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Neurochemical effects of the enantiomers of mirtazapine in normal rats

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Abstract

The present study was designed to examine the neurochemical effects of (±)-mirtazapine (10 mg kg^{-1} i.p.) and its enantiomers in rats. Male Sprague–Dawley rats received either (+)-mirtazapine, (−)-mirtazapine, (±)-mirtazapine or vehicle, by intraperitoneal injection for two weeks. Maximum change in temperature from baseline, following a single dose of the 5-HT_{1A} receptor agonist 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (0.15 mg kg^{-1} s.c.), was used to assess the function of the 5-HT_{1A} receptors. Chronic drug treatment potentiated this response, with (±)-mirtazapine > (−)-mirtazapine > (+)-mirtazapine. Receptor changes were also observed with a slight decrease in β_1-adrenoceptor density, although this failed to reach significance. A significant decrease in β_1-adrenoceptor affinity was observed following (−)-mirtazapine treatment. All drugs tested significantly reduced the density of the 5-HT_2 receptors. Results of the present study suggest that in so far as alterations in these receptor populations are important for the therapeutic action of antidepressants, neither of the enantiomers appear to be more active than the racemic mixture.

Keywords: Mirtazapine; 5-HT_{1A} receptor; β-Adrenoceptor; 5-HT_2 receptor; 8-OH-DPAT; 8-Hydroxy-2-(di-*n*-propylamino)tetralin; Hypothermia

1. Introduction

Despite their identical structures, enantiomers tend to interact differently with receptors which are usually enantioselective, preferring to interact with one enantiomer over the other (Pang, 1989). Very often enantiomers of a compound have different effects with one enantiomer responsible for the therapeutic action and the other for the side effects (Ariens, 1984).

Enantiomeric effects have been observed amongst antidepressant drugs. For instance, *S*-(+)-mianserin is 200–300 times more potent as a noradrenaline re-uptake inhibitor than the *R*-(−)-enantiomer (Coutts and Baker, 1989). Its ability to block α_2-adrenoceptors is also enantioselective, with its activity again residing predominantly with the (+)-enantiomer (Gower et al., 1988; Coutts and Baker, 1989). In the case of fluoxetine, the *R*(−)-enantiomer is 23 times more potent than the *S*(+)-enantiomer at blocking serotonin (5-hydroxytryptamine, 5-HT) reuptake (Wong et al., 1991). Similarly, in the case of norfluoxetine, *R*-norfluoxetine was 22 times less potent at inhibiting 5-HT uptake (Wong et al., 1993). The *S*- and *R*-enantiomers of citalopram and of its metabolites demethylcitalopram and didemethylcitalopram (Hyttel et al., 1992, 1995) are selective serotonin reuptake inhibitors, but due to its potency, the main pharmacologically active (as an antidepressant) compound is probably *S*-citalopram (Baumann, 1996).

Mirtazapine belongs to a class of antidepressant drugs that are distinct from the classical tricyclic antidepressants. As with the tricyclic antidepressants, mirtazapine interacts with both noradrenergic and serotonergic systems, but has a reduced side effect profile (De Boer et al., 1995). Mirtazapine enhances noradrenergic release in vivo, by blockade of α_2-autoreceptors and serotonin release by blockade of α_2-heteroceptors and by increasing 5-HT cell firing (De Boer et al., 1994, 1995, 1996; Haddjeri et al., 1996). It has been suggested that its pharmacological profile is characterised by α_2-adrenoceptor, 5-HT_2, 5-HT_3 and histamine H_1 receptor antagonist properties, it is devoid of anticholinergic activity and has no effect on monoamine re-uptake (Nickolson et al., 1992; De Boer et al., 1988).

Mirtazapine is an optically active compound and it has been suggested that both enantiomers contribute to its

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therapeutic effect (De Boer et al., 1995). The α_2-autoreceptor and 5-HT_2 blocking effects of mirtazapine are believed to reside predominantly in the (+)-enantiomer, while the (−)-enantiomer is believed to be responsible for the α_2-heteroreceptor blockade and 5-HT_3 receptor antagonism (De Boer et al., 1988; Kooyman et al., 1994).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats were housed four per cage with access to food and water ad lib. The animals were maintained on a 12:12 light–dark cycle (lights on at 0800; lights off at 2000).

2.2. Chemicals and radioligands

(−)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimdazol-2-one (CGP-12177, [5,7-^{3}H]- (specific activity 42.5 Ci/mmol) and [^{3}H]Ketanserin (specific activity 80.0 Ci/mmol) were obtained from Dupont (Australia). 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was purchased from Australian Laboratory supply. Oxytetracycline powder, Tris, sodium chloride, potassium chloride, copper sulphate, sodium hydroxide and sodium tartrate were purchased from Sigma (Melbourne, Australia). (±)-Mirtazapine and its enantiomers were kindly provided by Organon (Oss, Netherlands).

2.3. Drug treatment

Drugs were dissolved in dimethylsulphoxide (DMSO) in an injection volume of 1 ml kg^{-1} ((+)-mirtazapine (10 mg kg^{-1}), (−)-mirtazapine (10 mg kg^{-1}) and (±)-mirtazapine (10 mg kg^{-1})). Drugs were administered once daily i.p. for a period of two weeks. Controls received injections of vehicle (DMSO) alone.

2.4. 8-OH-DPAT

8-OH-DPAT (0.15 mg kg^{-1}) was administered s.c. 1 h after the last antidepressant treatment. Rectal temperatures were recorded prior to and at 30, 60 90 and 120 min following 8-OH-DPAT.

2.5. Binding experiments

2.5.1. Tissue preparation

Rats were sacrificed by decapitation 24 h after the last dose. The brains were removed, rapidly dissected on ice and the frontal cortex was stored at −70°C for binding studies. One cortical hemisphere was used for β_1 and the other cortical hemisphere for 5-HT_2 receptor binding. The tissue was homogenised for approximately 5 s in 500 μl of ice cold Tris–HCl buffer (50 mM Tris, pH 7.4). The samples were then centrifuged at 30 000 × *g* for 10 min at 4°C. The supernatant was discarded and the pellet of particulate membrane resuspended in 500 μl of ice cold Tris–HCl incubation buffer and recentrifuged as before. Following a further resuspension the supernatant was then decanted and the resulting pellet resuspended in ice cold Tris–HCl buffer (50 mM, pH 7.4) to form the final membrane suspension.

Specific β-adrenoceptor binding was assessed using [^{3}H]CGP-12177, [5,7-^{3}H]- as radioligand. All binding was carried out in triplicate by incubation of 200 μl of tissue suspension with radioligand at six concentrations (0.025–0.8 nM) in incubation buffer with atenolol (1 mM) or isoproterenol (1 mM) as the displacing agents, for 2 h at room temperature. Membrane bound radioactivity was determined by filtration under vacuum through Whatman GF/B glass fibre filters presoaked in Tris buffer (50 mM Tris containing 0.1% bovine serum albumin, pH 7.7) using a Brandell cell harvester. Filters were washed with 5 ml ice cold Tris buffer. Following three washes with buffer, the filter containing the membrane was transferred into 5 ml of Ready Protein scintillation fluid and counted using a Packard Scintillation counter. Specific binding was defined as the difference between total binding and the binding observed in the presence of atenolol or isoproterenol. In order to obtain β_1- and β_2-adrenoceptor binding the binding in the presence of isoproterenol and atenolol were subtracted from the total β-adrenoceptor binding counts, respectively.

Specific 5-HT_2 receptor binding was assessed using [^{3}H]Ketanserin. All binding was carried out in triplicate by the incubation of the membrane suspension in radioligand, at six concentrations 0.05–12 nM, in incubation buffer in the presence or absence of Methysergide (1 mM) for 1 h at 37°C. Membrane bound radioactivity was determined by filtration under vacuum through Whatman GF/B glass fibre filters presoaked in Tris buffer (50 mM Tris containing 0.1% bovine serum albumin, pH 7.7) using a Brandell cell harvester. Filters were washed with 5 ml ice cold Tris buffer. Following three washes with 5 ml of Tris bovine serum albumin buffer, the filter containing the membrane was transferred into 5 ml of Ready Protein scintillation fluid and counted using a Packard Scintillation counter.

2.6. Protein determination

The method used for protein determination was based on the method of Lowry et al. (1951) using bovine serum albumin as a standard in a concentration range of 10–60 μg/ml.

2.7. Statistical analysis

The temperature responses were analysed using an analysis of variance followed by Student–Newman–Keuls

Table 1
The effect of chronic pretreatment with mirtazapine on 8-OH-DPAT induced hypothermia in the rat

Treatment	T0–T30	T0–T60	T0–T90	T0–T120
Control	−0.34 ± 0.219	−0.05 ± 0.16	−0.26 ± 0.14	0.30 ± 0.08
(−)-mirtazapine	−1.06 ± 0.345[a]	−0.89 ± 0.42[a]	−0.40 ± 0.30[ac]	−0.16 ± 0.21[b]
(+)-mirtazapine	−0.82 ± 0.15	−0.06 ± 0.17	−0.15 ± 0.17	0.56 ± 0.13[c]
(±)-mirtazapine	−0.53 ± 0.13	−1.19 ± 0.14[a]	−1.12 ± 0.12[a]	−0.63 ± 0.15[a]

Results are tabulated as group mean change from baseline ± S.E.M. $n = 7–12$. [a]$P < 0.01$ vs. control, [b]$P < 0.05$; [c]$P < 0.01$ vs. racemate.

post-hoc test. Binding data were analysed by scatchard analysis using the EBDA program (McPherson, 1985) to provide K_d and B_{max} values. Differences between groups for K_d and B_{max} were analysed using an analysis of variance.

3. Effect of 8-OH-DPAT on rectal temperature

No significant differences in core body temperature were evident between the groups prior to the 8-OH-DPAT challenge. Results of a repeated measures analysis of variance (ANOVA) revealed a significant effect of drug on core body temperature ($F(3,28) = 9.09$, $P < 0.0002$) and a significant effect of time ($F(3,84) = 134.675$, $P < 0.0001$). Post-hoc analysis revealed that mirtazapine administration potentiated the hypothermic response to 8-OH-DPAT with racemate > (−)-enantiomer > (+)-enantiomer (Table 1).

4. [^{3}H]CGP 12177 binding

4.1. β-adrenoceptor density

The specific binding of CGP-12177, [5,7-^{3}H]- was determined in the cortex of the animals following two weeks of drug or vehicle treatment. A one-way ANOVA failed to show a significant effect of drug treatment on β_1-adrenoceptor density. Although this decrease failed to reach significance, there was a trend towards a reduction in β_1-adrenoceptor density following chronic treatment with the racemate and both enantiomers. No effect of chronic treatment was found on β_2-adrenoceptor populations with the racemate or either isomer (Fig. 1)

4.2. β-adrenoceptor affinity

A one-way ANOVA revealed a significant effect of drug treatment on β_1-adrenoceptor affinity [$F(3,16) = 3.723$, $P < 0.03$]. A Student–Newman–Keuls post-hoc analysis revealed a significant decrease in β_1-adrenoceptor affinity following chronic (−)-mirtazapine treatment when compared to the other groups ($P < 0.01$) (Fig. 2)

4.3. [^{3}H]Ketanserin binding

A two-way ANOVA revealed a significant effect of drug treatment on the density of 5-HT$_2$ receptor binding in the frontal cortex ($F(3,13) = 6.391$, $P < 0.006$). Chronic treatment with all forms tested significantly reduced the density of the 5-HT$_2$ receptors when compared with vehi-

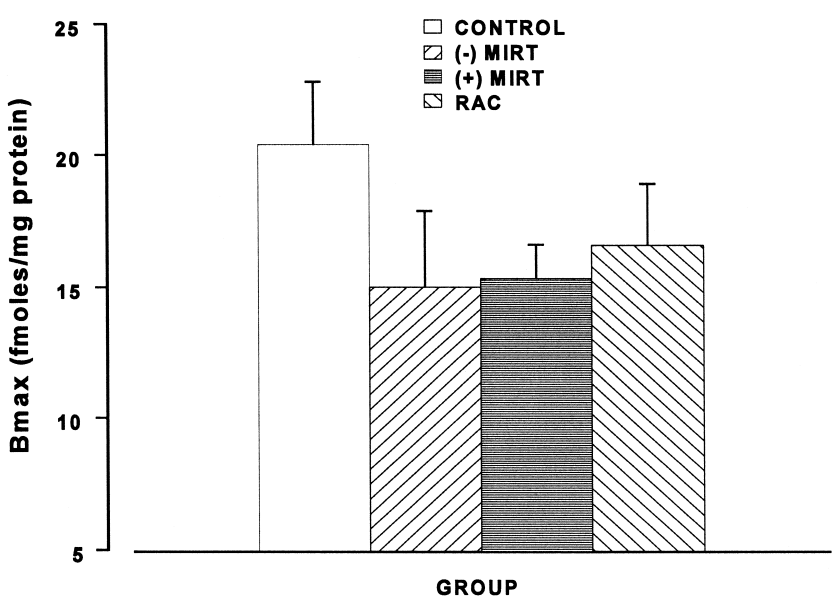

Fig. 1. The effect of chronic mirtazapine treatment on β_1 receptor density in the rat. Data are represented as group mean ± S.E.M. $n = 4–6$.

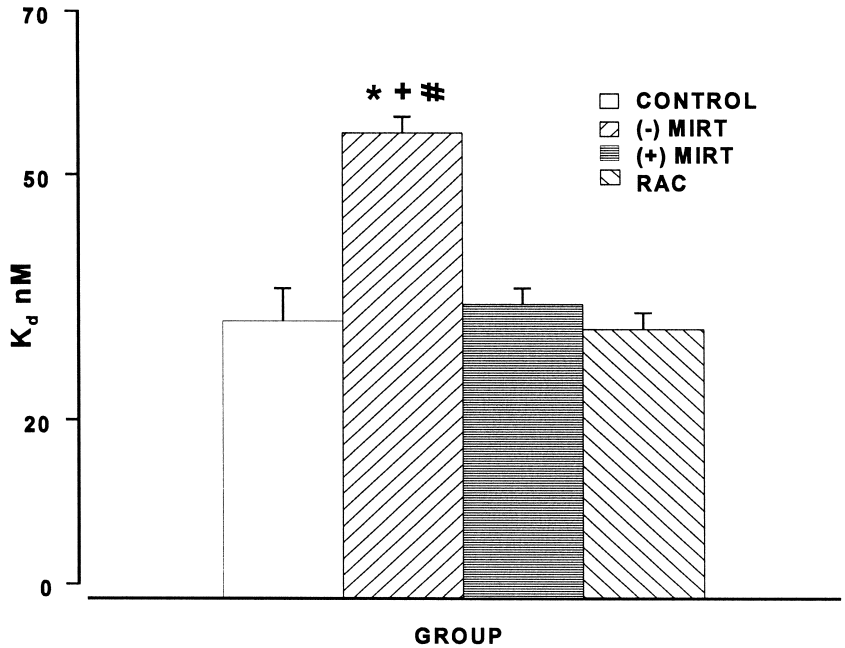

Fig. 2. The effect of chronic mirtazapine treatment on β_1 receptor affinity in the rat. Data are represented as group mean ± S.E.M. $n = 4–6$. $* P < 0.05$ vs. control, $^{+} P < 0.01$ vs. (+)-mirtazapine, $^{\#} P < 0.05$ vs. racemate.

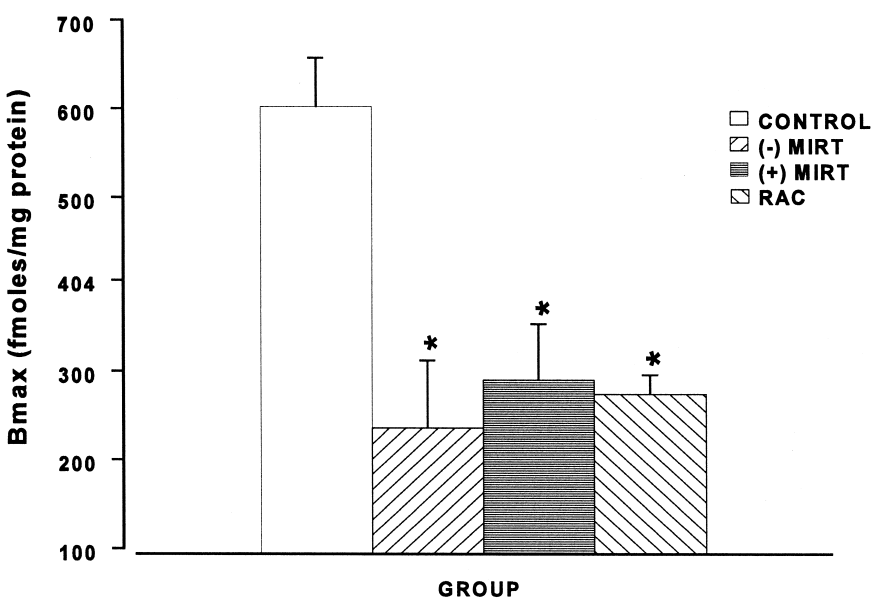

Fig. 3. The effect of chronic mirtazapine treatment on 5-HT_2 receptor density in the rat. Data are represented as group mean ± S.E.M. $n = 4–6$. $* P < 0.01$ vs. control.

cle animals (Student–Newman–Keuls post-hoc analysis). No effect of drug treatment was observed on the affinity of these receptors (Fig. 3).

5. Discussion

The effect of antidepressant treatment on the hypothermic response induced by the acute administration of 8-OH-DPAT has often been used as a measure of 5-HT_{1A} receptor function (Hamon et al., 1987). Chronic antidepressant treatment with most antidepressants has been shown to attenuate the hypothermic response to 8-OH-DPAT in rats (Goodwin, 1989; Frazer and Hensler, 1990), although controversial findings have been reported, with some antidepressants having no effect on the hypothermic response (Yamada et al., 1994) and others potentiating this response (McGrath et al., unpublished observation). In the present study, chronic treatment with mirtazapine or its enantiomers did not attenuate the hypothermic response to

8-OH-DPAT and in fact, mirtazapine treatment appears to have potentiated the hypothermic response, with racemate > (−)-enantiomer > (+)-enantiomer. This finding suggests that chronic mirtazapine treatment (either as the racemic mixture or the individual enantiomers), at least as measured by this functional test, does not appear to have direct effects on the 5-HT_{1A} receptor.

This finding suggests that an increase in 5-HT neurotransmission, as evidenced by an attenuation of the hypothermic response to 8-OH-DPAT, does not appear to be involved in mediating the antidepressant action of mirtazapine.

An alteration in the density or responsiveness of the 5-HT_2 receptors has also been implicated in the pathophysiology of depression. 5-HT_2 receptor density has been shown to be reduced by chronic treatment with a variety of antidepressant drugs such as the tricyclic antidepressants and the monoamine oxidase inhibitors (Charney et al., 1981; Fuxe et al., 1983). In the present study chronic treatment with mirtazapine and its enantiomers significantly reduced the density of 5-HT_2 receptors in the frontal cortex, with no change in the affinity of these receptors. There is, however, some controversy surrounding the importance of 5-HT_2 receptor down regulation in the therapeutic effect of antidepressants since chronic treatment with some selective serotonin re-uptake inhibitors such as fluoxetine has been shown to upregulate these receptors (Hrdina and Vu, 1993). An upregulation of these receptors has also been reported to occur following electroconvulsive therapy (Kellar et al., 1981).

Mirtazapine and its enantiomers administered to rats once daily produced a reduction in the density of the β_1-adrenoceptors in the frontal cortex, although this failed to reach statistical significance. The lack of effect in this study questions the power of the study to detect a difference if $n = 4$. In addition, chronic antidepressant treatment has been reported to preferentially decrease β_1-adrenoceptors in the amygdala (Ordway et al., 1991). It is possible therefore that the failure of mirtazapine and its enantiomers to significantly reduce the density of the β_1-adrenoceptors in the present study could be a reflection of the brain region studied. Assessment of the chronic treatment with (−)-mirtazapine also produced a significant decrease in the affinity of the β_1-adrenoceptors in the frontal cortex, while (+)-mirtazapine and the racemate had no effect. This finding of a decrease in the density of β_1-adrenoceptors following mirtazapine treatment is in agreement with previous research which has shown down regulation of the β-adrenoceptors following chronic treatment with a variety of antidepressants including tricyclic antidepressants, monoamine oxidase inhibitors and electroconvulsive shock (Banarjee et al., 1977; Sugrue, 1983; Dennis et al., 1994). Interestingly, chronic treatment with mianserin, which also blocks α_2-adrenoceptors either failed to produce a down regulation of the β-adrenoceptors (Mishra et al., 1980) or produced a slight but insignificant reduction in these receptors (Clements-Jewery, 1978) reducing them to 82% of controls.

Results of the present study suggest in so far as down regulation of the β_1-adrenoceptors and 5-HT_2 receptors are important for the therapeutic action of antidepressant drugs, neither of the enantiomers of mirtazapine appear to be more active than the racemic mixture.

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