Histamine Release Induced by Immobilization, Gentle Handling and Decapitation From Mast Cells and Its Inhibition by Nedocromil in Rats

Zhi-Li Huang[#,+], Takatoshi Mochizuki[#], Hirokazu Watanabe and Kazutaka Maeyama*

Department of Pharmacology, Ehime University School of Medicine, Shigenobu-cho, Onsen-gun, Ehime 791–0295, Japan

Received January 27, 1999 Accepted April 12, 1999

ABSTRACT—The effect of immobilization, gentle handling and decapitation on the level of plasma histamine in Wistar rats was investigated. Mast cell deficient (Ws/Ws) rats were used to characterize the source of elevated histamine in plasma by stress, and the effect of nedocromil, a mast cell stabilizer, on histamine release was assessed in these models in vivo. The plasma histamine concentration of freely moving rats was 93.0±2.3 pmol/ml. Gentle handling produced a transient increase in plasma histamine level by 1.9-fold, whereas immobilization resulted in a longer-lasting elevation by 2.6-fold compared to that in the freely moving rats. Decapitation increased the plasma histamine level by 10- to 16-fold compared with that in the freely moving rats. No increase in plasma histamine was found in Ws/Ws rats exposed to stress. Nedocromil inhibited the increase in plasma histamine level induced by stress in a dose-dependent manner. These findings suggest that stress induces histamine release from mast cells in Wistar rats and the extent of this histamine release increases with the severity of stress. Nedocromil proved to be a good pharmacological tool to inhibit stress-induced release of mediators from mast cells.

Keywords: Immobilization stress, Gentle handling, Decapitation, Histamine release, Nedocromil

Stress is a basic response to diverse real or perceived threatening stimuli (1) and can affect illness (2), especially autoimmune and neuroinflammatory syndromes. Stress participates in or worsens certain neuroinflammatory conditions such as migraines (3), neurogenic pruritus (4) and interstitial cystitis (5, 6), all of which have been associated with mast cell activation. When activated, mast cells release histamine and other mediators that induce anaphylactic responses including vasodilation, increased vascular permeability and contraction of smooth muscle.

The classic pathway of mast cell activation is initiated upon interaction of a multivalent antigen (allergen) with its specific IgE antibody attached to the cell membrane via its high affinity receptor, FcεRI (7). Mast cells may also be activated by nonimmunologic stimuli such as neuropeptides (8, 9), corticotropin-releasing hormone (10), as well as electrical stimulation of nerves in the dura (11) and in the skin (12, 13). Moreover, mast cells have also been found in close apposition to neurons (14–16). These findings suggest participation of peripheral histamine in the expression of physiological and pathological responses to stress.

Measurement of the plasma level of histamine can provide unique information about the activation of histamine-storing cells in vivo. A previous study in our laboratory showed that water immersion stress for 6 hr results in a biphasic increase in plasma histamine level (17), where the initial acute increase originated from mast cells and the second sustained release was attributed to enterochromaffin-like cells. This stress, extremely potent stimuli that are used to make gastric ulcer models, contains two components of stressor; i.e., restraint in a stainless steel mesh and immersion in a water bath. The effect of only immobilization stress on the plasma level of histamine, however, still remains unclear. It is worthwhile to confirm this effect because a short period of immobilization or gentle handling is needed for administering drugs. The reported "baseline" values of the histamine level in rats, especially when the animals are pretreated by handling or decapitation which includes handling and

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

+ Visiting scholar from Department of Pharmacology, Wannan Medical College, Wuhu, Anhui 241001, P.R. China.

* To whom correspondence should be addressed.

sacrifice of animals, vary widely. No data are available, however, on whether handling or decapitation affects the level of plasma histamine.

Nedocromil, a mast cell stabilizer, inhibits mediator release from inflammatory cells involved in allergic reactions and is widely used for prophylaxis of asthma (18). It remains unclear whether nedocromil can inhibit the stress-induced increase in plasma histamine level. If nedocromil can inhibit stress-induced release of mediators from mast cells, it would be a candidate for the treatment of many inflammatory conditions related to the activation of mast cells.

In the present study, effects of stress commonly encountered in the laboratory, i.e., immobilization, gentle handling and decapitation of Wistar rats, on the level of plasma histamine were investigated. Blood samples from the control rats were obtained in a freely moving condition. To characterize the sources of plasma histamine during stress exposure, we applied immobilization, gentle handling and decapitation to genetically mast-cell-deficient (Ws/Ws) rats (19). Furthermore, both the immobilization and gentle handling stress models were used to evaluate the pharmacological effect of nedocromil in vivo on the release of histamine from mast cells in rats exposed to stress.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 240−250 g, were purchased from CLEA Japan (Osaka) and kept in our laboratory for 1 week before exposing them to stress. Male and female Ws/+ rats, both of the Donryu strains, were crossed to obtain male Ws/Ws rats which are deficient in mast cells, using the procedure described by Niwa et al. (19). Each Ws/Ws rat had a weight of 230−250 g. All of the rats were housed at a constant temperature (24±2℃), with a humidity of 55±10%, on an automatically controlled, 12/12-hr 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 hr before they were exposed to stress, but were permitted intake of water ad libitum. The protocol was approved by the Animal Care and Use Committee of Ehime University.

Chemicals

Histamine and *o*-phthaladehyde were obtained from Wako Company (Osaka). Nedocromil, the disodium salt of a pyranoquinoline dicarboxylic acid, was supplied by Fujisawa-Fisons Pharmaceuticals (Osaka). All other chemicals were of the highest grade commercially available.

Cannulation procedure

Cannulation was carried out as described by Huang et al. (17). 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 stress was applied.

Immobilization procedure and blood sampling

Fasted Wistar or Ws/Ws rats were subjected to immobilization stress or gentle handling beginning at around 10:00 a.m. For the immobilization stress study, each rat was removed from the home cage environment and restrained firmly with a stainless steel mesh, and the immobilized rats were placed at a room temperature of 24℃ for 2 hr. Blood samples, at a volume of 0.15 ml each, were drawn from the jugular catheter at 0, 5, 15, 30, 45, 60, 90 and 120 min during the stress. The collected blood samples were immediately centrifuged ($800 \times g$) at 4℃ for 15 min. Fifty microliters of the plasma of each blood sample was stored at −84℃ until histamine determination.

The control rats were not subjected to stress. Blood samples were obtained from the rats under the freely moving condition during a 2-hr period at the time points noted above. Each rat was placed in an acrylic cage, and the outlet of the catheter was connected to a liquid swivel attached to the balanced arm (Tsumura, Tokyo). This system enabled us to obtain plasma samples from freely moving animals without exposing them to any type of stress.

Handling

After collection of the control blood sample via the catheter connected to the Tsumura freely moving system, Wistar or Ws/Ws rats were handled by being lifted by the tail so that the front paws could barely touch the floor of the cage for 1 min. A blood sample was obtained immediately at the end of the handling (1 min) and at 5, 15, 30, 45, 60, 90 and 120 min after the animal was returned to its cage. Control rats were used to obtain samples in the freely moving condition.

The effect of nedocromil on the increase in plasma histamine level induced by immobilization or gentle handling

Nedocromil was dissolved in saline and given at doses of 0.1, 1 and 10 mg/kg intravenously at volumes of 1 ml/kg. The same volume of saline was injected into the control rats. Two hours after nedocromil administration, the rats were subject to immobilization stress for 2 hr or

gentle handling for 1 min according to the procedure described above, and plasma samples were obtained from the catheter indwelled 3 days prior.

Decapitation

Other unanesthetized Wistar and Ws/Ws rats into which catheters were not implanted, were decapitated by a guillotine (Natsume, Tokyo), and three sequential 3-ml samples (a total of 8 to 10 ml per rat) of blood from the severed neck of the body were collected in plastic tubes containing 2000 I.U. of heparin powder. Drainage of blood from the trunk required up to 1 min, with the first 3 ml obtained within 10 sec and the second 3 ml during a 20-sec interval. In the control experiment, blood samples were obtained through indwelling catheters on postoperative day 3 in the freely moving condition.

Determination of histamine content by high-performance liquid chromatography (HPLC)-fluorometry

The concentration of histamine in the plasma was determined by the HPLC-fluorometry technique (20, 21). Fifty microliters of each plasma sample was diluted with 350 μl of 0.46 M perchloric acid and centrifuged at 10,000$\times g$ for 15 min at 4℃. Fifty microliters of the supernatant was injected directly into a column packed with the TSKgel SP2SW cation exchanger (150$\times$6 mm i.d.) (Tosoh, Tokyo). 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), using excitation and emission wavelengths of 360 and 450 nm, respectively.

Statistical analyses

Results are presented as the mean$\pm$S.E.M. Differences in plasma histamine level of the stress-exposed and freely moving rats were analyzed by Fisher's PLSD after two-factor analysis of variance (ANOVA). The effect of decapitation on plasma histamine was analyzed by Student's *t*-test. Significance was accepted at $P<0.05$.

RESULTS

Effect of immobilization on histamine release in Wistar rats

In Wistar rats exposed to immobilization for a 2-hr period, the plasma histamine level increased rapidly as shown in Fig. 1a. The maximal elevation, reached at 5 min after immobilization, was 2.6-fold higher than the basal level in the freely moving rats. Thereafter, the level of histamine declined but was sustained up to at least 90 min between 130–190 pmol/ml, which is significantly higher than that of the control. In contrast, the mean basal level of plasma histamine of the control rats in the freely moving condition was 93.0$\pm$2.3 pmol/ml. The level was not affected by the procedure of sampling.

Effect of handling on histamine release in Wistar rats

Gentle handling for 1 min increased the plasma level of histamine significantly in the samples drawn immediately after the handling. The peak level of histamine, reached 15 min after the handling, was 1.9-fold the plasma histamine concentration in the freely moving rats. The plasma histamine level then decreased and returned to the basal level at 30 min after handling (Fig. 2a).

Effect of immobilization and handling on histamine release in Ws/Ws rats

In Ws/Ws rats, the basal plasma histamine level was 51.1$\pm$1.0 pmol/ml, which is half the value in the Wistar rats. In addition, the rapid increase in plasma histamine level was not observed during the immobilization stress (Fig. 1b) or after gentle handling (Fig. 2b).

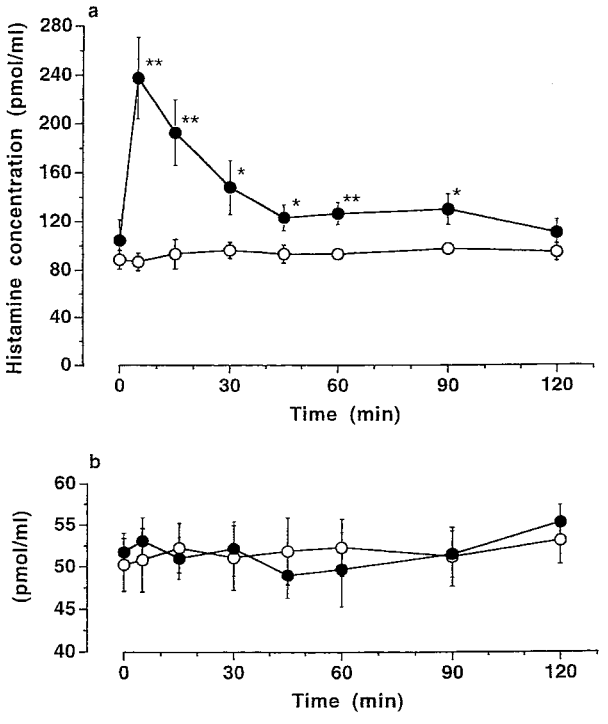

Fig. 1. Plasma concentration of histamine in Wistar (a) and Ws/Ws (b) rats exposed to immobilization stress for 2 hr (●) and under the freely moving condition (○). Freely moving rats served as the control group. Each data point represents the mean$\pm$S.E.M. (n=5 or 6 for Wistar rats and n=4 or 5 for Ws/Ws rats). **$P<0.01$, *$P<0.05$, compared to the respective value in the control group.

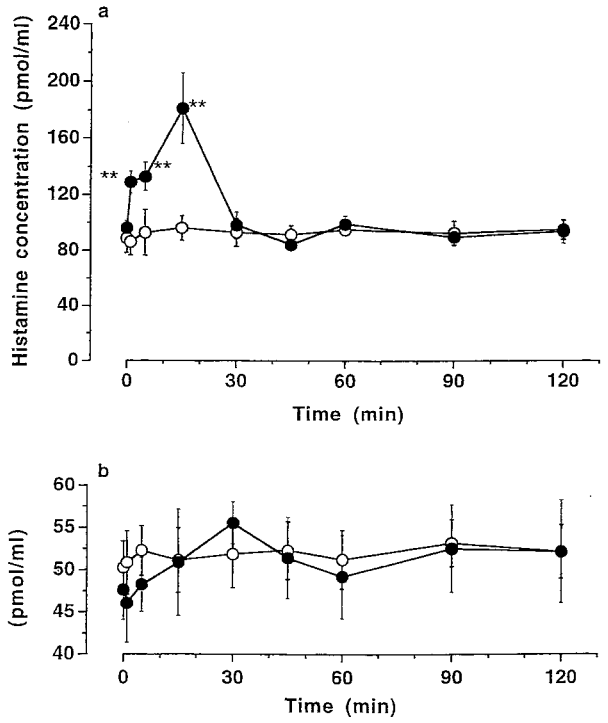

Fig. 2. Plasma concentration of histamine in Wistar (a) and Ws/Ws (b) rats exposed to gentle handling for 1 min (●) and under the freely moving condition (○). Freely moving rats served as the control group. Each data point represents the mean±S.E.M. (n=5 or 6 for Wistar rats and n=4 or 5 for Ws/Ws rats). **P<0.01, *P<0.05, compared to the respective value in the control group.

Effect of nedocromil on the increase in plasma histamine level induced by immobilization and gentle handling

The effect of various doses of nedocromil on the increase in plasma histamine level induced by immobilization is shown in Fig. 3. In the saline group, the plasma histamine level increased dramatically, peaking at a level about 2.8 times higher than the basal level, and thereafter rapidly decreased but sustained a level higher than the basal level. When 0.1 mg/kg of nedocromil was administered, the stress-induced elevation in plasma histamine was significantly inhibited at 5, 15, 60 and 90 min during the stress, and this inhibition became more marked as the dose of nedocromil was increased. The inhibitory effect of nedocromil at 1 mg/kg was much greater than that at 0.1 mg/kg. A dose of 10 mg/kg nedocromil eliminated the stress-induced histamine elevation, and the basal level of plasma histamine was reduced to 72% of the control level.

Similar results were observed after gentle handling (Fig. 4). Stress-induced elevation of the plasma histamine level appeared within 30 min after handling. The three dosages of nedocromil each inhibited the increase in histamine level at the end of handling and 15 min after

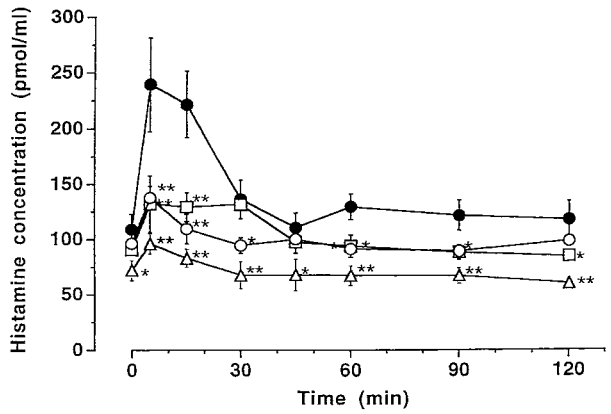

Fig. 3. Effect of various doses of nedocromil on the plasma concentration of histamine in Wistar rats exposed to immobilization for 2 hr. Nedocromil was administered intravenously 2 hr before immobilization. Each point represents the mean±S.E.M. (n=4 or 5). **P<0.01, *P<0.05, compared to the respective value in the saline group. ●: saline; △: nedocromil, 10 mg/kg; ○: nedocromil, 1 mg/kg; □: nedocromil, 0.1 mg/kg.

the stress. At 5 min after the stress, nedocromil at doses of 0.1 and 1 mg/kg did not inhibit the elevation in plasma histamine level but did at a dose of 10 mg/kg of nedocromil.

Effect of decapitation on histamine release in rats

After decapitation, the level of plasma histamine in Wistar rats was elevated throughout the blood collection period and reached the maximal level, a 16-fold increase, by the second 3-ml sample. Even in the first sample obtained after decapitation, the level of plasma histamine

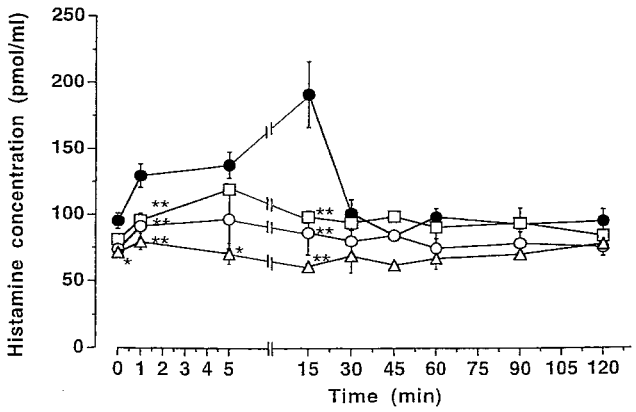

Fig. 4. Effect of various doses of nedocromil on the plasma concentration of histamine in Wistar rats exposed to gentle handling for 1 min. Nedocromil was administered intravenously 2 hr before gentle handling. Each point represents the mean±S.E.M. (n=4 or 5). **P<0.01, *P<0.05, compared to the respective value in the saline group. ●: saline; △: nedocromil, 10 mg/kg; ○: nedocromil, 1 mg/kg; □: nedocromil, 0.1 mg/kg.

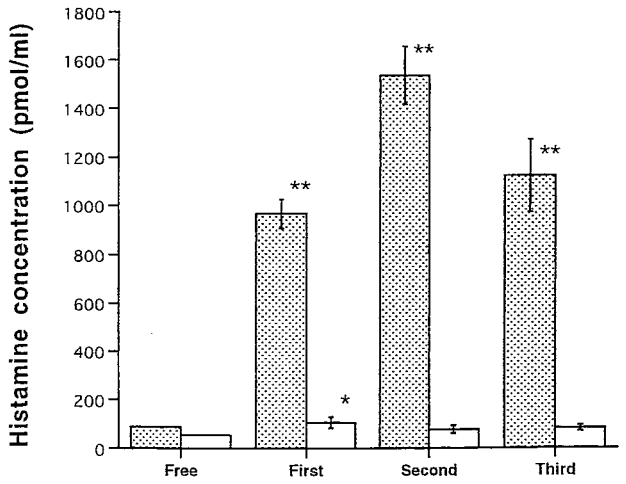

Fig. 5. Effect of decapitation on the plasma histamine concentrations in Wistar (close bar, n=7) and Ws/Ws (open bar, n=4) rats. In the control experiment, plasma samples were obtained in the freely moving condition by catheter on postoperative day 3. In the decapitation experiment, three sequential 3-ml samples of blood were collected from the trunk of the body. Each column represents the mean±S.E.M. of plasma histamine concentration. $^{**}P<0.01$, $^{*}P<0.05$, compared to the respective value in the freely moving rats.

had sharply increased to an amount that was 10-fold the level of plasma histamine in the freely moving rats, and 5- and 4-fold the peak level after gentle handling and the peak level during immobilization, respectively. In the third sample, the plasma histamine level was nearly the same as that in the first sample. In the first sample obtained after decapitation of Ws/Ws rats, the plasma histamine level was 90% higher than that in the freely moving rats and a little hematocytolysis was observed, while the plasma histamine level of the second and third samples did not differ from that of the freely moving rats (Fig. 5).

DISCUSSION

Water immersion stress, which has been regarded as a very useful experimental procedure to study stress-induced gastric ulcer in this past decade (22), induced a 4-fold transient increase in plasma histamine level (17). Immobilization which restrains animals without water immersion, however, is one of the commonly used experimental methods to study stress (23). The present study clearly demonstrates that both immobilization stress and gentle handling for just 1 min rapidly increased the plasma histamine level by 2.6- and 1.9-fold in Wistar rats, respectively (Figs. 1a and 2a). The acute increase in plasma histamine level induced by the two kinds of stress in Wistar rats, was not seen in the Ws/Ws rats that lack the connective tissue mast cells (Figs. 1b and 2b). These findings indicate that the increase in plasma histamine level observed in Wistar rats after exposure to stress is due to the release of histamine from mast cells and that the amount of histamine released from mast cells depends on the type and degree of stimulus. In the periphery, histamine is mainly stored in mast cells distributed throughout the body, basophils in the blood and enterochromaffin-like (ECL) cells in the stomach. Ws/Ws rats are genetically deficient in mast cells, but contain basophils and ECL cells. Previous studies showed that the number of basophils in the blood of Ws/Ws rats is 4×10^6/l, which is the same as that in wild type rats (24), and that the histamine content and histidine decarboxylase activity in the gastric mucosa, where ECL cells are localized, of Wistar rats and Ws/Ws rats do not differ (17). Taken these findings together, this suggests that basophils and ECL cells are not involved in the acute increase in plasma histamine level by stress (Figs. 1b and 2b).

Mast cells are localized predominantly in association with blood vessels in the subepithelial connective tissue of the bronchi, conjunctiva, gut, skin and the peritoneal cavity (25). Although we could not localize the mast cells that degranulated and released histamine in response to these stresses, previous results (17) showed that there is an immediate decrease in the histamine content of the dorsal skin of Wistar rats by 20% during exposure to water immersion stress, suggesting that skin mast cells dramatically respond to stress and release histamine. We might suppose that mast cells at least in the skin, contribute to the increase in the plasma histamine level caused by stress.

In the present experiments, no exogenous antigen was administered to the rats exposed to stress. Thus, the stress facilitated mast cell degranulation via a non-immunological process. Neuropeptides such as substance P and neurokinin A released from sensory nerves have been suggested to initiate histamine release from connective tissue mast cells (26–28). There are some reports that support this idea; mast cells are in direct anatomical contact with neuropeptide-containing sensory nerves (11–13), and electrical stimulation of the sciatic nerve induces an increase in histamine release in the paw of rats by microdialysis (Z.-L. Huang et al., unpublished data). Degranulation of mast cells in the dura and the bladder of rats was morphologically recognized after an acute immobilization stress (5, 29). The recent studies (30) showed that stress resulted in histamine release from mast cells, whereas the degeneration of sensory nerves in the rats pretreated with capsaicin significantly inhibited this response, suggesting that activation of sensory nerves participates in stress-induced histamine release from mast cells. These findings suggest that histamine release from mast cells is under neuronal control, and the present results provide direct evidence of this event in vivo. The

present method may be useful for examining the mechanism underlying the allergic response related to the central and peripheral nervous system.

Our finding that immobilization and gentle handling rapidly increase the plasma level of histamine is similar to the report showing that immobilization or gentle handling rapidly increases the plasma level of catecholamines, the catecholamine precursor 3,4-dihydroxyphenylalanine and metabolites of norepinephrine and epinephrine (31, 32). Among catecholamines, the neurotransmitter norepinephrine, which is mainly released from sympathetic nerve terminals, responds to stress extremely rapidly and reaches a peak level in plasma within 1 min of immobilization or gentle handling. Further study is needed to clarify whether the sympathetic neurotransmitters are involved in the induction of histamine release from mast cells in rats exposed to stress.

This finding was further confirmed pharmacologically by the administration of nedocromil prior to the stress. In vitro experiments showed that nedocromil has a high degree of specificity in its action, inhibiting immunological histamine release from serosal, but not intestinal, mast cells of rats, and it was completely ineffective against human basophils (33, 34). These findings indicate that nedocromil inhibits histamine release from connective tissue-type mast cells, but has no effect on mucosal mast cells and basophils. In this study, nedocromil inhibited histamine release induced by either immobilization or gentle handling in a dose-dependent manner. When the dose reached 10 mg/kg, not only did it inhibit the stress-induced increase of plasma histamine level, but it also reduced the basal level of plasma histamine. Taken together, the transient increase of plasma histamine level in Wistar rats occurred by the induction of histamine release from mast cells. Nedocromil may be a good pharmacological tool to inhibit the release of mast cell mediators induced by stress, and it might be used for prophylaxis and treatment of many inflammatory conditions such as migraines, neurogenic pruritus and interstitial cystitis.

In the present study, the use of indwelling jugular catheters and freely moving equipment permitted measurement of circulating histamine levels in rats not disturbed by the handling and/or restraint usually required to obtain samples of blood. In the blood of conscious unrestrained rats obtained through indwelling catheters, the normal plasma histamine level of 93.0 pmol/ml, is considerably lower than the 20 and 70 ng/ml which have been previously reported in the rat (35, 36).

In the freely moving condition, plasma histamine levels were stable. Relatively gentle handling in which the rats were suspended by the tail while remaining in their cage is probably less disturbing than the handling required for an i.p. or i.m. injection, but produced a 1.9-fold increase in circulating histamine. This finding that even a brief period of gentle handling can increase the plasma level of histamine, may help to explain the commonly encountered problem of large variability in baseline plasma histamine level in studies on plasma histamine.

The high level of histamine found in blood obtained from the trunk of decapitated animals without catheters (the same high level was observed in the decapitated rats with catheters, data not shown) was much higher than the plasma histamine level during immobilization or after gentle handling and that in freely moving conditions in the present study, but is similar to that previously reported in rat plasma (35, 37). Thus, the high levels of plasma histamine previously reported in rats have apparently been due to the stress of laboratory manipulation associated with the methods of blood collection. We suggest that blood collection from the decapitated trunk is not appropriate for studying the effects of stress or chemicals on plasma histamine. In the Ws/Ws rats, plasma histamine in only the first sample was increased due to hematocytolysis which resulted in histamine release from basophils, but the plasma histamine level in the two other samples did not differ from that of the freely moving rats. We suggest that the plasma histamine increase after decapitation in Wistar rats is also attributed to the release of histamine from mast cells.

In conclusion, the plasma level of histamine rapidly increases by immobilization and even gentle handling in conscious rats, and the greater the stress is, the greater the extent of histamine release is. This increase was not observed in Ws/Ws rats and was inhibited by a mast cell stabilizer, indicating that the elevation of plasma histamine originated from mast cells in Wistar rats. Nedocromil inhibited the increase in stress-induced plasma histamine in a dose-dependent manner, suggesting that nedocromil may be a good pharmacological tool to inhibit stress-induced exocytosis of compounds from mast cells, and it might be used for prophylaxis and treatment of many inflammatory conditions related to the activation of mast cells.

Acknowledgments

This study was partly supported by a Grants-in-Aid from the Ministry of Education, Culture, Sports and Science of Japan (No. 07557011) and the Japan-China Medical Research Foundation. We are grateful to Prof. N. Ogawa for stimulating discussions and encouragement to the first author.

REFERENCES

1 Covelli V: What is stress? How does it correlate with the immune system? Ann NY Acad Sci **741**, 212–215 (1994)

2 Rabkin JG and Struening EL: Live events, stress, and illness.

Science **194**, 1013–1020 (1977)
3 Theoharides TC: Mast cells and migraines. Perspect Biol Med **26**, 672–675 (1983)
4 Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D and Murphy GF: Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. Proc Natl Acad Sci USA **88**, 4220–4224 (1991)
5 Spanos C, Pang X, Ligris K, Letourneau R, Alferes L, Alexacos N, Sant GR and Theoharides TC: Stress-induced bladder mast cell activation, implications for interstitial cystitis. J Urol **157**, 669–672 (1997)
6 Sant GR and Theoharides TC: The role of the mast cell in interstitial cystitis. Urol Clin North Am **21**, 41–53 (1994)
7 Metcalfe DD, Baram D and Mekori YA: Mast cells. Physiol Rev **77**, 1033–1079 (1997)
8 Church MK, Lowman MA, Rees PH and Benyon RC: Mast cells, neuropeptides and inflammation. Agents Actions **27**, 8–16 (1989)
9 Foreman JC: Neuropeptides and the pathogenesis of allergy. Allergy **42**, 1–11 (1987)
10 Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E and Chrousos G: Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects. Endocrinology **139**, 403–413 (1998)
11 Dimitriadou V, Buzzi MG, Moskowitz MA and Theoharides TC: Trigeminal sensory fiber stimulation induces morphological changes reflecting secretion in rat dura mater mast cells. Neuroscience **44**, 97–112 (1991)
12 Kowalski ML and Kaliner MA: Neurogenic inflammation, vascular permeability, and mast cells. J Immunol **140**, 3905–3911 (1988)
13 Oura H, Takeda K and Daikoku S: Blood vessels and immunoreactive substance P-containing nerve fibers in rat skin treated topically with clobetasol propionate, a corticosteroid. J Dermatol **19**, 335–341 (1992)
14 Olsson Y: Mast cells in human peripheral nerve. Acta Neurol Scand **47**, 357–368 (1971)
15 Wiesner-Menzel L, Schulz B, Vakilzadeh F and Czarnetzki BM: Electron microscopical evidence for a direct contact between nerve fibres and mast cells. Acta Derm Venereol **61**, 465–469 (1981)
16 Williams RM, Bienenstock J and Stead RH: Mast cells, the neuroimmune connection. Chem Immunol **61**, 208–235 (1995)
17 Huang ZL, Mochizuki T, Watanabe H, Kagoshima M and Maeyama K: Biphasic elevation of plasma histamine induced by water immersion stress, and their sources in rats. Eur J Pharmacol **360**, 139–146 (1998)
18 Enerback L and Bergstrom S: Effect of nedocromil sodium on the compound exocytosis of mast cells. Drugs **37**, S44–S50; discussion 69–77 (1989)
19 Niwa Y, Kasugai T, Ohno K, Morimoto M, Yamazaki M, Dohmae K, Nishimune Y, Kondo K and Kitamura Y: Anemia and mast cell depletion in mutant rats that are homozygous at "white spotting (Ws)" locus. Blood **78**, 1936–1941 (1991)
20 Yamatodani A, Fukuda H, Wada H, Iwaeda T and Watanabe T: 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 (1985)
21 Guo Y, Mochizuki T, Morii E, Kitamura Y and Maeyama K: Role of mast cell histamine in the formation of rat paw edema: a microdialysis study. Eur J Pharmacol **331**, 237–243 (1997)
22 Arakawa H, Kodama H, Matsuoka N and Yamaguchi I: Stress increases plasma enzyme activity in rats: differential effects of adrenergic and cholinergic blockades. J Pharmacol Exp Ther **280**, 1296–1303 (1997)
23 Puri S, Ray A, Chakravarty AK and Sen P: Role of histaminergic mechanisms in the regulation of some stress responses in rats. Pharmacol Biochem Behav **39**, 847–850 (1991)
24 Kasugai T, Okada M, Morimoto M, Arizono N, Maeyama K, Yamada M, Tei H, Dohmae K, Onoue H, Newlands GFJ, Watanabe T, Nishimune Y, Miller HRP and Kitamura Y: Infection of Nippostrongylus brasiliensis induces normal increase of basophils in mast cell-deficient Ws/Ws rats with a small deletion at the kinase domain of c-kit. Blood **81**, 2521–2529 (1993)
25 Leung KB and Pearce FL: A comparison of histamine secretion from peritoneal mast cells of the rat and hamster. Br J Pharmacol **81**, 693–701 (1984)
26 Joos GF, Kips JC and Pauwels RA: 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 (1994)
27 Horsmanheimo L, Harvima IT, Harvima RJ, Ylonen J, Naukkarinen A and Horsmanheimo M: 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 (1996)
28 Hua XY, Back SM and Tam EK: Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea. Am J Physiol **270**, L985–L991 (1996)
29 Theoharides TC, Spanos C, Pang X, Alferes L, Ligris K, Letourneau R, Rozniecki JJ, Webster E and Chrousos GP: Stress-induced intracranial mast cell degranulation, a corticotropin-releasing hormone-mediated effect. Endocrinology **136**, 5745–5750 (1995)
30 Huang ZL, Mochizuki T, Watanabe H and Maeyama K: The activation of sensory nerves participates in stress-induced histamine release from mast cells. Jpn J Pharmacol **79**, Supp **I**, 44P (1999)
31 Kvetnansky R, Goldstein DS, Weise VK, Holmes C, Szemeredi K, Bagdy G and Kopin IJ: Effects of handling or immobilization on plasma levels of 3,4-dihydroxyphenylalanine, catecholamines, and metabolites in rats. J Neurochem **58**, 2296–2302 (1992)
32 Kvetnansky R, Armando I, Weise VK, Holmes C, Fukuhara K, Deka-Starosta A, Kopin IJ and Goldstein DS: Plasma dopa response during stress, dependence on sympathoneural activity and tyrosine hydroxylation. J Pharmacol Exp Ther **261**, 899–909 (1992)
33 Pearce FL, Al-Laith M, Bosman L, Brostoff J, Cunniffe TM, Flint KC, Hudspith BN, Jaffar ZH, Johnson NM and Kassessinoff TA: Effects of sodium cromoglycate and nedocromil sodium on histamine secretion from mast cells from various locations. Drugs **37**, S37–S43; discussion 69–77 (1989)
34 Kivity S, On A, Agami O, Levo Y and Fireman E: A comparison of the inhibitory effect of cromoline and nedocromil Na on

histamine release from airway metachromatic cells and from peripheral basophils. Immunol Lett **53**, 147–151 (1996)

35 Carraway R, Cochrane DE, Lansman JB, Leeman SE, Paterson BM and Welch HJ: Neurotensin stimulates exocytotic histamine secretion from rat mast cells and elevates plasma histamine levels. J Physiol (Lond) **323**, 403–414 (1982)

36 Green MD, Simon ML and Lomax P: Histamine as a neurotransmitter in the central thermoregulatory pathways of the rat. Proc West Pharmacol Soc **18**, 110–113 (1975)

37 Oishi R, Itoh Y, Nishibori M and Saeki K: Feeding-related circadian variation in tele-methylhistamine levels of mouse and rat brains. J Neurochem **49**, 541–547 (1987)