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# Mirtazapine increases dopamine release in prefrontal cortex by 5-$HT_{1A}$ receptor activation

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**Abstract**

Mirtazapine has a low affinity for 5-$HT_{1A}$ receptors but shows 5-$HT_{1A}$-agonistic-like effects in behavioral pharmacology test. However, there is to date no clear evidence that mirtazapine enhances 5-$HT_{1A}$ neurotransmission. The object of the present study was to assess the effects of mirtazapine on dialysate levels of dopamine and 5-HT in the medial frontal cortex of freely moving rats and to determine whether this drug could modulate 5-$HT_{1A}$ neurotransmission. In vivo microdialysis was used to study the effects of mirtazapine on extracellular dopamine and 5-HT levels, and the effect of the 5-$HT_{1A}$ antagonist WAY100,356 on extracellular dopamine level increased by mirtazapine in the rat prefrontal cortex. Mirtazapine (4–16 mg/kg, i.p.) produced a dose-dependent increase in extracellular dopamine levels in the medial prefrontal cortex (mPFC) of freely moving rats without modifying those of 5-HT. In the presence of the selective 5-$HT_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazineyl]ethyl]-*N*-(pyridinyl)-cyclohexane-carboxamide (WAY100,635; 0.3 mg/kg; i.p.), the influence of mirtazapine on cortical levels of dopamine was markedly attenuated. These results indicate that mirtazapine induces the enhancement of the output of cortical dopamine mediated via blockade of $\alpha_2$-adrenergic receptors and facilitation of post-synaptic 5-$HT_{1A}$ function.
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*Keywords:* Mirtazapine; Dopamine; Serotonin; 5-$HT_{1A}$ agonist; Prefrontal cortex; Microdialysis

## 1. Introduction

Mirtazapine {1,2,3,4,10,14*b*-hexa-hydro-2-meth-ylpyrazino[2,1-*a*]pyrido[2,3-*c*]benzazepin} is a tetracyclic compound with antidepressant activity in human [6,14]. Mirtazapine has a unique mechanism of action, different from that of the classical tricyclic antidepressants, the serotonin selective reuptake inhibitors and monoamine oxidase inhibitors, and could be described as a noradrenergic and specific serotonergic antidepressant, abbreviated as NaSSA [10]. The pharmacological profile of mirtazapine is characterized by potent presynaptic $\alpha_2$-adrenergic antagonistic activity, 5-$HT_1$ agonistic activity, and potent 5-$HT_2$ and 5-$HT_3$ antagonistic activities, as well as by a potent $H_1$ antagonistic activity [11]. The blockade of presynaptic $\alpha_2$-adrenergic receptors is considered as a possible mechanism for antidepressant activity of mirtazapine. The interactions of mirtazapine with 5-HT receptors were studied in vivo in experiments measuring selective 5-HT receptor subtype-mediated behaviors [4]. Mirtazapine was found to induce lower lip retraction mediated by 5-$HT_{1A}$ receptors in rats [3]. Moreover, the taste aversion induced by mirtazapine was also prevented by pretreatment with the 5-$HT_{1A}$ agonist, 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT), indicating similar stimulus properties of mirtazapine and 8-OH-DPAT [3]. This indicates that mirtazapine exerts in vivo effects mediated by 5-$HT_{1A}$ receptor without high affinity for 5-$HT_{1A}$ receptors. There is to date no clear evidence that mirtazapine enhances 5-$HT_{1A}$ neurotransmission. The frontal cortex has been repeatedly proposed as an area involved in depression, since positron emission tomography studies revealed that depressed patients show functional changes in the frontal cortex [2,5,9]. It has been suggested that the property of stimulating dopamine transmission in the prefrontal cortex has a role in the antidepressant action [23,24].

In light of these observations, the aims of the study reported here were to assess the effects of mirtazapine on dialysate levels of dopamine and 5-HT in the medial

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prefrontal cortex (mPFC) of freely moving rats and to determine whether this drug could modulate 5-$HT_{1A}$ neurotransmission.

## 2. Material and methods

### 2.1. Animals

Male Wistar rats (Clea Japan Inc., Tokyo) weighing 220–300 g were used. Rats were housed in groups of five and maintained at a temperature 25 ± 2 °C on a 12-h-light/12-h-dark cycle with lights on between 7 am and 7 pm. Rats were fed a standard laboratory food and tap water ad libitum. The experimental procedures for animals were conducted in accordance with the guidelines of the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

### 2.2. Surgery

Rats were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.) and placed in a stereotaxis apparatus. Rats were implanted with a guide cannula in the mPFC (A 3.2, L 0.9, V –3.0 from dura, according to the atras of Paxinos and Watson) [20]. The animals were then allowed to recover from surgery for 7 days.

### 2.3. Microdialysis

Microdialysis and subsequent chromatographic analysis were performed using an automated on-line sample injection system. On the day of the experiment, rats were transferred to a plastic cage and a microdialysis probe (membrane length, 2 mm; molecular mass cutoff, 50 kDa; Eicom Co., Kyoto, Japan) was inserted into the guide cannula so that the final placement of the probe was in the mPFC. The probe was perfused at a rate of 2 μl/min with Ringer's solution (147 mM NaCl, 4.0 mM KCl, and 2.3 mM $CaCl_2$) and dialysate was collected at 20-min intervals. Dialysate samples were analyzed for serotonin and dopamine content using high performance liquid chromatography with electrochemical detector (ECD-300). The mobile phase consisted of 0.1 M phosphate buffer (pH 6.0) containing 500 mg/l sodium 1-octanesulfonate and 50 mg/l. EDTA-$Na_2$ was pumped at a rate of 2 ml/min through a reverse phase column (Eicompak CA-50DS, Eicom). After 2 h of perfusion, i.e. when basal release was stable, drugs were administered.

### 2.4. Drugs

Mirtazapine was obtained from Nippon Organon K.K., Japan. Mirtazapine was dissolved in a drop of glacial acetic acid, diluted to final volume with 0.9% saline and neutralized (pH 6–7) with solid $NaHCO_3$. WAY100,635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazineyl]ethyl]-*N*-(pyridinyl)-cyclohexanecarboxamide 3HCl) was purchased from Sigma Chemical Co., St. Louis, MO, USA. WAY100,635 was dissolved in saline. Drugs were administered intraperitonealy in a volume of 1.0 ml/kg.

### 2.5. Statistics

Data were presented as the percentage of the mean amount in the three samples preceding drug injection (mean ± S.E.M.). Statistical analysis of the experimental data was performed using one-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's test. Statistical significance at the 5% level was presented.

## 3. Results

The basal extracellular levels of dopamine in the dialysates of the medial prefrontal cortex, without considering probe recovery, were 0.42 ± 0.04 pg/sample. As shown in Fig. 1, the administration of mirtazapine at

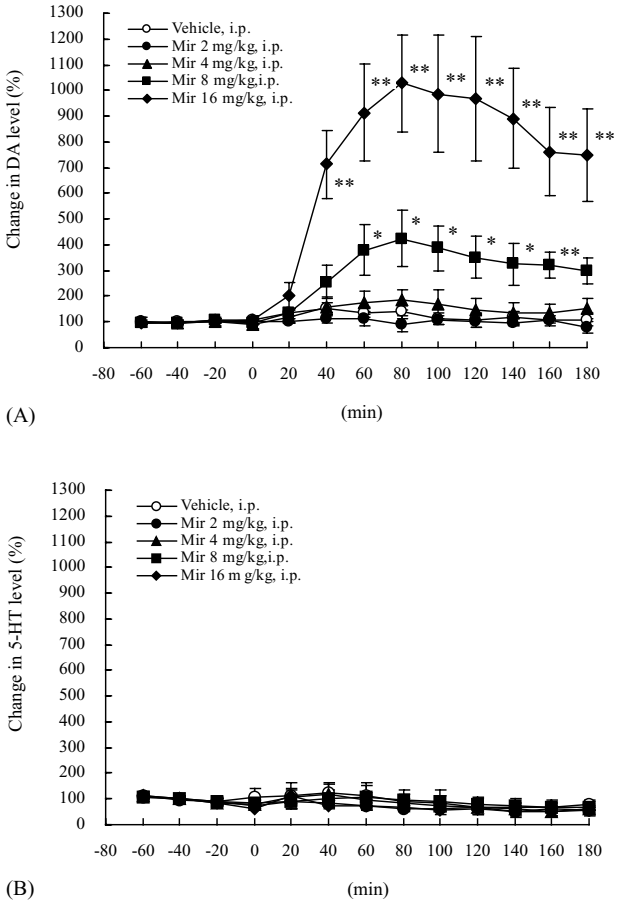


Fig. 1. Effect of mirtazapine (Mir) on extracellular DA (A) and 5-HT (B) level in the rat prefrontal cortex. Data are means ± S.E.M. of six rats per group and expressed as a percentage of basal values. * $P < 0.05$, ** $P < 0.01$ vs. vehicle group by Dunnett's test.

doses of 8 and 16 mg/kg, i.p. elicited a dose-dependent increase in extracellular levels of dopamine in dialysates of the medial prefrontal cortex, whereas its 2 and 4 mg/kg doses were ineffective. Forty minutes after administration of mirtazapine at a dose of 16 mg/kg, i.p., a significant increase in dopamine content was observed relative to vehicle-treated control. Extracellular dopamine content increased to a maximum of 1028 ± 87% of base-line values at 80 min post-injection. The increase in dopamine content persisted up to 180 min after administration of mirtazapine. The basal extracellular levels of 5-HT in dialysates of the medial prefrontal cortex were 1.41 ± 0.13 pg/sample. Mirtazapine at doses of 2, 4, 8, and 16 mg/kg, i.p. did not influence extracellular levels of 5-HT in dialysates of the medial prefrontal cortex. The 5-$HT_{1A}$ receptor antagonist WAY100,635 at 0.3 mg/kg, i.p. did not by itself affect the extracellular dopamine and 5-HT levels in the medial prefrontal cortex (data not shown). Simultaneous treatment with WAY100,635 at 0.3 mg/kg, i.p. reduced significantly the increase in dopamine content elicited by mirtazapine at 16 mg/kg, i.p. (Fig. 2).

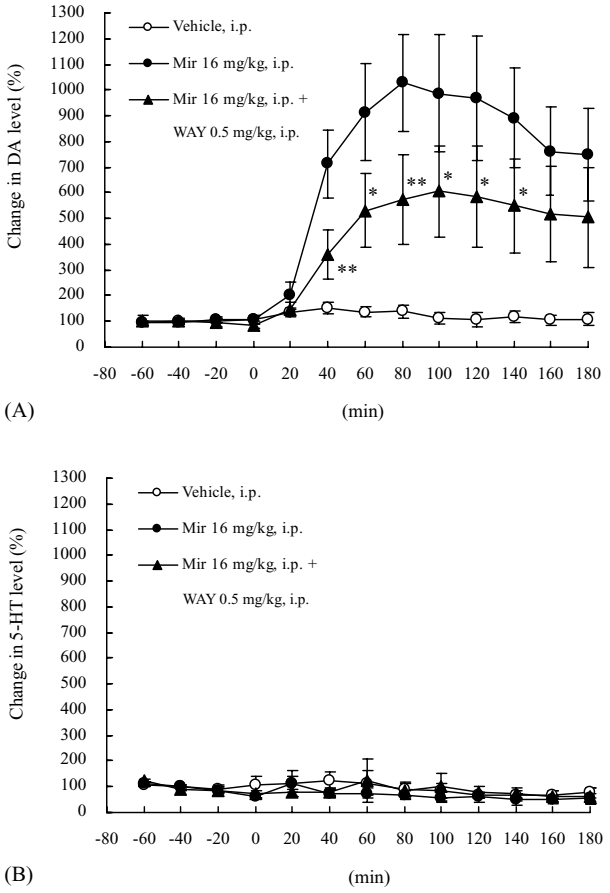


Fig. 2. Effect of WAY100,356 (WAY) on extracellular DA level increased by mirtazapine (Mir) (A) and on extracellular 5-HT level in the presence of Mir (B) in the rat prefrontal cortex. Data are means ± S.E.M. of six rats per group and expressed as a percentage of basal values. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle group by Tukey's test.

## 4. Discussion

The present results show that mirtazapine elicits increase in extracellular dopamine levels in the medial prefrontal cortex of freely moving rats. Neurochemical studies in vitro have shown that the affinity of mirtazapine for dopamine, 5-HT and noradrenaline uptake sites was negligible [19]. It has also been shown that mirtazapine manifests high affinity for in vitro study with recombinant human $\alpha_{1A}$-adrenergic, $\alpha_{2A}$-adrenergic, 5-$HT_{2A}$, 5-$HT_{2B}$, and 5-$HT_{2C}$ receptors. Antagonist action of mirtazapine at $\alpha_{2A}$-adrenergic receptors have shown both blockade of the influence of noradrenaline on the activity of pyramidal cells in the rat hippocampus [15] and blockade of clonidine-induced mydriasis in rats [11]. Hertel et al. reported that the $\alpha_2$-adrenoceptor antagonist idazoxan preferentially increases basal dopamine output in the medial prefrontal cortex through a local mechanism, an effect which appears largely independent of dopaminergic activity [16]. Furthermore, it has been suggested that the enhanced output of cortical dopamine may contribute to the purported augmentation by $\alpha_2$-adrenoceptor antagonist of the therapeutic effects of antidepressant drugs [16]. These findings suggest that the increase in extracellular dopamine content in the prefrontal cortex elicited by mirtazapine is the result of antagonism of $\alpha_2$-adrenergic receptors, and that this effect might be associated with an impairment of depressive symptoms.

On the other hand, this study demonstrates that the selective 5-$HT_{1A}$ antagonist WAY100,635 inhibits the stimulation of dopamine release in the prefrontal cortex elicited by mirtazapine. It has been reported that intact serotonergic neurons are required for the facilitatory effects of idazoxan, a selective $\alpha_2$-adrenergic receptor antagonist, on dopamine release in the rat prefrontal cortex [17]. Wedzony et al. have shown that 5-$HT_{1A}$ receptor agonists, such as ipsapirone and buspirone are capable of enhancing the outflow of dopamine in the rat prefrontal cortex, which suggests that these drugs may enhance dopaminergic neurotransmission in the rat prefrontal cortex [25]. It has also been indicated that the effect of ipsapirone is specific for activation of 5-$HT_{1A}$ receptors, since its effect is antagonized by WAY100,635. It has also been shown that conditioned stress in vivo enhances dopaminergic neurotransmission in the rat prefrontal cortex, this effect being attenuated by such novel anxiolytics as ipsapirone and buspirone, which operate via serotonergic 5-$HT_{1A}$ receptors [26]. Doherty et al. have shown that the 5-$HT_{1A}$ receptors are present in the ventral tegmental area, and may modulate dopaminergic activity in the ventral tegmental area both directly and indirectly [12]. There may be multiple potential sites for modulation of dopaminergic output by 5-$HT_{1A}$ receptor activation in the ventral tegmental area [12]. Moreover, it was found that 8-OH-DPAT, a 5-$HT_{1A}$ receptor agonist, enhanced the burst activity of dopaminergic neurons, especially those localized in the ventral tegmental area [1], i.e. the type of neuronal activity of dopaminergic neurons that was predominantly

linked to the release of dopamine from its terminals [13]. One possible underlying mechanism is a role of 5-$HT_{1A}$ autoreceptors, the activation of which may reverse inhibition of mesocortical dopaminergic pathways by removing a suppressive influence of 5-HT exerted via excitatory 5-$HT_{2C}$ receptors localized on GABAergic interneurones in the ventral tegmental area [18,21]. It is possible that 5-$HT_{1A}$ receptors might also be involved in the alteration of descending glutamatergic pathways making a direct contact with dopaminergic neurons innervating cortex, since such a descending glutamatergic projection in controlled by 5-$HT_{1A}$ receptors [7,8]. Mirtazapine has a low affinity for 5-$HT_{1A}$ receptors but shows 5-$HT_{1A}$-agonistic-like effects in a conditioned taste aversion test and by causing lower lip retraction in rats [4,11]. The 5-$HT_{2C}$ receptors antagonist has also been reported to increase diasylate levels of dopamine, but not 5-HT in the rat frontal cortex [18]. In fact, the present results show that mirtazapine did not influence extracellular levels of 5-HT in dialysates of the prefrontal cortex. These data show that mirtazapine increases dopamine release in rat prefrontal cortex in large part via activation of 5-$HT_{1A}$ receptors. Furthermore, the antagonist properties of mirtazapine at 5-$HT_{2C}$ receptors may be of importance to its facilitatory effect on dopamine release in the rat prefrontal cortex. The frontal cortex has been repeatedly proposed as an area involved in depression, since positron emission tomography studies revealed that depressed patients show functional changes in the frontal cortex [2,5,9]. There is evidence that dopamine $D_2/D_3$ receptor agonist ropinirole produces antidepressant-like effects in animal models [22]. From these results, it is suggested that mirtazapine induces the enhancement of the output of cortical dopamine mediated via blockade of $\alpha_2$-adrenergic receptors and facilitation of post-synaptic 5-$HT_{1A}$ function, and that this enhanced dopamine neurotransmission may underlie the antidepressant effect of mirtazapine.

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