Adjunctive $\alpha_2$-adrenoceptor blockade enhances the antipsychotic-like effect of risperidone and facilitates cortical dopaminergic and glutamatergic, NMDA receptor-mediated transmission

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Abstract

Compared to both first- and second-generation antipsychotic drugs (APDs), clozapine shows superior efficacy in treatment-resistant schizophrenia. In contrast to most APDs clozapine possesses high affinity for $\alpha_2$-adrenoceptors, and clinical and preclinical studies provide evidence that the $\alpha_2$-adrenoceptor antagonist idazoxan enhances the antipsychotic efficacy of typical $\mathrm{D}_2$ receptor antagonists as well as olanzapine. Risperidone has lower affinity for $\alpha_2$-adrenoceptors than clozapine but higher than most other APDs. Here we examined, in rats, the effects of adding idazoxan to risperidone on antipsychotic effect using the conditioned avoidance response (CAR) test, extrapyramidal side-effect (EPS) liability using the catalepsy test, brain dopamine efflux using $in$-$vivo$ microdialysis in freely moving animals, cortical $N$-methyl-$D$-aspartate (NMDA) receptor-mediated transmission using intracellular electrophysiological recording $in$-$vivo$, and $ex$-$vivo$ autoradiography to assess the $in$-$vivo$ $\alpha_{2A}$- and $\alpha_{2C}$-adrenoceptor occupancies by risperidone. The dose of risperidone needed for antipsychotic effect in the CAR test was $\sim 0.4 \, \text{mg/kg}$, which produced 11% and 17% $in$-$vivo$ receptor occupancy at $\alpha_{2A}$- and $\alpha_{2C}$-adrenoceptors, respectively. Addition of idazoxan (1.5 mg/kg) to a low dose of risperidone (0.25 mg/kg) enhanced the suppression of CAR, but did not enhance catalepsy. Both cortical dopamine release and NMDA receptor-mediated responses were enhanced. These data propose that the therapeutic effect of risperidone in schizophrenia can be enhanced and its EPS liability reduced by adjunctive treatment with an $\alpha_2$-adrenoceptor antagonist, and generally support the notion that the potent $\alpha_2$-adrenoceptor antagonistic action of clozapine may be highly important for its unique efficacy in schizophrenia.

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Introduction

Clinical studies have shown that clozapine exerts superior clinical efficacy compared to first-generation antipsychotic drugs (FGAs) in treatment-resistant schizophrenia, including positive, negative and cognitive symptoms (Kane et al. 1988; Rosenheck et al. 1997), and also, in comparison with second-generation antipsychotic drugs (SGAs) clozapine is the most effective drug for individuals with a poor symptom response to previous antipsychotic drug (APD) treatment (Davies et al. 2008; Lewis et al. 2006; McEvoy et al. 2006; Swartz et al. 2008). In addition, clozapine has shown superiority compared to FGAs and the SGA olanzapine in reducing suicidality in schizoaffective disorders and schizophrenia (Meltzer et al. 2003). Indeed, the efficacy of clozapine seems to be superior to both FGAs and most, if not all SGAs (Davis et al. 2003; Leucht, 2009). Besides its modest affinity for the $\mathrm{D}_2$ receptor, clozapine possesses high affinity for a...
number of other receptors, including a potent \(\alpha_2\)-adrenoceptor antagonistic action (Ashby & Wang, 1996; Shahid et al. 2009). Interestingly, adjunctive treatment with idazoxan, a selective \(\alpha_2\)-adrenoceptor antagonist, has been found to enhance the effect of FGAs in treatment-resistant schizophrenia (Litman et al. 1993, 1996).

Both negative symptoms and cognitive dysfunction in schizophrenia are thought to be related to a hypo-dopaminergic state in the prefrontal cortex (PFC; e.g. Stone et al. 2007). Prefrontal dopamine, specifically via dopamine \(D_1\) receptors, has been shown to be importantly involved with normal cognitive function (Goldman-Rakic et al. 2000). In schizophrenia, both clinical and preclinical results indicate an impairment of prefrontal dopamine function (see Tan et al. 2007; Weinberger et al. 2001), and \(D_1\) receptor dysregulation is suggested to be critically involved in the cognitive impairments associated with this disease (Abi-Dargham & Moore, 2003; Abi-Dargham et al. 2002). Experimentally, SGAs, e.g. clozapine, increase dopamine output preferentially in the PFC, whereas FGAs, e.g. haloperidol, induce higher dopamine output in subcortical areas such as the nucleus accumbens (NAC) and the striatum (Kuroki et al. 1999; Moghaddam & Bunney, 1990; Nomikos et al. 1994; Svensson, 2003). In accordance with these experimental observations some clinical studies suggest that SGAs, but not FGAs, may improve various aspects of cognitive dysfunction in schizophrenia (Davis et al. 2003; Meltzer & McGurk, 1999).

Glutamate, the main transmitter in cortical pyramidal cells, is involved in higher mental functions, such as cognition, memory and learning. Thus, antagonists at the glutamatergic \(N\)-methyl-\(D\)-aspartate (NMDA) receptors impair learning and memory, while NMDA receptor agonists and facilitators may improve memory (Francis, 2003; Francis et al. 1993). In addition, NMDA receptor antagonists have, in healthy volunteers, been shown to be capable of inducing both positive and negative symptoms as well as the formal thought disorder that is a distinct feature of schizophrenia (Javitt & Zukin, 1991), and to generate cognitive impairment, e.g. verbal working memory (Honey et al. 2003). Therefore, a glutamatergic synaptic hypofunction in schizophrenia has been proposed (see Coyle et al. 2003), a notion recently supported by a reduced prefrontal expression of the NMDA receptor subunits NR1, NR2A and NR2C in schizophrenia (Beneyto & Meador-Woodruff, 2008). Significantly, SGAs, but not FGAs have been found to facilitate cortical NMDA receptor-mediated transmission (Jardemark et al. 2002; Ninan et al. 2003).

Although the \(5\)-HT_{2A}/D_{2} ratio has been proposed as a critical determinant of atypicality among the SGAs (Meltzer, 1989; Meltzer et al. 1989), which may have a bearing on their extrapyramidal side-effect (EPS) liability, little attention has so far been focused on the \(\alpha_2/D_2\) ratio of various APDs (but see Nutt, 1994). Previous work from our group has shown that the \(\alpha_2\)-adrenoceptor antagonist idazoxan enhances the effect of a low dose of raclopride, a selective \(D_2/3\) receptor antagonist, on conditioned avoidance response (CAR), a preclinical test with high predictive validity for clinical antipsychotic effect (Wadenberg & Hicks, 1999), and similarly with SGAs, but not FGAs, produces an increased dopamine efflux in the medial PFC (mPFC; Hertel et al. 1999a). Both clozapine and the combination of idazoxan + raclopride have also been shown to enhance cortical NMDA receptor-mediated transmission, effects mediated by dopamine acting at \(D_1\) receptors, as well as to reverse cognitive impairment in rats induced by NMDA receptor blockade (Marcus et al. 2005). Addition of idazoxan to low doses of the FGA haloperidol and the SGA olanzapine, both possessing low affinity for the \(\alpha_2\)-adrenoceptor (Schotte et al. 1996; Shahid et al. 2009), also enhances the effect of these APDs in the CAR test as well as on cortical dopamine release (Wadenberg et al. 2007).

Risperidone possesses lower affinity for the \(\alpha_2\)-adrenoceptor than clozapine but higher than most other SGAs (Schotte et al. 1996; Shahid et al. 2009). Therefore, in the present study, we investigated the significance of additional \(\alpha_2\)-adrenoceptor blockade by adding idazoxan to a low dose of risperidone and examined the effect on antipsychotic efficacy using the CAR test, EPS liability using a catalepsy test, dopamine efflux in the mPFC and NAc using the microdialysis technique in freely moving rats, and cortical NMDA receptor-mediated transmission, using intracellular electrophysiological recording in pyramidal neurons \textit{in vitro}. In addition, we assessed the \textit{in-vivo} \(\alpha_{2A}\) and \(\alpha_{2C}\)-adrenoceptor occupancies of risperidone, using \textit{ex-vivo} autoradiography.

Methods

Animals

Adult male Wistar rats (200–270 g upon arrival) were used for behavioural and microdialysis experiments, whereas male Sprague–Dawley rats (~70 g upon arrival; B&K Universal, Sweden) were used for \textit{in-vitro} electrophysiological experiments. The animals were housed under standard laboratory conditions with food and water available \textit{ad libitum}. For the
behavioural tests the animals were kept on a reversed 12 h light/dark cycle (lights off 06:00 hours), whereas for the other experiments, animals were maintained on a 12 h light/dark cycle (lights on 06:00 hours). Experiments were approved by, and conducted in accordance with, the local Animal Ethics Committee, Stockholm North and the Karolinska Institutet, Sweden.

For the ex-vivo radioligand binding experiments male Wistar rats (180–220 g) were housed in a rat raising facility with food and water available ad libitum. All procedures were conducted in strict accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the animal care and use committee of Johnson and Johnson Pharmaceutical Research and Development.

**CAR behaviour**

Rats were trained and tested in conventional shuttle boxes (530 × 250 × 225 mm), divided into two compartments of equal size by a partition with an opening (Salmi et al., 1994). Upon presentation of the 80-dB white noise (Lafayette Instruments, USA) conditioned stimulus (CS), the rats had 10 s to move from one compartment of the shuttle box into the other. If the rat remained in the same compartment for more than 10 s, an intermittent electric shock (intershock interval 2.5 s, shock duration 0.5 s) of 0.4 mA, i.e. the unconditioned stimulus (UCS), was presented to the grid floor until an escape was performed. If the rat did not respond within 50 s, the trial was terminated, i.e. escape failure. Avoidance (response to CS within 10 s), escape (response to CS + UCS) and escape failure (failure to respond within 50 s) were recorded. The animals were trained for 5 d, each session consisted of ~20 trials randomly distributed over 15 min. Only animals reliably performing >85% avoidance were included in the study. Experiments were preceded by a pre-test and experiment sessions, lasting 10 min, were conducted at 20, 90 and 240 min. Experimental days were separated by at least two non-experimental days. The animals were tested in a counterbalanced change-over design serving as their own controls (Li, 1964).

**Catalepsy measurements**

Catalepsy was observed in a dimly lit room by placing the animals on an inclined grid (60°) for a maximum of 2.5 min. The animals were allowed 30 s of adaptation on the grid before observation started. The different treatments were unknown to the observer. Catalepsy was scored from 0 to 5, according to the time (square-root transformation) the rat remained immobile (min): 0 = 0.00–0.08; 1 = 0.09–0.35; 2 = 0.36–0.80; 3 = 0.81–1.42; 4 = 1.43–2.24; 5 ≥ 2.25 (Ahlenius & Hillegaart, 1986).

**In-vivo microdialysis**

Procedures for microdialysis were as previously described (Schilström et al., 1998). Rats were anaesthetized with a cocktail of Hypnorm® (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen-Cilag Ltd, UK) and Dormicum® (5 mg/ml midazolam; Roche AB, Sweden) diluted in distilled water [1:1:2; 5 ml/kg, intraperitoneal (i.p.)] mounted in a stereotaxic frame, and implanted with dialysis probes in the mPFC or NAc [AP (mm): +2.6, +1.4; ML: −0.6, −1.4; DV: −5.2, −8.2], respectively, relative to bregma and dural surface (Faxinos & Watson, 1998). Dialysis occurred through a semi-permeable membrane (Filtral AN69, Hospal Industrie, France) with an active surface length of 4 mm (mPFC) or 2.25 mm (NAc). Dialysis experiments were conducted ~48 h after surgery in awake, freely moving rats. The dialysis probe was perfused with a physiological perfusion solution [in mM: 147 NaCl, 3.0 KCl, 1.3 CaCl₂, 1.0 MgCl₂, 1.0 NaHPO₄ (pH 7.4)] at a rate of 2.5 μl/min, set by a microinfusion pump (Harvard Apparatus, USA). Dialysate samples were collected over 30 min (mPFC) or 15 min (NAc). Online quantification of dopamine was accomplished by high performance liquid chromatography (HPLC) coupled to electrochemical detection (ESA Bioscience, USA), with a detection limit of ~0.2 fmol/min. The injector (Valco Instruments, USA) was directed by a computerized system, Totalcrom WS version 6.3 (PerkinElmer, USA). Separation of dopamine and metabolites was achieved by reversed-phase liquid chromatography. The mobile phase consisted of 55 mM sodium acetate buffer (pH 4.0), 12% methanol and 0.55 mM octanesulfonic acid delivered by an HPLC pump (model 2150, Pharmacia LKB, Sweden) on a C-18 column (Nucleocil 150/75 × 4.6 mm, 5 μm), flow rate 0.8 ml/min. After separation, the analyte was passed through a guard cell with an applied oxidizing potential of 50 mV to reduce baseline. Samples were quantified by sequential oxidation and reduction in a high sensitive analytical cell (model 5011; ESA Bioscience) that was controlled by a potentiostat (Coulochem II model 5200; ESA Bioscience) with applied potentials of 400 mV and −200 mV for detection of metabolites and dopamine. Injection of drug was performed after a stable outflow (~10% variation) of dopamine and metabolites. Baseline was calculated as the average of
the last two (mPFC) or four (NAc) pre-injection values. The placement of the probe was later verified in slices stained with Neutral Red.

Electrophysiological experiments

Procedures for electrophysiological experiments were as previously described (Konradsson et al. 2006). Briefly, the rats were decapitated while under halothane anaesthesia (AstraZeneca AB, Sweden). The brains were quickly removed and cooled in ice-cold Ringer’s solution [in mM: 126 NaCl, 2.5 KCl, 2.4 CaCl₂, 1.3 MgCl₂, 1.2 NaH₂PO₄, 10 d-glucose, 18 NaHCO₃ (pH 7.4)] aerated by 95% O₂:5% CO₂. The brains were cut coronally into 450-μm slices, using a Vibroslice (Campden model MA 752, World Precision Instruments, USA), and kept submerged in aerated Ringer’s solution at room temperature for >1 h to allow for recovery. A slice containing mPFC (approximately AP +3.2 mm from bregma) was transferred to a recording chamber (32 °C), and held submerged between two nylon nets. The chamber was continuously perfused with aerated Ringer’s solution, flow rate 1.5–2.5 ml/min. Electrodes were pulled from borosilicate glass capillaries (i.d. 0.58 mm; Clark Electromedical Instruments, UK) by using a horizontal electrode puller (model P-87, Sutter Instruments, USA). Recording electrodes were filled with 2 M KAc, electrode puller (model P-87, Sutter Instruments, USA) by using a horizontal silicate glass capillaries (i.d. 0.58 mm; Clark Electromedical Instruments, UK) by using a horizontal electrode puller (model P-87, Sutter Instruments, USA).

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45–180 min for NAc. The overall effects were statistically evaluated by one-way ANOVA, followed by planned comparison test. One-way ANOVA was used to detect differences between baseline values. Statistical evaluation of the electrophysiological experiments was performed by one-way ANOVA followed by post-hoc least significant difference (LSD) test. In all statistical measures \( p < 0.05 \) was considered significant. The statistical evaluations were performed by using Statistica version 8.0 (StatSoft Inc., USA).

**Drugs**

Risperidone was provided by Johnson & Johnson Pharmaceutical Research & Development, division of Janssen Pharmaceutica NV, Belgium. Idazoxan HCl, bicuculline methiodide, glycine and NMDA were purchased from Sigma-Aldrich, USA. Tetrodotoxin was obtained from Tocris, UK. For in-vivo experiments risperidone was dissolved in a minimal amount of acetic acid with a 5.5% glucose solution added to volume and idazoxan was dissolved in saline (0.9% NaCl). For electrophysiological experiments, stock solutions of risperidone (dissolved in dimethyl sulfoxide) and idazoxan (dissolved in purified water) were prepared.

**Results**

**Effects of risperidone alone and in combination with idazoxan on CAR behaviour**

The dose–response relationship for the antipsychotic-like effect of risperidone was established. Risperidone (0.25, 0.3, 0.4, and 0.5 mg/kg, \( n = 11 \)) produced a suppression of CAR at 20 min: \( \chi^2(4) = 32.84, \quad p < 0.001 \) (Fig. 1a); 90 min: \( \chi^2(4) = 16.07, \quad p < 0.01 \), Fig. 1b) in a dose-dependent manner. All doses of risperidone produced statistically significant suppression of CAR at 20 min (\( p < 0.01–0.001 \)) and 0.4 and 0.5 mg/kg at 90 min (\( p < 0.05 \)). At 240 min all animals were back to baseline performance. The results show that the dose needed for antipsychotic effect, i.e. ~80% suppression of CAR (Wadenberg et al. 2001) is between 0.4 and 0.5 mg/kg. However, at 20 min both 0.4 and 0.5 mg/kg risperidone induced some escape failures, 4 and 5 of 11 rats, respectively, indicating that these doses also produce non-specific behavioural effects. ED\(_{50}\) was 0.28 mg/kg.

In another set of animals (\( n = 12 \)), pre-treatment with 1.5 mg/kg idazoxan was added to a dose of risperidone, that by itself did not produce a suppression of CAR sufficient for antipsychotic effect, i.e. 0.25 mg/kg. A statistically significant suppression of CAR was found at 20 min \( \chi^2(3) = 22.88, \quad p < 0.001 \), Fig. 2a), but not at 90 min (Fig. 2b). No escape failures were induced. Compared to vehicle, 0.25 mg/kg risperidone enhanced suppression of CAR \( (p < 0.01) \). Compared to risperidone alone, the combination of risperidone + idazoxan also significantly enhanced the suppression of CAR \( (p < 0.01) \).

The relatively transient effect of risperidone in the CAR test, i.e. that it declined after 90 min and returned to baseline at 240 min, is in all probability related to the concomitantly declining degree of D\(_2\) occupancy, since this has been shown to peak at 30 min and begin to decline after 1 h (Olsen et al. 2008).

**Fig. 1.** Effects of risperidone (0.25, 0.3, 0.4, and 0.5 mg/kg i.p.) on conditioned avoidance response (CAR) behaviour in rats at (a) 20 and (b) 90 min after administration of drug. The results are presented as median (avoidance %) ± semi-interquartile range. Animals \( (n = 11) \) are serving as their own control in a change-over design (Li, 1964). * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \) for vehicle vs. risperidone-treated animals.

**Effects of risperidone alone and in combination with idazoxan in the catalepsy test**

Risperidone (0.3, 0.5 and 1.0 mg/kg) was tested in the catalepsy test [30 min: \( \chi^2(3) = 9.50, \quad p < 0.05 \); 60 min: \( \chi^2(3) = 11.61, \quad p < 0.01 \); 120 min: \( \chi^2(3) = 2.58, \quad p = 0.46 \); Table 1a]. Compared to vehicle, risperidone (0.3 and 0.5 mg/kg) reached statistically significant levels at 30 min \( (p < 0.05–0.01) \), and 1.0 mg/kg risperidone at both 30 and 90 min \( (p < 0.05–0.01) \). However, the...
The mean ± S.E.M. basal dopamine level in the mPFC and the NAc were 0.34 ± 0.03 fmol/min (n = 24) and 3.18 ± 0.43 fmol/min (n = 25), respectively. No statistically significant differences between mean baseline concentrations of dopamine output were found between different treatment groups within the same brain region studied. Vehicle injections had no statistically significant effect on dopamine output.

Statistical evaluation for dopamine output in the mPFC (Fig. 3a) revealed a significant treatment effect, AUC (45–180 min), was statistically significant (F15,180 = 3.02, p < 0.001) as well as interaction effect (F24,180 = 3.02, p < 0.001). Compared to baseline, 0.25 mg/kg risperidone increased dopamine efflux in the mPFC at 60–90 and 150 min (p < 0.05–0.01), 1.5 mg/kg idazoxan at 60–150 min (p < 0.05) and the combination at 30–210 min (p < 0.05–0.01). For the interaction effect, there were a statistically significant difference between vehicle and risperidone at 90 and 150 min (p < 0.05), vehicle and idazoxan at 90 and 150 min (p < 0.05), vehicle and the combination at 60–180 min (p < 0.01–0.001). There was also a statistically significant difference between risperidone and the combination at 60 and 120 min (p < 0.05). In order to assess the effects of the two drugs in combination, we analysed the overall effect following injection of the second drug, AUC (60–180 min). The overall effect was statistically significant (F15,180 = 9.06, p < 0.001). Compared to saline/vehicle, all treatments were significantly higher (p < 0.01–0.001) and the combination of risperidone + idazoxan was statistically higher than both risperidone (p < 0.05) and idazoxan (p < 0.05), when given alone.

Statistical evaluation for dopamine output in the NAc (Fig. 3b) revealed a significant treatment (F14,150 = 4.73, p < 0.01), time (F14,150 = 14.77, p < 0.001) as well as interaction effect (F24,150 = 4.73, p < 0.001). Compared to baseline, 1.5 mg/kg idazoxan had no significant effect on dopamine efflux in the NAc, whereas 0.25 mg/kg risperidone increased dopamine efflux at 30–90 min (p < 0.05–0.01) and the combination at 15–180 min (p < 0.05–0.01). For the interaction effect, there was a statistically significant difference between vehicle and risperidone at 45–75 min (p < 0.05–0.001), vehicle and idazoxan at 15 min (p < 0.05), vehicle and the combination at 30–120 and 150–180 min (p < 0.05–0.001). There was also a statistically significant difference between risperidone and the combination at 60 min (p < 0.05) and between idazoxan and the combination at 45–120 and 165 min (p < 0.05–0.001). The overall effect, AUC (45–180 min), was statistically significant (F24,150 = 6.43, p < 0.01). Compared to saline/vehicle, only the combination of idazoxan + risperidone was significantly higher (p < 0.001), and the combination was statistically higher than idazoxan (p < 0.01), but not compared to risperidone.

**Effects of risperidone alone and in combination with idazoxan on dopamine output in the mPFC and the NAc**

The mean ± S.E.M. basal dopamine level in the mPFC and NAc were 0.34 ± 0.03 fmol/min (n = 24) and 3.18 ± 0.43 fmol/min (n = 25), respectively. No statistically significant differences between mean baseline concentrations of dopamine output were found between different treatment groups within the same brain region studied. Vehicle injections had no statistically significant effect on dopamine output.

Statistical evaluation for dopamine output in the mPFC (Fig. 3a) revealed a significant treatment effect (F15,180 = 8.07, p < 0.001), time (F15,180 = 22.00, p < 0.001) as well as interaction effect (F24,180 = 3.02, p < 0.001). Compared to baseline, 0.25 mg/kg risperidone increased dopamine efflux in the mPFC at 60–90 and 150 min (p < 0.05–0.01), 1.5 mg/kg idazoxan at 60–150 min (p < 0.05) and the combination at 30–210 min (p < 0.05–0.01). For the interaction effect, there were a statistically significant difference between vehicle and risperidone at 90 and 150 min (p < 0.05), vehicle and idazoxan at 90 and 150 min (p < 0.05), vehicle and the combination at 60–180 min (p < 0.01–0.001). There was also a statistically significant difference between risperidone and the combination at 60 and 120 min (p < 0.05). In order to assess the effects of the two drugs in combination, we analysed the overall effect following injection of the second drug, AUC (60–180 min). The overall effect was statistically significant (F15,180 = 9.06, p < 0.001). Compared to saline/vehicle, all treatments were significantly higher (p < 0.01–0.001) and the combination of risperidone + idazoxan was statistically higher than both risperidone (p < 0.05) and idazoxan (p < 0.05), when given alone.

Statistical evaluation for dopamine output in the NAc (Fig. 3b) revealed a significant treatment (F14,150 = 4.73, p < 0.01), time (F14,150 = 14.77, p < 0.001) as well as interaction effect (F24,150 = 4.73, p < 0.001). Compared to baseline, 1.5 mg/kg idazoxan had no significant effect on dopamine efflux in the NAc, whereas 0.25 mg/kg risperidone increased dopamine efflux at 30–90 min (p < 0.05–0.01) and the combination at 15–180 min (p < 0.05–0.01). For the interaction effect, there was a statistically significant difference between vehicle and risperidone at 45–75 min (p < 0.05–0.001), vehicle and idazoxan at 15 min (p < 0.05), vehicle and the combination at 30–120 and 150–180 min (p < 0.05–0.001). There was also a statistically significant difference between risperidone and the combination at 60 min (p < 0.05) and between idazoxan and the combination at 45–120 and 165 min (p < 0.05–0.001). The overall effect, AUC (45–180 min), was statistically significant (F24,150 = 6.43, p < 0.01). Compared to saline/vehicle, only the combination of idazoxan + risperidone was significantly higher (p < 0.001), and the combination was statistically higher than idazoxan (p < 0.01), but not compared to risperidone.

**Effects of risperidone alone and in combination with idazoxan on NMDA-induced currents in pyramidal cells**

Intracellular voltage-clamp recordings were performed in pyramidal cells in layers V and VI in the
prelimbic region of the rat mPFC. The characterization of pyramidal cells was performed according to criteria previously described (see above).

Concentration–response curves for both clozapine and risperidone on NMDA-induced currents have previously been established in our laboratory (Konradsson et al. 2006), showing biphasic response curves with a maximal effect at 100 nM for clozapine [214 ± 16% (mean ± S.E.M.)] and 20 nM for risperidone (147 ± 26%), i.e. a concentration that has been found to generate functionally effective α2-adrenoceptor blockade in rat cortical slices (Leysen et al. 1988). For comparative reasons these data are included in Fig. 4. To investigate the effect of adjunctive α2-blockade, a submaximal concentration of risperidone was chosen, i.e. 10 nM. Here we show (Fig. 4) that whereas neither 1 μM idazoxan (96 ± 8%, n = 4) nor 10 nM risperidone (101 ± 7%, n = 6) had any effect on their own, the combination of 1 μM idazoxan + 10 nM risperidone enhanced NMDA-induced currents (177 ± 26%, n = 5). Group comparison (F2,12 = 7.55, p < 0.01) revealed a statistically significant difference between risperidone and the combination of risperidone + idazoxan (p < 0.01) and between idazoxan and the combination of risperidone + idazoxan (p < 0.01).

### Discussion

The present study shows that adjunctive treatment with the selective α2-adrenoceptor antagonist idazoxan when added to a low dose of risperidone significantly enhances the risperidone-induced suppression of CAR in rats, indicating an enhanced antipsychotic effect. In addition, both increased cortical dopamine release and enhanced NMDA receptor-mediated responses in cortical pyramidal cells were obtained. Previous studies suggest that such effects may be associated with an improvement of negative symptoms and cognitive impairment in schizophrenia. Our data generally propose that the α2/D2 ratio for risperidone is not necessarily optimal and that its therapeutic effect in schizophrenia can be enhanced by adjunctive treatment with an α2-adrenoceptor antagonist such as idazoxan in spite of a dose reduction of risperidone.

The CAR test provides a highly predictive and reliable animal assay of antipsychotic potential (Wadenberg & Hicks, 1999). Thus, within a certain dose range, all APDs so far tested have been found to effectively suppress CAR without affecting escape capability, whereas compounds with a general sedative but not antipsychotic action do not show this selectively at any dose. Previous data also show that the CAR test does not reflect amnesic effects, since anticholinergic drugs such as scopolamine, which induces amnesia in animals and causes memory disturbances in man, do not suppress CAR (Shannon et al. 1999).

Dopamine D2 receptor blockade is a common denominator of all clinically available APDs. However, the degree of receptor blockade differs between

### Table 1. Effects of (a) risperidone and (b) idazoxan + risperidone on catalepsy in rats

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<th>Score at 30 min</th>
<th>Score at 60 min</th>
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<tr>
<td><strong>(a) Effects of risperidone (0.3, 0.5. 1.0 mg/kg i.p.)</strong></td>
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<td>Vehicle</td>
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<tr>
<td>Risperidone 0.3 mg/kg</td>
<td>0.5 ± 0.5*</td>
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<td>Risperidone 0.5 mg/kg</td>
<td>1.5 ± 1.5**</td>
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<tr>
<td>Risperidone 1.0 mg/kg</td>
<td>0.5 ± 1.5*</td>
<td>1.5 ± 1.0**</td>
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<td><strong>(b) Effects of idazoxan (1.5 mg/kg s.c.) + risperidone (0.25 mg/kg i.p.)</strong></td>
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<tr>
<td>Saline + vehicle</td>
<td>0 ± 0.25</td>
<td>1 ± 0.5</td>
<td>1 ± 0.5</td>
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<tr>
<td>Saline + risperidone 0.25 mg/kg</td>
<td>0 ± 0.5</td>
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<tr>
<td>Idazoxan (1.5 mg/kg) + vehicle</td>
<td>0.5 ± 0.5</td>
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<tr>
<td>Idazoxan (1.5 mg/kg) + risperidone (0.25 mg/kg)</td>
<td>0 ± 0</td>
<td>0 ± 0.5</td>
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The results are shown as medians ± semi-interquartile range based on observations of eight animals per treatment group.

*p < 0.5, **p < 0.01 vehicle vs. risperidone-treated animals.
different APDs. Whereas FGAs, such as haloperidol, typically produce a D₂ receptor occupancy of ~70%, clozapine generates only ~45% at clinically effective dosage (Farde & Nordström, 1992; Farde et al., 1989). The D₂ receptor occupancy generated by risperidone is approximately in the same range as that produced by FGAs (Kapur et al., 1995, 1999; Nyberg et al., 1999).

In rodents, a dose of clozapine that shows a robust antipsychotic effect in the CAR test, i.e. 5 mg/kg (Olson et al., 2006), produces ~45% D₂, 65% α₂A- and 95% α₂C-receptor occupancy (Marcus et al., 2005). In the present study, the dose of risperidone required to achieve a robust antipsychotic-like effect was between 0.4 and 0.5 mg/kg, corresponding to ~55% D₂ receptor occupancy (Wadenberg et al., 2001) and to ~12% α₂A- and ~19% α₂C-adrenoceptor occupancy.

![Fig. 3](image_url)

Fig. 3. The effects of risperidone (0.25 mg/kg i.p.) administration on dopamine output in (a) the mPFC and (b) the NAc, respectively, in rats pretreated with saline or idazoxan (1.5 mg/kg s.c.). Arrows indicate saline/idazoxan and vehicle/risperidone injections. The results are presented as mean ± S.E.M. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to vehicle, # p < 0.05 risperidone vs. idazoxan + risperidone (n=5–7).

![Fig. 4](image_url)

Fig. 4. (a) The effects of 10 nM risperidone, 1 μM idazoxan and the combination of 10 nM risperidone + 1 μM idazoxan on NMDA-induced currents in pyramidal cells of the mPFC. The results are presented as mean ± S.E.M. ** p < 0.01 between different treatments. For comparison, the maximal effects of clozapine (100 nM) and risperidone (20 nM; Konradsson et al., 2006) are included in the figure. (b) Representative traces illustrating NMDA-induced inward currents in a pyramidal cell of the mPFC, before (left) and after (right) administration of the combination of 10 nM risperidone + 1 μM idazoxan.

![Fig. 5](image_url)

Fig. 5. In-vivo α₂A-adrenoceptor (□) and α₂C-adrenoceptor (●) occupancy measured by ex-vivo autoradiography 1 h after subcutaneous administration of risperidone.
However, already at this dose level some escape failures were registered, indicating a contribution of unspecific effects of the drug. Significantly, the combination of a low dose of risperidone (0.25 mg/kg), which produces ~50% D2 receptor occupancy (Wadenberg et al. 2001), and idazoxan (1.5 mg/kg), which generates 75% a2A- and 90% a2C-adrenoceptor occupancy (Marcus et al. 2005), thus resulted in receptor occupancies that are similar to those of clozapine and also produced a robust antipsychotic effect in the CAR test. Given the superior efficacy of clozapine in schizophrenia, it is therefore of considerable interest that we here, by adding idazoxan, obtained a robust antipsychotic activity by a low dose of risperidone, which by itself was not sufficient to generate such an effect. Yet, no catalepsy was observed with this drug combination, thus suggesting a very low EPS liability.

Besides being an a2-adrenoceptor antagonist, idazoxan also has affinity for 5-HT1A and imidazoline receptors. However, its affinity for a2-adrenoceptors is much higher than for both 5-HT1A and imidazoline receptors (Ernsberger et al. 1990; Kleven et al. 2005), indicating that the effects seen with idazoxan are, in all probability, largely due to a2-adrenoceptor blockade.

Addition of idazoxan to a low dose of risperidone significantly enhanced dopamine output in the mPFC, and also in the NAc. Addition of idazoxan to a selective D2 antagonist and also to haloperidol has previously been shown to increase dopamine output in the mPFC but not in NAc (Hertel et al. 1999a; Wadenberg et al. 2007). However, the effect in the NAc by the combination of idazoxan + risperidone is comparable to the effects produced by adding idazoxan to olanzapine, which also produced a marked increase in prefrontal dopamine output as well as an enhanced antipsychotic-like effect (Wadenberg et al. 2007). Both olanzapine and risperidone possess higher affinity for serotonergic receptors, e.g. 5-HT1A and 5-HT2C, than for D2 receptors (Shahid et al. 2009). Clinically, ritanserin, a 5-HT2A/2C receptor antagonist has been found to enhance the effect of FGAs in schizophrenia (Gelders et al. 1986; Reyntjens et al. 1986). However, when added to the selective D2 receptor antagonist raclopride, ritanserin not only enhanced the antipsychotic-like effect (Wadenberg et al. 1996) but also increased the raclopride-induced dopamine outflow in both the mPFC and NAc (Andersson et al. 1995). Moreover, asenapine, a SGA with higher affinity for a range of serotonergic receptors and a2-adrenoceptors than for D2 receptors (Shahid et al. 2009) increases dopamine output in mPFC as well as in both NAc and striatum (Frånberg et al. 2008). Thus, both preclinical and clinical data seem to indicate that the increased accumbal dopamine output induced by adding a 5-HT2A/C receptor antagonist or idazoxan to various APDs does not prevent a concomitantly enhanced antipsychotic effect of the drug combinations. Indeed, previous data propose that a marked enhancement of dopamine outflow in the mPFC per se is sufficient to produce an enhanced antipsychotic effect of APDs (Eltabb et al. 2005; Hertel et al. 1999a), a notion that is indirectly supported by several studies showing that cortical dopamine may regulate and functionally inhibit subcortical dopamine transmission (Murase et al. 1993; Saunders et al. 1998). An enhanced dopamine output in the NAc might seem counteractive to the antipsychotic-like effect of APDs, which has been shown to be mediated in the ventral striatum (Wadenberg et al. 1990). The increased dopamine output in the NAc does not seem to have any functional consequences, at least it does not prevent the antipsychotic-like effect seen in this model. The increased dopamine outflow in striatal areas might still contribute to reduce catalepsy. The combination of idazoxan + risperidone, in concentrations that had no effect by themselves, facilitated NMDA receptor-induced currents in the mPFC. Thus, both enhanced cortical dopaminergic and glutamatergic NMDA receptor-mediated transmission were observed following combined treatment with risperidone + idazoxan. In our previous study (Marcus et al. 2005), the facilitation of cortical NMDA receptor-mediated neurotransmission by the combination of D2 receptor and a2-adrenoceptor blockade was found to be mediated by dopamine and executed via the D1 receptor. Moreover, evidence for dopaminergic D2 dysregulation as well as glutamatergic NMDA receptor hypofunction in schizophrenia has been reported (see Introduction) and cognitive processes per se seem to be dependent on normally functioning D1 receptors as well as NMDA receptors (Aura & Riekkinen, 1999; Goldman-Rakic et al. 2000). Consequently, the facilitation of cortical NMDA receptor-mediated transmission generated by a combination of idazoxan and a low dose of risperidone might contribute to improve cognitive deficits, e.g. impaired working memory, in schizophrenia, an effect that may contribute to improve treatment outcome (Green, 1996).

Clozapine has been shown to reduce suicidality in schizophrenia and schizoaffective disorder (see Introduction). The a2-adrenoceptor blocking effect of clozapine may well contribute to its suicide-preventing effect, since a2-adrenoceptor blockade represents one of the mechanisms of action of clinically effective antidepressant drugs such as mianserin and...
mirtazapine, and blockade of α₂-autoreceptors on nerve terminals results in an increased release of both noradrenaline and serotonin in the brain (de Boer, 1995; Haddjeri et al. 1995; Hertel et al. 1997, 1999b). Indeed, the antidepressant drug mianserin has been found to enhance the effect of FGAs on positive and negative symptoms as well as cognitive dysfunction in schizophrenia (cf. above), thereby generating a clozapine-like effect. Therefore, adjunct treatment with an α₂-adrenoceptor antagonist may improve the efficacy of risperidone on depressive symptoms in schizophrenia and potentially even reduce suicidality.

Consequently, the enhanced prefrontal dopaminergic as well as cortical NMDA receptor-mediated transmission induced by adjunct idazoxan when added to a low dose of risperidone may improve its effect on negative symptoms and cognitive deficits in schizophrenia. In addition, the lower D₂ receptor occupancy, which is achieved by a reduced dose of risperidone, might in itself contribute to such improvement, since a high degree D₂ blockade per se has been shown to generate detrimental effects on both cognition and mood in healthy volunteers (Saeedi et al. 2006). In fact, a high degree of D₂ blockade may in itself serve to exacerbate cognitive as well as negative symptoms in schizophrenia (Carpenter, 1996). Finally, a reduced EPS liability would, in principle, also be expected by the combination of idazoxan and a low dose of risperidone. The present results provide further evidence for the notion that the potent α₂-adrenoceptor antagonistic action of clozapine may significantly contribute to its unique efficacy in schizophrenia, as originally proposed by Natt (1994).

Acknowledgements

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Statement of Interest

Dr X. Langlois is an employee of Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium.

References


