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Effect of citalopram, a selective serotonin reuptake inhibitor, on the acquisition of conditioned freezing

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Abstract

The present study examined the effects of the selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor citalopram on the acquisition of conditioned freezing, an index of anxiety. Acute treatment with citalopram (1–10 mg/kg) dose dependently prevented the acquisition of conditioned freezing, while acute treatment with noradrenaline or dopamine reuptake inhibitors failed. The acute effect of citalopram was not antagonized by the 5-HT_{1A} receptor antagonist NAN190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimmido)butyl]piperazine or the 5-$HT_{2A/2C}$ receptor antagonist ICI169,369, 2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride. These results indicate that selective 5-HT reuptake inhibitors reduce not only the expression of conditioned freezing as reported previously, but also the acquisition of conditioned freezing. Both these effects of selective 5-HT reuptake inhibitors may be related to their clinical efficacy in the treatment of anxiety disorders.

Keywords: Anxiety; Conditioned fear stress; Freezing behavior; 5-HT (5-hydroxytryptamine, serotonin) reuptake inhibitor, selective; Citalopram

1. Introduction

Conditioned fear stress (exposure to an environment paired previously with inescapable electric footshock) induces a well-characterized defensive response termed conditioned freezing (Fanselow, 1980). The duration of conditioned freezing increases reliably with shock intensity and has proved to index an animal's degree of fear and anxiety (Fanselow, 1980). Conditioned freezing has been used as an animal model of anxiety in order to evaluate several classes of anxiolytics or candidate anxiolytics and to measure the neurochemical changes during anxiety (Herman et al., 1982; Fanselow and Helmstetter, 1988; Rittenhouse et al., 1992; Swiergel et al., 1993; Inoue et al., 1993, 1994; Yoshioka et al., 1995; Hashimoto et al., 1996; Inoue et al., 1996a). The expression of conditioned freezing is attenuated by not only the classical anxiolytic benzodiazepines (Fanselow and Helmstetter, 1988; Inoue et al., 1996a), but also new non-benzodiazepine serotonergic anxiolytics, such as the 5-HT_{1A} receptor agonist ipsapirone and the 5-HT reuptake inhibitors citalopram and fluvoxamine (Rittenhouse et al., 1992; Hashimoto et al., 1996; Inoue et al., 1996a). These effects of selective 5-HT reuptake inhibitors on the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996a) are consistent with recent clinical evidence that several serotonergic agents are effective in the treatment of human anxiety disorders (Erikkson and Humble, 1990). They also suggest that conditioned freezing is a useful model for detecting the anxiolytic potential of 5-HT reuptake inhibitors and other clinically effective serotonergic anxiolytics (Erikkson and Humble, 1990).

Several lines of evidence have shown that selective 5-HT reuptake inhibitors are effective in the treatment of anxiety disorders, such as panic disorder, social phobia and posttraumatic stress disorder (Den Boer and Westenberg, 1988; Humble and Wistedt, 1992; Van der Kolk et al., 1994; Van Vliet et al., 1994; Katzelnick et al., 1995). In social phobia and posttraumatic stress disorder, anxiety is cued by social situations and stimuli recalling the stressor, respectively (DSM-IV, 1994). Although the panic disorder definition requires that at least some of the panic attacks be unexpected, individuals with panic disorder are frequently reported to have situation-bound attacks, particu-

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larly later in the course of the disorder (DSM-IV, 1994). If a panic attack occurred in a certain situation, similar situations will cause anticipatory anxiety, which then can set off another panic attack (Jacob and Rapport, 1984). Thus, fear and anxiety conditioned to some stimuli also play an important role in the course and exacerbation of a variety of anxiety disorders. Although our previous studies demonstrated the anxiolytic effect of selective 5-HT reuptake inhibitors on the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996a), the effect of selective 5-HT reuptake inhibitors on the acquisition of conditioned freezing, i.e. conditioning of conditioned fear stress, which may also be important for the treatment of anxiety disorders, has never been reported.

The purpose of this study is to assess the effects of acute administration of the selective 5-HT reuptake inhibitor citalopram (Hyttel, 1982; Richelson and Pfenning, 1984) on the acquisition of conditioned fear stress-induced freezing behavior in rats. In addition, the selective noradrenaline reuptake inhibitor ORG4428, *cis*-1,2,3,4,4a, 13b-hexahydro-2,10-dimethyldibenz[2,3:6,7]oxepino[4,5-*c*]pyridin-4a-ol (Organon Japan Co., ORG4428 Investigator Brochure, p. 14, 1993), and the selective dopamine reuptake inhibitor GBR12909, 1-(2-(bis(4-fluorphenyl)-methoxy)-ethyl)-4-(3-phenyl-propyl)piperazine (Andersen, 1989) were evaluated in this paradigm. Moreover, the effects of 5-HT receptor antagonists on the effect of citalopram were also studied.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan), weighing 250–300 g, were housed in groups of four and maintained in a 12-h L : 12 h D (light phase; 06:30–18:30 h), temperature-controlled environment (22 ± 1°C) with free access to food and water. Experiments began after a 2-week period of acclimatization. Rats were tested between 8:00 and 13:00 h.

2.2. Drugs

Citalopram hydrobromide (H. Lundbeck A/S, Copenhagen, Denmark) and ICI169,369, 2-(2-dimethylaminoethylthio)-3-phenylquinoline hydrochloride (Imperical Chemical Industries, Macclesfield, UK) were dissolved in 0.9% sterile saline. ORG4428 (Organon Japan Co., Japan) and GBR12909 (Gist-Brocades Co., Netherlands) were suspended in 0.5% sodium carboxymethyl cellulose (CMC). NAN190, 1-(2-methoxyphenyl)-4-[4-(2-phthalimmido)butyl]piperazine (RBI, Natick, USA) was suspended in 1% Tween 80. All drugs were injected subcutaneously (s.c.) in a volume of 1 ml/kg.

2.3. Procedures

2.3.1. Conditioned fear stress-induced freezing

Thirty minutes after a single injection of vehicle, citalopram, ORG4428 and GBR12909, rats were subjected to inescapable electric footshock for a total of 2.5 min [2.5 mA scrambled shock (10 ms shock every 100 ms), with a shock duration of 30 s × 5 and a variable-interval schedule with a mean intershock interval of 60 s (35–85 s)] in a chamber with a grid floor (19 × 22 × 20 cm, Medical Agent Co., Japan). In the experiments which examined the effect of ICI169,369 and NAN190 on the citalopram-induced reduction in the acquisition of conditioned freezing, ICI169,369 and NAN190 were administered 40 min before electric footshock, i.e. 10 min before citalopram injection. Electric shock was provided by a Model SGS-02D Shock Generator (Medical Agent Co., Japan). This provides a high-voltage, high-resistance circuit with resistance controlled by dial settings calibrated by the manufacturer in a short circuit current. At the setting of 2.5 mA, this generator actually gives a shock with an intensity of 0.2 mA to rats. Twenty-four hours after footshock, the rats were again placed in the shock chamber and observed for 5 min without shocks. During the observation period, the duration of freezing behavior was recorded using a time-sampling procedure (Fanselow, 1980). Every 10 s, the behavior in which the animal was currently engaged was classified as either freezing or activity. Freezing was defined as the absence of all observable movement of the skeleton and the vibrissae, except those related to respiration. All other behavior was scored as activity. The percentage scores for the duration of freezing behavior (% freezing) were calculated for a 5-min observation period. These procedures were approved by the Hokkaido University School of Medicine Animal Care and Use Committee, and were in compliance with the Guide for the Care and Use of Laboratory Animals, Hokkaido University School of Medicine.

2.3.2. Pain

Effects of citalopram on footshock-induced pain were examined. Four behaviors, such as vocalization, limb withdrawal of the forepaw, limb withdrawal of the hindpaw and jumping, were used as being indicative of nociception. Generally, they have been used as endpoints in the hot plate procedure (Carter, 1991). Thirty minutes after drug injection (citalopram 10 mg/kg, s.c.), rats were individually placed in a shock chamber with a grid floor (19 × 22 × 20 cm). After a 5-min adaptation period, rats were subjected to 15 series of scrambled electric footshocks. Each series was of 10-s duration and spaced at 40-s intervals, ranging from 0.4 mA to 3.2 mA in 0.2 mA steps, presented in ascending order. The response of rats to each shock was recorded, and minimal intensities of electric footshocks, at which each four behaviors appeared, were determined.

2.4. Data analysis

All the data are presented as the means ± S.E.M. of the individual values of the rats from each group. Statistical differences between two groups were analyzed using an unpaired *t*-test (two-tailed). Multiple group comparisons were made using a one-way analysis of variance (ANOVA) followed by Duncan's test. When two drugs were given, the data were analyzed by a 2-way ANOVA.

3. Results

The selective 5-HT reuptake inhibitor citalopram (1–10 mg/kg) dose dependently reduced the acquisition of conditioned freezing [$F(3,60) = 5.557$, $P < 0.01$] (Fig. 1A). The possibility that the effect of citalopram administered 30 min before footshock was still present during testing 24 h later and directly affected the expression of conditioned freezing can be obviated, because 10 mg/kg of citalopram 5 min after footshock failed to reduce the acquisition of conditioned freezing (data not shown). The selective noradrenaline reuptake inhibitor ORG4428, or the selective dopamine reuptake inhibitor GBR12909 had no effect on the acquisition of conditioned freezing [ORG4428, $F(3,68) = 0.726$, $P = 0.54$; GBR12909, $F(3,28) = 0.828$, $P = 0.49$] (Fig. 1B,C).

As shown in Fig. 2, the effect of citalopram (10 mg/kg) on the acquisition of conditioned freezing was not affected by 1 mg/kg of NAN190 [effect of citalopram, $F(1,28) = 12.214$, $P < 0.01$; effect of NAN190, $F(1,28) = 3.205$, $P = 0.08$; interaction of NAN190 with citalopram, $F(1,28) = 0.028$, $P = 0.87$] or 10 mg/kg of ICI169,369 [effect of citalopram, $F(1,76) = 6.703$, $P < 0.02$; effect of ICI169,369, $F(1,76) = 1.067$, $P = 0.305$; interaction of ICI169,369 with citalopram, $F(1,76) = 0.122$, $P = 0.728$] (Fig. 2A,B).

Table 1 shows the effects of citalopram (10 mg/kg) on pain-related behaviors induced by footshock. Minimal intensities of electric footshocks at which pain-related behaviors first appeared, i.e. pain thresholds, were not different between the citalopram group and vehicle controls.

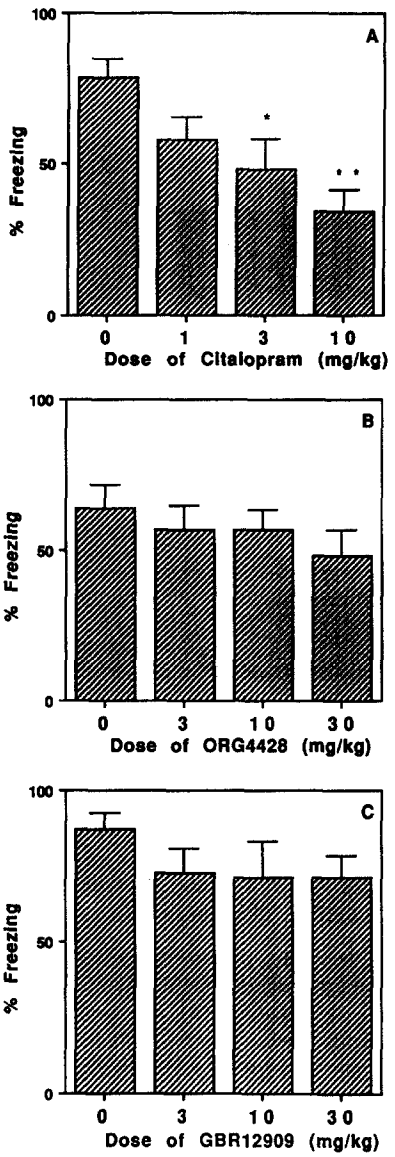

Fig. 1. Effects of the selective serotonin reuptake inhibitor citalopram (A), selective noradrenaline reuptake inhibitor ORG4428 (B), and selective dopamine reuptake inhibitor GBR12909 (C) on the acquisition of conditioned freezing. Thirty minutes after a single injection of drugs or the vehicle (s.c.), rats were individually subjected to 2.5 mA footshock for 5 min. Twenty-four hours after footshock, rats were placed in the shock chamber without shocks and observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. The number of rats/group for each experiment were: citalopram, 16; ORG4428, 16-24; GBR12909, 8. * $P < 0.05$, * * $P < 0.01$ vs. vehicle controls.

4. Discussion

In the present study, the selective 5-HT reuptake inhibitor citalopram reduced the acquisition of conditioned freezing, unlike noradrenaline and dopamine reuptake inhibitors. We have already reported that acute treatment with selective 5-HT reuptake inhibitors decreased the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996a). Taken together, selective 5-HT reuptake inhibitors reduce both the expression and acquisition of conditioned freezing, as did the classical anxiolytic benzodiazepines and a new anxiolytic 5-HT_{1A} receptor agonist in previous studies (Fanselow and Helmstetter, 1988; Rittenhouse et al., 1992; Inoue et al., 1996a,b; our unpublished data).

Since citalopram did not affect the threshold of footshock-induced pain, consistent with the previous studies (Hyttel, 1982), the blocking effect of citalopram on the acquisition of conditioned freezing cannot be attributed to a reduced sensitivity to electric footshock. In addition, citalopram administration 5 min after footshock failed to

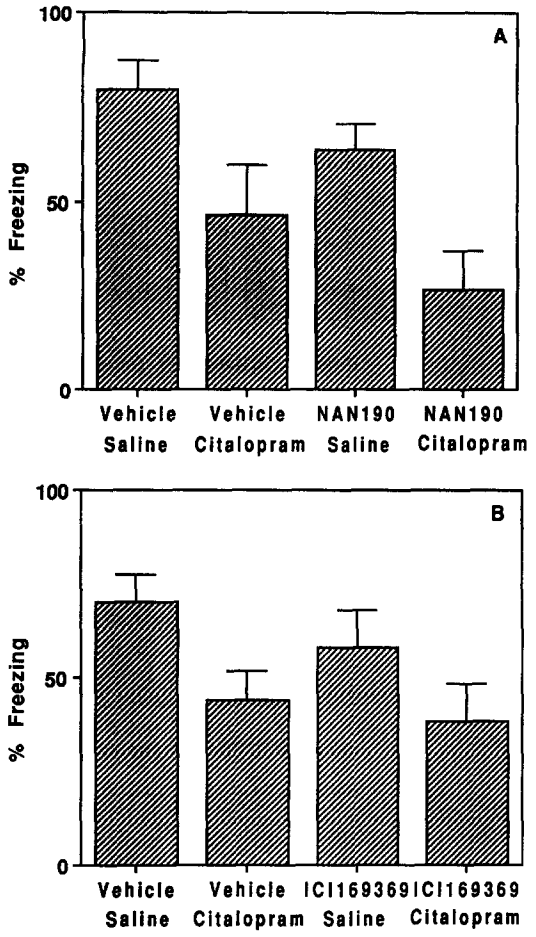

Fig. 2. Effects of the 5-HT_{1A} receptor antagonist NAN190 (1 mg/kg, A) and 5-$HT_{2A/2C}$ receptor antagonist ICI169,369 (10 mg/kg, B) on the blocking effect of citalopram (10 mg/kg) on the acquisition of conditioned freezing. Forty minutes after NAN190 or ICI169,369 s.c. and 30 min after citalopram s.c., rats were individually subjected to 2.5 mA footshock for 5 min. Twenty-four hours after footshock, rats were placed in the shock chamber without shocks and observed for 5 min. Represented are the mean percentages ± S.E.M. of freezing scored for a 5-min observation period. The number of rats/group for each experiment were: NAN190, 8; ICI169,369, 16–24. Significant F values by a 2-way ANOVA: NAN190, main effect of citalopram, $F(1,28) = 12.214$, $P < 0.01$; ICI169,369, main effect of citalopram, $F(1,76) = 6.703$, $P < 0.02$.

reduce the acquisition of conditioned freezing. Therefore, the possibility that the effect of citalopram administered 30 min before footshock was still present during testing 24 h later and directly affected the expression of conditioned freezing is unlikely. Furthermore, previous papers showed that selective 5-HT reuptake inhibitors did not impair memory in humans (Linnoila et al., 1993), and indeed enhanced learning and memory in rodents (Flood and Cherkin, 1987; Meneses and Hong, 1995). These results suggest that citalopram reduces aversive conditioning without affecting pain sensitivity or memory.

Table 1
Effect of citalopram (10 mg/kg s.c.) on pain-related behavior induced by footshock. Data are represented as the means ± S.E.M. of pain threshold (mA), at which each behavior first appeared. $n = 8$. n.s., not significant

Behavior	Saline group	Citalopram group	
Withdrawal of forepaws	0.80 ± 0.05	0.85 ± 0.07	n.s.
Withdrawal of hindpaws	1.05 ± 0.06	1.18 ± 0.10	n.s.
Vocalization	1.40 ± 0.08	1.65 ± 0.09	n.s.
Jumping	2.54 ± 0.18	2.47 ± 0.25	n.s.

Several clinical trials have reported that selective 5-HT reuptake inhibitors, including citalopram, are effective in the treatment of anxiety disorders, such as panic disorder, social phobia and posttraumatic stress disorder (Den Boer and Westenberg, 1988; Humble and Wistedt, 1992; Van der Kolk et al., 1994; Van Vliet et al., 1994; Katzelnick et al., 1995). Den Boer and Westenberg (1988) obtained a much better effect with another selective 5-HT reuptake inhibitor fluvoxamine than with the noradrenaline reuptake inhibitor maprotiline in the treatment of panic disorder. This clinical evidence is consistent with our present and previous results (Hashimoto et al., 1996) that selective 5-HT reuptake inhibitors, but not noradrenaline reuptake inhibitors, were effective in reducing both the acquisition and expression of conditioned freezing. While the effect of citalopram on the expression of conditioned freezing clearly shows its anxiolytic activity, the effect of citalopram on the acquisition of conditioned freezing suggests that it affects the conditional stimulus-unconditional stimulus formation or the perception of noxious footshock (unconditional stimulus). As we indicated in the Introduction, aversive conditioning to some stimuli may play an important role in the course and exacerbation of a variety of anxiety disorders. Accordingly, the effect of a selective 5-HT reuptake inhibitor on the acquisition of conditioned freezing may be important for the treatment of anxiety disorders, as well as its effect on the expression of conditioned freezing.

A recent in vivo microdialysis study demonstrated that citalopram (10 mg/kg, i.p.) increased extracellular 5-HT concentrations in the frontal cortex of freely moving rats (Invernizzi et al., 1992). The question may arise as to which 5-HT receptor subtypes are involved in the citalopram-induced inhibition of the acquisition of conditioned freezing. Our previous study, which showed that the 5-HT_{1A} receptor agonist ipsapirone reduced the acquisition of conditioned freezing, suggests that the effect of citalopram may be attributable to activation of 5-HT_{1A} receptors (Inoue et al., 1996b). The present study tried to clarify the role of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors in the effect of citalopram on the acquisition of conditioned freezing, by using the purported 5-HT_{1A} receptor antagonist NAN190 and the 5-$HT_{2A/2C}$ receptor antagonist ICI169,369 (Middlemiss and Tricklebank, 1992; Hoyer et al., 1994). Neither drug, however, altered the effect of citalopram on the acquisition of conditioned freezing. Other studies suggest that NAN190 is not an ideal 5-HT_{1A} receptor antagonist, but a very weak 5-HT_{1A} receptor agonist (Middlemiss and Tricklebank, 1992). Accordingly, the present results cannot lead to the conclusion that 5-HT_{1A} receptor activation is

not related to the effect of citalopram. Further investigations with selective 5-HT_{1A} receptor antagonists, which have no agonist effect on 5-HT neuronal firing, are necessary to verify the mechanism of the effect of citalopram on the acquisition of conditioned freezing. Alternatively, the effect of citalopram on the acquisition of conditioned freezing may be due to the consequence of interactions between several different 5-HT receptor subtypes activated by elevated synaptic 5-HT, or activation of other 5-HT receptor subtypes.

The 5-$HT_{2A/2C}$ receptor antagonist ICI169,369 or the 5-HT_{1A} receptor antagonist NAN190 alone did not affect the acquisition of conditioned freezing. Some previous studies reported anxiolytic effects of 5-$HT_{2A/2C}$ receptor antagonists and 5-HT_{1A} receptor antagonists in animal models, but this anxiolytic activity of 5-$HT_{2A/2C}$ (or 5-HT_{2C}) and 5-HT_{1A} receptor antagonists in animals has not been consistently observed by several investigators (Chopin and Briley, 1987; Chojnacka-Wójcik and Przegalinski, 1991; Handley and McBlane, 1993; Rodgers and Cole, 1994; Kennett et al., 1994). The differences between studies in the anxiolytic effects of 5-$HT_{2A/2C}$ and 5-HT_{1A} receptor antagonists may be attributable to differences between paradigms. However, clinical studies have not ascertained the anxiolytic effects of 5-$HT_{2A/2C}$ and 5-HT_{1A} receptor antagonists yet (Kahn et al., 1988; Erikkson and Humble, 1990).

The present study showed the acute effect of citalopram on the acquisition of conditioned freezing. Our previous studies also reported that acute treatment with SSRI were effective in reducing the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996a). However, clinical studies have suggested that chronic but not acute treatment with these agents reduces anxiety (Den Boer and Westenberg, 1988). Apparently, there is a discrepancy between our experimental results and clinical findings. A possible explanation is that our anxiety model produces acute anxiety, while the anxiety of clinical anxiety disorders is chronic. If this is the case, animal models of chronic anxiety might be necessary to understand this discrepancy between our model and human anxiety disorders in the response to treatment with SSRIs. Another explanation is that more doses of SSRIs might be necessary to achieve a rapid anxiolytic effect in clinical anxiety disorders since the present study showed a clear dose-dependent effect of citalopram.

In conclusion, the selective 5-HT reuptake inhibitor citalopram was effective in reducing the acquisition of conditioned fear stress-induced freezing behavior, while noradrenaline or dopamine reuptake inhibitors were ineffective. These results are consistent with recent clinical evidence. In addition to the previous findings that selective 5-HT reuptake inhibitors reduce the expression of conditioned freezing (Hashimoto et al., 1996; Inoue et al., 1996a), the effect of a selective 5-HT reuptake inhibitor on the acquisition of conditioned freezing may be involved in the therapeutic effects of selective 5-HT reuptake inhibitors in anxiety disorders.

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References

Andersen, P.H., 1989, The dopamine uptake inhibitor GBR12909: selectivity and molecular mechanism of action, Eur. J. Pharmacol. 166, 493.

Carter, R.B., 1991, Differentiating analgesic and non-analgesic drug activities on rat hot plate: effect of behavioral endpoint, Pain 47, 211.

Chojnacka-Wójcik, E. and E. Przegalinski, 1991, Evidence for the involvement of 5-HT_{1A} receptors in the anticonflict effect of ipsapirone in rats, Neuropharmacology 30, 703.

Chopin, P. and M. Briley, 1987, Animal models of anxiety: the effects of compounds that modify 5-HT neurotransmission, Trends Pharmacol. Sci. 8, 383.

Den Boer, J.A. and H.G.M. Westenberg, 1988, 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.

DSM-IV, 1994, Diagnostic and Statistical Manual of Mental Disorders, 4th edn., The American Psychiatric Association, Washington, DC).

Erikkson, E. and M. Humble, 1990, Serotonin in psychiatric pathophysiology: a review of data from experimental and clinical research, in: Progress in Basic Clinical Pharmacology, Vol. 3. The Biological Basis of Psychiatric Treatment, eds. R. Pohl and S. Gershon (Karger, Basel) p. 66.

Fanselow, M.S., 1980, Conditional and unconditional components of post-shock freezing, Pavlov. J. Biol. Sci. 15, 177.

Fanselow, M.S. and F.J. Helmstetter, 1988, Conditional analgesia, defensive freezing, and benzodiazepines, Behav. Neurosci. 102, 233.

Flood, J.F. and A. Cherkin, 1987, Fluoxetine enhances memory processing in mice, Psychopharmacology 93, 36.

Handley, S.L. and J.W. M^{c}Blane, 1993, 5HT drugs in animal models of anxiety, Psychopharmacology 112, 13.

Hashimoto, S., T. Inoue and T. Koyama, 1996, Serotonin reuptake inhibitors reduce conditioned fear stress-induced freezing behavior in rats, Psychopharmacology 123, 182.

Herman, J.P., D. Guillonneau, R. Dantzer, B. Scatton, L. Semerdjian-Rouquier and M. Le Moal, 1982, Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat, Life Sci. 30, 2207.

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxena and P.P.A. Humphrey, 1994, VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46, 157.

Humble, M. and B. Wistedt, 1992, Serotonin, panic disorder and agoraphobia: short-term and long-term efficacy of citalopram in panic disorders, Int. Clin. Psychopharmacol. 6 (Suppl. 5), 21.

Hyttel, J., 1982, Citalopram. Pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity, Prog. Neuropsychopharmacol. Biol. Psychiatry. 6, 277.

Inoue, T., T. Koyama and I. Yamashita, 1993, Effect of conditioned fear stress on serotonin metabolism in the rat brain, Pharmacol. Biochem. Behav. 44, 371.

Inoue, T., K. Tsuchiya and T. Koyama, 1994, Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain, Pharmacol. Biochem. Behav. 49, 911.

Inoue, T., K. Tsuchiya and T. Koyama, 1996a, Serotonergic activation reduces defensive freezing in the conditioned fear paradigm, Pharmacol. Biochem. Behav. 53, 825.

Inoue, T., K. Tsuchiya and T. Koyama, 1996b, Effects of typical and atypical antipsychotic drugs on freezing behavior induced by conditioned fear, Pharmacol. Biochem. Behav. (in press).

Invernizzi, R., S. Belli and R. Samanin, 1992, Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex, Brain Res. 584, 322.

Jacob, R.G. and M.D. Rapport, 1984, Panic disorder: medical and psychological parameters, in: Behavioral Theories and Treatment of Anxiety, ed. S.M. Turner (Plenum Press, New York) p. 187.

Kahn, R.S., H.M. Van Praag, S. Wetzler, G.M. Asnis and G. Barr, 1988, Serotonin and anxiety revisited, Biol. Psychiatry 23, 189.

Katzelnick, D.J., K.A. Kobak, J.H. Greist, J.W. Jefferson, J.M. Mantle and R.C. Serli, 1995, Sertraline for social phobia: a double-blind, placebo-controlled crossover study, Am. J. Psychiatry 152, 1368.

Kennett, G.A., K. Pittaway and T.P. Blackburn, 1994, Evidence that 5-HT2C receptor antagonists are anxiolytic in the rat Geller-Seifter model of anxiety, Psychopharmacology 114, 90.

Linnoila, M., J.M. Stapleton, D.T. George, E. Lane and M.J. Eckardt, 1993, Effects of fluvoxamine, alone and in combination with ethanol, on psychomotor and cognitive performance and on autonomic nervous system reactivity in healthy volunteers, J. Clin. Psychopharmacol. 13, 175.

Meneses, A. and E. Hong, 1995, Effect of fluoxetine on learning and memory involves multiple 5-HT systems, Pharmacol. Biochem. Behav. 52, 341.

Middlemiss, D.N. and M.D. Tricklebank, 1992, Centrally active 5-HT receptor agonists and antagonists, Neurosci. Biobehav. Rev. 16, 75.

Richelson, E. and M. Pfenning, 1984, Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: most antidepressants selectively block norepinephrine uptake, Eur. J. Pharmacol. 104, 277.

Rittenhouse, P.A., E.A. Bakkum, P.A. O'Connor, M. Carnes, C.L. Bethea and L.D. Van De Kar, 1992, Comparison of neuroendocrine and behavioral effects of ipsapirone, a 5-HT1A agonist, in three stress paradigm: immobilization, forced swim and conditioned fear, Brain Res. 580, 205.

Rodgers, R.J. and J.C. Cole, 1994, Anxiolytic-like effect of (S)-WAY 100135, a 5-HT1A receptor antagonist, in the murine elevated plus-maze test, Eur. J. Pharmacol. 261, 321.

Swiergel, A.H., L.K. Takahashi and N.H. Kalin, 1993, Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat, Brain Res. 623, 229.

Van der Kolk, B.A., D. Dreyfuss, M. Michaels, D. Shera, R. Berkowitz, R. Fisler and G. Saxe, 1994, Fluoxetine in posttraumatic stress disorder, J. Clin. Psychiatry 55, 517.

Van Vliet, I.M., J.A. Den Boer and H.G. Westenberg, 1994, Psychopharmacological treatment of social phobia; a double blind placebo controlled study with fluvoxamine, Psychopharmacology 15, 128.

Yoshioka, M., M. Matsumoto, H. Togashi and H. Saito, 1995, Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex, Pharmacol. Biochem. Behav. 51, 515.