ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 53, No. 4, pp. 825–831, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02084-5

Serotonergic Activation Reduces Defensive Freezing in the Conditioned Fear Paradigm

TAKESHI INOUE,[1] KIYOSHI TSUCHIYA AND TSUKASA KOYAMA

Department of Psychiatry, Hokkaido University School of Medicine, North 15, West 7, Kita-ku, Sapporo 060, Japan

Received 12 August 1994

INOUE, T., K. TSUCHIYA AND T. KOYAMA. *Serotonergic activation reduces defensive freezing in the conditioned fear paradigm.* PHARMACOL BIOCHEM BEHAV **53**(4) 825–831, 1996.—Our previous study showed that conditioned fear stress (CFS) increased serotonin (5-HT) metabolism in the medial prefrontal cortex and induced freezing behavior. Although these results could support the 5-HT hypothesis of anxiety, the functional significance of the 5-HT response to stress is unclear. In this study, the effects of 5-HT reuptake inhibitors, agonists, antagonists, and diazepam on freezing behavior induced by CFS were examined using a time-sampling procedure. Various doses of test compounds were administered subcutaneously to rats 24 h after the last session of repeated foot-shock for 5 days. Rats were again placed in the shock chamber without shocks 20 min after injections of drugs, and observed. Diazepam (1 mg/kg) and the 5-HT_{1A} agonist ipsapirone (0.5–10 mg/kg) significantly inhibited freezing behavior. L-5-Hydroxytryptophan (with benserazide) and the selective 5-HT reuptake inhibitor citalopram (10 mg/kg) reduced freezing behavior. The 5-HT_2 antagonists ICI169,369 and ketanserin failed to change freezing behavior. *p*-Chlorophenylalanine (200 mg/kg) administered 15 h before the test did not affect freezing. The effect of ipsapirone was not modified in rats with lesions of 5-HT neurons, produced by *p*-chloroamphetamine (2 × 10 mg/kg). In conclusion, these results suggest the anxiolytic potential of ipsapirone and citalopram, and support the hypothesis that the facilitation of 5-HT neurotransmission decreases anxiety.

Serotonin Conditioned fear stress Freezing behavior Anxiety Anxiolytic Ipsapirone Citalopram 5-HT_{1A} receptor

RECENTLY, we have found that conditioned fear stress (CFS), an animal model of anxiety without physical stimuli, selectively increased serotonin (5-HT) metabolism in the medial prefrontal cortex (mPFC) and produced marked freezing behavior, regarded as an index of fear (26). Furthermore, in another study, we showed that the augmentation of CFS increased 5-HT metabolism not only in the mPFC, but also in the nucleus accumbens and amygdala, and enhanced fear-related behavior, such as freezing (27). These findings support the hypothesis that the 5-HT neuron system is closely related to anxiety, and are consistent with the clinical evidence that various 5-HT-related drugs are effective in anxiety disorders (13,29). However, it is not clear whether CFS-induced brain 5-HT activation reflects emotional reaction (anxiety) to, or coping with, stress.

It is widely accepted that 5-HT neurons promote anxiety in humans as well as in animal models (20). However, this classical hypothesis of 5-HT function in anxiety is inconsistent with a large number of recent clinical and animal data, which indicate that facilitation of 5-HT neurotransmission prevents anxiety (13). Several clinical placebo-controlled studies have consistently revealed that drugs assumed to facilitate 5-HT neurotransmission, such as serotonin reuptake inhibitors (SRI), monoamine oxidase inhibitors (MAOI), and 5-HT precursors, are effective in the treatment of anxiety disorders (8,13,28,29). These clinical data support the hypothesis that facilitation of serotonergic nerve transmission decreases anxiety. Such a hypothesis makes it possible to interpret CFS-induced brain 5-HT activation as reflecting coping with stress rather than an emotional reaction to stress.

On the other hand, new anxiolytic 5-HT_{1A} receptor agonists such as buspirone and ipsapirone have complex actions on 5-HT_{1A} receptors, which are located on both presynaptic and postsynaptic neurons (18). Some authors have suggested that the ability of 5-HT_{1A} receptor agonists to decrease central 5-HT function via presynaptic 5-HT_{1A} receptors is related to

[1] To whom requests for reprints should be addressed.

their antianxiety properties (5,7,10–12,22,37). However, as mentioned before, this classical hypothesis conflicts with the clinical effects of SRI and MAOI in the treatment of anxiety. Furthermore, there are some data implicating postsynaptic 5-HT_{1A} receptors in the anxiolytic effects of these agonists (6,10,30,34,36). Unfortunately, because there are few studies that have demonstrated the anxiolytic effects of both 5-HT_{1A} receptor agonists and SRI in the same animal model of anxiety (7), it is difficult to explain these differences between studies.

In an attempt to clarify the relationship between 5-HT neurotransmission and anxiety, the present study examined the effects of diazepam and various 5-HT-related agents, such as a 5-HT_{1A} agonist, selective 5-HT reuptake inhibitor, 5-HT precursor, and 5-HT_2 antagonists (31), on CFS-induced freezing behavior. Moreover, the anxiolytic effect of a 5-HT_{1A} agonist was also studied in rats with lesions of central 5-HT neurons induced by *p*-chloroamphetamine (PCA). Preliminary results of the present experiments have been reported previously (25).

METHODS

Animals

Male Sprague-Dawley rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan), weighing 250–300 g, were used. The rats were housed four per cage and maintained in a 12 L : 12 D, temperature-controlled environment, with free access to food and water. All experiments were performed between 0800 and 1300 h.

Drugs

The following drugs were used diazepam (Yamanouchi Pharmaceutical Co., Tokyo, Japan); ipsapirone HCl (Bayer Yakuhin Ltd., Japan); L-5-hydroxytryptophan (L-5-HTP; Tokyokasei, Tokyo, Japan); benserazide HCl (Hoffman-La Roche, Basal, Switzerland); citalopram HBr (H. Lundbeck A/S, Copenhagen, Denmark); ICI 169,369 HCl (Imperical Chemical Industries, Macclesfield, UK); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); DL-*p*-chlorophenylalanine methyl ester HCl (PCPA; Sigma, St. Louis, MO); and *p*-chloroamphetamine (PCA; Sigma). Ipsapirone, ICI169,369, citalopram, L-5-HTP, benserazide, ketanserin, PCPA, and PCA were dissolved in 0.9% NaCl solution. Diazepam was used as the commercially available solution Horizon diluted with 40% propylene glycol. Drugs were injected subcutaneously (SC) in a volume of 1 ml/kg except for PCA and PCPA, which were injected intraperitoneally.

Conditioned Fear

Rats were individually subjected to 30 min inescapable electric foot-shock [2.5 mA of scrambled shock, on a variable interval schedule with a mean intershock interval of 60 s (35–85 s) and shock duration of 30 s] in a chamber with a grid floor (19 × 22 × 20 cm; Medical Agent Co., Kyoto, Japan) for 5 days. Twenty-four hours after the last session, the rats were again placed in the shock chamber and observed for 10 min without shocks. Drugs or vehicle were administered 20 min before testing except for L-5-HTP (30 min) and PCPA (15 h). L-5-HTP was injected 30 min after peripheral amino acid decarboxylase inhibition by means of benserazide (SC, 25 mg/kg). During these observational periods, freezing behavior was recorded using a time-sampling procedure (15). Every 10 s, the behavior that the animal was currently engaged in was classified as either freezing or activity. Freezing was defined as the lack of all observable movements of the body and the vibrissae except those related to respiration. The percentage scores for freezing were calculated for a 10-min observation period. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee.

Open Field Activity

The open field consisted of a square arena 1.22 × 1.22 m with opaque walls 45 cm high. The floor was divided by lines into 16 equal squares. Twenty minutes after an SC injection of citalopram (1 mg/kg) and ipsapirone (1 mg/kg) or 30 min after an SC injection of L-5-HTP (20 mg/kg, 30 min after benserazide, 25 mg/kg, SC), rats were taken from their cages and placed in a corner of the open field facing the apparatus wall. The number of squares crossed during 5 min was taken as a measure of locomotor activity. After removal of each animal, the open field was thoroughly cleaned.

Biochemical Determinations

The following brain regions were punched out with small stainless-steel needles according to the method of Palkovits et al. (33): mPFC, amygdala and hippocampus. 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine, and noradrenaline were determined by high-pressure liquid chromatography with electrochemical detection (HPLC-ECD), as described previously (26,27).

Data Analysis

All data are presented as the means ± SEM of the individual values of the rats from each group. Statistical differences between two groups were made using an unpaired *t*-test (two-tailed). Multiple group comparisons were made using one-way analysis of variance followed by Duncan's test.

RESULTS

Conditioned fear stress induced marked freezing behavior (Block 1, 72.1 ± 12.2%; Block 2, 58.8 ± 14.2%), whereas freezing was not observed during a 10-min observation period in the control group [Block 1, $p < 0.001$; Block 2, $p < 0.01$ (unpaired *t*-test)].

Diazepam (1 mg/kg) produced a significant reduction in the duration of freezing induced by conditioned fear [$F(3, 31) = 3.93$, $p < 0.02$] (Fig. 1). The 5-HT_{1A} agonist ipsapirone (0.5–10 mg/kg) dose-dependently reduced freezing [$F(4, 35) = 19.99$, $p < 0.0002$] (Fig. 1). Citalopram (10 mg/kg) significantly attenuated freezing behavior [$F(5, 58) = 3.53$, $p < 0.01$] (Fig. 2). In addition, when a 10-min observation period was further divided into two 5-min periods, citalopram (1, 2, and 10 mg/kg) had a significant effect on freezing on the final 5-min block [$F(5, 58) = 5.01$, $p < 0.001$] (Fig. 2). The administration of L-5-HTP (20 mg/kg, after benserazide) significantly reduced freezing [$F(4, 39) = 5.16$, $p < 0.002$] (Fig. 2), whereas L-5-HTP (20 mg/kg) without benserazide did not change freezing (data not shown). The 5-HT_2 antagonists ICI169,369 (5–20 mg/kg) and ketanserin (0.1–5 mg/kg) had no effect on freezing at any dose tested (data not shown). PCPA (200 mg/kg) did not change freezing (data not shown). PCPA reduced the cortical concentrations of 5-HT and 5-HIAA by about 40–50% and reduced the cortical dopamine concentrations by about 30%, but not the brain noradrenaline concentrations (data not shown).

The effect of ipsapirone (1 mg/kg) was not modified in animals with lesions of 5-HT neurons produced by PCA (2 × 10 mg/kg) (Fig. 3). PCA reduced the 5-HT and 5-HIAA

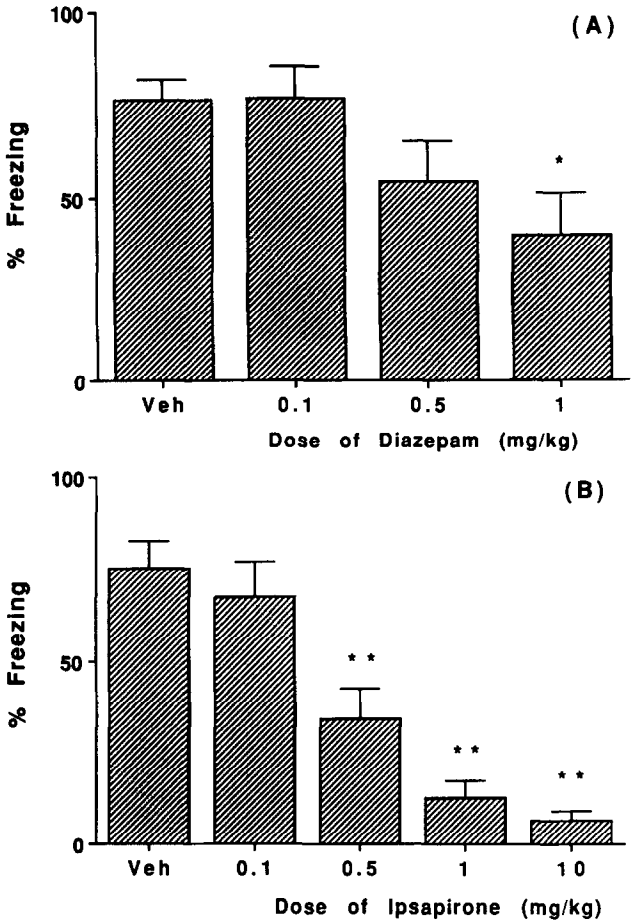

FIG. 1. Effects of diazepam (A) and ipsapirone (B) on freezing. Data represent the mean percentages ± SEM of freezing scored for a 10-min observation period. Behavior was sampled at 10-s intervals. The number of rats per group for each experiment were: diazepam, 7–12; ipsapirone, 8; $*p < 0.05$; $**p < 0.01$ vs. vehicle controls.

concentrations in the mPFC, amygdala, and hippocampus by about 70–80% (data not shown), but did not affect the dopamine and noradrenaline concentrations in any of the brain regions (data not shown).

In the open field test, effective doses in the conditioned fear test of ipsapirone (1 mg/kg), citalopram (1 mg/kg), and L-5-HTP (20 mg/kg after benserazide) were administered to rats. These drugs did not affect locomotion compared with vehicle controls (Table 1).

DISCUSSION

A number of paradigms have been developed to evaluate the anxiolytic potential of new compounds in animals. Among them, conflict tests are very sensitive to benzodiazepines and have been widely used for screening tests of anxiolytic agents. However, there are some problems with the conflict tests (38). First, demonstrating anticonflict activity for the 5-HT_{1A} agonists selective SRI and MAOI was not always possible (7,10,20); these have been found to be effective agents in the treatment of anxiety. In particular, there have been only a few animal studies concerning the anxiolytic effects of selective SRI and MAOI until now (20). The second problem is that the conflict tests are inappropriate for investigating the relationship between the neurochemical changes and anxiety, because animals receive nonspecific physical stimuli (electric shocks) in these models. For these reasons, to investigate the anxiolytic effects of various 5-HT compounds, we chose the conditioned fear model, which has no physical stimuli at time of testing and does not use appetitive or consumatory behaviors.

Several studies have shown that CFS produces a number of neurochemical, endocrinologic, and behavioral changes, which are related to anxiety and fear (2,3,9,15,21,26,27,39). Until now, there have been only two studies that used CFS to investigate the anxiolytic effects of benzodiazepines and a 5-HT_{1A} agonist (16,35). The present study also demonstrated that the classical anxiolytic diazepam reduced CFS-induced freezing behavior, which is considered to be an excellent index of fear and anxiety (2,3). These results are consistent with those of Fanselow and Helmstetter (16), who found that benzodiazepines (midazolam, chlordiazepoxide, and diazepam) decreased conditioned freezing.

The present study showed that both the selective SRI citalopram (24) and the 5-HT precursor L-5-HTP reduced conditioned freezing. In other animal models of anxiety, there have only been a few studies investigating the anxiolytic effects of selective SRIs (7). Handley and McBlane (20) reported that acute treatment with fluoxetine was anxiolytic in the conflict test but anxiogenic in the elevated X-maze test, whereas other studies failed to show the anxiolytic effects of selective SRIs in the conflict test and elevated plus-maze test (7). Previous studies have shown both anxiolytic and anxiogenic effects of L-5-HTP in conflict tests (13). Thus, the previous data concerning the effects of L-5-HTP and selective SRIs obtained in animal models of anxiety have been contradictory. However, clinical placebo-controlled studies have demonstrated that selective SRIs and L-5-HTP are effective in the treatment of anxiety disorders (1,8,28). Our results are consistent with these clinical data and suggest that facilitation of 5-HT neurotransmission decreases anxiety, because citalopram and L-5-HTP are assumed to increase the output from the serotonin synapse.

L-5-HTP after benserazide (25 mg/kg) significantly reduced conditioned freezing, whereas L-5-HTP (20 mg/kg) alone (without benserazide pretreatment) did not change conditioned freezing. Because a low dose of benserazide was reported to enhance and reduce L-5-HTP-induced increases in 5-HT levels in the brain and the periphery, respectively (14), the present results suggest that the effects of L-5-HTP on conditioned freezing are mediated by central mechanisms but not peripheral mechanisms.

5-HT_{1A} agonists reduce anxiety in animal and human studies without producing sedative side effects (10,19,23). In the present study, the selective 5-HT_{1A} agonist ipsapirone (31) produced a dose-dependent reduction in conditioned freezing, consistent with the results of Rittenhouse et al. (35). Furthermore, that a high dose of ipsapirone (10 mg/kg) also reduced freezing behavior is consistent with the clinical findings that ipsapirone has a negligible effect on psychomotor performance (23). Ipsapirone is a partial 5-HT_{1A} agonist, and its intrinsic activity is lower than that of 5-HT (18,31). In our unpublished data, the full 5-HT_{1A} receptor agonist 8-OH-DPAT also reduced conditioned freezing, whereas the selective 5-HT_{1A} receptor antagonist WAY100135 did not change it. These suggest that ipsapirone's effect on conditioned freezing is due to its agonistic activity at 5-HT_{1A} receptors. In addition, in the open field test, effective doses in the conditioned fear test of ipsapirone, citalopram, and L-5-HTP did not af-

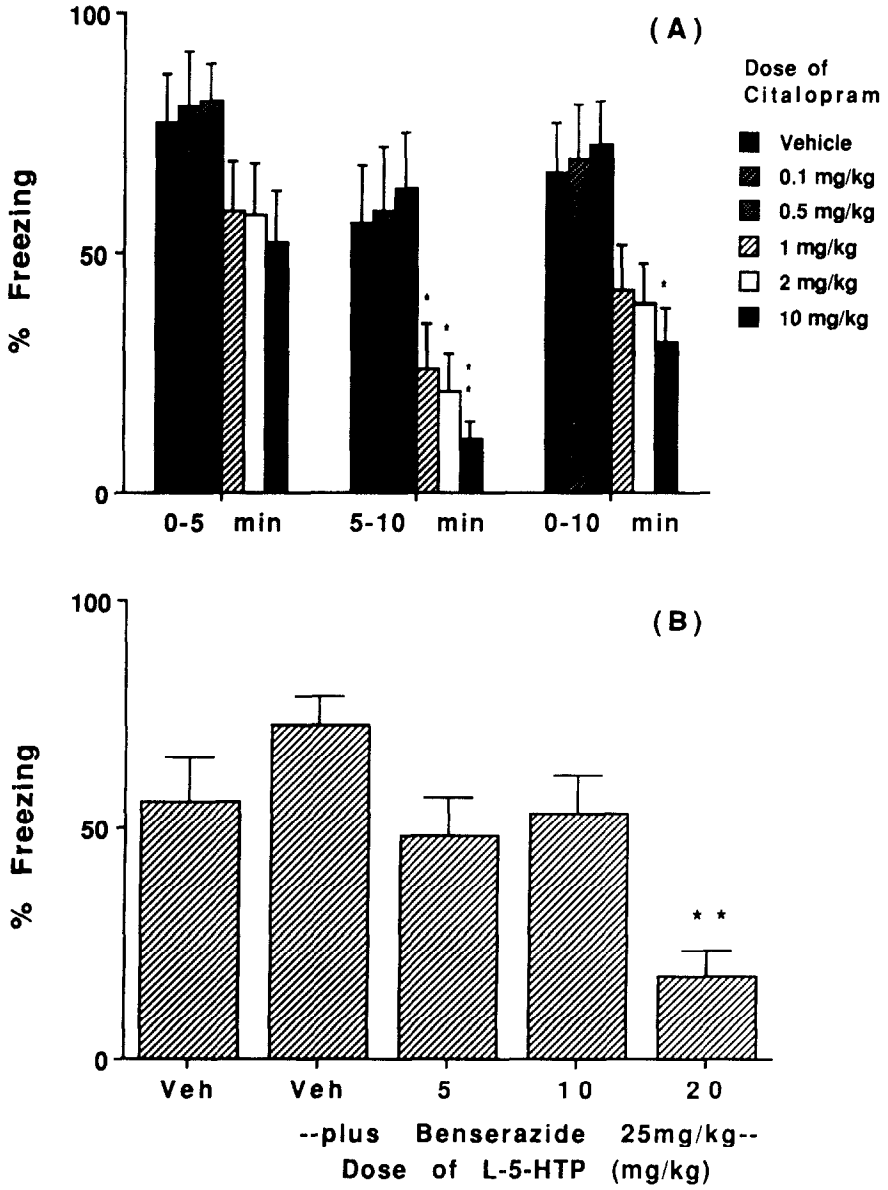

FIG. 2. Effects of citalopram (A) and L-5-hydroxytryptophan (L-5-HTP) (B) with benserazide on freezing. Data represent the mean percentages ± SEM of freezing scored for a 10-min observation period. Behavior was sampled at 10-s intervals. The number of rats per group for each experiment were: citalopram, 8–12; L-5-HTP, 8. $*p < 0.05$; $**p < 0.01$ vs. vehicle controls.

fect locomotion compared with vehicle controls. These findings suggest that the reduction in freezing observed with these agents appears to be independent of any effects on motor activity at doses required to reduce freezing significantly.

The present study showed acute effects of citalopram and ipsapirone on conditioned fear-induced freezing. However, clinical studies have suggested that chronic but not acute treatment with these agents reduces anxiety, whereas benzodiazepine efficacy is observed earlier (4). Unfortunately, several previous studies have reported that acute treatment with 5-HT_{1A} agonists is effective in animal models of anxiety; there are few studies to investigate the chronic effects of these agents (7,10,20). Therefore, animal models of chronic anxiety might be necessary to understand possible reasons for the differences between animal models of anxiety and human anxiety disorders in response to treatment with serotonergic agents.

The 5-HT_2 antagonists ICI169,369 and ketanserin were not effective in the conditioned fear paradigm. Other previous studies reported the effects of 5-HT_2 antagonists on animal models of anxiety, but the anxiolytic activity of 5-HT_2 antagonists in animals has not been consistently observed by several investigators (7). The differences between studies in the anxiolytic effects of 5-HT_2 antagonists may be attributable to differences between paradigms. However, clinical studies have not yetascertained the anxiolytic effects of 5-HT_2 antagonists (13,29).

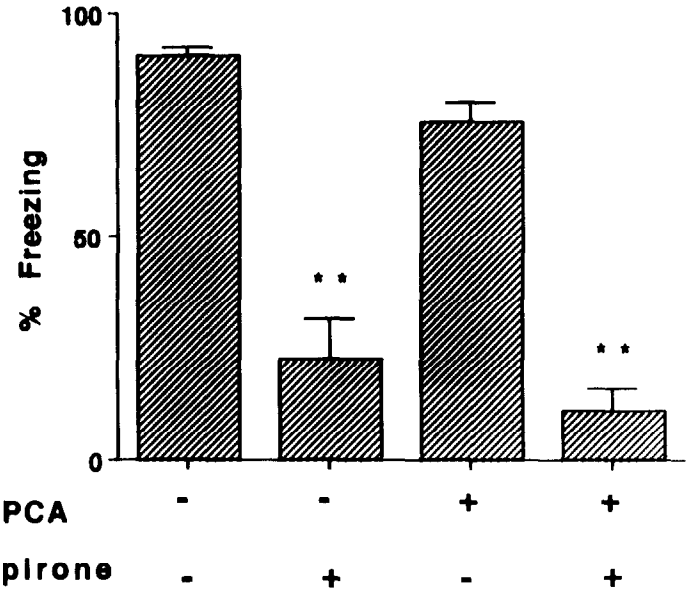

FIG. 3. Effect of *p*-chloroamphetamine (PCA) on the anxiolytic action of ipsapirone. PCA (2 × 10 mg/kg) was given intraperitoneally 12 and 11 days before the test. Ipsapirone (1 mg/kg) was subcutaneously injected 20 min before the test. Data represent the mean percentages ± SEM of freezing scored for a 5-min observation period. Behavior was sampled at 10-s intervals. $^{**}p < 0.01$ vs. control group. N = 6–8 rats per group.

Several studies have investigated the mechanism of action of anxiolytic effects of 5-HT_{1A} agonists (10). A number of studies of the role of serotonergic transmission have been related to the question of the relative contribution of pre- and postsynaptic 5-HT_{1A} receptors to the anxiolytic effects of these compounds. However, whether presynaptic or postsynaptic 5-HT_{1A} receptors are involved in the anxiolytic effects of these drugs remains a matter of controversy (10). In some models, it is suggested that the anxiolytic effects of 5-HT_{1A} agonists are mediated by both pre- and postsynaptic 5-HT_{1A} receptors (10). On the other hand, Eriksson and Humble (13) concluded that a large number of clinical studies, which demonstrated the anxiolytic effects of selective SRI, MAOI, 5-HTP, and 5-HT_{1A} agonists, indicate that the facilitation of 5-HT neurotransmission decreases anxiety. In the present study, the administration of PCA, which reduced the concentrations of 5-HT and 5-HIAA in the mPFC, amygdala, and hippocampus by about 70–80%, did not modify the anxiolytic effect of ipsapirone. Because PCA causes degeneration of 5-HT axon terminals arising from dorsal raphe nuclei but not from median raphe nuclei (17), it is likely that the 5-HT_{1A} receptors in the dorsal raphe are not associated with the effect of ipsapirone on conditioned freezing. Our finding is in line with the results of Chojnacka-Wójcik et al. (6), who reported the lack of effect of the 5-HT lesion induced with PCA on the anticonflict action of ipsapirone; however, it is inconsistent with other studies (10). Taken together, these effects of ipsapirone, citalopram, and L-5-HTP suggest that the anxiolytic effect of ipsapirone in the conditioned fear model might be mediated by postsynaptic 5-HT_{1A} receptors, and support the hypothesis of Eriksson and Humble (13).

TABLE 1

EFFECT OF IPSAPIRONE, CITALOPRAM, AND L-5-HYDROXYTRYPTOPHAN (L-5-HTP) ON LOCOMOTION IN THE OPEN FIELD TEST

	Drug Dose (mg/kg)	No. of Rats	Locomotion (No. of Squares Crossed)
Saline	(1 ml/kg)	16	45.1 ± 5.0
Ipsapirone	1	8	41.6 ± 9.5
Citalopram	1	16	59.9 ± 6.9
Benserazide + vehicle		8	73.6 ± 7.6
Benserazide + L-5-HTP	20	8	65.6 + 5.1

In no case were statistical significant differences observed.

Our previous studies demonstrated that CFS produced 5-HT activation in the mPFC (26), and the enhancement of CFS intensity further activated the 5-HT metabolism in the mPFC, nucleus accumbens, and amygdala (27). The functional significance of this 5-HT activation during CFS remains unclear. Nevertheless, because the present results suggest that facilitation of 5-HT neurotransmission decreases conditioned fear, it is possible to postulate the hypothesis that 5-HT activation in those regions during CFS reflects coping with stress rather than an emotional reaction to stress. This idea could explain the present result that PCA did not reduce conditioned freezing despite a significant reduction in brain 5-HT levels (up to 70–80%), indicating that 5-HT lesions produced by PCA did not reduce anxiety. On the other hand, the finding that PCA did not enhance freezing suggests that the extent of 5-HT activation during conditioned fear is not enough to attenuate freezing acutely in this experimental condition. Interestingly, in line with this view, a recent study showed that

mPFC lesions interfered with the extinction of conditioned fear responses elicited by conditioned stimuli (32). However, this hypothesis obviously needs further experimental support such as lesion studies.

In summary, the present results showed that diazepam, ipsapirone, citalopram, and L-5-HTP are effective in reducing conditioned freezing, an index of fear and anxiety. These are consistent with clinical evidence that these drugs are effective in the treatment of anxiety. Thus, CFS is a simple and useful model for detecting the anxiolytic potential of 5-HT-related drugs and investigating the relationship between 5-HT neurotransmission and anxiety. Furthermore, the present data support the hypothesis that facilitation of 5-HT neurotransmission decreases anxiety.

ACKNOWLEDGEMENTS

This work was supported in part by Grant-in-Aid for Scientific Research No. 05454308 (T.K.) and No. 06770740 (T.I.) from the Japanese Ministry of Education, Science, and Culture.

REFERENCES

1. Black, D. W.; Wesner, R.; Bowers, W.; Gabel, J. A comparison of fluvoxamine, cognitive therapy, and placebo in the treatment of panic disorder. Arch. Gen. Psychiatry 50:44-50; 1993.
2. Blanchard, D. C.; Blanchard, R. J. Crouching as an index of fear. J. Comp. Physiol. Psychol. 67:370-375; 1969.
3. Bouton, M. E.; Bolles, R. C. Conditioned fear assessed by freezing and by the suppression of three different baselines. Anim. Learn. Behav. 8:429-434; 1980.
4. Brown, C. S.; Rakel, R. E.; Wells, B. G.; Downs, J. M.; Akiskal, H. S. A practical update on anxiety disorders and their pharmacological treatment. Arch. Intern. Med. 151:873-884; 1991.
5. Carli, M.; Prontera, C.; Samanin, R. Evidence that central 5-hydroxytryptaminergic neurones are involved in the anxiolytic activity of buspirone. Br. J. Pharmacol. 96:829-836; 1989.
6. Chojnacka-Wójcik, E.; Przegalinski, E. Evidence for the involvement of 5-HT_{1A} receptors in the anticonflict effect of ipsapirone in rats. Neuropharmacology 30:703-709; 1991.
7. Chopin, P.; Briley, M. Animal models of anxiety: The effects of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. 8:383-387; 1987.
8. Den Boer, J. A.; Westenberg, H. G. M. Effect of a serotonin and noradrenaline uptake inhibitor in panic disorder: A double-blind comparative study with fluvoxamine and maprotiline. Int. Clin. Psychopharmacol. 3:59-74; 1988.
9. Deutch, A. Y.; Tam, S.-Y.; Roth, R. H. Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Res. 333:143-146; 1985.
10. De Vry, J. M.; Schreiber, R.; Glaser, T.; Traber, J. Behavioral pharmacology of 5-HT_{1A} agonists: Animal models of anxiety and depression. In: Stahl, S. M.; Gastpar, M.; Keppel Hesselink, J. M.; Traber, J., eds. Serotonin$_{1A}$ receptors in depression and anxiety. New York: Raven Press; 1992:55-81.
11. Dourish, C. T.; Hutson, P. H.; Curzon, G. Putative anxiolytics 8-OH-DPAT, buspirone and TVXQ7821 are agonists at 5-HT_{1A} autoreceptors in the raphe nuclei. Trends Pharmacol. Sci. 7:212-214; 1986.
12. Eison, A. S.; Eison, M. S.; Stanley, M.; Riblet, L. A. Serotonergic mechanisms in the behavioral effects of buspirone and gepirone. Pharmacol. Biochem. Behav. 24:701-707; 1986.
13. Erikkson, E.; Humble, M. Serotonin in psychiatric pathophysiology: A review of data from experimental and clinical research. In: Pohl, R.; Gershon, S., eds. Progress in basic clinical pharmacology, vol. 3: The biological basis of psychiatric treatment. Basel: Karger; 1990:66-119.
14. Ervin, G. N.; Carter, R. B.; Webster, E. L.; Moore, S. I.; Cooper, B. R. Evidence that taste aversion learning induced by L-5-hydroxytryptophan is mediated peripherally. Pharmacol. Biochem. Behav. 20:799-802; 1984.
15. Fanselow, M. S. Conditional and unconditional components of postshock freezing. Pavlov. J. Biol. Sci. 15:177-182; 1980.
16. Fanselow, M. S.; Helmstetter, F. J. Conditional analgesia, defensive freezing, and benzodiazepines. Behav. Neurosci. 102:233-243; 1988.
17. Fuller, R. W. Effects of *p*-chloroamphetamine on brain serotonin neurons. Neurochem. Res. 5:449-456; 1992.
18. Glaser, T.; De Vry, J. M. Neurobiology of 5-HT_{1A} receptors. In: Stahl, S. M.; Gastpar, M.; Keppel Hesselink, J. M.; Traber, J., eds. Serotonin$_{1A}$ receptors in depression and anxiety. New York: Raven Press; 1992:35-53.
19. Goudie, A. J.; Leathley, M. J. Evaluation of the dependence potential of the selective 5-HT_{1A} agonist ipsapirone in rats and of its effects on benzodiazepine withdrawal. Psychopharmacology 103:529-537; 1991.
20. Handley, S. L.; McBlane, J. W. 5-HT drugs in animal models of anxiety. Psychopharmacology 112:13-20; 1993.
21. Herman, J. P.; Guillonneau, D.; Dantzer, R.; Scatton, B.; Semerdjian-Rouquier, L.; Le Moal, M. Differential effects of inescapable foot shocks and of stimuli previously paired with inescapable foot shocks on dopamine turnover in cortical and limbic areas of the rat. Life Sci. 30:2207-2214; 1982.
22. Higgins, G. A.; Jones, B. J.; Oakley, N. R. Effect of 5-HT_{1A} receptor agonists in two models of anxiety after dorsal raphe injection. Psychopharmacology 106:261-267; 1992.
23. Hindmarch, I.; Shillingford, J.; Kerr, J. S.; Keppel Hesselink, J. M. The comparative psychopharmacology of 5-HT_{1A} agonists. In: Stahl, S. M.; Gastpar, M.; Keppel Hesselink, J. M.; Traber, J., eds. Serotonin$_{1A}$ receptors in depression and anxiety. New York: Raven Press; 1992:109-117.
24. Hyttel, J. Citalopram. Pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. Prog. Neuropsychopharmacol. Biol. Psychiatry 6:277-295; 1982.
25. Inoue, T.; Koyama, T.; Yamashita, I. Serotonin$_{1A}$ agonist reduced conditioned freezing behavior. Soc. Neurosci. Abstr. 18: 1530; 1992.
26. Inoue, T.; Koyama, T.; Yamashita, I. Effect of conditioned fear stress on serotonin metabolism in the rat brain. Pharmacol. Biochem. Behav. 44:371-374; 1993.
27. Inoue, T.; Tsuchiya, K.; Koyama, T. Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. Pharmacol. Biochem. Behav. 49:911-920; 1994.
28. Kahn, R. S.; Westenberg, H. G. M.; Verhoeven, W. M. A.; Gispen-de Wied, C. C.; Kamerbeek, W. D. J. Effect of a serotonin precursor and uptake inhibitor in anxiety disorders: A double-blind comparison of 5-hydroxytryptophan, clomipramine and placebo. Int. Clin. Psychopharmacol. 2:33-45; 1987.
29. Kahn, R. S.; van Praag, H. M.; Wetzler, S.; Asnis, G. M.; Barr, G. Serotonin and anxiety revisited. Biol. Psychiatry 23:189-208; 1988.
30. Kostowski, W.; Plaznik, A.; Stefanski, R. Intra-hippocampal buspirone in animal models of anxiety. Eur. J. Pharmacol. 168: 393-396; 1989.
31. Middlemiss, D. N.; Tricklebank, M. D. Centrally active 5-HT receptor agonists and antagonists. Neurosci. Biobehav. Rev. 16: 75-82; 1992.
32. Morgan, M. A.; Romanski, L. M.; LeDoux, J. E. Extinction of emotional learning: Contribution of medial prefrontal cortex. Neurosci. Lett. 163:109-113; 1993.
33. Palkovits, M.; Brownstein, M. J. Maps and guide to microdissection of the rat brain. New York: Elsevier; 1988.
34. Przegalinski, E.; Chojnacka-Wójcik, E.; Filip, M. Stimulation of

postsynaptic 5-HT_{1A} receptors is responsible for the anticonflict effect of ipsapirone in rats. J. Pharm. Pharmacol. 44:780–782; 1992.
35. Rittenhouse, P. A.; Bakkum, E. A.; O'Connor, P. A.; Carnes, M.; Bethea, C. L.; van de Kar, L. D. Comparison of neuroendocrine and behavioral effects of ipsapirone, a 5-HT_{1A} agonist, in three stress paradigms: Immobilization, forced swim and conditioned fear. Brain Res. 580:205–214; 1992.
36. Stefanski, R.; Palejko, W.; Bidzinski, A.; Kostowski, W.; Plaznik, A. Serotonergic innervation of the hippocampus and nucleus accumbens septi and the anxiolytic-like action of midazolam and 5-HT_{1A} receptor agonists. Neuropharmacology 32:977–985; 1993.
37. Traber, J.; Glaser, T. 5-HT_{1A} receptor-related anxiolytics. Trends Pharmacol. Sci. 8:432–437; 1987.
38. Treit, D. Animal models for the study of anti-anxiety agents: A review. Neurosci. Biobehav. Rev. 9:203–222; 1985.
39. Tsuda, A.; Tanaka, M.; Ida, Y.; Tsujimaru, S.; Ushijima, I.; Nagasaki, N. Effects of preshock experience on enhancement of rat brain noradrenaline turnover induced by psychological stress. Pharmacol. Biochem. Behav. 24:115–119; 1986.