Role of different monoamine receptors controlling MK-801-induced release of serotonin and glutamate in the medial prefrontal cortex: relevance for antipsychotic action

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Abstract

Several studies have demonstrated that systemically administered N-methyl-D-aspartate (NMDA) receptor antagonists increase serotonin (5-HT) and glutamate release in the medial prefrontal cortex (mPFC). Previously we showed that the perfusion of clozapine in the mPFC prevented the MK-801-induced increase in extracellular glutamate and 5-HT whereas haloperidol blocked only the effect of MK-801 on glutamate. To study the contribution of different monoaminergic receptors (for which clozapine and haloperidol exhibit distinct affinities) to these effects, here we used in-vivo microdialysis to examine the role of local blockade of dopamine D2, 5-HT2A and α1-adrenergic receptors as well as agonism at dopamine D1 and 5-HT1A receptors in the mPFC on the increased efflux of glutamate and 5-HT elicited by MK-801. The results show that M100907 (5-HT2A antagonist), BAY x 3702 (5-HT1A agonist) and prazosin (α1-adrenergic antagonist) blocked the MK-801-induced increase of 5-HT and glutamate in the mPFC. However, raclopride, eticlopride (dopamine D2 antagonists) and SKF-38393 (dopamine D1 agonist) were able to prevent the increased efflux of glutamate (but not that of 5-HT) elicited by MK-801. We propose that D2 receptor antagonists and D1 agonists would act predominantly on a subpopulation of GABAergic interneurons of the mPFC, thus leading to an enhanced cortical inhibition that would prevent an excessive glutamatergic transmission. On the other hand, atypical antipsychotic drugs might further act upon 5-HT2A, 5-HT1A and α1-adrenoceptors present in pyramidal cells (including those projecting to the dorsal raphe nucleus), which would directly inhibit an excessive excitability of these cells.

Key words: MK-801, dopamine D1/D2 receptors, 5-HT2A receptor, 5-HT1A receptor, α1-adrenoceptor.

Introduction

Non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) and ketamine have been used as a pharmacological model of schizophrenia because they can mimic psychotic and negative symptoms as well as cognitive impairment in healthy individuals (Javitt and Zukin, 1991; Krystal et al., 1994). In rats, NMDA antagonists have also been shown to increase extracellular glutamate (Calcagno et al., 2006; Ceglia et al., 2004; López-Gil et al., 2007; Lorrain et al., 2003; Moghaddam et al., 1997), dopamine (Adams and Moghaddam, 1998; Schmidt and Fadayel, 1996), and serotonin (5-HT) (Adams and Moghaddam, 2001; Amargo’s-Bosch et al., 2006; Calcagno et al., 2006; Ceglia et al., 2004; López-Gil et al., 2007; Millan et al., 1999) in the medial prefrontal cortex (mPFC). It has been proposed that non-competitive NMDA receptor antagonists might impair GABAergic inhibition of glutamatergic neurons in the mPFC (Homayoun and Moghaddam, 2007b; Krystal et al., 2003; Moghaddam et al., 1997), leading to downstream changes in other transmitters. Indeed, GABAergic interneurons in limbic cortex and...
hippocampus are more sensitive to the action of NMDA receptor antagonists than pyramidal neurons (Grunze et al., 1996).

Numerous studies have demonstrated that dopamine, 5-HT and glutamate play an important role in schizophrenia and that receptors for these transmitters are involved in the action of antipsychotic drugs. It is generally accepted that all available antipsychotic drugs possess some degree of dopamine D_2 receptor antagonism and that blockade of limbic D_2 receptors improves positive symptoms (Kapur et al., 2000). However, this has been challenged recently by the finding that drugs that attenuate glutamate release without acting directly on dopamine receptors are beneficial for positive and negative symptoms (Patil et al., 2007). With regard to clozapine, the prototypical atypical antipsychotic drug, it has been suggested that its superior efficacy and tolerability is partly due to its lower occupancy of D_2 receptors (Kapur et al., 1999) together with an additional interaction with 5-HT_A receptors (Lundberg et al., 1996). Therefore, it has been proposed that a weak D_2 receptor blockade coupled to a relatively stronger 5-HT_A inhibition is the key feature of atypical antipsychotic drugs (see Meltzer, 2004 for review), responsible for causing fewer extrapyramidal side-effects (EPS). On the other hand, the relationship found between cognitive/negative symptoms in schizophrenia and a reduction of prefrontal dopamine D_2 receptor binding (Okubo et al., 1997) prompted some investigators to suggest that dopamine D_2 receptor agonists might be helpful in alleviating negative symptoms and cognitive deficits of schizophrenia (see Goldman-Rakic et al., 2004 for review). There is also evidence underpinning the importance of 5-HT_A receptors in certain aspects of the pharmacotherapy of schizophrenia (Millan, 2000; Sumiyoshi et al., 2001). Furthermore, 5-HT_A receptor agonists reduce the incidence of EPS in schizophrenia patients treated with haloperidol (Goff et al., 1991; Moss et al., 1993). Finally, many clinically effective antipsychotic drugs (both classical and atypical) exhibit a1-adrenergic receptor antagonism (Arnt and Skarsfeldt, 1998; Bymaster et al., 1996), which has been postulated to be involved in their clinical action (Svensson, 2003). However, although central a1-adrenoceptors may regulate sensorimotor gating altered in schizophrenia (Alsene et al., 2006), there are no studies dealing with the occupancy of a1-adrenoceptors in individuals under antipsychotic treatment.

In the preclinical setting, it has been shown that the local administration of antipsychotic drugs decrease extracellular 5-HT in the mPFC (Amargós-Bosch et al., 2003, 2007). Activation of 5-HT_A receptors and blockade of 5-HT_A receptors might contribute to this effect since both types of compounds were able to prevent the increases in 5-HT and glutamate, as well as cognitive deficits induced by NMDA receptor antagonists (Calcagno et al., 2006; Carli et al., 2006; Ceglia et al., 2004). Furthermore, clozapine and haloperidol attenuated PCP-induced increase in cortical glutamate (Abekawa et al., 2006) as well as the elevated firing of a population of mPFC pyramidal neurons elicited by PCP or MK-801 (Homayoun and Moghaddam, 2007a; Kargieman et al., 2007). Interestingly, clozapine exhibited a levelling effect on the firing of pyramidal cells in the mPFC (Homayoun and Moghaddam, 2007a). This fine-tuning effect might contribute to the unique therapeutic efficacy of clozapine in schizophrenia.

In a previous study we showed that the perfusion of clozapine in the mPFC prevented the MK-801-induced increase in extracellular glutamate and 5-HT whereas haloperidol blocked only the effect of MK-801 on glutamate (López-Gil et al., 2007). To study the contribution of different monoaminergic receptors (for which clozapine and haloperidol exhibit distinct affinities) to these effects, in the present study we examined the role of blockade of dopamine D_2, 5-HT_A and a1-adrenergic receptors as well as agonism at dopamine D_1 and 5-HT_A receptors in the mPFC on the increased efflux of glutamate and 5-HT elicited by MK-801.

**Materials and methods**

**Animals**

Male Wistar rats (Charles River Laboratories, Cerdanyola del Valles, Spain) weighing 250–280 g were used. They were maintained on a 12 h light/dark cycle (lights on 07:00) and housed three per cage before surgery and individually after surgery. Food and water were available ad libitum. All experimental procedures followed European Union regulations (Official Journal of the European Communities L358/1, 18 December 1986), and were approved by the Institutional Animal Care and Use Committees. To reduce the influence of between-day variations on drug effects, most experimental groups were not completed at once, but throughout the duration of the whole work instead.

**Drugs and reagents**

All the HPLC reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). Dizocilpine maleate (MK-801), 5-hydroxytryptamine
oxalate, glutamate, and ready-made o-phthalaldialdehyde (OPA) reagent, made up of 1 mg OPA per ml solution with 2-mercaptoethanol as the sulphhydril moiety, were purchased from Sigma-Aldrich (Tres Cantos, Spain). Raclopride, eticlopride hydrochloride, prazosin hydrochloride, and SKF-38393 were from Tocris (Bristol, UK). Citalopram hydrobromide, BAY x 3707, and M100907 (formerly MDL-100907) were generously donated by H. Lundbeck A/S (Copenhagen-Valby, Denmark), Bayer AG (Wuppertal, Germany) and Pierre-Fabre (Castres, France), respectively.

MK-801 was dissolved in saline for intraperitoneal (i.p.) administration. The dose of MK-801 was taken from our previous study (López-Gil et al., 2007). It is possible that lower doses of MK-801 are sufficient to induce stereotypes and increases in pyramidal cell firing (Jackson et al., 2004), but they failed to induced changes in extracellular glutamate (López-Gil et al., 2007). All other drugs were dissolved in the perfusion fluid for local application through dialysis probes. Concentrated solutions (1 mM) were stored at −80 °C and working solutions were prepared immediately before the experiments by dilution in the perfusion fluid. Concentrations are expressed as free bases. Because of the difficulty in obtaining the real extracellular concentration of the drugs perfused through the dialysis fluid, we tested the least effective concentrations (i.e. within the low micromolar range) in order to gain selectivity for the specific receptors studied.

**Microdialysis procedures**

Concentric microdialysis probes were constructed with a 4-mm-long membrane. Following anaesthesia with sodium pentobarbital (60 mg/kg i.p.), rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA), and dialysis probes were implanted in the mPFC and secured to the skull with anchor screws and dental cement. Stereotaxic coordinates from Bregma and skull surface were: AP +3.2 mm, L −0.8 mm, DV −6.0 mm; according to Paxinos and Watson (1986). Microdialysis experiments were conducted 20–24 h after surgery in freely moving rats by continuously perfusing probes with a fluid containing 125 mM NaCl, 2.5 mM KCl, 1.26 mM CaCl₂, 1.18 mM MgCl₂, and 1 μM citalopram. The addition of a 5-HT uptake blocker to the perfusion fluid is used in some microdialysis experiments to reduce clearance from the extracellular space, thus enhancing the release component of extracellular 5-HT (Adell et al., 2002). On the other hand, low concentrations of 5-HT uptake blockers, such as used in the present study, are without effect on dialysate glutamate in vivo (Langman et al., 2006; C. M. T. Queiroz and F. Artigas, unpublished results). Furthermore, voltammetric studies have shown that MK-801 may interact with the serotonin transporter (Callado et al., 2000; Iravani et al., 1999), thereby underscoring the importance of including citalopram in the perfusion fluid.

The artificial cerebrospinal fluid was perfused at 1.5 μl/min with a Harvard model 22 syringe pump (Harvard Apparatus, South Natick, MA, USA) attached to an overhead liquid swivel (Instech, Plymouth Meeting, PA, USA). Dialysate samples of 30 μl were collected every 20 min and divided into two fractions for the determination of 5-HT (20 μl) and glutamate (10 μl). The in-vitro dialysis probe recoveries for 5-HT and glutamate were 16% and 17%, respectively. Owing to the incidental occurrence of chromatographic problems, for some rats data on only one transmitter (glutamate or 5-HT) were available. After an initial 1 h sample of dialysate was discarded, four samples were collected to establish stable baseline levels of 5-HT and glutamate (expressed as concentration of transmitter in a 30-μl sample) before any pharmacological treatment. At the completion of dialysis experiments, rats were given an overdose of sodium pentobarbital and a Fast Green solution was perfused through the dialysis probes to stain the surrounding tissue for subsequent histological examination.

**Biochemical determinations**

The concentration of 5-HT in dialysate samples was determined by an HPLC system consisting of a Waters 717plus autosampler (Waters Cromatografia, Cerdanyola, Spain), a Hewlett-Packard series 1050 pump (Agilent Technologies, Las Rozas, Spain), a 3 μ octadeckylsilica (ODS) column (7.5 cm × 0.46 cm; Beckman, San Ramon, CA, USA), and an amperometric detector Hewlett-Packard 1049 (Agilent Technologies) set at an oxidation potential of 0.6 V. The mobile phase consisted of 0.15 M NaH₂PO₄, 1.8 mM octyl sodium sulfate, 0.2 mM EDTA (pH 2.8, adjusted with phosphoric acid), and 30% methanol, and was pumped at 0.7 ml/min (Lopez-Gil et al., 2007). For the determination of glutamate, another HPLC system was used, which consisted of a Waters 717plus autosampler, a Waters 600 quaternary gradient pump, and a Nucleosil 5 μ ODS column (10 cm × 0.4 cm; Teknokroma, Spain). Dialysate samples were precolumn-derivatized with OPA reagent and the entire process was carried out by the autosampler. Briefly, 90 μl distilled water was added to the 10 μl dialysate sample
and this was followed by the addition of 15 μl OPA reagent. After 2.5 min reaction, 80 μl of this mixture was injected into the column. Detection was carried out with a Waters 470 scanning fluorescence detector using excitation and emission wavelengths of 360 nm and 450 nm, respectively. The mobile phase was pumped at 0.8 ml/min and consisted of two components (Calcagno et al., 2006): solution A, made up of 0.05 m Na₂HPO₄, 28% methanol, adjusted to pH 6.4 with 85% H₃PO₄, and solution B, made up of 100% methanol/H₂O (8:2 ratio). After the elution of glutamate peak at 3 min with 100% solution A, a gradient was established going from 100% solution A to 100% solution B in 2 min. After washing out late-eluting peaks (3 min), mobile phase returned to initial conditions (100% solution A) in 2 min. The detection limits for 5-HT and glutamate were 4 fmol and 0.2 pmol, respectively (signal-to-noise ratio 3). Quantification of 5-HT and glutamate was carried out by comparison to a daily standard curve comprising the concentrations of transmitters expected in dialysate samples.

Statistics

Data (mean ± S.E.M.) are expressed as fmol/30 μl for 5-HT and pmol/30 μl for glutamate, and shown in the figures as percentages of basal values, averaged from four fractions collected before treatment. The changes in dialysate 5-HT and glutamate for each drug were analysed by two-way repeated-measures analysis of variance (ANOVA) with time and treatment as factors. When significant effects were found, post-hoc Newman–Keuls (NK) multiple comparison tests were used to compare effects of different treatment groups. The level of significance was set at p < 0.05.

Results

The basal (pre-drug) concentrations of 5-HT and glutamate in dialysate samples of the mPFC, not corrected for in-vitro recovery, were 49.8 ± 1.2 pmol/30 μl (n = 168) and 28.3 ± 3.7 pmol/30 μl (n = 168), respectively. Although the basal concentration of glutamate is higher than that in our previous study (López-Gil et al., 2007), it falls within the range reported by other authors (Adams and Moghaddam, 2001; Lorrain et al., 2003; Moghaddam et al., 1997). The systemic administration of MK-801 to rats increased the extracellular concentration of 5-HT in comparison to the saline-injected group, as demonstrated by the significant effect of treatment (F₁,₁₆₈ = 33.91, p < 0.0001), time (F₁₅,₁₆₈ = 7.99, p < 0.00001), and treatment × time interaction (F₁₅,₁₆₈ = 8.65, p < 0.00001). MK-801 also elevated extracellular glutamate in the mPFC as shown by the significant effect of treatment (F₁,₁₃ = 31.12, p < 0.0001), time (F₁₅,₁₃₈ = 7.18, p < 0.00001), and treatment × time interaction (F₁₅,₁₃₈ = 7.16, p < 0.00001).

Effects of dopamine D₂ antagonists

Two different D₂-like antagonists were used, raclopride and eticlopride, which possesses 10-fold greater affinity than raclopride for dopamine D₂/D₃ receptors (Assié et al., 2006). Raclopride, which by itself had no effect on 5-HT and glutamate in saline-injected animals, was unable to block the MK-801-induced increase in dialysate 5-HT (Figure 1a). However, it prevented the effect of MK-801 on glutamate (F₁,₂₁ = 12.08, p < 0.0001; Figure 1b) in a concentration-dependent manner. Post-hoc comparisons showed...
that only the concentration of 30 μM raclopride significantly abolished the increase in cortical glutamate elicited by MK-801 (p < 0.002, NK test). At 100 μM, raclopride was also unable to block the MK-801-induced increase in cortical 5-HT (data not shown).

In a similar way, eticlopride did not alter basal levels of 5-HT and glutamate when perfused in saline-injected animals and failed to reduce the effects of MK-801 on cortical 5-HT (Figure 2a). It blocked only the effects of MK-801 on glutamate (F_{15.15} = 5.22, p < 0.00001; Figure 2b). Both concentrations of eticlopride were equally effective in this respect (p < 0.0005, NK test).

Effects of 5-HT_{2A} antagonists and 5-HT_{1A} agonists

In contrast to dopamine D₂ antagonists, the selective 5-HT_{2A} antagonist, M100907, prevented the increase in 5-HT (F_{3,20} = 15.08, p < 0.0001; Figure 3a) and glutamate (F_{3,20} = 15.72, p < 0.00002; Figure 3b) elicited by MK-801. Post-hoc comparisons showed that both concentrations of M100907 had similar effects on 5-HT (p < 0.02, NK test) and glutamate (p < 0.0005; Newman-Keuls test). When administered to saline-injected animals, M100907 did not change the basal extracellular concentration of 5-HT, but a significant increase in cortical glutamate was observed (F_{2,14} = 10.67, p < 0.002) although this was only statistically significant after the perfusion of 10 μM M100907 (p < 0.003, NK test).

The selective 5-HT_{1A} agonist, BAY x 3702, also abolished the increase in 5-HT (F_{4,14} = 12.92, p < 0.0001; Figure 4a) and glutamate (F_{4,18} = 20.30, p < 0.00001; Figure 4b) elicited by MK-801. Post-hoc comparisons showed that both concentrations of BAY x 3702 had
comparable effects on 5-HT ($p < 0.002$, NK test) and glutamate ($p < 0.0005$, NK test). Although it seemed that the concentration of 1 µM BAY x 3702 was more effective than that of 30 µM in blocking the MK-801-induced increase of 5-HT (Figure 4a), this difference was not statistically significant. When applied alone in saline-injected animals, BAY x 3702 failed to change the concentration of glutamate, but reduced that of 5-HT ($F_{2,19} = 8.24$, $p < 0.005$; Figure 4a). Post-hoc comparisons showed that both concentrations of BAY x 3702 were equally effective ($p < 0.02$, NK test).

Effects of $\alpha_1$-adrenergic antagonists

Similar to 5-HT$_{1A}$ antagonists and 5-HT$_{1A}$ agonists, the intra-mPFC perfusion of the selective $\alpha_1$-adrenergic antagonist, prazosin, blocked the increases in 5-HT ($F_{3,19} = 10.38$, $p < 0.003$; Figure 5a) and glutamate ($F_{3,28} = 11.44$, $p < 0.001$; Figure 5b) evoked by MK-801, in a concentration-dependent manner. Post-hoc comparisons showed that only 10 µM prazosin was required to prevent the MK-801-induced increases in 5-HT ($p < 0.02$, NK test) and glutamate ($p < 0.005$, NK test). When applied alone in saline-injected animals, prazosin did not alter the concentration of glutamate, but reduced that of 5-HT ($F_{2,18} = 5.69$, $p < 0.02$; Figure 5a). Post-hoc comparisons showed that only the concentration of 10 µM prazosin reduced basal 5-HT levels in the mPFC ($p < 0.02$, NK test).

Effects of dopamine D$_1$ agonists

SKF-38393 has similar affinity for dopamine D$_1$ and D$_3$ receptors. Like dopamine D$_2$ antagonists, the dopamine D$_1$ agonist, SKF-38393, which by itself had
no effect on 5-HT and glutamate in saline-injected animals, was unable to block the MK-801-induced increase in 5-HT, whereas haloperidol blocked only the effects on glutamate (López-Gil et al., 2007). In view of those results we proposed that serotonergic transmission in the mPFC is regulated by multiple monoamine receptors (for which clozapine exhibits moderate to high affinity), whereas glutamatergic transmission is regulated predominantly by dopamine D₂ receptor blockade (a key feature of antipsychotic action that prevails in typical antipsychotic drugs such as haloperidol). The present work was therefore aimed at testing the veracity of this proposition, using selective agonists and antagonists for monoamine receptors.

**Discussion**

In a previous study, we showed that the perfusion of clozapine in the mPFC prevented the MK-801-induced increase in glutamate and 5-HT, whereas haloperidol blocked only the effects on glutamate (López-Gil et al., 2007). In view of those results we proposed that serotonergic transmission in the mPFC is regulated by multiple monoamine receptors (for which clozapine exhibits moderate to high affinity), whereas glutamatergic transmission is regulated predominantly by dopamine D₂ receptor blockade (a key feature of antipsychotic action that prevails in typical antipsychotic drugs such as haloperidol). The present work was therefore aimed at testing the veracity of this proposition, using selective agonists and antagonists for monoamine receptors.

**Effects of dopaminergic drugs**

In very good accordance with our hypothesis, two different dopamine D₂ receptor antagonists, raclopride and eticlopride, replicate the results of haloperidol, i.e. both drugs prevented the MK-801-induced increase of glutamate, but not that of 5-HT. Because only ~5% of projection neurons in layer V of the mPFC project to the dorsal raphe nucleus (Gabbott et al., 2005), it is conceivable that, under conditions of increased 5-HT and glutamate transmission in the mPFC following MK-801 administration, blockade of D₂ receptors by raclopride and eticlopride might be able to inhibit cortical output (blockade of increased glutamate efflux), although sparing cortico-raphe projections.

Tyrosine hydroxylase-positive terminals are in apposition with GABA interneurons (Benes et al., 2000; Sesack et al., 1998), and dopamine D₂ receptors are abundant in GABA interneurons of deep cortical layers of the rat (Khan et al., 1998; Le Moine and Gaspar, 1998; Vincent et al., 1995). Furthermore, dopamine exerts a tonic inhibitory effect on fast-spiking GABA cells that target the perisomatic domain of pyramidal cells through D₂ receptors (Gao et al., 2003; Seamans et al., 2001). Therefore, an excessive release of dopamine following MK-801 administration (Schmidt and Fadayel, 1996) might lead to a further reduction in GABAergic inhibition, which would result in an impairment of the intrinsic cortical circuitry. In fact, this has been postulated to occur in the schizophrenia brain (Beasley et al., 2002; Benes, 1997; Egan and Weinberger, 1997). Dopamine D₂ antagonists would relieve the inhibitory action of dopamine overflow on GABA-containing neurons, thus promoting GABA release and reducing cortical glutamatergic output induced by MK-801. An important question that can be raised here is whether dopamine D₂ antagonists would act on the same population of GABAergic neurons impaired by MK-801 and/or on another...
subset of interneurons. Further research is needed to answer this question.

The regulation of prefrontal function by dopamine involves not only D₂ but also D₁ receptors. Our results show that dopamine D₁ agonism elicits comparable effects to those of dopamine D₂ antagonists. It is difficult to reconcile the effects of SKF-38393 with an action on D₁ receptors located on pyramidal neurons (Bergson et al., 1995; Davidoff and Benes, 1998) because this receptor is excitatory. Since mPFC D₁ receptors are also present on inhibitory GABA interneurons (Davidoff and Benes, 1998; Le Moine and Gaspar, 1998), one possibility for the effect of SKF-38393 is activation of GABAergic inhibition, which would block the increase in glutamate efflux induced by MK-801. The lack of effects of raclopride, eticlopride and SKF-38393 on mPFC 5-HT suggests that these two dopamine receptors could regulate distinct inhibitory circuits, except those that control the prefrontal projection to the raphe nuclei, at least in the conditions of the present study.

Altogether our results suggest that dopamine can regulate the efflux of glutamate by means of an action on GABAergic interneurons in the mPFC. Because of the different nature of dopamine D₁ (excitatory) vs. D₂ (inhibitory) receptors, D₂ antagonists and D₁ agonists might end up with the same final response, i.e. to restore cortical GABA efflux in order to block an excessive glutamatergic transmission following MK-801 administration. Furthermore, our results are also coincident with the proposal that D₁ receptor activation requires phasic dopamine release whereas D₂ receptors are continuously driven by basal, tonic dopamine release (Grace, 1991).

Effects of serotonergic and adrenergic drugs

In contrast to dopaminergic compounds, the selective 5-HT₁₄ receptor agonist, M100907, was able to prevent the increase of 5-HT and glutamate evoked by MK-801, which is in line with previous work using a competitive NMDA antagonist (Ceglia et al., 2004). This effect can be accounted for by a reduction of the increased excitability of prefrontal pyramidal neurons (including those that project to the dorsal raphe nucleus) produced by an exacerbated glutamatergic transmission. In good accord with the present work, previous studies from our laboratory have demonstrated that M100907 also blocked the increased serotonergic transmission caused by the intra-mPFC perfusion of S-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate (S-AMPA), 2,5-dimethoxy-4-iodoamphetamine (DOI) and the α₁-adrenoceptor antagonist cirazoline (Amargós-Bosch et al., 2003, 2004), as well as thalamic disinhibition (Amargós-Bosch et al., 2007). 5-HT₁₄ receptors are predominantly localized to apical dendrites of pyramidal neurons (Cornea-Hébert et al., 1999; Willins et al., 1997), a cellular zone that receives inputs from different cortical layers, allowing cross-layer integration. Consequently, 5-HT₁₄ antagonists are in a unique position to cancel the increased cortico-cortical transmission that probably occurs after MK-801 administration and the pharmacological conditions mentioned above. Although there is evidence that 5-HT₁₄ receptors are also present in cortical GABAergic interneurons of the rat mPFC (Santana et al., 2004; Willins et al., 1997), our results points to a predominant effect of M100907 on those receptors located in pyramidal neurons following MK-801 administration.

The local perfusion of BAY x 3702 in the mPFC also prevented the increased efflux of 5-HT and glutamate following MK-801 administration with a potency similar to that of M100907. Similarly, the 5-HT₁₄ receptor agonist, 8-OH-DPAT, prevents the increase in extracellular 5-HT and glutamate evoked by a competitive NMDA antagonist (Calcagno et al., 2006). The reduction of 5-HT is probably accounted for by a decrease in the activity of pyramidal cells (Araneda and Andrade, 1991; Ashby et al., 1994), which would result in a reduction of a tonic excitatory input on the dorsal raphe nucleus, thereby decreasing the activity of 5-HT cells (Celada et al., 2001). Similarly to the action of 5-HT₁₄ receptor antagonists (see above), previous work has also shown that 5-HT₁₄ agonists reverse the increased cortical 5-HT efflux induced by intra-mPFC perfusion of S-AMPA, DOI and cirazoline (Amargós-Bosch et al., 2003, 2004), as well as thalamic disinhibition (Amargós-Bosch et al., 2007). In the present study we show that BAY x 3702 also blocks the effects of MK-801 on glutamate. This suggests that 5-HT₁₄ receptor activation in the mPFC potently attenuates the action of agents that increase the activity of pyramidal neurons, an effect shared by several different treatments and involving the stimulation of AMPA/kainate receptors in the mPFC (Katayama et al., 2007; López-Gil et al., 2007). Furthermore, the pivotal localization of 5-HT₁₄ receptors in the perisomatic region of cortical pyramidal neurons of the rat (Czyrak et al., 2003) might be the basis of the powerful effect of 5-HT₁₄ receptor agonism. In addition, the high level of expression of 5-HT₁₄ and 5-HT₁₃ receptors in pyramidal cells labelled by vGluT1 (Santana et al., 2004) together with the high co-localization of both receptors in the mPFC (~80%; Amargós-Bosch et al., 2004) provides further support for the neurochemical
changes described. Although 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors are also expressed in cortical GABAergic interneurons of the rat (Aznar et al., 2003; Santana et al., 2004; Willins et al., 1997), they do not seem to play a role in the control of cortical 5-HT and glutamate in the conditions of the present work.

The local perfusion of the $\alpha_1$-adrenergic antagonist, prazosin, in the mPFC was also able to prevent the MK-801-induced increase in 5-HT and glutamate. The effect of prazosin probably involves the blockade of $\alpha_1$-adrenoceptors located on pyramidal neurons, including those in layer V that project to the dorsal raphe nucleus, as previously observed for 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors. The mechanism of action of prazosin is probably similar to that of M100907 inasmuch as both 5-HT$_{2A}$ and $\alpha_1$-adrenoceptors share the same signal transduction mechanism and mediate the excitatory actions of 5-HT and noradrenaline, respectively, on pyramidal neurons of the mPFC (Araneda and Andrade, 1991; Marek and Aghajanian, 1999). Moreover, they are localized to the same cortical areas (Day et al., 1997; Domyancic and Morilak, 1997; Pieribone et al., 1994). In line with the present work, previous studies in our laboratory have demonstrated that intra-mPFC perfusion of prazosin reversed the increase of 5-HT elicited by the cortical application of cirazoline, DOI and S-AMPA (Amargós-Bosch et al., 2003), as well as thalamic disinhibition (Amargós-Bosch et al., 2007).

Altogether these effects need to be interpreted at the cellular (pyramidal) and not at receptor level because all these compounds do not interact directly with each other’s receptors. It is therefore suggested that antagonism at $\alpha_1$-adrenergic and 5-HT$_{2A}$ receptors, as well as agonism at 5-HT$_{1A}$ receptors are able to prevent an excessive glutamatergic transmission in the mPFC produced by different treatments and conditions.

**Implications for antipsychotic action**

The findings of the present and previous (López-Gil et al., 2007) work from our laboratory suggest that excessive glutamate transmission in the mPFC, secondary to a NMDA receptor blockade, may be associated with some positive symptoms of schizophrenia, particularly those that are better responsive to dopamine D$_2$ receptor antagonism (a feature shared by most antipsychotic drugs). In contrast, an impairment of serotonergic pathways in the mPFC might rather be related to negative symptoms and/or cognitive deficits, conditions for which clozapine and some other atypical antipsychotics posses superior efficacy. It remains to be established, however, whether this antiserotonergic effect may confer the atypical profile of such drugs. There is a general consensus that dopamine D$_2$ blockers (typical neuroleptics) are less effective in treating negative/cognitive symptoms, which, are better palliated by drugs with a strong antagonism at 5-HT$_{2A}$ receptors and a weak blockade of dopamine D$_2$ receptors (see Meltzer, 2004 for review). Thus, there is a clear need for targeting different transmitter receptors to achieve an effective treatment for schizophrenia. Our results indicate that 5-HT$_{2A}$, 5-HT$_{1A}$, dopamine D$_2$ and $\alpha_1$-adrenergic receptors may play an important role. Drugs showing 5-HT$_{2A}$ antagonism and/or 5-HT$_{1A}$ agonism, such as clozapine, increase dopamine efflux in the mPFC of rodents (Assié et al., 2005; Ichikawa et al., 2001; Pehek and Yamamoto, 1994; Rollema et al., 1997), an effect that appears to be dependent on the presence of intact 5-HT$_{1A}$ receptors (Bortolozzi et al., 2007; Díaz-Mataix et al., 2005). This selectivity on cortical dopamine transmission together with a lesser occupancy of limbic dopamine D$_2$ receptors (Kapur et al., 1999) appears to confer a better side-effect profile of antipsychotics. However, selective 5-HT$_{1A}$ receptor agonists do not appear to provide a distinct antipsychotic-like action, and their possible benefit in the clinic may rather be related to a lower appearance of EPS (Millan, 2000).

The intra-mPFC administration of M100907, BAY × 3702 and prazosin prevents MK-801-induced increase in 5-HT and glutamate, effects that are comparable to those obtained with clozapine (López-Gil et al., 2007). It is possible that this may relate to a better treatment of most symptoms of schizophrenia. In fact, preclinical studies have shown that antagonism at 5-HT$_{2A}$ receptors and agonism at 5-HT$_{1A}$ receptors can ameliorate cognitive deficits induced by NMDA receptor antagonists (Carli et al., 2006). Because each of these receptor components do not confer antipsychotic properties individually, it is conceivable that a combined effect is necessary to achieve this goal.

Dopamine D$_3$ receptors are associated with sustained activity of mPFC neurons and have been postulated to be essential for working-memory performance (see Goldman-Rakic et al., 2004 for review). Clozapine may enhance dopamine D$_3$ receptor-mediated neurotransmission (Ahlenius, 1999; Oerther and Ahlenius, 2000). Therefore, the pro-cognitive action of clozapine could also result from its action on dopamine D$_3$ receptors or, alternatively, through potentiation of NMDA transmission (see Millan, 2005 for review). If psychosis is modelled in animals by an increased glutamatergic transmission in the mPFC...
(Adams and Moghaddam, 2001; López-Gil et al., 2007; Lorrain et al., 2003; Moghaddam et al., 1997), the blockade of the effect of MK-801 by SKF-38393 (present study) may argue in favour of the use of dopamine D1 agonists in the treatment of the illness.

In summary, our results suggest that the combination of blockade of dopamine D2, 5-HT1A and $\alpha_1$-adrenergic receptors as well as agonism at dopamine D4 and 5-HT1A receptors in the mPFC may be beneficial in antipsychotic-like action. It appears that dopamine D4 antagonists and dopamine D1 agonists would activate cortical GABA inhibition. The lack of effect of dopaminergic compounds on the efflux of 5-HT implies that not all subtypes of GABAergic interneurons are regulated in a similar way by dopamine (Seamans and Yang, 2004). Atypical antipsychotic drugs might further act upon 5-HT1A and $\alpha_1$-adrenoceptors present in pyramidal cells (including those projecting to the dorsal raphe nucleus), which would inhibit directly an excessive excitability of these cells. This would contribute to the fine-tuning of discrete cortical circuits impaired in schizophrenia. As we have suggested previously, it is possible that an excessive glutamate transmission in the mPFC may be related with positive symptoms of schizophrenia that are generally alleviated by an antagonism at D2 receptors whereas an impaired serotonergic transmission might rather be associated to negative symptoms and/or cognitive impairment.

The conclusions raised in the present study are based on the analogies between the action of antipsychotic drugs and that of selective agonists and antagonists for monoaminergic receptors. However, it remains to be determined if there is any possible effect resulting from a combination of multiple interactions among these receptors.

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Statement of Interest

None.

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