 \times g$ for 15 min at 4°C. The supernatant was obtained, and 100 μl of the supernatant was diluted 10 times with 5 mM Na_2EDTA. Fifty microliters of this solution was injected into the HPLC system.

2.7. Determination of the level of histidine decarboxylase activity

The remaining homogenate of each of the gastric mucosa preparations, was used to determine the level of histidine decarboxylase activity, using the methods described by Watanabe et al. (1980), and Sugimoto et al. (1995). Eight hundred microliters of the homogenate from each preparation, was centrifuged twice at $10\,000 \times g$ for 15 min at 4°C. The resultant supernatant was dialyzed against 100 volumes of the potassium phosphate buffer at 4°C; this procedure was carried out three times. The enzyme solution was incubated with 0.25 mM L-histidine for 1 h at 37°C. The amount of histamine was quantified in the HPLC-fluorometry system. The protein content of each enzyme solution was measured using a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA); bovine serum albumin was used as the standard.

2.8. Statistical analysis

The concentration of histamine in the plasma, is expressed as pmol/ml. The histamine content of the skin and the gastric mucosa, is expressed as nmol/g wet tissue weight. All data are expressed as means ± S.E.M. ($n = 5$ in each experimental group of rats). Two-way analysis of variance, followed by the Scheffe test, were used to determine if the difference between two groups is statistically significant. A probability value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effect of water immersion stress on the plasma histamine concentration of the Wistar rats

The mean basal level of histamine in the plasma of the freely moving Wistar rats, was 100 ± 4 pmol/ml. This level was maintained throughout the sampling period, as shown in Fig. 1.

In the rats exposed to water immersion stress for a 6 h period, the plasma histamine level increased four-fold in the first 15 min. The plasma histamine level then decreased, although it did not return to the basal level. Thereafter, a sustained, elevated plasma histamine level, was observed over the entire 6 h period of stress exposure. Thus, there are two components to the stress-induced increase in plasma histamine level. The first is an acute, initial elevation in the plasma concentration of histamine, which reached a maximal value at 15 min after initiation of water immersion stress. The second is a sustained elevation in plasma histamine concentration, at a concen-

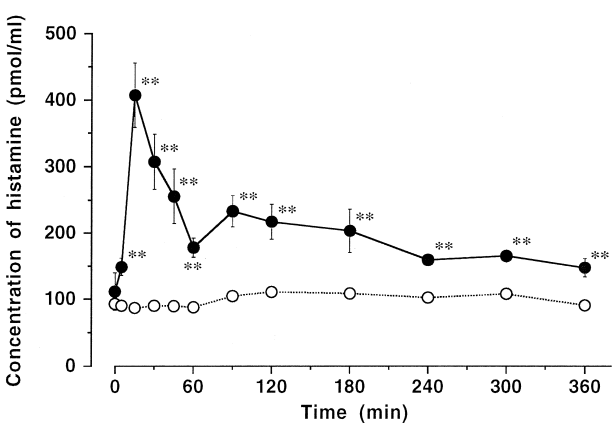

Fig. 1. Plasma concentration of histamine in Wistar rats exposed to water immersion stress for 6 h (●), and under the freely moving condition (○). The freely moving rats served as the control group. Each data point represents the mean ± S.E.M. ($n = 5$). $** P < 0.01$ compared to the respective value in the control group.

tration which was two-fold higher than the basal level in the freely moving rats.

3.2. Effect of water immersion stress on the plasma histamine concentration in the +/+ and Ws/Ws rats

Fig. 2a shows the effects of water immersion stress on the plasma histamine level in the +/+ rats. The basal level of plasma histamine concentration in the +/+ rats, was 91 ± 10 pmol/ml under the freely moving condition. This is nearly equivalent to that of the Wistar rats. Water immersion stress induced a change in plasma histamine concentration in the +/+ rats, similar to that seen in the Wistar rats. That is, during stress exposure, there was an initial, acute increase in plasma histamine concentration by four-fold, and a second, lower, sustained elevation.

In the Ws/Ws rats, the basal level of plasma histamine concentration, was 52 ± 3 pmol/ml, which is half the value in the Wistar and +/+ rats. In addition, the initial, acute increase in plasma histamine concentration was not observed (Fig. 2b). There was only a relatively small, sustained elevation in the plasma histamine level during the 6 h period of stress exposure, compared to that in the Wistar and +/+ rats.

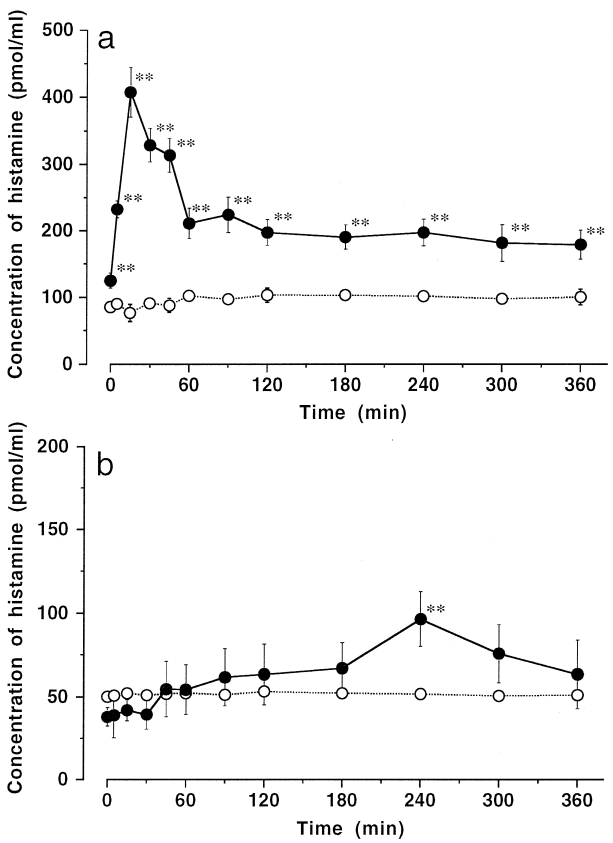

Fig. 2. Plasma concentration of histamine in +/+ (a) and Ws/Ws (b) rats exposed to water immersion stress for 6 h (●), and under the freely moving condition (○), respectively. The freely moving rats served as the control group. Each data point represents the mean ± S.E.M. ($n = 5$). $** P < 0.01$ compared to the respective value in the control group.

Table 1
Histamine content of the dorsal skin of Wistar and Ws/Ws rats exposed to water immersion stress for various lengths of time

	Wistar	Ws/Ws
Control	234.34 ± 16.15	0.46 ± 0.02
Stress for 15 min	188.72 ± 20.88[a]	ND
Stress for 1 h	187.54 ± 21.43[a]	ND
Stress for 4 h	170.93 ± 17.73[a]	0.47 ± 0.04

Data represent the means ± S.E.M. ($n = 5$), in nmol/g wet tissue weight.
[a] $P < 0.05$, compared to the value in the respective control group.
ND, not determined.

3.3. Effect of water immersion stress on the histamine content of the skin of the Wistar and Ws/Ws rats

Table 1 shows the effect of water immersion stress on the histamine content of the dorsal skin of the Wistar and Ws/Ws rats. The histamine content of the skin of Wistar rats not exposed to stress, was 234.34 ± 16.15 nmol/g wet tissue weight. In Wistar rats which were sacrificed after being exposed to stress for 15 min, the histamine content of the skin was 20% lower than that in the freely moving rats. In Wistar rats which were sacrificed after being exposed to stress for 1 and 4 h, no further decrease in the histamine content of the skin, was seen.

The histamine content of the skin of the Ws/Ws rats, was only 0.2% of that of the Wistar rats. In addition, the histamine content of the skin of Ws/Ws rats exposed to stress for 4 h, had not changed.

3.4. Effect of water immersion stress on the plasma histamine concentration of the gastrectomized Wistar and gastrectomized Ws/Ws rats

The basal level of plasma histamine in the gastrectomized Wistar rats under the freely moving condition, was 94 ± 2 pmol/ml. This value does not differ significantly from that of the non-gastrectomized Wistar rats. In the gastrectomized Wistar rats which were exposed to water immersion stress, an initial, acute increase in plasma histamine concentration by 2.5-fold, was seen. Thereafter, the plasma histamine concentration decreased to the basal level by 1 h, and it remained at the basal level up through 6 h (Fig. 3a).

In the gastrectomized Ws/Ws rats, the basal level of plasma histamine concentration was very low at 24 ± 1 pmol/ml. Upon exposing these rats to stress, neither the

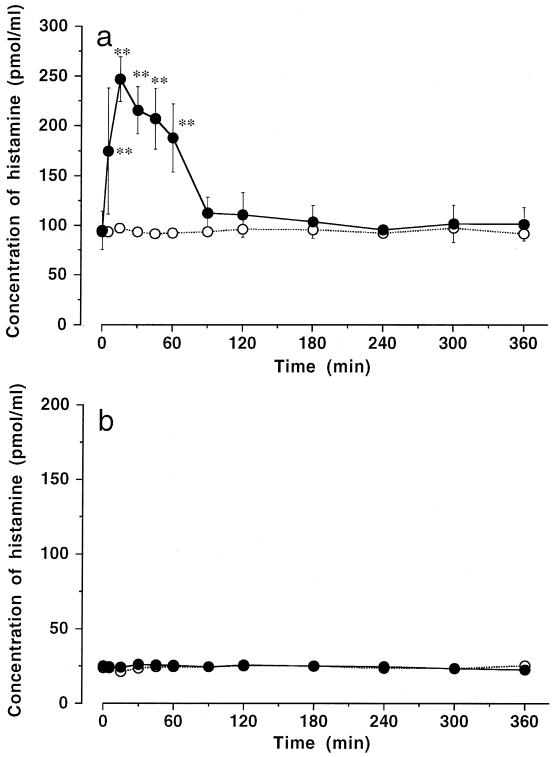

Fig. 3. Plasma concentration of histamine in gastrectomized Wistar (a) and Ws/Ws (b) rats exposed to water immersion stress for 6 h (●), and under the freely moving condition (○), respectively. The freely moving rats served as the control group. Each data point represents the mean ± S.E.M. ($n = 5$). $**$ $P < 0.01$ compared to the respective value in the control group.

initial, acute increase, nor the second, sustained increase in plasma histamine level, was observed (Fig. 3b).

3.5. Effect of water immersion stress on the histamine content and level of histidine decarboxylase activity in the gastric mucosa of the Wistar and Ws / Ws rats

Table 2 shows the histamine content, and level of histidine decarboxylase activity, in the gastric mucosa of Wistar rats and Ws/Ws rats exposed to water immersion stress for 4 h. The histamine content of the gastric mucosa of the Wistar rats not exposed to stress, was 501.95 ± 31.83 nmol/g wet tissue weight. The histamine content of the gastric mucosa of Wistar rats exposed to stress for 4 h, was 24% lower than that in the Wistar rats not exposed to stress. Histidine decarboxylase activity in the mucosa of the Wistar rats exposed to stress for 4 h, was 2.2-fold higher than that in the Wistar rats not exposed to stress.

Similar results were obtained in the gastric mucosa of the Ws/Ws rats. The histamine content of the gastric mucosa of the Ws/Ws rats not exposed to stress, was in a similar range at 597.53 ± 44.99 nmol/g wet tissue weight. In the Ws/Ws rats exposed to stress for 4 h, the histamine content in the gastric mucosa was 20% lower, and the level of histidine decarboxylase activity was 2.2-fold higher, than the respective value in the Ws/Ws rats not exposed to stress.

4. Discussion

The present study clearly shows that exposing Wistar and +/+ rats to water immersion stress induced a biphasic increase in the plasma histamine level over 6 h. The basal plasma histamine level in both the Wistar and +/+ rats under the freely moving condition, were equivalent. The plasma histamine level of both the Wistar and +/+ rats, changed in a similar manner upon exposure to water immersion stress (Figs. 1 and 2a). Thus, there is no fundamental difference in the response of the plasma histamine level to water immersion stress between these two strains. However, when Ws/Ws rats, which lack connective tissue-type mast cells, were exposed to stress, the initial, acute increase in plasma histamine level, was not seen (Fig. 2b). This indicates that, upon exposure to stress, the initial increase in plasma histamine is due to release of histamine from mast cells.

This finding was further confirmed by the results obtained from the gastrectomized animals. In gastrectomized Wistar rats, two types of cells in the periphery store histamine, mast cells and basophils. In gastrectomized rats, the initial, acute increase in plasma histamine concentration upon exposure to stress, should be derived from these two stores. Furthermore, in gastrectomized Ws/Ws rats, only basophils in the periphery contain histamine; upon exposure to stress, no response in the plasma histamine level was seen (Fig. 3b). These results clearly indicate that,

Table 2
Histamine content and level of histidine decarboxylase (HDC) activity in the gastric mucosa of Wistar and Ws/Ws rats

	Wistar		Ws/Ws	
	Histamine content	HDC activity	Histamine content	HDC activity
Control	501.95 ± 31.83	4.69 ± 0.76	597.53 ± 44.99	6.13 ± 0.65
Stress for 4 h	383.67 ± 21.51[a]	10.22 ± 0.76[a]	486.55 ± 24.18[a]	13.16 ± 2.29[a]

Data represent the means ± S.E.M. ($n = 5$).
Histamine content is expressed as nmol/g wet tissue weight.
Histidine decarboxylase activity is expressed as pmol/min/mg protein.
[a] $P < 0.05$, compared to the value in the respective control group.

upon exposure to water immersion stress, the initial, acute increase in plasma histamine concentration in Wistar rats, is due to induction of histamine release from mast cells; it is unlikely that basophils are involved in the increase in plasma histamine level.

Mast cells are localized predominantly in association with the blood vessels in the subepithelial connective tissue of the bronchi, conjunctiva, gut, skin and the peritoneal cavities (Leung and Pearce, 1984). Although the mast cells which underwent degranulation and released histamine in response to stress, could not be localized, we confirmed that the histamine content in the dorsal skin of Wistar rats decreased by 30% while exposed to water immersion stress for 4 h (Table 1). The histamine content of the skin of Ws/Ws rats was 0.2% of that obtained from the Wistar rats. This result is in accordance with that reported by Onoue et al. (1993). In the Ws/Ws rats exposed to water immersion stress for 4 h, no reduction in the histamine content of the skin was observed. These findings suggest that mast cells, at least in the skin, contributed to the increase in the plasma histamine level caused by stress.

It is well known that the release of histamine and other chemical mediators from mast cells is caused by an immunological reaction; that is, an antigen interacts with IgE bound to the surface of mast cells. In the present study, however, no exogenous antigen was administered to the animals during the exposure to stress. Thus, water immersion stress facilitated mast cell degranulation and histamine release through a non-immunological reaction. In our previous study, stimuli including the insertion of injection needles, and also the infusion of saline into the subplantar space of the rat hind paws, increased the subcutaneous level of histamine, which was measured by an in vivo microdialysis method (Guo et al., 1997). This strongly suggests a functional link between the mast cells and the peripheral nervous system. Some previous studies support this idea. Mast cells are in direct anatomical contact with neuropeptide-containing sensory nerves, and electrical stimulation of these nerve fibers induces the degranulation of mast cells (Dimitriadou et al., 1991; Oura et al., 1992). In addition, it has been suggested that neuropeptides, such as substance P and neurokinin A, which are released from sensory neurons, stimulate the release of histamine from mast cells (Joos et al., 1994; Horsmanheimo et al., 1996; Hua et al., 1996). Furthermore, it has also been reported that application of a cold water stress to rats, induces the release of substance P into the peritoneal cavity (Zhu et al., 1996). It has also been shown morphologically that an acute immobilization stress induces the degranulation of mast cells in the dura (Theoharides et al., 1995), and in the bladder (Spanos et al., 1997), of rats. All of these findings suggest the existence of neuronal control of histamine release from mast cells. The results from the present study provide direct evidence of this. The method used in this study may be useful for studying the mechanism underlying the allergic response, which is caused by the central and peripheral nervous system.

The second, sustained elevation in plasma histamine concentration, was seen in the Wistar rats (Fig. 1), +/+ rats (Fig. 2a), and the Ws/Ws rats (Fig. 2b), but not in the gastrectomized rats (Fig. 3a and b). These results indicate that the second phase of histamine increase, originated from gastric histamine-storing cells, probably ECL cells. After exposure to stress for 4 h, the histamine content in the gastric mucosa, where ECL cells are localized, had decreased by 20%, while histidine decarboxylase activity was enhanced by two-fold, in both the Wistar and Ws/Ws rats (Table 2). Asahara et al. (1996), reported that, in rats exposed to water immersion stress for 6 h, the level of histidine decarboxylase mRNA in the rat stomach was five times higher than that in the rats not exposed to stress. These findings suggest that, during stress exposure, ECL cells are stimulated to produce histidine decarboxylase and increase the amount of histamine released.

It is believed that ECL cells are activated by mechanisms mediated by gastrin/cholecystokinin B receptors, muscarinic M_1 receptors, β-adrenoceptors, and vasoactive intestinal polypeptide receptors (Sandor et al., 1996). Ma et al. (1993), reported that the serum concentration of gastrin in rats, increased significantly during the application of an immobilization stress for 1 h. In our previous study, we found that an infusion of pentagastrin into the jugular vein of rats, increased the concentration of plasma histamine in a dose-dependent manner, and enhanced histidine decarboxylase activity in the glandular stomach (Watanabe et al., 1996). A similar effect of gastrin on histamine release has been reported in studies on the oxyntic mucosa (Gerber and Payne, 1992), and the isolated ECL cells of rats (Chen et al., 1994). However, the peritoneal mast cells of rats (Watanabe et al., 1996), and mucosal mast cells prepared from the canine oxyntic mucosa (Soll et al., 1988), did not show any response to gastrin. These findings suggest that gastrin is a candidate hormone which mediates the activation of ECL cells induced by stress.

There was, however, a smaller increase in plasma histamine concentration in Ws/Ws rats exposed to water immersion stress, compared to that in the Wistar and +/+ rats (Fig. 2b). The ulcer index in the gastric mucosa of the Ws/Ws rats, is also much smaller than the ulcer index of the Wistar rats (data not shown). However, the histamine content and level of histidine decarboxylase activity in the gastric mucosa of the Wistar and Ws/Ws rats, before and after exposure to stress, were in the same range (Table 2). A possible explanation for the effect of water immersion stress on histamine release and gastric mucosal damage in Ws/Ws rats, would be the contribution of mast cells to gastric mucosal damage. It is clear that mast cells released histamine soon after the rats were exposed to stress, and that mast cells are responsible for the initial, acute increase in plasma histamine level. If we

assume that the initial, acute increase in plasma histamine level led to the development of gastric mucosal damage, the extent of gastric mucosal damage might be smaller in the Ws/Ws rats, because of the absence of the initial, acute increase in plasma histamine level in the Ws/Ws rats exposed to stress. Moreover, the initial, acute increase in the plasma histamine level of the gastrectomized Wistar rats (Fig. 3b), is smaller than that observed in the non-gastrectomized Wistar rats, although the basal level of plasma histamine concentration in these two groups of rats, are in the same range. Thus, there may be a considerable number of connective tissue-type mast cells in the stomach, and not in the mucosa, which could degranulate and release histamine immediately after exposure to stress. In a previous study, we compared the histamine content of the stomach fundus of +/+ and Ws/Ws rats, and found that almost half of the histamine content of the stomach fundus originated from connective tissue-type mast cells (Onoue et al., 1993). Therefore, the local concentration of histamine in the stomach would be affected by the degranulation of mast cells induced by stress. Taking these findings into consideration, we rather suppose that the second, sustained elevation in plasma histamine level, includes not only the histamine released from ECL cells in response to water immersion stress, but also the histamine which leaks from ECL cells that are damaged during exposure to stress. This is similar to the mechanism proposed in the study on gastric mucosal damage caused by administration of acetic or salicylic acid (Johnson and Overholt, 1967). Damage to the gastric mucosa would begin immediately after the onset of water immersion stress, and mucosal damage would develop gradually over several hours. The sustained increase in plasma histamine concentration, would be a result of the damage to ECL cells. This idea would account for the smaller increase in plasma histamine concentration, and the lesser degree of damage to the gastric mucosa of Ws/Ws rats, compared to that in the wild-type animals.

In conclusion, the present study showed that water immersion stress caused a biphasic increase in plasma histamine concentration in normal rats. The initial, acute increase in plasma histamine concentration originated from mast cell degranulation, and the second, sustained elevation originated from ECL cells. We suggest that histamine released in the periphery, participates in the physiologic and pathogenic process induced by stress, and in the development of gastric mucosal damage.

Acknowledgements

This study was partly supported by a Grants-in-Aid from the Ministry of Education, Culture and Science of Japan (No. 07557011), and the Japan–China Medical Research Foundation.

References

Ader, R., Cohen, N., Felten, D., 1995. Psychoneuroimmunology: interactions between the nervous system and the immune system. Lancet 345, 99–103.

Asahara, M., Mushiake, S., Shimada, S., Fukui, H., Kinoshita, Y., Kawanami, C., Watanabe, T., Tanaka, S., Ichikawa, A., Uchiyama, Y., Narushima, Y., Takasawa, S., Okamoto, H., Tohyama, M., Chiba, T., 1996. Reg gene expression is increased in rat gastric enterochromaffin-like cells following water immersion stress. Gastroenterology 111, 45–55.

Chen, D., Monstein, H.J., Nylander, A.G., Zhao, C.M., Sundler, F., Håkanson, R., 1994. Acute responses of rat stomach enterochromaffin-like cells to gastrin: secretory activation and adaptation. Gastroenterology 107, 18–27.

Cho, C.H., Koo, M.W.L., Garg, G.P., Ogle, C.W., 1992. Stress-induced gastric ulceration: its aetiology and clinical implications. Scand. J. Gastroenterol. 27, 257–262.

Dimitriadou, V., Buzzi, M.G., Moskowitz, M.A., Theoharides, T.C., 1991. Trigeminal sensory fiber stimulation induces morphological changes reflecting secretion in rat dura mater mast cells. Neuroscience 44, 97–112.

Gerber, J.G., Payne, N.A., 1992. The role of gastric secretagogues in regulating gastric histamine release in vivo [see comments]. Gastroenterology 102, 403–408.

Guo, Y., Mochizuki, T., Morii, E., Kitamura, Y., Maeyama, K., 1997. Role of mast cell histamine in the formation of rat paw edema: a microdialysis study. Eur. J. Pharmacol. 331, 237–243.

Håkanson, R., Vallgren, S., Ekelund, M., Rehfeld, J.F., Sundler, F., 1984. The vagus exerts trophic control of the stomach in the rat. Gastroenterology 86, 28–32.

Honda, K., Fukuda, S., Ishikawa, S.E., Kuzuya, T., Saito, T., 1994. Role of endogenous vasopressin in development of gastric ulcer induced by restraint and water immersion. Am. J. Physiol. 266, R1448–R1453.

Horsmanheimo, L., Harvima, I.T., Harvima, R.J., Ylonen, J., Naukkarinen, A., Horsmanheimo, M., 1996. Histamine release in skin monitored with the microdialysis technique does not correlate with the weal size induced by cow allergen. Br. J. Dermatol. 134, 94–100.

Hua, X.Y., Back, S.M., Tam, E.K., 1996. Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea. Am. J. Physiol. 270, L985–L991.

Johnson, L.R., Overholt, B.F., 1967. Release of histamine into gastric venous blood following injury by acetic or salicylic acid. Gastroenterology 52, 505–509.

Joos, G.F., Kips, J.C., Pauwels, R.A., 1994. In vivo characterization of the tachykinin receptors involved in the direct and indirect bronchoconstrictor effect of tachykinins in two inbred rat strains. Am. J. Respir. Crit. Care. Med. 149, 1160–1166.

Knigge, U., Matzen, S., Warberg, J., 1990. Histamine as a neuroendocrine regulator of the stress-induced release of peripheral catecholamines. Endocrinology 126, 1430–1434.

Kvetnansky, R., Goldstein, D.S., Weise, V.K., Holmes, C., Szemeredi, K., Bagdy, G., Kopin, I.J., 1992. Effects of handling or immobilization on plasma levels of 3,4-dihydroxyphenylalanine, catecholamines, and metabolites in rats. J. Neurochem. 58, 2296–2302.

Leung, K.B., Pearce, F.L., 1984. A comparison of histamine secretion from peritoneal mast cells of the rat and hamster. Br. J. Pharmacol. 81, 693–701.

Ma, J., Aou, S., Matsui, H., Hori, T., 1993. Gastric vagus mediates immobilization-induced hypocalcemia in rats. Am. J. Physiol. 265, R609–R614.

Niwa, Y., Kasugai, T., Ohno, K., Morimoto, M., Yamazaki, M., Dohmae, K., Nishimune, Y., Kondo, K., Kitamura, Y., 1991. Anemia and mast cell depletion in mutant rats that are homozygous at 'white spotting (Ws)' locus. Blood 78, 1936–1941.

Onoue, H., Maeyama, K., Nomura, S., Kasugai, T., Tei, H., Kim, H.M.,

Watanabe, T., Kitamura, Y., 1993. Absence of immature mast cells in the skin of Ws/Ws rats with a small deletion at tyrosine kinase domain of the c-kit gene. Am. J. Pathol. 142, 1001–1007.

Oura, H., Takeda, K., Daikoku, S., 1992. Blood vessels and immunoreactive substance P-containing nerve fibers in rat skin treated topically with clobetasol propionate, a corticosteroid. J. Dermatol. 19, 335–341.

Pacak, K., McCarty, R., Palkovits, M., Cizza, G., Kopin, I.J., Goldstein, D.S., Chrousos, G.P., 1995. Decreased central and peripheral catecholaminergic activation in obese Zucker rats. Endocrinology 136, 4360–4367.

Pearce, F.L., 1989. Mast cells: function, differentiation and activation. Curr. Opin. Immunol. 1, 630–636.

Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H.F., Sachs, G., 1993. Histamine secretion from rat enterochromaffin-like cells. Gastroenterology 105, 449–461.

Sandor, A., Kidd, M., Lawton, G.P., Miu, K., Tang, L.H., Modlin, I.M., 1996. Neurohormonal modulation of rat enterochromaffin-like cell histamine secretion. Gastroenterology 110, 1084–1092.

Soll, A.H., Toomey, M., Culp, D., Shanahan, F., Beaven, M.A., 1988. Modulation of histamine release from canine fundic mucosal mast cells. Am. J. Physiol. 254 (1 Pt 1), G40–G48.

Spanos, C., Pang, X., Ligris, K., Letourneau, R., Alferes, L., Alexacos, N., Sant, G.R., Theoharides, T.C., 1997. Stress-induced bladder mast cell activation: implications for interstitial cystitis. J. Urol. 157, 669–672.

Sternberg, E.M., Chrousos, G.P., Wilder, R.L., Gold, P.W., 1992. The stress response and the regulation of inflammatory disease. Ann. Int. Med. 117, 854–866.

Sugimoto, K., Maeyama, K., Alam, K., Sakurai, E., Onoue, H., Kasugai, T., Kitamura, Y., Watanabe, T., 1995. Brain histaminergic system in mast cell-deficient (Ws/Ws) rats: histamine content, histidine decarboxylase activity, and effects of (S) alpha-fluoromethylhistidine. J. Neurochem. 65, 791–797.

Takagi, K., Kasaya, Y., Watanabe, K., 1964. studies on the drugs for peptic ulcer. A reliable method for producing stress ulcer in rats. Chem. Pharm. Bull. 12, 465–472.

Theoharides, T.C., Spanos, C., Pang, X., Alferes, L., Ligris, K., Letourneau, R., Rozniecki, J.J., Webster, E., Chrousos, G.P., 1995. Stress-induced intracranial mast cell degranulation: a corticotropin-releasing hormone-mediated effect. Endocrinology 136, 5745–5750.

Theoharides, T.C., Singh, L.K., Boucher, W., Pang, X., Letourneau, R., Webster, E., Chrousos, G., 1998. Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects. Endocrinology 139, 403–413.

Waldum, H.L., Sandvik, A.K., 1989. Histamine and the stomach. Scand. J. Gastroenterol. 24, 130–139.

Watanabe, T., Maeyama, K., Yamatodani, A., Yamada, M., Kitamura, Y., Wada, H., 1980. Histidine decarboxylase activities in mutant mice deficient in mast cells. Life Sci. 26, 1569–1574.

Watanabe, H., Mochizuki, T., Maeyama, K., 1996. Transient increase of blood histamine level induced by pentagastrin. Continuous monitoring by in vivo microdialysis. Scand. J. Gastroenterol. 31, 1144–1150.

Yamatodani, A., Fukuda, H., Wada, H., Iwaeda, T., Watanabe, T., 1985. High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. J. Chromatogr. 344, 115–123.

Zhu, G.F., Chancellor-Freeland, C., Berman, A.S., Kage, R., Leeman, S.E., Beller, D.I., Black, P.H., 1996. Endogenous substance P mediates cold water stress-induced increase in interleukin-6 secretion from peritoneal macrophages. J. Neurosci. 16, 3745–3752.