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ORIGINAL INVESTIGATION

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Different effects of 5-HT_{1A} receptor agonists and benzodiazepine anxiolytics on the emotional state of naive and stressed mice: a study using the hole-board test

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Abstract *Objectives:* The effects of 5-HT_{1A} receptor agonists on the emotional behavior of naive or stressed mice were examined and compared with those of benzodiazepine anxiolytics. *Methods:* Changes in the emotional state of mice were evaluated in terms of changes in exploratory activity, i.e. total locomotor activity, numbers and duration of rearing and head-dipping and latency to the first head-dipping, using an automatic hole-board apparatus. *Results:* The 5-HT_{1A} receptor full agonists flesinoxan (0.03–1 mg/kg, IP) and 8-OH-DPAT (0.03–1 mg/kg, IP), and the partial agonist buspirone (0.3–10 mg/kg, IP) dose-dependently decreased all of the exploratory behaviors. Significant decreases in both the number and duration of head-dips, and an increase in the latency to head-dipping were observed at 30 min after exposure to acute restraint stress (60 min). These emotional changes were scarcely improved by post-stress treatment with 5-HT_{1A} receptor agonists, at doses that alone did not produce a significant behavioral effect. In contrast, pretreatment with flesinoxan (0.1–1 mg/kg, IP) or 8-OH-DPAT (0.1–1 mg/kg, IP) 24 h prior to exposure to stress dose-dependently suppressed the decrease in various exploratory behaviors that was observed immediately after the exposure to acute restraint stress. Moreover, pretreatment with buspirone (1–10 mg/kg, IP) 24 h prior to exposure to stress also significantly suppressed the decrease in rearing behavior and the increase in head-dip latency. However, changes in the emotional response to stress stimuli were not observed in mice that had been pretreated with the benzodiazepine anxiolytics diazepam (0.1–1 mg/kg, IP) and chlordiazepoxide (2–8 mg/kg, IP). *Conclusions:* The present study clearly demonstrates that the behavioral effects of 5-HT_{1A} receptor agonists in both naive and stressed mice were quite different from those of benzodiazepine anxiolytics, as previously reported by us. Notably, 5-HT_{1A} receptor agonists but not benzodiazepine anxiolytics protect against various emotional changes produced by stress stimuli, and the results suggest that activation of 5-HT_{1A} receptors may facilitate some mechanism(s) involved in the recognition of and/or ability to cope with stressful situation.

Key words Hole-board · Emotional behavior · 5-HT_{1A} receptor agonists · Benzodiazepine anxiolytics · Restraint stress · Mouse

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Introduction

Most of the procedures used to assess anxiety in rodents are particularly adapted to detect anxiolytic activities. In general, animals are exposed to anxiogenic conditions, i.e. either a novel environment (elevated plus-maze test, social interaction test, open field test, hole-board test) or a conflict situation (Vogel punishment drinking test or Geller-Seifter test) (Geller and Seifter 1960; Vogel et al. 1971; File and Wardill 1975; File and Hyde 1978; Pellow et al. 1985), and these procedures have been used to study the anxiolytic properties of many compounds. The hole-board test, which was first introduced by Boissier and Simon (1962, 1964), offers a simple method for measuring the response of an animal to an unfamiliar environment. Previously, the hole-board test has been used to assess emotionality, anxiety and/or responses to stress in animals (Rodriguez Echandia et al. 1987). Some advantages of this test are that several behaviors can be readily observed and quantified, which makes possible a comprehensive description of the animal's behavior.

To establish a more detailed behavioral analysis, we recently developed an automatic hole-board apparatus (Takeda et al. 1998). We expect that this system will make it possible automatically to measure changes in various exploratory activities of animals, and therefore,

this system may be a useful tool for objectively estimating various emotional states of animals. However, this advantage is also a defect in that the behaviors affected by anxiety- and/or anxiogenic-relevant manipulations often vary between animals. Therefore, to overcome this problem, it is important to identify behavior(s) of animals that are affected by anxiety and/or an anxiolytic state. We previously examined the effects of benzodiazepine anxiolytics and anxiogenics or exposure to acute restraint stress on emotional behaviors of mice using our automatic hole-board apparatus (Takeda et al. 1998). In this experiment, we found that benzodiazepine anxiolytics and anxiogenics selectively increased and decreased the head-dipping behavior of mice, respectively. Moreover, a decrease in head-dipping behavior was also observed in mice that had been exposed to acute restraint stress, and this effect of stress was suppressed by post-stress treatment with diazepam, a typical benzodiazepine anxiolytic. Based on these observations, we speculated that changes in head-dipping behaviors in the hole-board test might reflect the anxiogenic and/or anxiolytic state of mice produced via a benzodiazepine-mediated mechanism.

Recent clinical and preclinical studies suggest that central serotonin (5-HT) neurotransmission may be involved in the etiology, expression and treatment of anxiety, impulsiveness and depression (Murphy 1990). The discovery of multiple 5-HT receptor subtypes and the development of various selective ligands for these receptors offer an opportunity to clarify the roles of 5-HT in these mental disorders and to treat them more effectively (Murphy 1990; Cowen 1991; Martin and Humphrey 1994). 5-HT_{1A} receptors have been of particular interest because it seems that they are involved in the regulation of emotional and behavioral processes (Murphy 1990; Barrett and Vanover 1993; Artigas 1996). Clinical studies involving the 5-HT_{1A} receptor partial agonist buspirone and the full agonist flesinoxan have shown promising results with regard to generalized anxiety disorder and depression (Murphy et al 1991; Grof et al 1993; Pitchot et al. 1995). On the contrary, in preclinical studies using various animal models of anxiety, 5-HT_{1A} receptor agonists do not exert anxiolytic activity in some paradigms used to detect the effects of benzodiazepine anxiolytics (Barrett 1991). These reports indicate the possibility that the reduction in anxiety observed with 5-HT_{1A} receptor agonists in the clinic differs qualitatively from that observed with classical benzodiazepine anxiolytics. The aim of the present study was to provide additional evidence to support this hypothesis. Thus, the effects of 5-HT_{1A} receptor agonists on various exploratory behaviors of naive and stressed mice were examined using our automatic hole-board apparatus and compared with those of benzodiazepine anxiolytics.

Materials and methods

The present studies were conducted in accordance with Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Tokyo Medical University and the Japanese Pharmacological Society.

Animals

Male ICR mice (Charles River, Japan) weighing 25–30 g were housed at a room temperature of 23±1°C with a 12-h light-dark cycle (light on 6:00 a.m. to 6:00 p.m.). Food and water were available ad libitum. All experiments were carried out in the light phase of the cycle.

Apparatus

The automatic hole-board apparatus (model ST-1; Muromachi Kikai Co., Ltd, Japan) consisted of a gray wooden box (50×50×50 cm) with four equidistant holes 3 cm in diameter in the floor. An infrared beam sensor was installed on the wall to detect the number and duration of rearing and head-dipping behaviors and the latency to the first head-dipping. Other behavioral parameters such as locus, distance and speed of movement of mice in the hole-board were recorded by an overhead color CCD camera; the heads of the mice were painted yellow and the color CCD camera followed their center of gravity. Data from the CCD camera were collected through a custom-designed interface (CAT-10; Muromachi Kikai) as a reflection signal. Head-dipping behaviors were double-checked via an infrared beam sensor and the overhead color CCD camera. Thus, only when both the head intercepted the infrared beam and the head was detected at the hole by the CCD camera was head-dipping behavior counted. All of the data were analyzed and stored in a personal computer using analytical software (Comp ACT HBS; Muromachi Kikai).

Behavioral procedure

Effects of 5-HT1A receptor agonists on the exploratory behavior of mice in the hole-board test

Groups of animals were injected with drugs or vehicle. Thirty minutes later, each animal was placed in the center of the hole-board, and allowed to freely explore the apparatus for 5 min. Total locomotor activity, number and duration of rearing and head-dipping and latency to the first head-dip were automatically recorded.

Effects of post-stress treatment with 5-HT1A receptor agonists on acute restraint stress-induced changes in exploratory behavior of mice in the hole-board test

Animals were restrained in a snug-fit apparatus (stressed group) or left in their home cage (non-stressed group) for 60 min. Each animal was then injected with drugs or vehicle, and the behavioral experiments were performed 30 min later.

Effects of pretreatment with 5-HT1A receptor agonists or benzodiazepine anxiolytics on behavioral response of mice to acute restraint stress 24 h later in the hole-board test

Groups of animals were injected with drugs or vehicle. Twenty-four hours later, animals were restrained in a snug-fit apparatus (stressed group) or left in their home cage (non-stressed group) for 60 min, and the behavioral experiments were performed immediately.

Effects of treatment with 5-HT1A receptor agonists on behavioral response of mice that habituated to the hole-board apparatus

Groups of animals allowed to freely explore the apparatus for 5 min once daily for 7 consecutive days, and exploratory behaviors were recorded every day. On day 7, animals were injected with drugs or vehicle immediately after the behavioral experiments. Twenty-four hours later, exploratory behaviors were recorded again for 5 min.

Drugs

The drugs used in the present study were diazepam (Wako Pure Chemical Industries Ltd, Japan), chlordiazepoxide hydrochloride (Sigma Chemical Co., St Louis, Mo., USA), flesinoxan hydrochloride (provided by Solvay, Netherlands), *R*(+)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide (8-OH-DPAT; Research Biochemicals, Natick, Mass., USA) and buspirone hydrochloride (Research Biochemicals). Diazepam and chlordiazepoxide were suspended by ultrasound in saline with a drop of Tween 20, and all other drugs were dissolved in saline.

Statistical analysis

The data are presented as the mean±SEM. Head-dip latency was tested for significance with the non-parametric Kruskal-Wallis test because Bartlett's test for homogeneity of variances suggested that variances of data in some groups are not equal. Other behavioral parameters were tested with one-way repeated measures analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test (P<0.05 and 0.01).

Results

Effects of 5-HT_{1A} receptor agonists on the exploratory behavior of mice in the hole-board test

The effects of 5-HT_{1A} receptor agonists on the exploratory behavior of mice are shown in Table 1. Flesinoxan (0.03–1 mg/kg, IP) and 8-OH-DPAT (0.03–1 mg/kg, IP), 5-HT_{1A} receptor full agonists, produced a dose-dependent decrease in locomotor activity and in the number and duration of rearing behaviors, and the differences were statistically significant at 0.3 and 1 mg/kg (P<0.05 and 0.01). The number and duration of head-dips were also dose-dependently decreased by treatment with both drugs, and the differences were statistically significant at 0.3 and 1 mg/kg (P<0.05 and 0.01). In contrast, the latency to head-dipping dose-dependently increased following drug treatments, and significant changes were observed at 1 mg/kg (P<0.01). Similar effects on the exploratory behaviors of mice were also observed by treatment with buspirone (0.3–10 mg/kg, IP), a 5-HT_{1A} receptor partial agonist.

Effects of post-stress treatment with 5-HT_{1A} receptor agonists and benzodiazepine anxiolytic on acute restraint stress-induced changes in the exploratory behavior of mice in the hole-board test

Effects of post-stress treatment with 5-HT_{1A} receptor agonists on acute restraint stress-induced changes in exploratory behavior of mice are shown in Table 2. A significant decrease in both the number and duration of head-dips (P<0.05 or 0.01), and an increase in the latency of head-dipping (P<0.05) were observed 30 min after exposure to acute restraint stress. Flesinoxan, at a dose which alone did not produce a significant behavioral effect (0.1 mg/kg, IP) (see Table 1), had a weak but significant suppressive effect on the restraint stress-induced decrease in head-dip duration (P<0.05). However, the decrease in head-dip count and the increase in the latency to head-dipping produced by restraint stress were not significantly affected by treatment with flesinoxan. Furthermore, neither 8-OH-DPAT (0.1 mg/kg, IP) nor buspirone (0.3 mg/kg, SC), at doses which alone did not pro-

Table 1 Effects of 5-HT_{1A} agonists on exploratory behavior of mice in the hole-board test. Drugs or saline was injected 30 min prior to the measurement of exploratory behavior. Each data point represents the mean with SEM of eight to ten mice

Drugs (mg/kg, IP)		Locomotion (cm)	Rearing		Head-dips		
			Counts	Duration (s)	Counts	Duration (s)	Latency (s)
Saline		2939.9±211.4	33.3±2.9	34.4±3.8	30.0±3.5	15.8±3.1	46.9±10.3
Flesinoxan	(0.03)	2451.6±180.5	32.8±2.9	38.2±3.8	23.4±4.3	10.5±2.3	40.2±12.2
	(0.1)	2418.0±123.9	33.4±2.6	44.9±3.9	19.1±3.3	9.2±2.4	32.5±4.0
	(0.3)	2079.9±141.2*	20.1±2.4**	20.2±2.5**	17.8±4.6	7.0±2.4*	48.7±13.3
	(1)	1800.3±255.0**	12.0±2.9**	12.0±3.6**	4.4±1.4**	1.1±0.4**	114.0±22.5**
Saline		2286.2±108.7	25.7±1.5	33.4±2.3	32.6±4.0	15.9±3.3	41.2±3.6
8-OH-DPAT	(0.03)	2106.8±114.5	25.2±3.0	30.2±3.5	25.8±2.7	10.8±1.8	45.6±7.5
	(0.1)	2054.6±94.9	24.9±2.8	31.3±3.7	22.1±2.8*	8.7±1.5*	47-6±8.6
	(0.3)	1712.9±138.6**	21.4±2.8	21.5±2.6*	16.5±2.9**	6.5±1.3**	87.8±28.4
	(1)	1355.2±128.1**	10.6±2.3**	8.9±2.2**	7.0±1.7**	2.2±0.6**	138.0±32.4*
Saline		2155.2±92.4	31.6±2.2	41.5±4.0	26.9±3.5	12.9±2.9	31.7±3.9
Buspirone	(0.3)	2109.1±124.7	25.4±3.1	30.6±4.1	23.1±4.0	9.1±1.9	51.7±7.6
	(1)	1757.2±89.9	25.8±3.3	32.3±4.5	14.8±2.5**	4.5±0.9**	64.3±10.5
	(3)	1813.2±99.6	24.3±3.4	27.7±4.1	9.9±1.6**	3.0±0.7**	73.2±25.9
	(10)	1345.7±148.3**	20.1±3.5*	19.9±3.5**	4.5±1.0**	1.3±0.3**	114.3±31.4*

*P<0.05, **P<0.01 vs saline-treated group

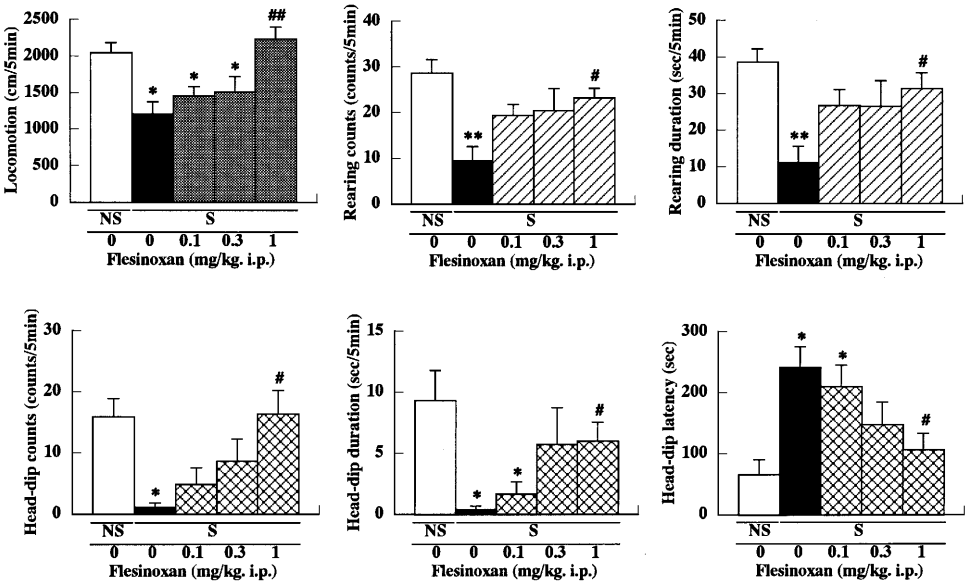

Fig. 1 Effects of pretreatment with flesinoxan on the behavioral responses of mice to acute restraint stress 24 h later in the hole-board test. Mice were pretreated with flesinoxan (0.1–1 mg/kg, IP) or saline (10 ml/kg, IP). Twenty-four hours later, mice were exposed to acute restraint stress (60 min), and exploratory behaviors on the hole-board were then measured for 5 min. Each column represents the mean with SEM of eight or nine mice. **P*<0.05, ***P*<0.01 versus saline plus non-stressed group (*open column*). #*P*<0.05, ##*P*<0.01 versus saline plus stressed group (*closed column*)

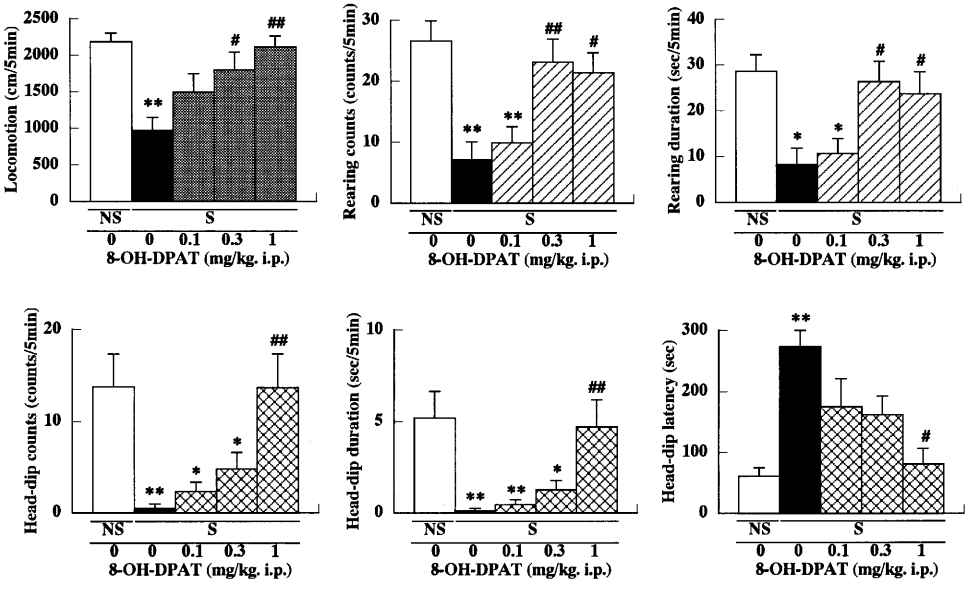

Fig. 2 Effects of pretreatment with 8-OH-DPAT on the behavioral responses of mice to acute restraint stress 24 h later in the hole-board test. Mice were pretreated with 8-OH-DPAT (0.1–1 mg/kg, IP) or saline (10 ml/kg, IP). Twenty-four hours later, mice were exposed to acute restraint stress (60 min), and exploratory behaviors on the hole-board were then measured for 5 min. Each column represents the mean with SEM of eight or nine mice. ***P*<0.01 versus saline plus non-stressed group (*open column*). #*P*<0.05, ##*P*<0.01 versus saline plus stressed group (*closed column*)

Table 2 Effects of post-stress treatment with 5-HT_{1A} agonists on acute stress-induced changes in exploratory behavior of mice tested in the hole-board test. Mice were exposed to acute restraint stress (60 min), and drugs or saline were then injected. Thirty minutes later, exploratory behavior was measured. Each data represents the mean with SEM of eight to ten mice

Treatment	Locomotion (cm)	Rearing		Head-dips		
		Counts	Duration (s)	Counts	Duration (s)	Latency (s)
Non-stress	2380.8±114.5	26.8±2.7	31.5±2.9	35.3±2.6	18.1±2.1	32.0±5.4
Stress	2038.6±83.6*	22.9±1.9	23.5±2.4	18.6±3.0**	6.7±1.4**	100.3±20.2*
Stress+Flesinoxan (0.1 mg/kg)	1745.2±64.2**	23.3±2.7	29.1±3.9	25.7±2.8*	13.6±2.1#	44.5±8.9
Non-stress	2199.2±161.7	24.1±1.8	29.8±3.9	38.4±2.0	21.7±2.5	34.2±9.5
Stress	1958.6±62.4	23.9±1.3	28.1±2.7	24.8±4.3**	10.9±2.2**	123.4±38.5*
Stress+8-OH-DPAT (0.1 mg/kg)	1954.3±54.5	22.1±2.0	25.7±2.9	25.0±3.0**	11.5±2.1**	38.9±6.9
Non-stress	2052.7±82.9	26.8±2.1	35.3±4.3	27.5±3.8	13.4±2.5	30.3±7.3
Stress	1800.1±118.6	29.1±2.0	34.4±2.6	14.9±2.7**	6.7±1.4*	68.1±13.4*
Stress+Buspirone (0.3 mg/kg)	1901.9±127.0	24.0±3.5	26.5±3.4	12.6±2.8**	5.3±1.9*	44.7±10.6

**P*<0.05, ***P*<0.01 vs non-stressed group, #*P*<0.05 vs stressed group

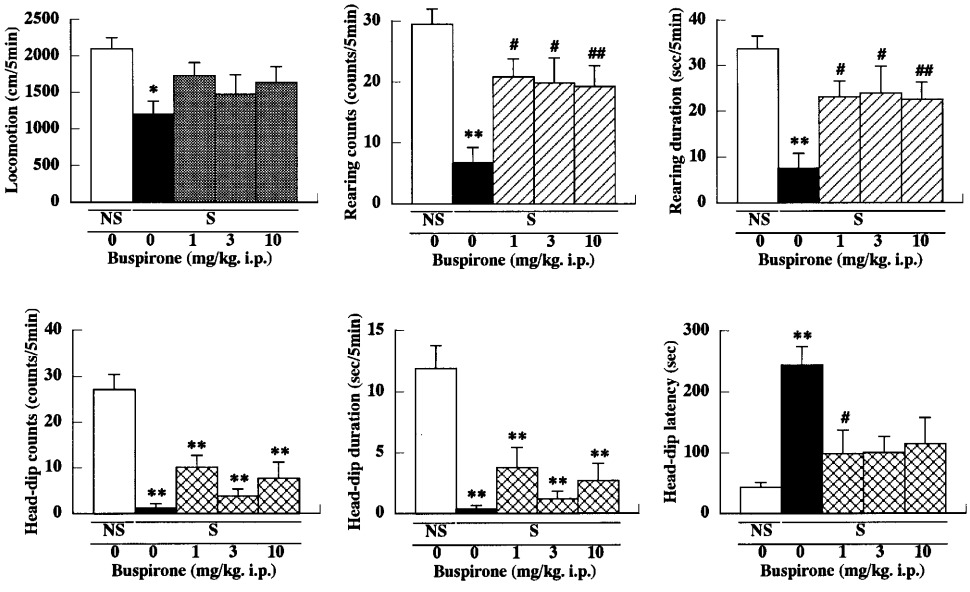

Fig. 3 Effects of pretreatment with buspirone on the behavioral responses of mice to acute restraint stress 24 h later in the hole-board test. Mice were pretreated with buspirone (1–10 mg/kg, IP) or saline (10 ml/kg, IP). Twenty-four hours later, mice were exposed to acute restraint stress (60 min), and exploratory behaviors on the hole-board were then measured for 5 min. Each column represents the mean with SEM of eight to ten mice. **P*<0.05, ***P*<0.01 versus saline plus non-stressed group (*open column*). ##*P*<0.01 versus saline plus stressed group (*closed column*)

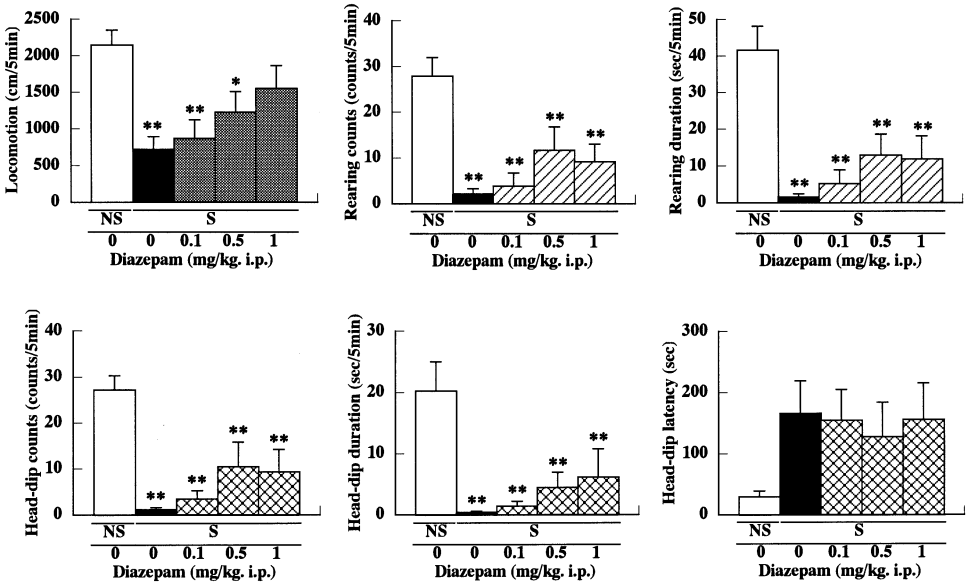

Fig. 4 Effects of pretreatment with diazepam on the behavioral responses of mice to acute restraint stress 24 h later in the hole-board test. Mice were pretreated with diazepam (0.1–1 mg/kg, IP) or saline (10 ml/kg, IP). Twenty-four hours later, mice were exposed to acute restraint stress (60 min), and exploratory behaviors on the hole-board were then measured for 5 min. Each column represents the mean with SEM of six mice. ***P*<0.01 versus saline plus non-stressed group (*open column*)

duce a significant behavioral effect, affected restraint stress-induced changes in head-dips. In contrast, the restraint stress-induced decrease in head-dipping behavior was reversed by post-stress treatment with the benzodiazepine anxiolytic chlordiazepoxide [24.7±3.9, 11.2±3.3 (*P*<0.05 versus non-stressed group) and 23.8±3.1 count (*P*<0.05 versus stressed group) (head-dip counts); 15.5±3.5, 4.5±1.5 (*P*<0.05 versus non-stressed group) and 11.3±2.0 s (*P*<0.05 versus stressed group) (head-dip duration) in non-stressed, stressed and stress plus chlordiazepoxide group, respectively].

Effects of pretreatment with 5-HT_{1A} receptor agonists and benzodiazepine anxiolytics on the behavioral responses of mice to acute restraint stress 24 h later in the hole-board test

The effects of pretreatment with 5-HT_{1A} receptor agonists and benzodiazepine anxiolytics on the behavioral responses of mice to acute restraint stress 24 h later are shown in Fig. 1, Fig. 2, Fig. 3, Fig. 4 and Fig. 5. A drastic decrease in various exploratory behaviors, i.e. locomotion and number and duration of rearing or head-dipping behaviors, and an increase in the latency of head-dipping were observed immediately after exposure to acute restraint stress. These changes in exploratory behaviors were dose-dependently suppressed by pretreatment with the 5-HT_{1A} receptor full agonists flesinoxan (0.1–1 mg/kg, IP) and 8-OH-DPAT (0.1–1 mg/kg, IP)

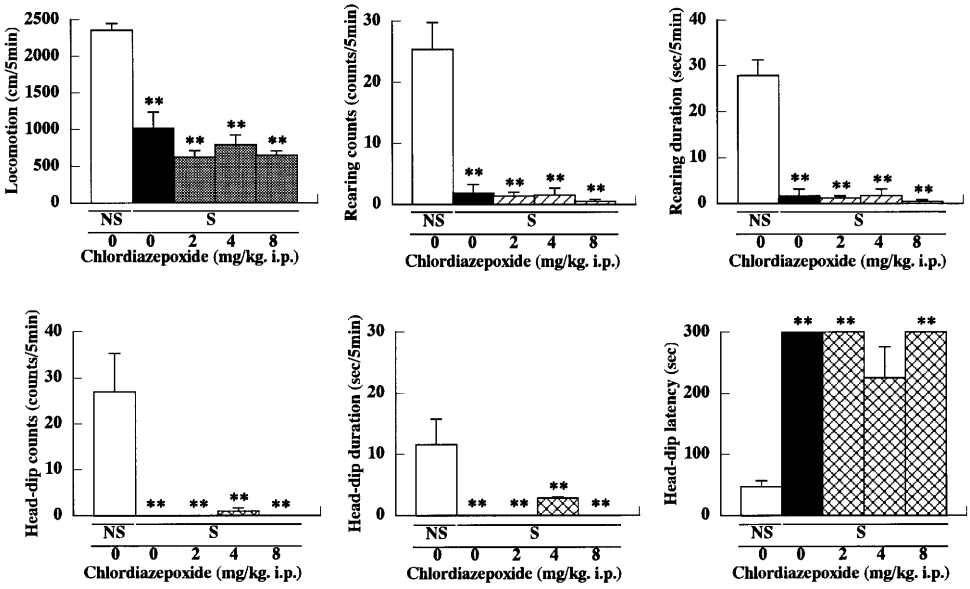

Fig. 5 Effects of pretreatment with chlordiazepoxide on the behavioral responses of mice to acute restraint stress 24 h later in the hole-board test. Mice were pretreated with chlordiazepoxide (2–8 mg/kg, IP) or saline (10 ml/kg, IP). Twenty-four hours later, mice were exposed to acute restraint stress (60 min), and exploratory behaviors on the hole-board were then measured for 5 min. Each column represents the mean with SEM of six mice. ***P*<0.01 versus saline plus non-stressed group (*open column*)

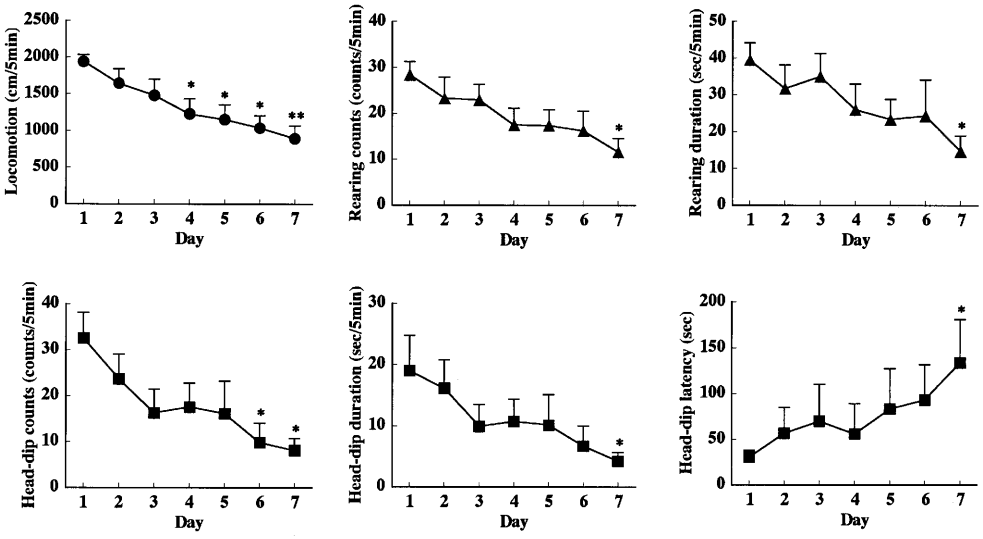

Fig. 6 Habituation pattern of mice to the environment of the hole-board apparatus. Mice were exposed to the hole-board apparatus once a daily for 7 consecutive days, and exploratory behaviors were measured for 5 min. Each point represents the mean with SEM of eight mice. **P*<0.05, ***P*<0.01 versus the value on day 1

24 h prior to exposure to acute restraint stress, and the effects were significant at 0.3 or 1 mg/kg (*P*<0.05 or 0.01) (Fig. 1 and Fig. 2). Pretreatment with buspirone (1–10 mg/kg, IP), a 5-HT_{1A} receptor full agonist, significantly suppressed only the decrease in rearing behavior and the increase in head-dip latency, but did not affect other behavioral changes (Fig. 3). In contrast, behavioral responses to acute restraint stress stimuli were not suppressed in mice that had been pretreated with either diazepam (0.1–1 mg/kg, IP) or chlordiazepoxide (2–8 mg/kg, IP), typical benzodiazepine anxiolytics (Fig. 4 and Fig. 5).

Effects of treatment with 5-HT_{1A} receptor agonists on behavioral response of mice that habituated to the hole-board apparatus

The effects of treatment with 5-HT_{1A} receptor agonists on behavioral response of mice that habituated to the hole-board apparatus are shown in Fig. 6 and Fig. 7. When mice were exposed to the hole-board apparatus once a day for 7 consecutive days, its exploratory behaviors, i.e. locomotor activity, rearing and head-dips, diminished depending on the times of exposure (Fig. 6). All of exploratory behaviors of mice on day 7 were significantly different from that on day 1 (*P*<0.05 or 0.01), suggesting that mice were habituated to the hole-board apparatus. Treatment with flesinoxan (1 mg/kg, IP), 8-OH-DPAT (1 mg/kg, IP) or buspirone (1–10 mg/kg, IP) to habituated mice 24 h prior to behavioral measurement did not exhibit the significant changes in exploratory behavior (Fig. 7).

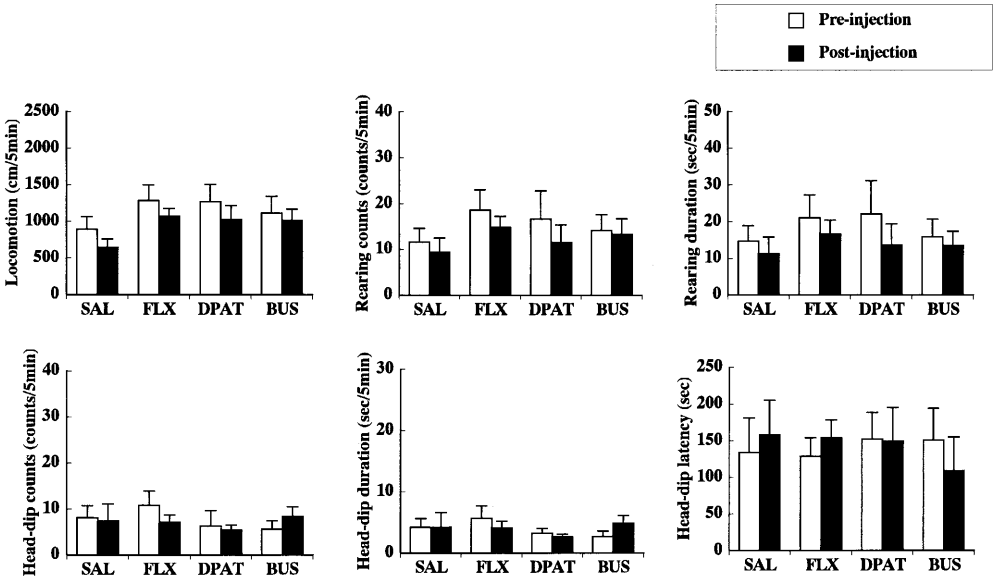

Fig. 7 Effects of treatment with 5-HT_{1A} receptor agonists on behavioral response of mice that habituated to the hole-board apparatus. Mice were exposed to the hole-board apparatus once a daily for 7 consecutive days. On day 7, exploratory behaviors of mice were measured for 5 min (pre-injection) and then animals were injected with flesinoxan (*FLX*; 1 mg/kg, IP), 8-OH-DPAT (*DPAT*; 1 mg/kg, IP), buspirone (*BUS*; 10 mg/kg, IP) or vehicle immediately. Twenty-four hours later, exploratory behaviors were recorded again for 5 min (post-injection)

Discussion

In the present study, the 5-HT_{1A} receptor full agonists flesinoxan and 8-OH-DPAT, and the partial agonist buspirone produced a decrease in locomotor activity as well as in rearing and head-dipping behaviors in the hole-board test. Similar behavioral suppression has been observed in previous studies using an open-field test or elevated plus-maze test (Ahlenius et al. 1991; Rodgers et al. 1994), indicating that 5-HT_{1A} receptor agonists reduce overall motility in a novel environment. Interestingly, these behavioral effects of 5-HT_{1A} receptor agonists are inconsistent with those of benzodiazepine anxiolytics that were observed in our previous studies (Takeda et al. 1998). We previously reported that typical benzodiazepine anxiolytics (diazepam and chlordiazepoxide) and anxiogenics (FG-7142 and β-CCM) have clear and consistent effects on head-dipping behavior in the hole-board test. Both the number and duration of exploratory head-dips were dose-dependently increased by treatment with diazepam and chlordiazepoxide at doses that did not produce sedation. This observation is consistent with previous reports of an increase in the frequency and duration of exploratory head-dips exhibited on a hole-board following the injection of non-sedative doses of either compound. (Nolan and Paekes 1973; Suzuki et al 1990). In contrast, benzodiazepine anxiogenics produced effects on head-dipping behavior that were opposite those of anxiolytics, i.e. both FG7142 and β-CCM dose-dependently decreased the number and duration of head-dips and increased the latency to the first head-dip. Based on these findings, we suggested that the head-dipping behavior of mice in the hole-board test is sensitive to changes in the emotional state modulated by benzodiazepine mechanisms, and that the anxiolytic effects of benzodiazepine can be detected by an increase in head-dipping behavior. The different behavioral effects of benzodiazepine anxiolytics and 5-HT_{1A} receptor agonists indicate that the mouse emotional state is variously modulated by each class of anxiolytic agents.

We previously reported that, similar to the effects of treatment with anxiogenics, exposure of mice to acute restraint stress produced a decrease in head-dipping behavior and an increase in the latency to head-dipping (Takeda et al. 1998). It has been previously reported that the exposure of animals to various stressful stimuli decreases some exploratory behaviors (Stone et al. 1984). In the hole-board test, a pronounced inhibition of head-dipping behavior was observed in animals that had been exposed to stressful stimuli (Rodriguez Echandia 1987). This report is consistent with our findings, and suggests that various stressful stimuli affect head-dipping behavior in animals. Moreover, we also found that the decrease in head-dipping behavior produced by acute restraint stress was reversed by post-stress treatment with diazepam at doses that alone did not produce significant behavioral effects (Takeda et al. 1998). These results were confirmed by additional present study using the benzodiazepine anxiolytic. These results suggest that acute restraint stress may produce anxiety and this emotional state of mice is improved by benzodiazepine anxiolytics. However, as shown in the present study, 5-HT_{1A} receptor agonists at doses which alone did not produce a significant decrease in emotional behavior (see Table 1) have hardly any capacity to improve the decrease in head-dipping behavior produced by acute restraint stress. These results indicate that 5-HT_{1A} receptor agonists have different effects than benzodiazepine anxiolytics in modulating the emotional state under stressful conditions. It has been previously reported that 5-HT_{1A} receptor agonists and benzodiazepine anxiolytics produce inconsistent effects on anxiety-related behaviors in various rodent models of anxiety (Barrett 1991), suggesting that the therapeutic effects of 5-HT_{1A} receptor agonists on anxiety disorders observed in the clinic differ qualitatively from those of benzodiazepine anxiolytics. The re-

sults of the present study may provide additional information to confirm this hypothesis.

An unexpected but important finding in the present study is that 5-HT_{1A} receptor agonists, but not benzodiazepine anxiolytics, have protective effects against various emotional changes produced by stress stimuli. Namely, pretreatment with the full agonist flesinoxan or 8-OH-DPAT 24 h prior to stress exposure suppressed the decrease in various emotional behaviors produced by acute restraint stress. In contrast, it is important to note that the exploratory behaviors of non-stressed mice previously habituated to the hole-board apparatus were unaffected by 24 h pretreatment with 5-HT_{1A} receptor agonists. These results indicate that general locomotor and/or exploratory behaviors of mice are not affected by 24 h pretreatment with 5-HT_{1A} receptor agonists, because any emotional abnormality such as anxiety of mice produced by placing them in a novel environment would be reduced by habituation to the hole-board apparatus. Thus, it is suggested that the decrease in behavioral response to restraint stress caused by 24 h pretreated with 5-HT_{1A} receptor agonists may be due to the changes in any emotional states related to stress stimuli but not in general motor activity. Moreover, preclinical studies of the pharmacokinetics of 8-OH-DPAT (Perry et al. 1989; Yu and Lewander 1997) and flesinoxan (unpublished observation, Solvay Duphar B.V., Weesp, The Netherlands) have indicated that these drugs disappear from the body within 24 h after administration. Therefore, the effects of any remaining drug also may be excluded. Previously, stress-induced decreases in various behaviors have been widely used as animal models of depression, and the effectiveness of 5-HT_{1A} receptor agonists has been demonstrated in these models (Schipper et al. 1991; Van Dijken et al. 1992; Hascoet et al. 1994). However, these previous experiments were performed under conditions in which the administered drugs remained in the body, i.e. behaviors were measured 30–60 min after drug injection. Therefore, the present effects of 5-HT_{1A} receptor agonists are different than those reported in previous reports. Several previous behavioral experiments have inspired the interesting interpretation that disappearance of the behavioral response to stress stimuli reflects the development of stress adaptation (Kennett et al 1985a, 1985b; Ohi et al. 1989). Accordingly, the present results imply the possibility that the short-term activation of 5-HT_{1A} receptors by agonists causes the facilitation of some adaptive mechanism(s) involved in the recognition of and/or ability to cope with stressful situations.

In contrast to treatment with flesinoxan and 8-OH-DPAT, only some of the behavioral response to stress stimuli, i.e. decrease in rearing behavior and increase in latency to head-dipping, was suppressed in mice that had been pretreated with the partial agonist buspirone, while the decreases in other parameters caused by exposure to stress were not affected. It is now known that buspirone, but not flesinoxan or 8-OH-DPAT, is a member of the azapirone family of anxiolytic drugs and has one major metabolite: 1-(2-pyrimidinyl)piperazine (1-PP) (Caccia et al. 1982). 1-PP is rapidly and abundantly formed in humans and rodents, and tends to accumulate in the brain (Caccia et al. 1985). 1-PP has been shown to act as an $alpha_2$-adrenoreceptor antagonist in vivo and in vitro (Gower and Tricklebank 1988; Gobbi et al 1990). Yohimbine, an antagonist at $alpha_2$-adrenoreceptor, has been shown to have anxiogenic effects in both humans and animals (Holmberg and Gershon 1961; Handley and Mithani 1984). Therefore, it has been suggested that 1-PP may diminish or even abolish the anxiolytic effects of its parent compounds (Martin 1991; Handley et al 1993; Kidd et al 1993; Matsuda et al 1995). Similarly, 1-PP may have altered the effects of buspirone in the present study.

Although the distinct mechanism(s) for the development of resistance to stress stimuli by short-term activation of 5-HT_{1A} receptors are unclear, one possible explanation is the desensitization of presynaptic 5-HT_{1A} autoreceptors. Indeed, the ability of a single pretreatment with 5-HT_{1A} receptor agonists at the doses used in the present study to produce desensitization of presynaptic 5-HT_{1A} autoreceptors is well documented (Kennett et al. 1987; Beer et al. 1990). The desensitization of presynaptic 5-HT_{1A} autoreceptors would presumably impair the feedback control of 5-HT release at terminals and hence increase 5-HT functional activity (Schlicker et al. 1985; Middlemiss 1986; Sprouse and Aghajanian 1987). Various lines of evidence have suggested that upregulation of the brain 5-HT system mediates short- and long-term adaptive or coping responses to aversive events such as stress stimuli (Kennett et al 1985a, 1985b, 1986; Ohi et al. 1989). We also previously reported the existence of differences in brain 5-HT dynamics between models with adaptability and non-adaptability to restraint stress (Takeda et al. 1996). Marked increases in 5-HT turnover in brain regions were observed in adaptive models, whereas these neurochemical changes were not observed in non-adaptive models. Similar neurochemical changes may occur with the single injection of 5-HT_{1A} receptor agonists. Moreover, desensitization of not only presynaptic but also postsynaptic 5-HT_{1A} receptor also has been demonstrated 24 h following single administration of 5-HT_{1A} receptor agonists (Foster et al. 1994). Thus, the possibility that decrease in function of postsynaptic 5-HT_{1A} receptor might be involved in the expression of present behavioral effects should also be excluded. Additional experiments to clarify the mechanism(s) underlying the present findings will provide new information to explain the processes of stress adaptation.

In conclusion, the present study demonstrated that 5-HT_{1A} receptor agonists have different effects on the emotionality of naive and stressed models than benzodiazepine anxiolytics. These results confirm a previous hypothesis that the therapeutic effects of 5-HT_{1A} receptor agonists on anxiety or depressive disorders observed in the clinic differ qualitatively from those of benzodiazepine anxiolytics. In particular, the protective effects of 5-HT_{1A} receptor agonists against various emotional changes produced by stress stimuli should be noted. These

suggest that the activation of 5-HT_{1A} receptors may play significant roles in the process of stress adaptation.

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