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In-vivo effects of the 1,2,4-piperazine derivatives MM5 and MC1, putative 5-HT agonists, on dopamine and serotonin release in rat prefrontal cortex

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Abstract

Two 1,2,4-substituted derivatives of piperazine were tested for their effect on dopamine and serotonin (5-HT) release in rat prefrontal cortex. Both compounds, 1-[4-(4-chinolin-2-yl-piperazin-1-yl)-butyl]piperidin-2-on (MM5) and 1-[4-(2-methyl-4-chinolin-2-yl-piperazin-1-yl)-butyl]-8-azaspiro [4.5]decano-7,9-dion (MC1), produced hypothermia in mice and showed affinity for 5-HT_{1A} receptors in-vitro. Like the selective 5-HT_{1A} agonist 8-OH-DPAT (0.1 mg kg^{-1}), MM5 given peripherally (30 mg kg^{-1}) decreased the extracellular 5-HT level in rat prefrontal cortex, while MC1 suppressed 5-HT release at a higher dose (40 mg kg^{-1}), but not at a lower one (30 mg kg^{-1}). The effect of both compounds on 5-HT release was abolished by WAY 100635 (0.3 mg kg^{-1}). MC1 (30 and 40 mg kg^{-1}), but not MM5, raised cortical dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and extracellular homovanillic acid (HVA) levels. The effect of MC1 on dopamine release was reversed by neither WAY 100635 nor the non-selective 5-HT_2 antagonist ritanserin (2 mg kg^{-1}). However, ritanserin prevented the effect of the higher dose of MC1 on 5-HT release. The results of this study suggest that MM5 exhibits the profile of a 5-HT_{1A} agonist devoid of dopaminergic activity. MC1 seems to possess moderate agonist activity at 5-HT_{1A} and 5-HT_{2A} receptors, while acting on 5-HT release in the rat prefrontal cortex. However, the facilitation of dopamine release by this compound does not seem to be related to its affinity for 5-HT_{1A} and 5-HT_{2A} receptors.

Introduction

The frontal cortex plays a crucial role in the process involved in the control of mood, affective processing, anxiety and stress (Millan et al 2000; Davidson 2002). The modulation of serotonergic, dopaminergic and noradrenergic pathways projecting to the prefrontal cortex by several classes of agents provides strategies for the treatment of psychiatric disorders. Direct interaction with serotonin 5-HT_{1A} receptors via selective agonists or antagonists may have a beneficial effect in the treatment of depression or anxiety (Chojnacka-Wójcik & Przegaliński 1991; Levine & Potter 1999; Barros et al 2003; Wesołowska et al 2003a, b). On the other hand, blockade of serotonin 5-HT_{2A} and dopamine D_2 receptors in addition to 5-HT_{1A} agonism is characteristic of atypical antipsychotic drugs, of which clozapine is the prototype (Ichikawa et al 2001). The blockade of serotonin 5-HT_{2A} and dopamine D_2 receptors by atypical antipsychotics, together with 5-HT_{1A} agonism, is regarded as a cause of the increased dopamine release in the prefrontal cortex (Ichikawa et al 2001, 2002; Jordan et al 2004). Moreover, 5-HT_{1A} agonists increase cortical dopamine release (Wędzony et al 1996; Ichikawa et al 2001; Ago et al 2003) with a concomitant decrease in cortical serotonin release (Gobert et al 1999; Ago et al 2003; Wesołowska et al 2003b). It is postulated that the facilitatory influence of 5-HT_{1A} agonists on cortical dopaminergic transmission is integrated in the ventral tegmental area (VTA) cell body region (Hajós et al 2003).

In this study, two novel 1,2,4-substituted piperazines were tested for their activity on dopamine and serotonin neurotransmission in rat prefrontal cortex. The two

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Table 1 Affinity of MM5 and MC1 for selected receptors in the rat striatal or cortical membranes

	K_i (nM)			
	5-HT_{1A}	5-HT_{2A}	D_1	D_2
MM5	65 ± 8	332 ± 32	> 10 000	> 10 000
MC1	24 ± 3	32 ± 3	> 10 000	> 10 000
8-OH-DPAT	7.8 ± 1.1	> 10 000	> 10 000	> 10 000
Ritanserin	> 10 000	1.1 ± 0.4	> 10 000	> 10 000

The data are means $\pm$ s.e.m., n = 3.

compounds, 1-[4-(4-chinolin-2-yl-piperazin-1-yl)-butyl]piperidin-2-on (MM5) and 1-[4-(2-methyl-4-chinolin-2-yl-piperazin-1-yl)-butyl]-8-azaspiro[4.5]decano-7,9-dion (MC1), showed high in-vitro affinity for 5-HT_{1A} and 5-HT_{2A} receptors (Table 1). In behavioural experiments, both compounds — like the selective 5-HT_{1A} receptor agonist 8-OH-DPAT — produced hypothermia in mice, which is regarded as 5-HT_{1A} presynaptic receptor agonist activity. The hypothermic response of MM5, but not MC1, was reversed by the selective 5-HT_{1A} antagonist WAY 100635 (Chilmonczyk et al 2002). Moreover, both compounds diminished the serotonin syndrome induced by 8-OH-DPAT (Iskra-Jopa et al 2002), that effect being accepted as an indication of 5-HT_{1A} postsynaptic receptor antagonist activity (Tricklebank et al 1984). We examined the impact of MM5 and MC1 on dopamine and serotonin release in rat prefrontal cortex using an in-vivo microdialysis technique. The role of 5-HT_{1A} or 5-HT_{2A} receptors in the effect of the two compounds was evaluated by blocking the changes induced by MM5 or MC1 in dopamine or serotonin release with the selective 5-HT_{1A} antagonist WAY 100635 or the 5-HT_2 antagonist ritanserin (Barnes & Sharp 1999).

Materials and Methods

Chemicals and drugs

All the chemicals used for HPLC were purchased from Merck (Warszawa, Poland). MM5 and MC1 (synthesized in Pharmaceutical Research Institute, Warszawa, Poland), WAY 100635, 8-OH-DPAT and ritanserin (Research Biochemicals Inc., Natick, USA) were dissolved in 0.9% NaCl and were given intraperitoneally, except for WAY 100635 and 8-OH-DPAT which were injected subcutaneously. Drug treatment details are given in figure captions.

Animals and surgery

Adult male Wistar rats, 280–300 g, were used. They were housed under conditions of constant temperature (20–22°C) and controlled light (12-h light–dark cycle), with free access to food and water. The experimental protocols were approved by the Animal Care and Use Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

The rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, USA). Transverse microdialysis probes (external diameter 0.2 mm, cut-off 50 000 Da), prepared according to the method of Imperato & Di Chiara (1984), were implanted in the prefrontal cortex (part of the cingulate, frontal and parietal cortices) at the following stereotaxic co-ordinates: A + 2.2 mm and H + 2.5 mm from the surface of the skull (Paxinos & Watson 1998). The microdialysis probes, made exclusively of dialysing fibre, were placed in the brain without guide cannulae only once and were thus not harmful to the surrounding tissue.

Microdialysis and an analytical procedure

On the following day, the microdialysis probes were perfused with artificial cerebrospinal fluid (aCSF) consisting (in mM) of NaCl 140, KCl 2.7, $CaCl_2$ 1.2, $MgCl_2$ 1, NaH_2PO_4 0.3, Na_2HPO_4 1.7, pH 7.4, at a flow rate of 2 μL min^{-1} with a CMA/100 microinfusion pump (CMA/Microdialysis, Stockholm, Sweden). Samples were collected from freely moving rats at 15-min intervals after a 3-h washout period. WAY 100635 or ritanserin were given 30 min before MM5 or MC1. Control rats received the appropriate volume of physiological solution (0.9% NaCl). The collection of samples started 15 min after administration of the drugs and continued for 2 h. The dialysates (20 μL) were immediately assayed for dopamine, serotonin, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC with an electrochemical detection (BAS 480; Bioanalytical Systems Inc., W. Lafayette, IN), equipped with an Inertsil-3 (3 μm, 4.6 × 125 mm) column (Varian, the Netherlands) and a glassy carbon electrode set at a potential of +800 mV versus an Ag/AgCl reference electrode. The mobile phase consisted of 0.1 M monochloracetic acid, pH 3.8, 25 mg L^{-1} of octane-1-sulfonic acid sodium salt, 0.4 mM EDTA, 8% methanol and 1% acetonitrile. The column temperature was set at 27°C, and the flow rate was 1 mL min^{-1}. Data were collected and analysed using BAS Inject V-1.27 software run on a PC computer. The probe recovery in-vitro was 10–15% for serotonin and dopamine.

At the end of the experiments, the brains were examined histologically for correct probe placement. Only data from rats in which the microdialysis probes were correctly located were included in the calculation of the results.

Statistics

For the statistical significance of differences in the measured substances after drug administration, compared with the baseline values, a one-way analysis of variance for repeated measures and Tukey's test were used. Changes in the cumulative amounts were analysed by a one-way analysis of variance, followed by Tukey's post-hoc test. $P \leq 0.05$ was considered to be statistically significant.

Radioligand binding studies

Dopamine D_1 and D_2 receptor binding assays

Both competition binding studies were conducted on rat striatal membranes prepared according to a previously published procedure (Ossowska et al 2001). The final tissue concentration was 3 mg of the original wet weight per millilitre. All assays were carried out in 50 mM potassium phosphate buffer (pH 7.4). The radioligands used were [^{3}H]-SCH 23390 (75.5 Ci mmol^{-1}; NEN Chemicals) and [^{3}H]-spiperone (15.70 Ci mmol^{-1}; NEN Chemicals) for D_1 and D_2 receptors, respectively. Displacement experiments were performed in a total volume of 1.2 mL. Assay tubes (in triplicate) containing 0.1 mL of a 1 nM specific radioligand, 0.1 mL of a competing drug or 0.1 mL of vehicle (total binding) and 1 mL of tissue were incubated at 30 °C for 60 min (D_1), or at 37°C for 30 min (D_2). Additionally, to prevent [^{3}H]-spiperone binding to 5-HT$_{2A}$ receptors, ketanserin (50 nM) was included in the D_2 assay buffer. Non-specific binding was determined using 5 μM *cis*-(Z)-flupentixol for D_1 receptors, or butaclamol (5 μM) for D_2 sites. The binding reaction was terminated by rapid filtration through Whatman GF/B filters, followed by three 4-mL washes with an ice-cold incubation buffer.

5-HT_{1A} and 5-HT_{2A} receptor binding assays

The affinity of MM5 and MC1 for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT (170 Ci mmol^{-1}) and [^{3}H]ketanserin (63.3 Ci mmol^{-1}; NEN Chemicals, Boston), respectively. Radioligand binding experiments were carried out on rat brain tissue collected from the cortex according to a previously published procedure (Bojarski et al 1993). Radioactivity was determined by liquid scintillation counting in Beckman LS 6500 apparatus. K_i values were determined from at least three experiments in which ten drug concentrations, run in triplicate, were used.

Results

Radioligand binding studies

Binding receptor studies showed high affinity of MC1 for both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors ($K_i = 24$ and 32 nM, respectively). The compound did not exhibit any affinity for D_1 or D_2 receptors. Also MM5 bound effectively to 5-HT$_{1A}$ receptors ($K_i = 65$ nM), but showed only moderate affinity for 5-HT$_{2A}$ receptors ($K_i = 332$ nM) (Table 1).

Effect of MM5 and MC1 on extracellular serotonin, dopamine, DOPAC and HVA levels in rat prefrontal cortex

Administration of the vehicle did not influence the extracellular levels of serotonin or dopamine (results not shown). 8-OH-DPAT (0.1 mg kg^{-1}), used as a reference drug, and MM5 (30 mg kg^{-1}) significantly decreased the extracellular level of serotonin ($F(8,27) = 6.84$, $P < 0.001$, $n = 4$ and $F(8,27) = 10.75$, $P < 0.001$, $n = 4$, respectively).

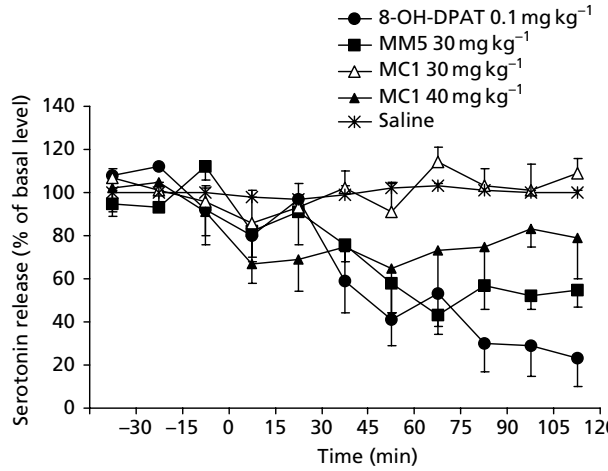

Figure 1 The effect of 8-OH-DPAT (0.1 mg kg^{-1}), MM5 (30 mg kg^{-1}) and MC1 (30 and 40 mg kg^{-1}) on serotonin release in rat prefrontal cortex. Basal extracellular concentrations of serotonin, in pg/20 μL of dialysate, were 5.70 ± 0.32, 5.54 ± 0.71, 6.77 ± 0.21, 5.29 ± 0.69, 6.56 ± 0.36 for saline, 8-OH-DPAT (0.1 mg kg^{-1}), MM5 (30 mg kg^{-1}), MC1 (30 mg kg^{-1}) and MC1 (40 mg kg^{-1}), respectively. The data, expressed as a percentage of basal level, are means ± s.e.m., $n = 4$/group.

MC1 elicited a decrease in serotonin level at a higher (40 mg kg^{-1}) dose only ($F(8,27) = 2.78$, $P < 0.02$, $n = 4$) but not at a lower one (30 mg kg^{-1}). The time-course of that effect is shown in Figure 1.

8-OH-DPAT (0.1 mg kg^{-1}) ($F(8,27) = 2.93$, $P < 0.02$, $n = 4$) and MC1 (30 and 40 mg kg^{-1}) elevated extracellular dopamine level ($F(8,27) = 4.34$, $P < 0.01$, $n = 4$ and $F(8,27) = 2.55$, $P < 0.03$, $n = 4$, respectively), but MM5 (30 mg kg^{-1}) had no effect on it. The time-course of that effect is shown in Figure 2. A successive increase of the dose of MM5 did not affect dialysate dopamine level (data not shown).

MC1 (30 and 40 mg kg^{-1}), but not 8-OH-DPAT (0.1 mg kg^{-1}) or MM5 (30 mg kg^{-1}), elicited a marked increase in extracellular DOPAC ($F(8,27) = 10.73$, $P < 0.001$, $n = 4$ and $F(8,27) = 16.74$, $P < 0.001$, $n = 4$, respectively) and HVA levels ($F(8,27) = 5.16$, $P < 0.001$, $n = 4$ and $F(8,27) = 5.65$, $P < 0.001$, $n = 4$, respectively) (Figure 2).

Antagonism towards the effect of MM5 and MC1 on extracellular serotonin, dopamine, DOPAC and HVA levels produced by WAY 100635 in rat prefrontal cortex

The selective 5-HT$_{1A}$ antagonist WAY 100635 (0.3 mg kg^{-1}) abolished the decrease in extracellular serotonin level induced by MM5 (30 mg kg^{-1}) and MC1 (40 mg kg^{-1}). WAY 100635 (0.3 mg kg^{-1}) by itself had no effect on serotonin level (Figure 3) and did not antagonize the increase in extracellular dopamine, DOPAC and HVA concentration induced by MC1 (30 mg kg^{-1}) (Figure 4).

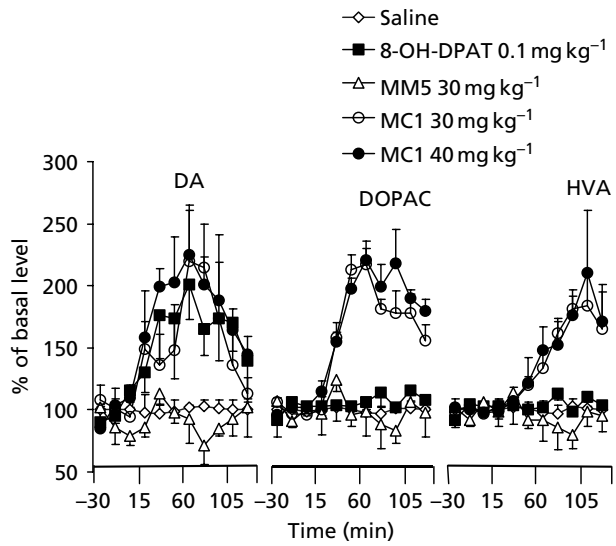

Figure 2 The effect of 8-OH-DPAT (0.1 mg kg^{-1}), MM5 (30 mg kg^{-1}) and MC1 (30 and 40 mg kg^{-1}) on extracellular dopamine (DA), DOPAC and HVA levels in rat prefrontal cortex. Basal extracellular concentrations of dopamine, DOPAC and HVA, in pg/20 μL of dialysate, were 2.69 ± 0.23, 315 ± 27 and 710 ± 21 for saline, 2.94 ± 0.31, 334 ± 38 and 806 ± 36 for 8-OH-DPAT (0.1 mg kg^{-1}), 2.71 ± 0.17, 384 ± 83 and 685 ± 87 for MM5 (30 mg kg^{-1}), 2.97 ± 0.21, 267 ± 27 and 686 ± 36 for MC1 (30 mg kg^{-1}) and 3.08 ± 0.17, 324 ± 26 and 770 ± 56 for MC1 (40 mg kg^{-1}), respectively. The data, expressed as a percentage of basal level, are means $\pm$ s.e.m., n = 4/group.

Antagonism towards the effect of MM5 and MC1 on extracellular serotonin, dopamine, DOPAC and HVA levels produced by ritanserin in rat prefrontal cortex

The non-selective 5-HT$_2$ receptor antagonist ritanserin (2 mg kg^{-1}) reversed the decrease in serotonin release induced by MC1 (40 mg kg^{-1}), but had no effect on the decrease in serotonin release induced by MM5 (30 mg kg^{-1}) (Figure 3). In contrast, ritanserin had no influence on the increase in dialysate dopamine, DOPAC and HVA levels induced by MC1 (30 mg kg^{-1}) (Figure 4). Ritanserin itself had no effect on extracellular serotonin, dopamine, DOPAC or HVA level (Figures 3 and 4).

Discussion

These findings indicate that two new 1,2,4-trisubstituted piperazine derivatives, with high affinity for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors, show a different profile when acting on serotonin and dopamine release in rat prefrontal cortex. Compound MM5 decreased serotonin release, but had no effect on the extracellular dopamine level in that brain area. On the other hand, compound MC1, which diminished serotonin release at the higher dose only, markedly raised the extracellular levels of dopamine and its metabolites DOPAC and HVA in rat prefrontal cortex. The effect of MM5, as well as that of the higher dose of MC1,

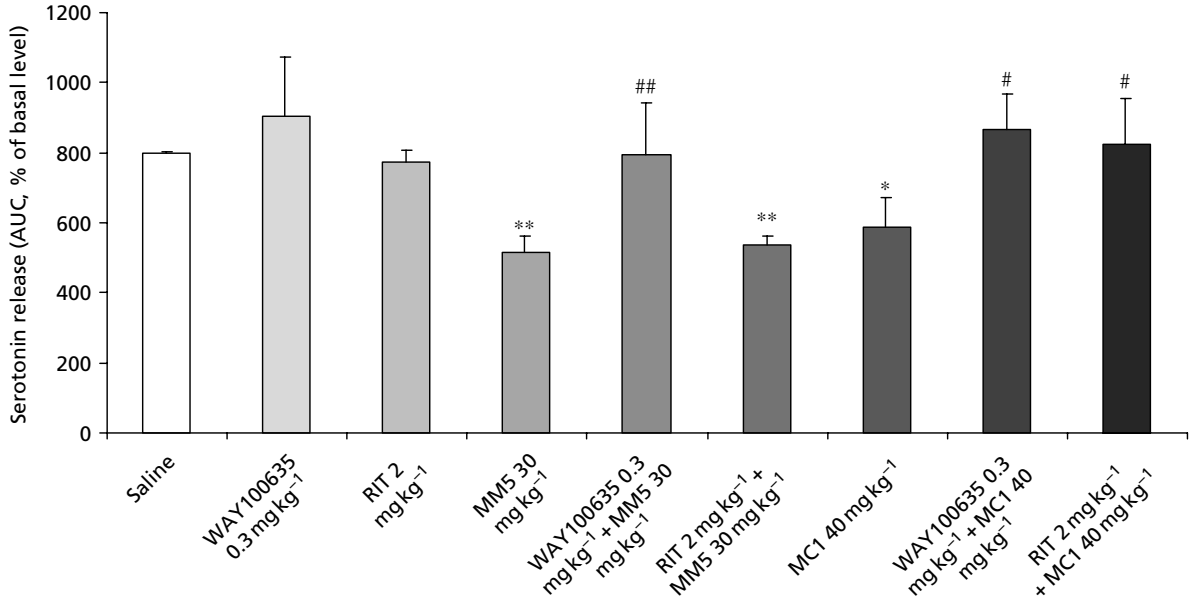

Figure 3 The cumulative effect of WAY 100635 (0.3 mg kg^{-1}) and ritanserin (2 mg kg^{-1}) on the decrease in extracellular serotonin level induced by MM5 (30 mg kg^{-1}) and MC1 (40 mg kg^{-1}) in rat prefrontal cortex, expressed as an area under the curve (AUC). WAY 100635 and ritanserin (RIT) were given 30 min before MM5 or MC1. The basal extracellular concentrations of serotonin, in pg/20 μL of the dialysate, were 4.48 ± 0.28 for saline, 4.00 ± 0.35 for WAY 100635 (0.3 mg kg^{-1}), 6.27 ± 0.11 for MM5 (30 mg kg^{-1}), 6.06 ± 0.16 for MC1 (40 mg kg^{-1}), 4.78 ± 0.40 for WAY 100635 (0.3 mg kg^{-1}) + MM5 (30 mg kg^{-1}), 4.24 ± 0.30 for WAY 100635 (0.3 mg kg^{-1}) + MC1 (40 mg kg^{-1}), 3.94 ± 0.15 for RIT (2 mg kg^{-1}), 5.96 ± 0.11 for RIT (2 mg kg^{-1}) + MM5 (30 mg kg^{-1}) and 4.72 ± 0.33 for RIT (2 mg kg^{-1}) + MC1 (40 mg kg^{-1}), respectively. The data, expressed as a percentage of the basal level, are means $\pm$ s.e.m., n = 4/group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with saline; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with MM5 or MC1, respectively (one-way analysis of variance, followed by Tukey's post-hoc test).

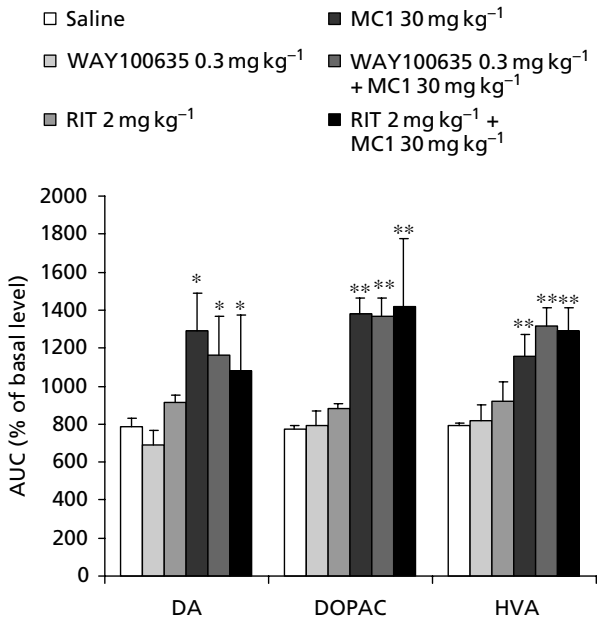

Figure 4 The cumulative effect of WAY 100635 (0.3 mg kg^{-1}) and ritanserin (2 mg kg^{-1}) on the increase in the extracellular levels of dopamine (DA), DOPAC and HVA, induced by MC1 (30 mg kg^{-1}) in rat prefrontal cortex. WAY 100635 and ritanserin (RIT) were given 30 min before MC1. Basal extracellular concentrations of dopamine, DOPAC and HVA, in pg/20 μL of dialysate, were 2.91 ± 0.17, 342 ± 50 and 578 ± 35 for saline, 3.07 ± 0.18, 297 ± 31 and 720 ± 41 for MC1 (30 mg kg^{-1}); 2.65 ± 0.17, 280 ± 15 and 684 ± 20 for WAY 100635 (0.3 mg kg^{-1}), 2.91 ± 0.31, 355 ± 20 and 730 ± 26 for WAY 100635 (0.3 mg kg^{-1}) + MC1 (30 mg kg^{-1}), 2.52 ± 0.31, 325 ± 20 and 635 ± 48 for RIT (2 mg kg^{-1}) and 2.92 ± 0.27, 367 ± 21 and 536 ± 13 for RIT (2 mg kg^{-1}) + MC1 (30 mg kg^{-1}), respectively. The data, expressed as a percentage of the basal level, are means $\pm$ s.e.m., n = 4/group. *$P < 0.05$, **$P < 0.01$ compared with saline (one-way analysis of variance, followed by Tukey's post-hoc test).

on serotonin release was attenuated by the selective 5-HT$_{1A}$ antagonist WAY 100635. However, attenuation of serotonin release by the higher dose of MC1, but not by MM5, was also reversed by the non-selective 5-HT$_2$ antagonist ritanserin. Neither antagonist of 5-HT receptors had any influence on the facilitation of dopamine, DOPAC and HVA release by MC1.

MC1 showed high affinity in-vitro for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors ($K_i = 24$ and 32 nM, respectively). MM5 was slightly less potent at 5-HT$_{1A}$ receptors ($K_i = 65$ nM) and about ten-fold weaker ($K_i = 332$ nM) in its binding to 5-HT$_{2A}$ receptors, compared with MC1. In behavioural experiments, both compounds – like the selective 5-HT$_{1A}$ agonist 8-OH-DPAT – produced hypothermia in mice (Iskra-Jopa et al 2002), regarded as activation of presynaptic 5-HT$_{1A}$ receptors (Martin & Heal 1991). In turn, the induction of lower lip retraction and behavioral syndrome (flat body posture and forepaw trading) in rats, which depends on the stimulation of postsynaptic 5-HT$_{1A}$ receptors (Tricklebank et al 1984; Berendsen et al 1990), indicated mixed agonist–antagonist activity of both those compounds at postsynaptic 5-HT$_{1A}$ receptors (Chilmonczyk et al 2002).

The modulation of serotonin and dopamine release by presynaptic and postsynaptic 5-HT$_{1A}$ receptors has been described in several earlier studies. Serotonin pathways project from the midbrain dorsal and median raphe nuclei to the medial prefrontal cortex (Steinbusch 1981), this region showing a high density of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (Pompeiano et al 1992, 1994; Willins et al 1997; Czyrak et al 2003). Moreover, the co-expression of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in the prefrontal cortex has been suggested to underlie the interaction between the prefrontal cortex and raphe nuclei, as a consequence of which the effect of 5-HT$_{2A}$ receptor activation is reversed by 5-HT$_{1A}$ agonists (Amaragós-Bosch et al 2004). The activation of pre- or postsynaptic 5-HT$_{1A}$ receptors has been reported to reduce serotonin release from cortical neuronal terminals (Casanovas & Artigas 1996; Casanovas et al 1999; Celada et al 2001; Ago et al 2003; Wesolowska et al 2003b). In our study, the decrease in serotonin release by MM5 and the reversal of that effect by the selective 5-HT$_{1A}$ antagonist WAY 100635 testified to the involvement of 5-HT$_{1A}$ receptors. MC1 was less potent in suppressing serotonin release, since only its higher dose was effective but, like MM5, its effect was also reversed by WAY 100635. The above findings indicate that MC1 exerts an inhibitory influence on serotonin neuronal terminals and acts as a 5-HT$_{1A}$ agonist in rat prefrontal cortex. Our in-vitro data show similar MC1 affinity for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors. It is likely that MC1 stimulates both these receptors with similar potency. In contrast to the suppressive effect mediated by 5-HT$_{1A}$ receptors, the activation of 5-HT$_{2A}$ receptors by MC1 may facilitate serotonin release, thus preventing the inhibitory influence mediated by 5-HT$_{1A}$ receptors (Amargós-Bosch et al 2004). Surprisingly, the effect of MC1, but not MM5, on serotonin release is also blocked by the non-selective 5-HT$_2$ antagonist ritanserin. Some evidence suggests that 5-HT$_{2A/2C}$ agonists mimic the effects of serotonin and may facilitate excitatory glutamatergic transmission in the prefrontal cortex (Marek et al 2000); moreover, since they are also located on GABAergic interneurons (Willins et al 1997), they may elevate extracellular gamma-aminobutyric acid (GABA) levels (Abi-Saab et al 1999). Hence, the ritanserin-sensitive inhibitory influence of MC1 on serotonin release is also likely to be indirectly mediated by GABA-ergic transmission.

The prefrontal cortex receives huge dopaminergic innervation from the pathways originating in the VTA (Descarries et al 1987) and is thus subjected to modulation by serotonergic pathways. The influence of serotonin on mesocortical dopaminergic pathways is mediated directly by the postsynaptic 5-HT receptors located on dopaminergic neurons in the VTA (Chen & Reith 1995; Hajós et al 2003) or indirectly via the modulation of activity of GABAergic interneurons (Millan et al 1998). 5-HT$_{1A}$ receptor agonists have been shown to preferentially increase dopamine release in the prefrontal cortex (Wędzony et al 1996; Gobert et al 1998; Lejeune & Millan 1998; Gobert & Millan 1999a; Ago et al 2003; Hajós et al 2003). Dopaminergic transmission is also

modulated through 5-HT_{2A} (Nocjar et al 2002) and 5-HT_{2C} receptors (Pompeiano et al 1994) localized in the VTA. It has been reported that, via 5-HT_{2A} receptors, serotonin enhances dopamine release in the rat, but inhibits it via 5-HT_{2C} receptors (Millan et al 1998; Gobert & Millan 1999b; Pehek et al 2001). In our study, compound MM5 (also when given in doses higher than that shown in Figure 2), despite its activity comparable with that of 8-OH-DPAT in suppressing serotonin release, did not affect dopamine release in rat prefrontal cortex. The reason for the lack of effect on dopamine transmission in the case of MM5 is unclear. However, it may be speculated that the MM5-induced stimulation of 5-HT_{1A} receptors in the region of dopamine cell bodies in the VTA is not sufficient to evoke changes in dopamine neuronal terminals in the prefrontal cortex. Such a mechanism of uncoupling somatodendritic and axonal dopamine release has been shown to occur in nigrostriatal neurons (Cobb & Abercrombie 2003). Hence, the activation of 5-HT_{1A} receptors by MM5 may inhibit dendritic regions in the VTA without affecting axon terminals in the prefrontal cortex. On the other hand, MC1 potently enhanced dopamine, DOPAC and HVA levels in rat prefrontal cortex, producing a weaker effect on serotonin release than did 8-OH-DPAT or MM5. The effect of MC1 on dopamine release is unlikely to depend on 5-HT_{1A} or 5-HT_2 receptor mediation, since it is not blocked by the antagonists of these receptors, WAY 100635 and ritanserin. The facilitating effect of MC1 on extracellular dopamine, DOPAC and HVA levels is difficult to explain on the basis of the presently available results. MC1 does not seem to exert direct or indirect influence on dopamine transmission via 5-HT_{1A}, 5-HT_{2A}, D_1 and D_2 receptors. Moreover, its action varies from that of a selective 5-HT_{1A} agonist, since in our study 8-OH-DPAT failed to affect DOPAC and HVA levels. The effect of MC1 on dopamine release may be mediated by other subtypes of 5-HT receptor (e.g. 5-$HT_{1B/D}$ or 5-HT_3) or by $\beta_{1/2}$- and $\alpha_{1,2}$-adrenergic receptors, which are potentially engaged in the regulation of frontocortical dopamine transmission (Ashby et al 1992; Iyer & Bradberry 1996; Gobert & Millan 1999a; Gobert et al 1999; Millan et al 2000). Furthermore, the involvement of synthesis pathway enzymes or neuronal transport proteins has also to be considered in the action of MC1 on dopamine release. The effect of an active MC1 metabolite should also be taken into account, as is the case with 1-PP, an active metabolite of another piperazine derivative, buspirone (Gobert et al 1999). Therefore, further studies are necessary to fully elucidate the mechanism of MC1 impact on frontocortical dopamine transmission.

Conclusions

Two new structural analogues of 1,2,4-piperazine, with in-vitro affinity for 5-HT_{1A} and 5-HT_{2A} receptors, revealed different pharmacological properties while acting on serotonin and dopamine transmission in rat prefrontal cortex. Like 8-OH-DPAT, compound MM5 suppressed serotonin release but was devoid of dopaminergic activity. In contrast, compound MC1 depressed to a lesser degree serotonin release, but markedly increased the extracellular levels of dopamine, DOPAC and HVA. The latter effect of MC1 does not seem to be mediated by 5-HT_{1A} or 5-HT_{2A} receptors, so further studies are necessary to fully elucidate the mechanism of its action on dopamine transmission in this brain structure.

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