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Experimental anxiety induced by histaminergics in mast cell-deficient and congenitally normal mice

Yasushi Ikarashi*, Mitsutoshi Yuzurihara

Kampo & Pharmacognosy Laboratories, Tsumura, 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan

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Abstract

To clarify the effect of mast cell-derived histamine release in the brain on anxiety, histaminergics-induced anxiety-like behaviors were examined by a light/dark test in mast cell-deficient (W/W^v) and congenitally normal (+/+) mice. In +/+ mice, when cimetidine (an H_2 receptor antagonist) was coadministered with thioperamide (a neuronal histamine releaser acting via inhibition of H_3 autoreceptors) or Compound 48/80 (C48/80, a selective histamine releaser from mast cells), the time spent in the light zone and the number of crossings between light and dark zones in a light/dark test decreased significantly, suggesting induction of anxiety. In W/W^v mice, however, experimental anxiety was induced by coadministration of thioperamide–cimetidine, but not C48/80–cimetidine. These results suggest that both nonneuronal mast cell-derived histamine and neuronal histamine play an important role in inducing experimental anxiety. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Anxiety; Cimetidine; Compound 48/80; Light/dark test; Mast cell-deficient (W/W^v) mice; Thioperamide

1. Introduction

Histamine is a decarboxylation product of the amino acid histidine and is widely distributed in the mammalian central nervous system (CNS) (Inagaki et al., 1990; Panula et al., 1989). The involvement of the histaminergic system in multifarious brain functions and various behaviors has been documented (Onodera and Miyazaki, 1999; Onodera et al., 1994). The clinically effective anxiolytic drugs—diazepam, which is a benzodiazepine (Chikai et al., 1993; Oishi et al., 1986), and buspirone, which is a serotonin (5-HT_{1A}) agonist (Oishi et al., 1992)—have been found to decrease the turnover rate of brain histamine in mice and rats. In addition, it has been demonstrated that experimental anxiety is induced by coinjecting a neuronal histamine releaser, thioperamide, with a histamine H_2 receptor antagonist such as zolantidine or cimetidine (Imaizumi and Onodera, 1993; Yuzurihara et al., 2000), or by coinjecting the histamine releaser with a histamine H_1 agonist, betahistine (Imaizumi et al., 1996). These findings suggest that cerebral histamine may play an important role in the regulation of anxiety.

Biochemical, pharmacological, and anatomical evidence suggests a dual localization of histamine in mast cells and neurons of the CNS (Bugajski et al., 1995; Oishi et al., 1988). The brain histamine level of mutant mice deficient in mast cells (W/W^v) is approximately 50% of that in congenitally (+/+) normal mice, in spite of the absence of mast cells in W/W^v mice (Grzanna and Shultz, 1982; Yamatodani et al., 1982). Compound 48/80 (C48/80) is a specific histamine releaser from mast cells that increases the histamine release in the rat brain (Russell et al., 1990), and brain histamine concentration falls by 50% (Bugajski et al., 1995). These findings suggest that approximately 50% of brain histamine is stored in neuronal and 50% in nonneuronal areas. Recently, we demonstrated that anxiety-like behavior was induced by coinjection of C48/80 with a histamine H_2 receptor antagonist, cimetidine (Yuzurihara et al., 2000), suggesting that mast cell-derived histamine as well as neuronal histamine, plays an important role in inducing experimental anxiety. However, further studies are necessary to confirm this hypothesis.

The purpose of the present study is to clarify the effects of mast cell-derived histamine on anxiety. For this purpose,

* Corresponding author. Fax: +81-298-89-2158.
E-mail address: ikarashi_yasushi@mail.tsumura.co.jp (Y. Ikarashi).

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histaminergics-induced anxiety-like effects in mast cell-deficient (W/W^v) and congenitally normal (+/+) mice were examined using a light/dark test.

2. Methods

2.1. Animals

Six-week-old male mutant (W/W^v) mice with deficient mast cells (Yamatodani et al., 1982) and congenitally normal (+/+) mice weighing 25–30 g were obtained from SLC (Hamamatsu, Japan). Animals were housed five per cage and allowed ad lib access to water and standard laboratory food (MF, Oriental Yeast, Tokyo, Japan). Animals were housed in a facility at a temperature of 24 ± 1 °C, relative humidity of 55 ± 5%, and controlled lighting with light on from 07:00 to 19:00 daily.

Experimental protocols met the "Guidelines for Animal Experimentation" approved by the Japanese Association of Laboratory Animal Science.

2.2. Drugs

C48/80 (Sigma, St. Louis, MO, USA) and thioperamide maleate (Research Biochemicals International, Natick, MA, USA) were dissolved in saline. Cimetidine (Sigma) was prepared in 0.1 N HCl, and the pH was subsequently adjusted to 7.0 with 0.1 N NaOH.

2.3. Experimental procedure

W/W^v ($n = 27$) and +/+ mice ($n = 27$) were divided into three groups, respectively: a sham-operated control group ($n = 9$), a thioperamide plus cimetidine-treated group ($n = 9$), and a C48/80 plus cimetidine-treated group ($n = 9$). In the sham-operated control groups of W/W^v and +/+ mice, 2.0 μl of saline was injected into the cerebral ventricle, immediately followed by intraperitoneal injection of saline (10.0 ml/kg). In the thioperamide plus cimetidine-treated groups, 10.0 μg/2.0 μl of cimetidine solution was intracerebroventricularly-injected, immediately followed by intraperitoneal injection of thioperamide (10.0 mg/10 ml/kg). In the C48/80 plus cimetidine-treated groups, 2.0 μl of a mixture solution containing 1.0 μg of C48/80 and 10.0 μg of cimetidine was intracerebroventricularly-injected, immediately followed by intraperitoneal injection of saline (10 ml/kg).

Sixty minutes after the injections (icv and ip) of these drugs, anxiety-like behaviors for each animal were measured for a period of 5 min using a light/dark test. All tests were carried out between 13:00 and 17:00.

2.4. Intracerebroventricular injection

Intracerebroventricular injection was carried out under a nonanaesthetized condition, according to the procedure described previously (Yuzurihara et al., 2000): In brief, 2.0 μl of drug or vehicle solution was injected into the right lateral ventricle (coordinates: posterior to bregma, 2 mm; right lateral, 2 mm; ventral, 3 mm from the outer surface of the cranium) through a 26-G needle using a microinjection pump over 10 s. Ten seconds after the injection, the needle was carefully withdrawn. A solution injected into the unilateral ventricle is distributed throughout the ventricles in the brain within a few minutes after the injection. This result was confirmed visually, in a preliminary experiment, on frontal sections of a formalin-fixed brain a few minutes after injection of a methylene blue solution into the unilateral ventricle using the same procedure.

2.5. Light/dark test

The apparatus (Tsumura, Tokyo, Japan) for the light/dark test consisted of two compartments: one light zone (30 (L) × 27 (W) × 27 (H) cm, 1000 lx) illuminated by a fluorescent light, and a dark zone (15 (L) × 27 (W) × 27 (H) cm, 5 lx) illuminated by dim red light. The two compartments were separated by a partition with an opening (7.5 × 7.5 cm). The animals were not habituated to the apparatus, and measurement began immediately after the mice were placed in the center of the light zone. Three parameters — locomotor activity, time spent in the light zone, and the number of crossings between light and dark zones — were measured for each animal for a period of 5 min. The locomotor activity was counted using an Animex (model MK-110, Muromachi Kikai, Tokyo, Japan), while the other two behaviors were analyzed from a video recording.

2.6. Statistical analysis

Factorial significance of the interaction between strain, treatment, and their interaction in each behavioral parameter was assessed using a two-way ANOVA. The statistical significance among the drug-treated groups in each parameter was evaluated by a one-way ANOVA followed by post hoc Schéffe's test. Significance was accepted at $P < .05$.

3. Results

The effect of thioperamide (10 mg/10 ml/kg ip) or C48/80 (1.0 μg/2 μl icv) with cimetidine (10 μg/2 μl icv) on each behavioral parameter of the light/dark test in +/+ and W/W^v mice is shown in Table 1.

A two-way ANOVA revealed that the time spent in the light zone was significantly affected by mouse strain [$F(1,48) = 6.364$, $P < .05$], drug treatment [$F(2,48) = 37.525$, $P < .001$], and their interaction [$F(2,48) = 12.294$, $P < .001$]. Post hoc analysis following a one-way ANOVA [$F(5,48) = 21.201$, $P < .001$] revealed significant decreases ($P < .001$) in both groups treated with the thioperamide plus cimetidine and the C48/80 plus cimetidine in +/+ mice when the value

Table 1
Effects of thioperamide or C48/80 coinjected with cimetidine on time spent in the light zone, number of crossings, and locomotor activity in congenitally normal (+/+) and mast cell-deficient (W/W^v) mice using a light/dark test

Parameter/group	+/+ mice	W/W^v mice
Time spent in the light zone (s/5 min)		
Sham-operated control	56.7 ± 2.9	53.3 ± 3.2
Thioperamide + cimetidine	28.8 ± 2.4***	27.1 ± 4.0***
C48/80 + cimetidine	27.9 ± 2.2***	52.2 ± 3.6†††
Number of crossings (times/5 min)		
Sham-operated control	13.0 ± 1.2	12.3 ± 1.3
Thioperamide + cimetidine	3.9 ± 0.6***	4.4 ± 0.7***
C48/80 + cimetidine	4.3 ± 0.7**	10.3 ± 0.6††
Locomotor activity (count/5 min)		
Sham-operated control	255.1 ± 10.9	247.6 ± 8.5
Thioperamide + cimetidine	233.0 ± 10.5	174.4 ± 8.3***,††
C48/80 + cimetidine	255.3 ± 7.6	227.9 ± 12.4

Each value is expressed as the mean ± S.E.M. ($n = 9$).
** $P < .01$ vs. sham-operated control group in +/+ mice.
*** $P < .001$ vs. sham-operated control group in +/+ or W/W^v mice.
†† $P < .01$ vs. corresponding group in +/+ mice.
††† $P < .001$ vs. corresponding group in +/+ mice.

was compared to that in the corresponding sham-operated control group. In W/W^v mice, coinjecting thioperamide with cimetidine significantly decreased this parameter ($P < .001$), but it was not affected by coadministration of C48/80 with cimetidine, in comparison with that in the corresponding sham-operated control group. Significant changes between strains were observed only in the C48/80 plus cimetidine-treated groups: The time spent in the light zone in W/W^v mice was significantly prolonged ($P < .001$) over that in +/+ mice. The prolonged time was almost same as that in the corresponding sham-operated control group.

A two-way ANOVA showed a significant effect of the strain [$F(1,48) = 6.914$, $P < .05$], the treatment [$F(2,48) = 44.153$, $P < .001$], and the interaction [$F(2,48) = 7.535$, $P < .01$] in the number of crossing between light and dark zones. Post hoc analysis following a one-way ANOVA [$F(5,48) = 22.058$, $P < .001$] revealed significant decreases in both groups treated with thioperamide plus cimetidine ($P < .001$) or C48/80 plus cimetidine ($P < .01$) in +/+ mice when the value was compared to that in the corresponding sham-operated control group. In W/W^v mice, coinjecting thioperamide with cimetidine significantly decreased this parameter ($P < .001$), but it was not affected by coadministration of C48/80 with cimetidine, in comparison with that in the corresponding sham-operated control group. Significant differences between strains were observed only in the C48/80 plus cimetidine-treated groups: The number of crossing in W/W^v mice increased significantly ($P < .01$) over that in +/+ mice. The increased number was almost the same as that in the corresponding sham-operated control group.

In the locomotor activity, a two-way ANOVA showed a significant effect of the strain [$F(1,48) = 16.153$, $P < .001$], the treatment [$F(2,48) = 14.004$, $P < .001$], and the interaction [$F(2,48) = 3.740$, $P < .05$]. Post hoc analysis following a one-way ANOVA [$F(5,48) = 10.328$, $P < .001$] revealed a significant reduction in only the thioperamide plus cimetidine group of W/W^v mice when the value was compared to those in the sham-operated group of the same strain ($P < .001$), and in the same-treated group of control (+/+) strain ($P < .01$). No significant changes in the locomotor activity were observed in any group of +/+ and W/W^v mice.

Behavioral abnormalities such as catalepsy and scratching were not observed in any animal during the experiment period.

4. Discussion

Mast cell-deficient W/W^v mice have been widely used for the analysis of mast cell function in vivo (Wershil and Galli, 1994). In spite of the absence of mast cells in W/W^v mice, the histamine concentration of the brain is approximately 50% of that in congenitally normal +/+ mice (Grzanna and Shultz, 1982; Yamatodani et al., 1982). Nowadays, the nonmast cell histamine is considered to be neuronal histamine (Inagaki et al., 1990; Onodera and Miyazaki, 1999; Onodera et al., 1994). Both C48/80 and thioperamide enhance the endogenous histamine release in the brain via different mechanisms: degranulation from non-neuronal mast cells in the case of C48/80 (Bugajski et al., 1995; Lewis et al., 1986) and inhibition of H_3 autoreceptors that regulate neuronal histamine release in the case of thioperamide (Mochizuki et al., 1991; Onodera and Watanabe, 1998). In the present study, the role of mast cell histamine or neuronal histamine in a state of anxiety was assessed by using C48/80 and thioperamide in W/W^v and compared to the results in control +/+ mice.

In a previous study using normal ddy mice (Yuzurihara et al., 2000), we examined the dose responses of H_1, H_2, and H_3 histamine receptor ligands and C48/80 for induction of anxiety-like behaviors, and demonstrated that anxiety-like behaviors are induced when cimetidine (10.0 μg/2.0 μl icv) is coinjected with thioperamide (10.0 mg/kg ip) or C48/80 (1.0 μg/2.0 μl icv). In the present study, the same doses of the ligands were used to induce anxiety-like behaviors in W/W^v and +/+ mice. The light/dark test for assessment of anxiety was carried out for 5 min at 60 min after injection of drugs, according to our previously reported procedure (Yuzurihara et al., 2000). The time intervals were selected on the basis of the following findings: Thioperamide causes an approximately double the histamine release in rat brain 30–60 min after intraperitoneal injection, and the increased levels are maintained for at least 4 h (Itoh et al., 1991; Mochizuki et al., 1991). Intracerebroventricular injection of C48/80 causes a significant increase in histamine release in the brain 60 min after the injection (Bugajski et al., 1995). Effects of intracerebroventricularly-injected histamine receptor antagonists on C48/80-induced pituitary–adrenocortical activity have been evaluated 75 min postinjection (Gadek-Michalska et al., 1991). These findings suggest that it is

possible to evaluate these drug effects 60 min after intraperitoneal or intracerebroventricular injection.

In the light/dark test, a state of anxiety is evaluated using the rodent's propensity for exploratory behavior and preference for a dark location. Anxiolytics have been found to increase the time spent in the light zone and the number of crossings between the dark and light zones, while anxiogenics decrease these parameters (De Angelis, 1995; Imaizumi et al., 1994; Young and Johnson, 1991), suggesting that both behaviors are good indicators of anxiety. In addition, Imaizumi and Onodera (1993) reported that a decrease in locomotor activity in the light zone indicates experimental anxiety, because the aversion for anxiety in the light decreases in locomotion as well as time spent in the light zone. In the present study, a significant reduction of locomotor activity was observed in only the thioperamide plus cimetidine-treated W/W^v mice, but not in the other groups of +/+ and W/W^v mice. Because locomotor activity in the present study was measured as total locomotor activity in both zones, we could not clarify the relationship between the changes in locomotor activities in the respective zones and the state of anxiety.

Histamine is also thought to be an important mediator of itchiness. For example, subcutaneous or intradermal injection of C48/80 has been reported to cause mice to scratch (Sugimoto et al., 1998). Lewis et al. (1986) and Onodera and Shinoda (1991) have demonstrated that sedation and catalepsy are induced by high concentrations of histamine (100 μg icv) or C48/80 (15 and 25 μg icv). Thus, the aversion induced by itching, and the depression of motor activity induced by catalepsy may affect the anxiety-like behavior. However, in the present study, video recording did not reveal any abnormal behaviors in drug-treated groups, including C48/80, suggesting that higher doses than those used in this study or peripheral injection of C48/80 may induce the abnormal behaviors. Therefore, we concluded that changes in the two anxious behaviors were not due to the behavioral toxicity.

In the present study, both behavioral parameters — the time spent in the light zone and the number of crossings — showed a significant decrease when cimetidine was coinjected with thioperamide or C48/80 in congenitally normal +/+ mice. These results were the same as those reported in our previous study using normal ddy mice (Yuzurihara et al., 2000). These results also suggest that not only were the experimental conditions appropriate for the induction of experimental anxiety in the present study, but also that both neuronal- and mast cell-derived histamine may induce anxiety-like behaviors in normal +/+ mice. In contrast, the injection of a selective mast cell histamine releaser (C48/80) with an H_2 blocker (cimetidine) into mast cell-deficient W/W^v mice did not induce the anxiety-like behaviors although the injection of a neuronal histamine releaser (thioperamide) with cimetidine did. These results clearly suggest that mast cell-derived histamine also plays an important role in inducing experimental anxiety. Furthermore, our results demonstrated that it was necessary to inject an H_2 blocker with a histamine releaser to induce experimental anxiety. Imaizumi et al. (1996) have demonstrated that injection of an H_1 agonist with a histamine releaser induces anxiety-like behaviors. We have previously demonstrated that an H_1 blocker, mepyramine, antagonizes the anxiety-like behaviors induced by the same procedure used in the present study (Yuzurihara et al., 2000). These findings, taken together, suggest that H_1 receptors have an anxiogenic effect, while H_2 receptors have an anxiolytic effect: namely, experimental anxiety is induced when histamine released from mast cells or histaminergic nerve terminals in response to the stimuli activates H_1 receptors more than H_2 receptors.

On the other hand, thioperamide has been reported to affect not only histaminergic neurotransmission through H_3 autoreceptors but also serotonergic, noradrenergic, and cholinergic neurotransmissions through the heteroreceptors in learning and memory (Onodera and Watanabe, 1998; Onodera et al, 1994). These findings assume that histamine released by C48/80 may affect various neuronal systems through the heteroreceptors. The interaction of various neuronal transmissions with histaminergic receptors in anxiety should be considered in future studies.

In conclusion, C48/80-induced experimental anxiety was found in normal +/+ mice, but not in mast cell-deficient W/W^v mice. These results suggest that mast cell-derived histamine plays an important role in inducing experimental anxiety. Our findings lay the foundation for further investigations to assess the effect of cerebral histamine on anxiety.

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