Pharmacology, Biochemistry and Behavior 92 (2009) 393–398

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

# Anxiolytic-like profile of mirtazapine in rat conditioned fear stress model: Functional significance of 5-hydroxytryptamine 1A receptor and α1-adrenergic receptor

Nobukazu Kakui [a,*], Fumikazu Yokoyama [a], Miki Yamauchi [a], Koichi Kitamura [a], Taiichiro Imanishi [a], Takeshi Inoue [b], Tsukasa Koyama [b]

[a] Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd. 760, Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan
[b] Department of Psychiatry, Neural Function, Hokkaido University Graduate School of Medicine, North 15, West 7, Kita-ku, Sapporo, 060-8638, Japan

ARTICLE INFO

*Article history:*
Received 22 October 2008
Received in revised form 22 December 2008
Accepted 29 December 2008
Available online 11 January 2009

*Keywords:*
Mirtazapine
Buspirone
Mianserin
5-$HT_{1A}$ receptor
α1-adrenergic receptor
Anxiolytic

ABSTRACT

Mirtazapine is an antidepressant with a unique mechanism of action and has been categorized as a Noradrenergic and Specific Serotonergic Antidepressant (NaSSA). Although numerous clinical trials suggested the usefulness of mirtazapine for not only major depressive disorders but also a variety of anxiety disorders, efficacy studies in animal anxiety models have been rarely reported. The present study investigated a potential anxiolytic-like profile of mirtazapine in rat conditioned fear stress model. A 5-hydroxytryptamine (5-HT) 1A receptor partial agonist, buspirone (1–5 mg/kg) exhibited a significant reduction in freezing time, and its maximal effect was reversed by a selective 5-$HT_{1A}$ antagonist, WAY-100635 (1 mg/kg). Mirtazapine (1–10 mg/kg) also reduced the freezing time in a dose-related fashion, a substantial proportion (approx. 50%) of which was likewise antagonized by WAY-100635 (1 mg/kg). Mianserin (1–30 mg/kg), a structural analogue for mirtazapine, was ineffective. Furthermore, co-administration of α1 adrenoceptor antagonist, prazosin (0.03 mg/kg) completely reversed mirtazapine (10 mg/kg)-induced reduction of freezing time. These findings represent the first demonstration that the anxiolytic-like action of mirtazapine involves activation of 5-$HT_{1A}$ receptor and α1 adrenoceptor to different extents, and are compatible with one aspect of mirtazapine's pharmacological profile as NaSSA.



## 1. Introduction

Mirtazapine, a novel 'atypical' antidepressant, has a unique mechanism of action. The pharmacological profile of mirtazapine is characterized by a potent antagonism of presynaptic α2-adrenergic receptor on both noradrenaline and serotonin (5-hydroxytryptamine: 5-HT) neurons and by a potent antagonism of postsynaptic 5-$HT_2$ and 5-$HT_3$ but not 5-$HT_{1A}$ receptor (de Boer, 1996). Unlike many currently available antidepressants, this drug does not affect serotonergic and noradrenergic transporters in a significant manner (de Boer, 1996). The net outcome of these actions is enhanced noradrenergic and serotonergic neurotransmission, in particular via the 5-$HT_{1A}$ receptor, the profile being officially recognized as a Noradrenergic and Specific Serotonergic Antidepressant (NaSSA) (Sambunaris et al., 1997; Sartorius et al., 2007). Several clinical studies have demonstrated beneficial effects of mirtazapine for the treatment of major depressive disorders (Anttila and Leinonen, 2001; Holm and Markham, 1999; Szegedi and Schwertfeger, 2005). Phenotypic similarities to these findings have been reported in some rodent depression models (O'Connor and Leonard, 1986; Rauggi et al., 2005; Reneric et al., 2002). On the other hand, most recent clinical trials established a newer generation of antidepressants as an important therapeutic modality for various types of anxiety disorders, and mirtazapine is also not the exception (Falkai, 1999). The efficacy of this drug has been indicated in general anxiety disorder (Gambi et al., 2005), panic disorder (Sarchiapone et al., 2003), social anxiety disorder (Van Veen et al., 2002), and obsessive–compulsive disorder (Koran et al., 2005). However, in marked contrast to this, efficacy studies in animal anxiety models have been rarely performed.

Conditioned fear stress (CFS) test is an established murine anxiety model in which animals pre-exposed to inescapable electric foot shocks show a typical freezing behavior under the same condition despite the absence of the shock exposure (Fanselow and Helmstetter, 1988). In addition to classical anxiolytic benzodiazepines, serotonergic anxiolytics like ipsapirone (5-$HT_{1A}$ agonist) and selective serotonin reuptake inhibitor such as citalopram, and precursor of 5-HT, 5-hydroxy-L-tryptophan have been shown to be effective in reducing conditioned freezing in this model (Inoue et al., 1996a,b; Rittenhouse et al., 1992). As explicitly suggested from these findings, the CFS model is thought to be suitable for evaluation of drugs endowed with the ability of stimulating serotonergic neurotransmission.

In the present study, we aimed to assess the anxiolytic-like profile of mirtazapine in terms of its dependence on 5-$HT_{1A}$ receptor and to compare it with a 5-$HT_{1A}$ partial agonist, buspirone and a structural analogue of mirtazapine, mianserin (de Boer et al., 1988). For further

* Corresponding author. Tel.: +81 45 541 2521; fax: +81 45 545 3152.
*E-mail address:* nobukazu_kakui@meiji.co.jp (N. Kakui).

0091-3057/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.12.022

exploration of the in vivo function of mirtazapine, we focused on the role of central α1-adrenergic receptor in particular on the 5-HT neurons and assessed the effects of combined treatment with α1-selective antagonist, prazosin (Menkes et al., 1981).

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats (Charles River Japan, Atsugi, Japan) weighing 270–300 g at the beginning of the experiments were used. The rats were housed six per cage in a temperature- and humidity-controlled room (lights on 0700 h, lights off 1900 h). Water and chow were available ad libitum. Animal care was performed according to the protocols reviewed by the Ethical Committee for Animal Experiment in Meiji Seika Pharmaceutical Research Center.

### 2.2. Drugs

Mirtazapine (Tocris Cookson Inc., Ellisville, MO) was suspended in 0.5% methylcellulose. Buspirone hydrochloride and the selective 5-$HT_{1A}$ antagonist, WAY-100635 maleate (Sigma-Aldrich, St. Louis, MO) were dissolved in 0.9% saline. Mianserin hydrochloride (Tocris Cookson Inc.) was suspended in 0.9% saline. Prazosin hydrochloride (Sigma-Aldrich) was dissolved in 0.5% methylcellulose. All of the tested drugs were administered intraperitoneally (i.p.) at a volume of 1 ml/kg.

### 2.3. Conditioned fear stress (CFS) test

In this procedure, rats were conditioned to anticipate a foot shock. On the first day, they were individually subjected to inescapable electric foot shocks for a total of 5 min (1 mA of scrambled shock; shock duration of 30 s × 5; and an intershock interval of 30 s) in an operant chamber (31 × 25 × 31 cm, Muromachi Kikai, Tokyo, Japan) with a stainless grid floor. A scrambled current shock was delivered via a shock generator (Model SGS-002, Muromachi Kikai). The test session was performed about 24 h after the exposure to electric foot shock. The rats were again placed in the same box and observed for 5 min in the shock chamber with no current applied to the floor of the chamber. Freezing was defined as the absence of all observable movement of the skeleton and the vibrissae, except for those related to respiration. Automated records were taken for freezing time with a software program (*Freeze Frame*: Actimetrics, Wilmette, IL). Mirtazapine (0.3, 1, 3, and 10 mg/kg), buspirone (0.3, 1, 2, and 5 mg/kg), mianserin (1, 10, and 30 mg/kg), or each vehicle was injected 30 min before the measurement. In combined treatment tests, WAY-100635 (0.3 and 1 mg/kg), prazosin (0.03 mg/kg) or each of their respective vehicles were injected simultaneously with the above drugs.

### 2.4. Locomotion

The effects of each drug treatment on spontaneous locomotor activity were evaluated, coincident with the timing of CFS test. Thirty minutes after the drug administration, the rats were individually placed in a transparent plastic activity cage (31 × 40 × 17 cm). Locomotion measurement system (NS-AS01, Neuroscience, Tokyo) consisted of an infrared sensor positioned just above the cage and the application software (Act-1). The locomotor activity was recorded for 5 min and analyzed by the software.

### 2.5. Statistical analysis

All results were represented as mean ± S.E.M. Statistical significance of the differences among multiple groups was tested using one-way analysis of variance followed by Dunnett's multiple comparison test. A two-tailed Student's *t* test was used to evaluate the difference between two experimental groups. Values associated with a probability (*p* value) of $<0.05$ were considered significant.

## 3. Results

### 3.1. Anxiolytic-like effect of buspirone and its reversal by combined treatment with WAY-100635 in CFS test

Buspirone showed an anxiolytic-like effect and its maximal activity was observed at 1–2 mg/kg (freezing time: 105.3 ± 25.5 s at 1 mg/kg, $p<0.05$, 100.5 ± 19.3 s at 2 mg/kg, $p<0.01$, vs. 207.3 ± 21.2 s in vehicle group) (Fig. 1A). While WAY-100635 (1 mg/kg) was ineffective in altering basal freezing responses on its own, it reversed the anxiolytic-like effect of buspirone (2 mg/kg) in a dose-dependent manner (147.7 ± 22.1 s at

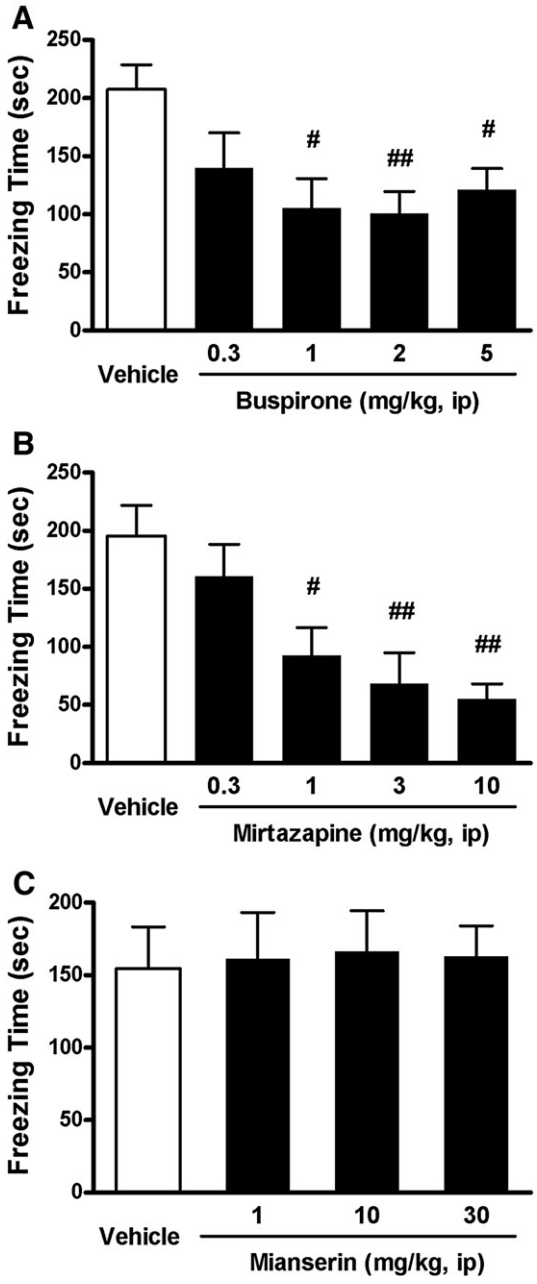


**Fig. 1.** Effect of single treatment with buspirone (A), mirtazapine (B), and mianserin (C) on the freezing time in rat CFS test. All tested drugs were administered 30 min before the measurements. Data are represented as mean ± SEM ($n = 9–10$). #$p<0.05$ and ##$p<0.01$, significantly different from the values in vehicle group.

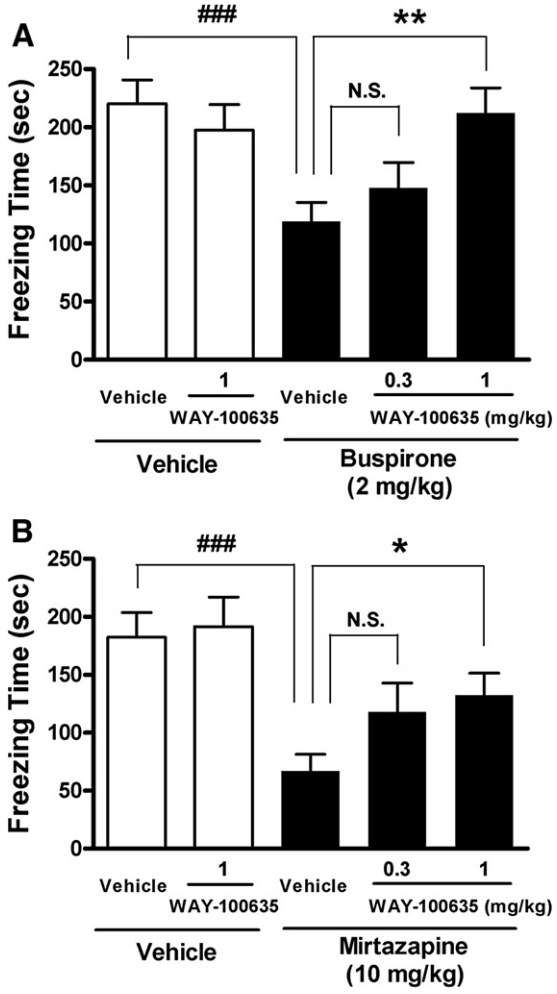


**Fig. 2.** Effect of concomitant treatment with WAY-100635 on buspirone (2 mg/kg)- (A) and mirtazapine (10 mg/kg)- (B) induced anxiolytic effects in rat CFS test. WAY-100635 (0.3–1 mg/kg) and each of the two drugs were co-administered 30 min before the measurements. Data are represented as mean ± SEM (A: $n = 10$–11, B: $n = 11$–12). ###$p<0.001$ and *$p<0.05$, **$p<0.01$, significantly different from the values in vehicle and drug-control group, respectively.

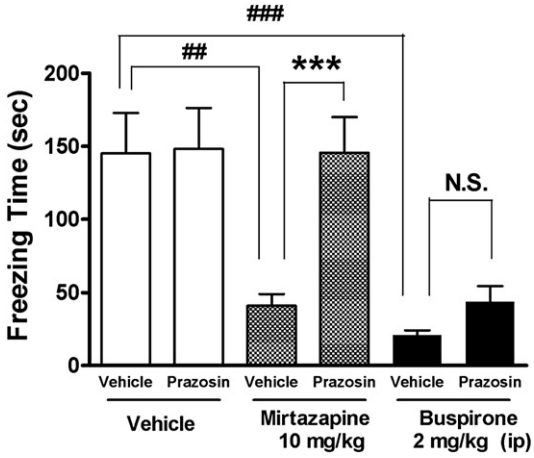


**Fig. 3.** Complete disappearance of anxiolytic action of mirtazapine by concomitant treatment with prazosin in rat CFS test. Prazosin (0.03 mg/kg) was co-administered with mirtazapine (10 mg/kg) or buspirone (2 mg/kg) 30 min before the measurements. Data are represented as mean ± SEM ($n = 10$). ##$p<0.01$, ###$p<0.001$ and ***$p<0.001$, significantly different from the values in vehicle and mirtazapine-control group, respectively.

0.3 mg/kg, $p>0.05$, 212.2 ± 21.7 s at 1 mg/kg, $p<0.01$, vs. 118.9 ± 16.5 s in vehicle group) (Fig. 2A).

### *3.2. Anxiolytic-like effect of mirtazapine and its reversal by combined treatment with WAY-100635 in CFS test*

Mirtazapine elicited a dose-dependent reduction in freezing time (92.4 ± 24.2 s at 1 mg/kg, $p<0.05$, 68.4 ± 26.4 s at 3 mg/kg, $p<0.01$, 55.3 ± 13.1 s at 10 mg/kg, $p<0.01$, vs. 195.5 ± 26.5 s in vehicle group) (Fig. 1B). Concomitant treatment with WAY-100635 (0.3 and 1 mg/kg) dose-dependently and partially antagonized the anxiolytic-like effects of mirtazapine at 10 mg/kg (118.0 ± 24.9 s at 0.3 mg/kg, $p>0.05$, 132.4 ± 19.0 s at 1 mg/kg, $p<0.05$, vs. 66.7 ± 14.7 s in vehicle group) (Fig. 2B).

### *3.3. Effect of mianserin on freezing behavior in CFS test*

Mianserin did not induce any significant changes in freezing behavior at doses tested in this study (1–30 mg/kg) (Fig. 1C).

### *3.4. Differential effects of prazosin on anxiolytic-like action of mirtazapine and buspirone in CFS test*

Although systemic administration of prazosin (0.03 mg/kg) by itself showed no significant changes in freezing time, it completely reversed the anxiolytic-like responses induced by mirtazapine at 10 mg/kg (145.5 ± 24.4 s in prazosin group, $p<0.001$, vs. 40.9 ± 8.1 s in vehicle group) (Fig. 3). By contrast, the co-administration of prazosin did not significantly alter the anxiolytic-like effects of buspirone at 2 mg/kg (Fig. 3).

### *3.5. Effect of tested drugs on locomotor activity*

Locomotion measurement studies clearly indicated that any single (buspirone at 2 mg/kg, mirtazapine at 10 mg/kg, WAY-100635 at 1 mg/kg, or prazosin at 0.03 mg/kg) and concomitant (with WAY-100635 or prazosin) treatments did not induce significant changes in spontaneous locomotor activities of animals (Table 1).

## 4. Discussion

The present pharmacological study clearly indicated the anxiolytic-like effects of mirtazapine (Fig. 1B), for the first time, in rat CFS model. The efficacy of mirtazapine was independent of changes in locomotor activities (Table 1) and partially reversed by combined treatment with WAY-100635 (Fig. 2B). Also the effects of this drug were completely sensitive to co-administered prazosin (Fig. 3). These results may well explain one aspect of mirtazapine's pharmacological profile as a noradrenergic and serotonergic antidepressant (Berendsen and Broekkamp, 1997; De Boer et al., 1994; de Boer et al., 1996).

**Table 1**
Effects of combined treatments with buspirone (2 mg/kg), mirtazapine (10 mg/kg), WAY-100635 (1 mg/kg), and prazosin (0.03 mg/kg) on spontaneous locomotor activities.

| Treatments | Locomotion (counts/5 min) | |
|---|---|---|
| Vehicle–vehicle | 213.0 ± 7.2 | |
| Vehicle–buspirone | 193.4 ± 20.5 | N.S. (vs. vehicle–vehicle) |
| WAY–vehicle | 215.6 ± 9.1 | N.S. (vs. vehicle–vehicle) |
| WAY–buspirone | 188.4 ± 14.5 | N.S. (vs. vehicle–vehicle) |
| Vehicle–vehicle | 208.4 ± 3.4 | |
| Vehicle–mirtazapine | 196.9 ± 13.2 | N.S. (vs. vehicle–vehicle) |
| WAY–vehicle | 211.1 ± 6.9 | N.S. (vs. vehicle–vehicle) |
| WAY–mirtazapine | 191.3 ± 18.3 | N.S. (vs. vehicle–vehicle) |
| Vehicle–vehicle | 207.1 ± 8.6 | |
| Prazosin–vehicle | 176.9 ± 14.7 | N.S. (vs. vehicle–vehicle) |
| Vehicle–mirtazapine | 188.4 ± 14.3 | N.S. (vs. vehicle–vehicle) |
| Prazosin–mirtazapine | 186.0 ± 8.5 | N.S. (vs. vehicle–vehicle) |

Data are expressed as mean ± SEM ($n = 7$).

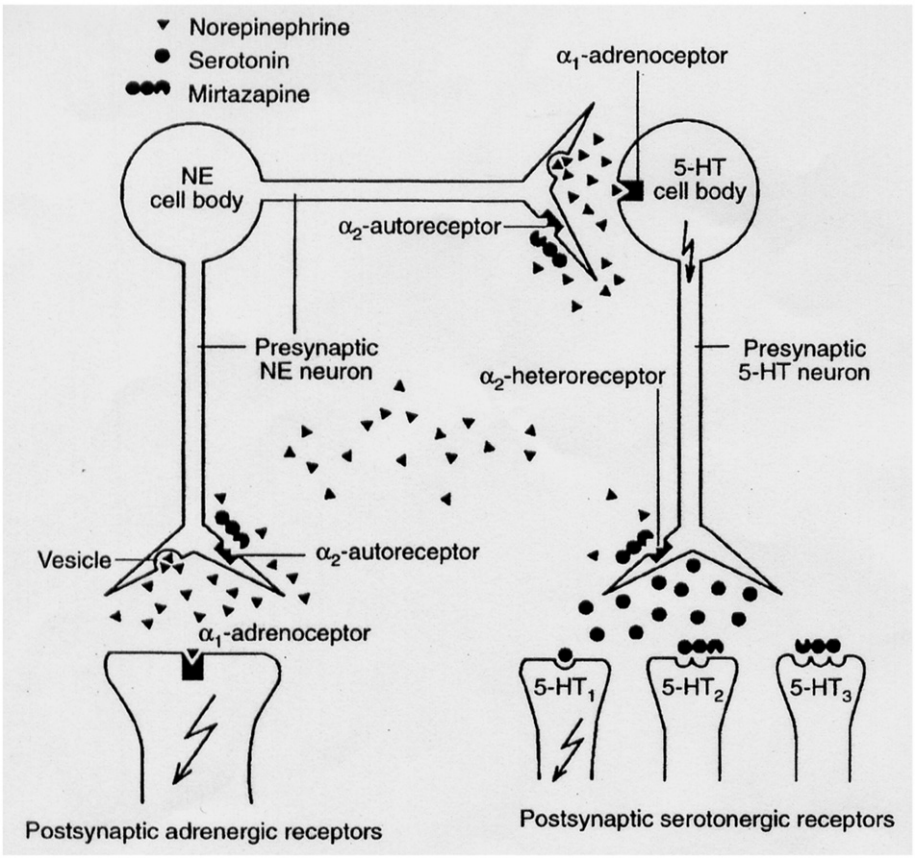


**Fig. 4.** Schematic diagram illustrating the 'NaSSA' concept for mirtazapine (from de Boer, 1996).

As a positive control, buspirone showed an anti-anxiety effect at 1–5 mg/kg (Fig. 1A). This was quantitatively consistent with the previous reports in the same model (Thompson and Rosen, 2006; Wislowska-Stanek et al., 2005). The anxiolytic-like effect of buspirone (2 mg/kg) was dose-dependently antagonized by WAY-100635 (Fig. 2A). With regard to 5-$HT_{1A}$ receptor pharmacology, functional heterogeneity in the pre- and post-synaptic receptors should always be taken into account. According to a recent study, a 5-$HT_{1A}$ agonist, flesinoxan exerts its anxiolytic-like action in rat CFS model via direct stimulation of postsynaptic 5-$HT_{1A}$ receptor in the hippocampus and amygdala (Li et al., 2006). Meanwhile, a differential action of WAY-100635 has been suggested in mouse depression model where it may preferentially bind to presynaptic 5-$HT_{1A}$ receptors at lower doses and the occupancy of postsynaptic receptors appears to be observed at higher doses (Harasawa et al., 2006). The existence of different dose-related pharmacological effects of 5-$HT_{1A}$ antagonists is also supported by two studies that used the CFS models. One study demonstrates that a lower dose (0.15 mg/kg) of WAY-100635 augmented anxiolytic-like effects of citalopram, suggesting that the blockade of 5-$HT_{1A}$ autoreceptor-mediated negative feedback may enhance the serotonergic neurotransmission and the relevant anxiolytic-like effects (Muraki et al., 2008). Likewise, NAN-190, the different 5-$HT_{1A}$ antagonist, strengthened at a low dose (0.1 mg/kg) anti-freezing effects of citalopram, while the treatments with higher doses (1–10 mg/kg) partially opposed the low dose response, probably due to the blockade of postsynaptic 5-$HT_{1A}$ receptor (Hashimoto et al., 1997). Corresponding to these concerns, the present result that the relatively higher dose (not 0.3 but 1 mg/kg) of WAY-100635 was required for complete blockade of the anxiolytic-like effects of buspirone is highly suggestive of the pathophysiological significance of postsynaptic 5-$HT_{1A}$ receptor in this anxiety model.

Despite the striking similarity of structural properties in general, mirtazapine and mianserin are pharmacologically different from each other (de Boer et al., 1988), and indeed displayed quite distinct anxiolytic-like profiles in this study; mirtazapine dose-dependently reduced the freezing time (Fig. 1B), while mianserin was completely ineffective in attenuating the responses (Fig. 1C). Mirtazapine primarily functions as an α2-autoreceptor antagonist to stimulate noradrenaline release from its synaptic terminals (Fig. 4), which subsequently triggers indirect activation of 5-HT neurons in the raphe nucleus via excitatory α1 receptor, because of the relatively weaker antagonistic activity of this drug for α1- compared with α2-receptor (4–15-fold difference in Kd values) (de Boer, 1996; Westenberg, 1999). Moreover, blockade by mirtazapine of α2-heteroreceptor located on serotonergic nerve terminals leads to a further increase in 5-HT neurotransmission by preventing the inhibitory effect of noradrenaline on 5-HT release (Fig. 4). In terms of the adrenoceptor binding properties, mianserin was found to be equally active as an antagonist for both α1 and α2 receptors (α1/α2 ratio [Kd value] = 0.66), and this drug was also a much more potent noradrenaline reuptake inhibitor than mirtazapine (de Boer et al., 1988). Reflecting these in vitro receptor binding profiles, previous electrophysiological studies demonstrated enhancement of the firing activity of 5-HT neurons in the treatment with mirtazapine (Haddjeri et al., 1996) but not with mianserin (Blier et al., 1984; Scuvee-Moreau and Dresse, 1979). Consistent with these pharmacological concepts are microdialysis studies dissociating the effects of mirtazapine and mianserin on serotonergic neurotransmission. Mirtazapine enhanced the amounts of both noradrenaline and 5-HT released in hippocampal dialysates, while mianserin only increased noradrenaline and failed to affect serotonergic neurotransmission (De Boer et al., 1994; de Boer et al., 1996). Given the facts that a variety of serotonergic agents exhibit anxiolytic-like effects in CFS model, (Inoue et al., 1996a,b; Rittenhouse et al., 1992) and that treatment with the selective noradrenergic reuptake inhibitor, reboxetine failed to reduce the expression of

anxiety (Inoue et al., 2006), the differences in anxiolytic-like efficacy between the two drugs can be accounted for by presence or absence of 5-HT-enhancing profiles. The effect of mirtazapine was partially but significantly reversed by 1 mg/kg of WAY-100635 (Fig. 2B), the dose of which fully corresponded to that with the maximal reversal of buspirone-induced reduction in freezing behavior (Fig. 2A). These findings strongly suggest the relative importance of serotonergic neurotransmission and at least in part, activation of postsynaptic 5-$HT_{1A}$ receptor in the anxiolytic-like action of mirtazapine. However, WAY-100635 did not completely reverse the effects of mirtazapine and this may suggest that mechanisms other than 5-$HT_{1A}$ receptor activation also contribute to the anxiolytic-like effects of mirtazapine. It has been reported that a selective 5-$HT_{2C}$ receptor antagonist, SB242084 is effective in certain animal tests of anxiety (Kennett et al., 1997). In this connection, our independent studies revealed that a single treatment with SB242084 at its typical dose (0.3 mg/kg) does not affect the freezing time (unpublished data), suggesting that 5-$HT_{2C}$ receptor antagonism of mirtazapine does not directly contribute to its anxiolytic-like effects in this model.

To substantiate the differences in pharmacological mechanisms of anxiolytic-like actions between mirtazapine and mianserin, further experiments focusing on the physiological role of α1 receptor were performed. The selective and centrally active α1 antagonist, prazosin (Menkes et al., 1981), completely suppressed mirtazapine-induced anxiolytic-like responses at the dose being ineffective on its own (Fig. 3), accompanied by no significant changes in locomotor activities (Table 1). The dose of prazosin used in this study was within the reported range (Rouquier et al., 1994) and did not alter the anxiolytic-like effects of buspirone in a significant manner (Fig. 3), which may help guarantee the specificity of α1-receptor blockade. These findings probably underscore the importance of α1 receptor-mediated activation of 5-HT neurons in the mirtazapine-induced anxiolytic-like actions. Previous studies have demonstrated that noradrenaline neurons extend their axons to the raphe region where they exert a tonic α1 receptor-mediated excitatory influence on 5-HT neurons (Fig. 4) projecting to the forebrain (Baraban and Aghajanian, 1980, 1981). Therefore, changes in the afferent noradrenergic activities at the level of the dorsal and median raphe nucleus may modulate 5-HT neurotransmission in several brain regions highly implicated in fear conditioning and anxiety such as the frontal cortex, hippocampus, and amygdala (Bechara et al., 1995; Inoue et al., 2004; Morgan and LeDoux, 1995). In fact, systemic administration of prazosin evoked a marked reduction in the firing rate of dorsal raphe 5-HT neurons (Lejeune et al., 1994), and decreased 5-HT release in the rat hippocampus (Hjorth et al., 1995; Rouquier et al., 1994). More direct evidence comes from the studies in which mirtazapine-induced 5-HT release in median raphe nucleus and hippocampus was blocked by co-treatment with prazosin (Westenberg, 1999). Collectively, these observations document the causal involvement of α1 receptor in the anxiolytic-like actions induced by mirtazapine, and the key difference in pharmacological profile from mianserin may be attributed to the extent of antagonistic activity for this receptor.

A phenotype of interest in this study was anxiolytic-like profiles of mirtazapine and the comparators by their acute treatments. The fact that clinically useful and late-onset anxiolytics such as selective serotonin reuptake inhibitor, citalopram (Inoue et al., 1996a) and 5-$HT_{1A}$ receptor agonist, buspirone (Figs. 1 and 2A; Thompson and Rosen, 2006; Wislowska-Stanek et al., 2005) stably show their acute effects may suggest a predictive validity of this model. However, further studies testing efficacies of drugs including mirtazapine after long term treatments should also be taken into account for an appropriate extrapolation of the preclinical data to substantial clinical evidence, particularly in terms of the maintenance of efficacy.

In the present study, we demonstrated the anxiolytic-like profile of mirtazapine, but not of mianserin, in animal model of anxiety. The anxiolytic-like action of mirtazapine was significantly reversed by the 5-$HT_{1A}$ receptor antagonist WAY-100635 and α1 receptor antagonist prazosin to varying degrees, highlighting the importance of α1 receptor in the enhanced 5-HT neurotransmission and of postsynaptic 5-$HT_{1A}$ receptor in the actual therapeutic efficacy of mirtazapine in the CFS model. Taken as a whole, these findings are in general agreement with 'NaSSA' concept for mirtazapine and also support clinical efficacy of this drug in a variety of anxiety disorders.

## References


Anttila SA, Leinonen EV. A review of the pharmacological and clinical profile of mirtazapine. CNS Drug Rev 2001;7:249–64.

Baraban JM, Aghajanian GK. Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. Neuropharmacology 1980;19: 355–63.

Baraban JM, Aghajanian GK. Noradrenergic innervation of serotonergic neurons in the dorsal raphe: demonstration by electron microscopic autoradiography. Brain Res 1981;204:1-11.

Bechara A, Tranel D, Damasio H, Adolphs R, Rockland C, Damasio AR. Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. Science 1995;269:1115–8.

Berendsen HH, Broekkamp CL. Indirect in vivo 5-HT1A-agonistic effects of the new antidepressant mirtazapine. Psychopharmacology (Berl) 1997;133:275–82.

Blier P, de Montigny C, Tardif D. Effects of the two antidepressant drugs mianserin and indalpine on the serotonergic system: single-cell studies in the rat. Psychopharmacology (Berl) 1984;84:242–9.

de Boer T. The pharmacologic profile of mirtazapine. J Clin Psychiatry 1996;57(Suppl 4):19–25.

de Boer TH, Maura G, Raiteri M, de Vos CJ, Wieringa J, Pinder RM. Neurochemical and autonomic pharmacological profiles of the 6-aza-analogue of mianserin, Org 3770 and its enantiomers. Neuropharmacology 1988;27:399–408.

De Boer T, Nefkens F, Van Helvoirt A. The alpha 2-adrenoceptor antagonist Org 3770 enhances serotonin transmission in vivo. Eur J Pharmacol 1994;253:R5–6.

de Boer TH, Nefkens F, van Helvoirt A, van Delft AM. Differences in modulation of noradrenergic and serotonergic transmission by the alpha-2 adrenoceptor antagonists, mirtazapine, mianserin and idazoxan. J Pharmacol Exp Ther 1996;277:852–60.

Falkai P. Mirtazapine: other indications. J Clin Psychiatry 1999;60(Suppl 17):36–40.

Fanselow MS, Helmstetter FJ. Conditional analgesia, defensive freezing, and benzodiazepines. Behav Neurosci 1988;102:233–43.

Gambi F, De Berardis D, Campanella D, Carano A, Sepede G, Salini G, et al. Mirtazapine treatment of generalized anxiety disorder: a fixed dose, open label study. J Psychopharmacol 2005;19:483–7.

Haddjeri N, Blier P, de Montigny C. Effect of the alpha-2 adrenoceptor antagonist mirtazapine on the 5-hydroxytryptamine system in the rat brain. J Pharmacol Exp Ther 1996;277:861–71.

Harasawa T, Ago Y, Itoh S, Baba A, Matsuda T. Role of serotonin type 1A receptors in fluvoxamine-induced inhibition of marble-burying behavior in mice. Behav Pharmacol 2006;17:637–40.

Hashimoto S, Inoue T, Koyama T. Effects of the co-administration of 5-HT1A receptor antagonists with an SSRI in conditioned fear stress-induced freezing behavior. Pharmacol Biochem Behav 1997;58:471–5.

Hjorth S, Bengtsson HJ, Milano S, Lundberg JF, Sharp T. Studies on the role of 5-HT1A autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release—I. BMY7378 and prazosin. Neuropharmacology 1995;34:615–20.

Holm KJ, Markham A. Mirtazapine: a review of its use in major depression. Drugs 1999;57:607–31.

Inoue T, Hashimoto S, Tsuchiya K, Izumi T, Ohmori T, Koyama T. Effect of citalopram, a selective serotonin reuptake inhibitor, on the acquisition of conditioned freezing. Eur J Pharmacol 1996a;311:1–6.

Inoue T, Tsuchiya K, Koyama T. Serotonergic activation reduces defensive freezing in the conditioned fear paradigm. Pharmacol Biochem Behav 1996b;53:825–31.

Inoue T, Li XB, Abekawa T, Kitaichi Y, Izumi T, Nakagawa S, et al. Selective serotonin reuptake inhibitor reduces conditioned fear through its effect in the amygdala. Eur J Pharmacol 2004;497:311–6.

Inoue T, Nakagawa S, Izumi T, Kitaichi Y, Koyama T. Effect of combined treatment with noradrenaline and serotonin reuptake inhibitors on conditioned freezing. Eur J Pharmacol 2006;540:91–5.

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, et al. SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 1997;36:609–20.

Koran LM, Gamel NN, Choung HW, Smith EH, Aboujaoude EN. Mirtazapine for obsessive–compulsive disorder: an open trial followed by double-blind discontinuation. J Clin Psychiatry 2005;66:515–20.

Lejeune F, Audinot V, Gobert A, Rivet JM, Spedding M, Millan MJ. Clozapine inhibits serotoninergic transmission by an action at alpha 1-adrenoceptors not at 5-HT1A receptors. Eur J Pharmacol 1994;260:79–83.

Li X, Inoue T, Abekawa T, Weng S, Nakagawa S, Izumi T, et al. 5-HT1A receptor agonist affects fear conditioning through stimulations of the postsynaptic 5-HT1A receptors in the hippocampus and amygdala. Eur J Pharmacol 2006;532: 74–80.

Menkes DB, Baraban JM, Aghajanian GK. Prazosin selectively antagonizes neuronal responses mediated by alpha1-adrenoceptors in brain. Naunyn-Schmiedeberg's Arch Pharmacol 1981;317:273–5.

Morgan MA, LeDoux JE. Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. Behav Neurosci 1995;109:681–8.

Muraki I, Inoue T, Koyama T. Effect of co-administration of the selective 5-HT1A receptor antagonist WAY 100,635 and selective 5-HT1B/1D receptor antagonist GR 127,935 on anxiolytic effect of citalopram in conditioned fear stress in the rat. Eur J Pharmacol 2008;586:171–8.

O'Connor WT, Leonard BE. Effect of chronic administration of the 6-aza analogue of mianserin (Org. 3770) and its enantiomers on behaviour and changes in noradrenaline metabolism of olfactory-bulbectomized rats in the "open field" apparatus. Neuropharmacology 1986;25:267–70.

Rauggi R, Cassanelli A, Raone A, Tagliamonte A, Gambarana C. Study of mirtazapine antidepressant effects in rats. Int J Neuropsychopharmacol 2005;8:369–79.

Reneric JP, Bouvard M, Stinus L. In the rat forced swimming test, NA-system mediated interactions may prevent the 5-HT properties of some subacute antidepressant treatments being expressed. Eur Neuropsychopharmacol 2002;12:159–71.

Rittenhouse PA, Bakkum EA, O'Connor PA, Carnes M, Bethea CL, van de Kar LD. Comparison of neuroendocrine and behavioral effects of ipsapirone, a 5-HT1A agonist, in three stress paradigms: immobilization, forced swim and conditioned fear. Brain Res 1992;580:205–14.

Rouquier L, Claustre Y, Benavides J. Alpha 1-adrenoceptor antagonists differentially control serotonin release in the hippocampus and striatum: a microdialysis study. Eur J Pharmacol 1994;261:59–64.

Sambunaris A, Hesselink JK, Pinder R, Panagides J, Stahl SM. Development of new antidepressants. J Clin Psychiatry 1997;58(Suppl 6):40–53.

Sarchiapone M, Amore M, De Risio S, Carli V, Faia V, Poterzio F, et al. Mirtazapine in the treatment of panic disorder: an open-label trial. Int Clin Psychopharmacol 2003;18:35–8.

Sartorius N, Baghai TC, Baldwin DS, Barrett B, Brand U, Fleischhacker W, et al. Antidepressant medications and other treatments of depressive disorders: a CINP Task Force report based on a review of evidence. Int J Neuropsychopharmacol 2007;10(Suppl 1):S1-S207.

Scuvee-Moreau JJ, Dresse AE. Effect of various antidepressant drugs on the spontaneous firing rate of locus coeruleus and dorsal raphe neurons of the rat. Eur J Pharmacol 1979;57:219–25.

Szegedi A, Schwertfeger N. Mirtazapine: a review of its clinical efficacy and tolerability. Expert Opin Pharmacother 2005;6:631–41.

Thompson BL, Rosen JB. Immediate-early gene expression in the central nucleus of the amygdala is not specific for anxiolytic or anxiogenic drugs. Neuropharmacology 2006;50:57–68.

Van Veen JF, Van Vliet IM, Westenberg HG. Mirtazapine in social anxiety disorder: a pilot study. Int Clin Psychopharmacol 2002;17:315–7.

Westenberg HG. Pharmacology of antidepressants: selectivity or multiplicity? J Clin Psychiatry 1999;60(Suppl 17):4–8.

Wislowska-Stanek A, Zienowicz M, Lehner M, Taracha E, Bidzinski A, Maciejak P, et al. Buspirone attenuates conditioned fear-induced c-Fos expression in the rat hippocampus. Neurosci Lett 2005;389:115–20.