Enhanced efficacy of both typical and atypical antipsychotic drugs by adjunctive $\alpha_2$ adrenoceptor blockade: experimental evidence

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Abstract

Adjunctive treatment with the selective $\alpha_2$ adrenoceptor antagonist idazoxan augments the effect of conventional antipsychotics in treatment-resistant schizophrenics comparing favourably with clozapine. Clozapine has high affinity for $\alpha_2$ adrenoceptors. Previously, we found that adjunctive idazoxan treatment to the dopamine (DA) $D_2/D_3$ antagonist raclopride enhanced raclopride-induced effects in an animal model of antipsychotic activity (conditioned avoidance response, CAR) and, similarly to clozapine, reversed the disruption of working memory induced by N-methyl-D-aspartate receptor blockade in rats with a concomitant increase in prefrontal DA efflux. To further investigate the significance of $\alpha_2$ adrenoceptor affinity for antipsychotic efficacy, we here investigated, in rats, the effects of adjunctive idazoxan treatment to low doses of a typical (haloperidol) and an atypical (olanzapine) antipsychotic drug, both lacking appreciable $\alpha_2$ adrenoceptor affinity, on (i) CAR; (ii) catalepsy; and (iii) DA output in the prefrontal cortex and the nucleus accumbens using microdialysis. Adjunctive treatment with idazoxan to haloperidol or olanzapine enhanced suppression of CAR to a level predicting sufficient antipsychotic activity, increased DA output preferentially in the prefrontal cortex, and reversed haloperidol-induced catalepsy. Our data confirm and extend our previous findings as well as clinical observations, and suggest that adjunctive $\alpha_2$ adrenoceptor blockade of both typical and atypical antipsychotic drugs, lacking appreciable affinity for the $\alpha_2$ adrenoceptor, may contribute to a more advantageous therapeutical profile of these drugs in schizophrenia treatment, allowing for reduced DA $D_2$ occupancy and reduction of unwanted side-effects.

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Introduction

Compared with typical antipsychotic drugs (APDs), clozapine (Clz) shows superior therapeutic efficacy in treatment-refractory patients (Kane et al., 1988, 2001) and is superior, in comparison with the atypical APD olanzapine (Olz), against suicidality in patients with schizophrenia and schizoaffective disorder (Meltzer et al., 2003). Beneficial effects of Clz on some aspects of cognitive impairment have also been suggested (Meltzer and McGurk, 1999).

Prefrontal dopamine (DA) is important for normal cognitive functioning (Goldman-Rakic et al., 2000), and the DA $D_1$ receptor in particular appears to be of major importance in this respect (Castner et al., 2000). While recent data indeed lend more direct support for the hypothesis of a specific DA mesolimbic over-reactivity in schizophrenia (see e.g. Laruelle et al., 1996), negative symptoms and cognitive disturbances may conversely be due to a hypodopaminergic state in prefrontal cortical brain areas (Weinberger et al., 2001). Moreover, a dysregulation of DA $D_1$ receptors is suggested in cognitive impairment in schizophrenia (Abi-Dargham et al., 2002), and in addition a hypofunction of the glutamatergic N-methyl-D-aspartate (NMDA) receptor may also contribute to cognitive disturbances (Emamian et al., 2004). In support of this notion, NMDA receptor antagonists can produce...
psychosis-like symptoms in healthy humans (Javitt and Zukin, 1991), and disruptions of, for example working memory in rodents (Levin et al., 1998) with a concomitant erratic locomotor activity pattern.

Experimentally, Clz and other atypical, but not typical, APDs produce a marked increase in DA output in the rat medial prefrontal cortex (mPFC) (Ichikawa et al., 2002; Nomikos et al., 1994). This effect is thought to be important for therapeutic efficacy in particular against negative symptoms and cognitive impairment in schizophrenia. While 5-HT2 receptor blockade in combination with selective DA D2 receptor antagonists has been shown experimentally to enhance their antipsychotic-like effect and to increase prefrontal DA output (Andersson et al., 1995), other pharmacological properties should also be considered in this respect. Clz is a potent antagonist for D2 adrenoceptors (Ashby and Wang, 1996), while most other atypical APDs, with the exception of risperidone, have relatively low affinity for this receptor. Clinical studies have previously shown that, compared with antipsychotic treatment alone, adjunctive treatment with the selective D2 adrenoceptor antagonist idazoxan (Ida) (Dabiré, 1986) significantly augmented the effect of a conventional APD (fluphenazine) in schizophrenic patients, meeting the criteria for treatment resistance, with a reduction of psychosis and negative symptoms, as well as a significant effect on thought disorders and withdrawal retardation (Litman et al., 1993, 1996). Litman et al. also noted that the add-on treatment with Ida to fluphenazine, but not fluphenazine alone, compared favourably with Clz. Improvements in cognitive domains such as verbal fluency, attention and planning by treatment with Ida alone have also been reported in patients with frontal lobe dementia (Coull et al., 1996; Sahakian et al., 1994).

Experimentally, we have previously shown that adjunctive Ida treatment to a selective DA D2/3 receptor antagonist, raclopride (Rac), markedly enhanced a modest antipsychotic-like effect of a low dose (0.05 mg/kg) of Rac in the conditioned avoidance response (CAR) test in rats for antipsychotic activity, as well as producing a substantial increase in prefrontal DA as assessed by microdialysis (Hertel et al., 1999a). Recently, using the eight-arm radial maze test we showed that, similarly to Clz, combined treatment with the same doses of Ida and Rac respectively, completely reversed the disruption of working-memory performance induced by the selective NMDA receptor antagonist MK-801 in rats. Furthermore, in an in-vitro electrophysiological experimental paradigm, run in parallel, combined Ida + Rac also caused a DA D1 receptor-mediated facilitation of glutamatergic transmission in rat prefrontal pyramidal cells, similarly to Clz, but not to typical APDs (Marcus et al., 2005).

Clz also shows little, if any, extrapyramidal side-effects (EPS) both experimentally (Hoffman and Donovan, 1995) and in humans (Casey, 1989; Iqbal et al., 2003). Again, α2 adrenoceptor antagonism might well be a contributory factor. Thus, the α2 adrenoceptor antagonist yohimbine reverses animal catalepsy induced by haloperidol (Hal) or loxapine (Kalkman et al., 1998), and Ida was recently reported to reverse Hal-induced catalepsy (Invernizzi et al., 2003). Taken together, the available data suggest that adjunctive α2 adrenoceptor blockade might significantly augment therapeutic efficacy of typical APDs, lacking appreciable α2 affinity, while at the same time reducing EPS liability.

Therefore, using the CAR test for antipsychotic activity, we investigated whether adjunctive Ida treatment might enhance the effects of low doses of a typical (Hal) and an atypical (Olz) APD respectively, both reportedly having relatively low affinity for the α2 adrenoceptor (see e.g. Bymaster et al., 1999). The CAR test has consistently shown high predictive validity (Arnt, 1982; Wadenberg and Hicks, 1999). All effective APDs have the ability to selectively suppress avoidance behaviour in rats in doses that correlate closely with their clinical doses (Arnt, 1982; Seeman, 1992). The effects of adjunctive Ida treatment to Hal or Olz respectively, were also assessed in the catalepsy test for EPS liability (Sanberg et al., 1988; Wadenberg, 1996). DA output in the mPFC, as well as in the nucleus accumbens (NAc), following combined Ida + Hal or Ida + Olz treatment, was assessed by means of in-vivo microdialysis.

Methods

Animals

Adult male Wistar rats (BKl:WR, Wistar), with an average weight of 200–225 g upon arrival, were purchased from B & K Universal, Sollentuna, Sweden. The animals were housed 3–5 per cage (Makrolon® IV) under standard laboratory conditions with a temperature of 21.0 ± 0.4 °C, and a relative humidity of 55–65%. Animals used for behavioural studies were kept on a reversed 12/12 h light–dark cycle (lights off at 07:00 hours) and animals used for microdialysis were kept on a normal 12/12 h light–dark cycle (lights off at 19:00 hours). Food (R34, Ewos, Södertälje, Sweden) and tap water were available ad libitum. The animals were acclimatized for at least 1 wk prior to...
training or experiments. Animals were transferred from the animal quarters to the laboratory 1 h before behavioural experiments were started, and were housed in a ventilated cabinet between observations. All experiments were performed between 08:00 and 18:00 hours.

The studies were approved by the Local Animal Ethics Committee, Stockholm North.

**Drugs**

Olz (Eli Lilly, Indianapolis, IN, USA), Hal and Ida (Sigma, Stockholm, Sweden) were used. Ida was dissolved in physiological saline. Olz and Hal were dissolved in a minimal amount of glacial acetic acid and made up to volume with 5.5% glucose (pH for Olz and Hal was ~5). The compounds were given subcutaneously (s.c.; Hal and Ida) or intraperitoneally (i.p.; Olz) in a volume of 2 ml/kg body weight.

**CAR behaviour**

Rats were trained and tested in a conventional, computer-assisted, two-way active avoidance (shuttle-box) apparatus equipped with photocells and a grid floor connected to a high resistance power supply (see Salmi et al., 1994). Upon presentation of the 80 dB white noise conditioned stimulus (CS), the animals had 10 s to move from one compartment of the shuttlebox into the other. If the rat remained in the same compartment for more than 10 s, an intermittent electric shock of ~0.3 mA [the unconditioned stimulus (UCS)], was presented to the grid floor until an escape was performed. If the animal did not respond within 50 s of the shock period, the trial was terminated (escape failure). The following variables were recorded: *avoidance* (response to CS within 10 s); *escape* (response to CS + UCS); *escape failures* (failure to respond within 50 s); and *inter-trial crosses*. The animals were trained for five consecutive days. Training consisted of one session (with a total average of 20 trials) per animal and day. Experimental manipulations were always preceded by a pretest. The animals were tested in a counterbalanced change-over design serving as their own controls (Li, 1964). Experimental days were always separated by at least two non-experimental days.

**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 observations started. The 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 min, i.e. if the rat remained immobile for 0.08 min, it was scored as 0, etc. (see Ahlenius and Hildegaard, 1986).

**Microdialysis**

The probe implantation and dialysis procedure, as well as the biochemical analyses, were similar to those previously described (see e.g. Schilstrom et al., 1998). Rats were anaesthetized with a cocktail of Hypnorm\(^w\) (0.315 mg/ml fentanyl citrate, and 10 mg/ml fluainsone, Janssen-Cilag Ltd, Saunderton, UK) and Dormikum\(^w\) (5 mg/ml midazolam, Roche AB, Stockholm, Sweden) diluted in distilled water (1:1:2; 5 ml/kg i.p.), mounted in a stereotaxic frame, and implanted with dialysis probes in the mPFC or NAc [AP: +2.6, +1.4; ML: −0.6, −1.4; DV: −5.2, −8.2] respectively, relative to bregma and dural surface (Paxinos and Watson, 1998). Dialysis occurred through a semi-permeable membrane (Filtral AN69, Hospal Industrie, Meyzieu, France) with an active surface length of 2.25 mM for the NAc and 4 mM for the mPFC. Dialysis experiments were conducted ~48 h after surgery in awake and freely moving rats. The dialysis probe was perfused with a physiological perfusion solution [147 mM sodium chloride, 3.0 mM potassium chloride, 1.3 mM calcium chloride, 1.0 mM magnesium chloride, and 1.0 sodium phosphate (pH 7.4), Apoteksbolaget, Solna, Sweden] at a rate of 2.5 μl/min set by a microinfusion pump (Harvard Apparatus, Holliston, MA, USA). The sampling period was 15 min or 30 min for the NAc and mPFC respectively. Online quantification of DA in the dialysate was accomplished by high performance liquid chromatography coupled to electrochemical detection (ESA, Chelmsford, MA, USA). The detection limit for DA was ~0.2 fmol/min, or 3 fmol/sample in the NAc and 6 fmol/sample in the mPFC. The location of the probe was later verified in slices stained with Neutral Red.

Results are expressed as changes of basal DA output over time. Baseline (=100%) was defined as the average of the last two (mPFC) or four (NAc) pre-injection values.

**Statistics**

Statistical evaluation was performed by means of the Friedman two-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test (CAR) or the Kruskal–Wallis one-way ANOVA, followed by the Mann–Whitney U test (catalepsy) (Siegel and
Castellan, 1988). Since the study involves small samples with subjects from a population that may not necessarily show a normal distribution, and quantification used scoring methods that are of ranking type (rather than truly numerical), non-parametric statistical procedures were deemed the most appropriate choice (see e.g. Siegel and Castellan, 1988).

Statistical evaluation of microdialysis data was performed by means of a two-way (treatment × time) ANOVA for repeated measures followed by the Newman–Keuls test for multiple comparisons (STATA vers. 7.1; StatSoft Inc., Tulsa, OK, USA).

Results

Effects of Hal alone, and in combination with Ida, on CAR in rats

Hal (0.025 mg/kg s.c.) produced a significant ($p < 0.01$) suppression of CAR 20 min after administration compared with vehicle-treated animals. Pretreatment with Ida (1.5 mg/kg s.c.) significantly ($p < 0.05$) enhanced the Hal (0.025 mg/kg)-induced suppression of CAR 20 min after Hal administration compared to animals treated with Hal alone. This effect was also still present 90 min after Hal administration. All effects were gone 240 min after Hal administration (Figure 1). In another set of animals, Hal (0.1 mg/kg s.c.) produced a significant ($p < 0.01$) suppression of CAR 20 min after administration compared with vehicle-treated animals. Pretreatment with Ida (1.5 mg/kg s.c.) significantly ($p < 0.01$) enhanced the Hal (0.1 mg/kg)-induced suppression of CAR 20 min after Hal administration compared with animals treated with Hal alone. Effects of treatment with Hal (0.1 mg/kg) alone, as well as with the combination of Ida + Hal, lasted for at least 4 h but were not significantly different from each other at any of those observation times (Figure 2). Ida itself had no effects on CAR.

Effects of Olz alone, and in combination with Ida, on CAR in rats

Olz (1.25 or 2.5 mg/kg i.p.) produced a significant ($p < 0.05$ and $p < 0.01$ respectively) suppression of CAR 20 min after Olz administration. Pretreatment with Ida (1.5 mg/kg s.c.) significantly ($p < 0.01$) enhanced the suppression of CAR induced by 2.5 mg/kg Olz compared with animals treated with that dose of Olz alone. All effects were gone at 240 min after Olz administration (Figure 3). Ida itself had no effects on CAR.

No escape failures were recorded in the CAR experiments at any observation time or in any treatment

Figure 1. Effects of haloperidol (0.025 mg/kg s.c.) or idazoxan (1.5 mg/kg s.c.), alone and in combination, on conditioned avoidance response in rats at (a) 20 min, (b) 90 min and (c) 240 min after vehicle or haloperidol administration. Shown are median percent avoidance ($\pm$ semi-interquartile range) based on repeated observations of the same animals ($n = 8$) serving as their own controls. Statistical evaluation performed by means of the Friedman two-way ANOVA, (a) $\chi^2(3) = 13.96$, $p < 0.01$, followed by the Wilcoxon matched-pairs signed-ranks test (Siegel and Castellan, 1988). ** $p < 0.01$ compared with vehicle treatment groups; * $p < 0.05$ compared with animals treated with haloperidol alone. □, Saline; ■, 1.5 mg/kg idazoxan.
Figure 2. Effects of haloperidol (0.1 mg/kg s.c.) or idazoxan (1.5 mg/kg s.c.), alone and in combination, on conditioned avoidance response in rats at (a) 20 min, (b) 90 min and (c) 240 min after vehicle or haloperidol administration. Shown are median percent avoidance (± semi-interquartile range) based on repeated observations of the same animals (n = 8) serving as their own controls. Statistical evaluation performed by means of the Friedman two-way ANOVA, (a) $\chi^2(3) = 23.22$, $p < 0.001$, followed by the Wilcoxon matched-pairs signed-ranks test (Siegel and Castellan, 1988). ** $p < 0.01$ compared with vehicle treatment groups; ++ $p < 0.01$ compared with animals treated with haloperidol alone. □, Saline; ■, 1.5 mg/kg idazoxan.

Figure 3. Effects of olanzapine (1.25 or 2.5 mg/kg i.p.) or idazoxan (1.5 mg/kg s.c.), alone and in combination, on conditioned avoidance response in rats at (a) 20 min, (b) 90 min and (c) 240 min after vehicle or olanzapine administration. Shown are median percent avoidance (± semi-interquartile range) based on repeated observations of the same animals (n = 9) serving as their own controls. Statistical evaluation performed by means of the Friedman two-way ANOVA. (a) $\chi^2(3) = 10.97$, $p < 0.05$ (1.25 mg/kg olanzapine + idazoxan); $\chi^2(3) = 26.05$, $p < 0.001$ (2.5 mg/kg olanzapine + idazoxan), followed by the Wilcoxon matched-pairs signed-ranks test (Siegel and Castellan, 1988). * $p < 0.05$, ** $p < 0.01$ compared with vehicle treatment groups; ++ $p < 0.01$ compared with animals treated with olanzapine alone. □, Saline; ■, 1.5 mg/kg idazoxan.
group. That is, any decrease in CAR behaviour resulted in a corresponding increase in escape behaviour.

**Intertrial crosses (ICs)**

Consistent with earlier literature, the drug-induced suppression of CAR in the present study was accompanied by a concomitant decrease in locomotor activity as measured by the number of ICs. The decrease in ICs was statistically significant following treatment with (i) Hal (0.1 mg/kg) alone, (ii) Hal (0.025 or 0.1 mg/kg) in combination with Ida (1.5 mg/kg), (iii) Olz (2.5 mg/kg) alone, and (iv) Olz (1.25 or 2.5 mg/kg) in combination with Ida (1.5 mg/kg). Ida (1.5 mg/kg) alone produced a statistically significant decrease in the number of ICs (30 min after Ida administration) in the set of animals used in the Olz study only (data not shown).

**Effects of Hal alone, and in combination with Ida, in the catalepsy test for rats**

Hal (0.1 mg/kg s.c.) produced significant ($p < 0.01$) catalepsy 1 and 2 h after administration. Pretreatment with Ida (1.5 mg/kg s.c.) significantly ($p < 0.01$, 1 h; $p < 0.05$, 2 h) reversed the Hal-induced catalepsy compared to animals treated with Hal alone. The lower dose of Hal (0.025 mg/kg) produced significant ($p < 0.01$) catalepsy 2 h after administration. This effect was somewhat attenuated by pretreatment with Ida. Ida itself had no effects in the catalepsy test (Figure 4).

**Effects of Olz alone, and in combination with Ida, in the catalepsy test for rats**

Olz (2.5 mg/kg i.p.) or Ida (1.5 mg/kg s.c.), alone or in combination, did not produce catalepsy (Figure 5).

**Effects of Hal alone, and in combination with Ida, on DA output in the mPFC and the NAc respectively in rats**

Hal (0.025 mg/kg s.c.) alone did not significantly alter DA output in the mPFC or in the NAc. The combined treatment with Ida (1.5 mg/kg s.c.) and Hal (0.025 mg/kg), however, produced a robust and significant ($p < 0.001$) increase in DA output in the mPFC. In the NAc, combined treatment with Ida+Hal produced a slight increase in DA output that was significant ($p < 0.05$) only at one time-point (Figure 6).

**Effects of Olz alone, and in combination with Ida, on DA output in the mPFC and NAc respectively in rats**

Olz (2.5 mg/kg i.p.) alone produced a significant increase in DA output in the mPFC ($p < 0.01$) and

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**Figure 4.** Effects of haloperidol (0.025 or 0.1 mg/kg, s.c.) or idazoxan (1.5 mg/kg, s.c.), alone and in combination, on catalepsy in rats at (a) 30 min, (b) 60 min and (c) 120 min after vehicle or haloperidol administration. Shown are median catalepsy score (± semi-interquartile range) based on repeated observations of eight animals per treatment group. Statistical evaluation performed by means of the Kruskal–Wallis one-way ANOVA, followed by the Mann–Whitney U test (Siegel and Castellan, 1988). ** $p < 0.01$ compared with vehicle treatment groups; + $p < 0.05$, ++ $p < 0.01$ compared with animals treated with haloperidol alone. □, Saline; ■, 1.5 mg/kg idazoxan.
the NAc ($p<0.001$). The combined treatment with Ida (1.5 mg/kg s.c.) and Olz (2.5 mg/kg), however, produced a robust and highly significant ($p<0.001$) increase in DA output in the mPFC and also when compared to animals treated with Olz alone ($p<0.001$). In the NAc, combined treatment with
Ida + Olz produced an increase in DA output that was significant \( p < 0.001 \) (Figure 7).

**Discussion**

The present study showed that adjunctive treatment with the selective \( \alpha_2 \) adrenoceptor antagonist Ida to relatively low doses of a typical (Hal), as well as an atypical (Olz), APD lacking appreciable affinity for the \( \alpha_2 \) adrenoceptor: (i) significantly enhanced the suppression of CAR in rats to a level that would predict sufficient clinical antipsychotic activity; (ii) produced a substantial increase in DA output in the mPFC compared with a more modest, similar effect in the NAc; and (iii) significantly reversed Hal (0.1 mg/kg)-induced catalepsy. Our data confirm and extend a previous report by Hertel et al. (1999a), and lend further experimental support for the significance of \( \alpha_2 \) adrenoceptor blockade in APD efficacy as, for example, reported previously by Litman et al. (1993, 1996), as well as for a favourable EPS profile similar to Clz, that indeed has high affinity for the \( \alpha_2 \) adrenoceptor (see Introduction). Furthermore, together with recent data from our laboratory (Marcus et al., 2005), showing that combined treatment with Ida + Rac, similarly to Clz, fully reversed impairment of working memory induced by the NMDA receptor antagonist MK-801 in rats, our present data suggest that adjunctive \( \alpha_2 \) adrenoceptor blockade to APDs, lacking appreciable affinity for the \( \alpha_2 \) adrenoceptor, may not only offer a reduced DA \( D_2 \) receptor occupancy with retained sufficient antipsychotic efficacy, but may possibly also allow for enhanced therapeutic efficacy against some aspects of cognitive impairment in schizophrenia.

Our present study also extends our previous work in the sense that we now show that previously reported antipsychotic-like effects in the CAR test by adjunctive \( \alpha_2 \) adrenoceptor blockade (using Ida) to an experimental drug, the selective DA \( D_2/3 \) receptor antagonist Rac (Hertel et al., 1999a), can also be reproduced using this same behavioural test when Ida is given as an adjunct to either a typical or an atypical APD lacking appreciable affinity for the \( \alpha_2 \) adrenoceptor.

Previously published data (Wadenberg et al., 2001) have demonstrated that an effective suppression of CAR, i.e. 80–100%, in the rat requires \( \sim 0.1 \) mg/kg Hal and 5 mg/kg Olz. These doses of Hal and Olz in rats would, according to previous findings, produce a striatal \( D_2 \) receptor occupancy of around 88% and 94% respectively, thus reaching levels where signs of EPS begin to emerge or, as shown in the present study, are even significant (Wadenberg et al., 2001). Here, we show that adjunctive Ida treatment to either Hal or Olz demonstrates an antipsychotic-like suppression of
CAR of equal magnitude using only 0.025 mg/kg Hal or 2.5 mg/kg Olz with estimated striatal D$_2$ occupancies subsequently reduced to 56% and 80% respectively. Thus, our present data provide experimental evidence that the doses of Hal or Olz required to obtain a clinically sufficient antipsychotic effect might be reduced by almost 75% and 50% respectively, by adjunctive Ida treatment. The dose of Ida (1.5 mg/kg) used in the present study (and throughout previous studies in our laboratory) produces an $\alpha_2$A/C receptor occupancy of ~75% (Marcus et al., 2005). For comparison, the dose of Clz (5 mg/kg) that produces antipsychotic-like effects in CAR (Wadenberg et al., 1993), as well as reversing MK-801-induced impairment in the radial maze test for working memory (Marcus et al., 2005), produces an $\alpha_2$A/C receptor occupancy of around 60% ($\alpha_{2A}$) and 90% ($\alpha_{2C}$) respectively (Marcus et al., 2005). The lower D$_2$ occupancy thus obtained should allow for a reduced incidence of EPS, and, as shown in the present study and elsewhere (cf. Introduction), additional $\alpha_2$ adrenoceptor blockade per se is also likely to further contribute to a reduction of EPS liability. A lower D$_2$ occupancy might, in addition, contribute to an amelioration of cognitive impairment, since it has been suggested that D$_2$ blockade could exacerbate cognitive, as well as negative, symptoms in schizophrenia (Carpenter, 1996).

The present study also showed that adjunctive Ida treatment to Hal or Olz, using the same doses as in the behaviourial part of the study, produced a substantial increase in DA output in the mPFC compared with the effects of Hal or Olz when administered alone. Such an effect was also found in the study by Hertel et al. (1999a) following combined treatment with Ida + Rac, but not with Rac alone. In comparison, with regard to Olz, it has previously been shown (Li et al., 1998) that as much as 10 mg/kg is needed for Olz to produce an increase in prefrontal DA of similar magnitude as shown in the present study with adjunctive Ida treatment to the low (2.5 mg/kg) dose of Olz. Furthermore, while Hal (0.1 mg/kg) induced a modest, non-significant increase in prefrontal DA release, a 10-fold higher dose (1 mg/kg) of Hal had no effect at all on prefrontal DA release (Bonaccorso et al., 2002).

Using an in-vitro electrophysiological paradigm, Marcus et al. (2005) found that in the case of combined Ida + Rac treatment, the increase in prefrontal DA output is associated with a facilitatory effect on glutamatergic neurotransmission in this brain region, and that this glutamatergic potentiation is mediated by DA via its D$_1$ receptor. Considering the reported evidence for both a DA D$_1$ dysregulation and a glutamatergic NMDA receptor hypofunction in schizophrenia (see Introduction), and the fact that cognitive processes do indeed seem to be dependent on normally functioning D$_2$, as well as NMDA, receptors (Aura and Riekkinen, 1999; Goldman-Rakic et al., 2000), these deficiencies may well contribute to the cognitive disturbances seen in schizophrenia. Thus, against this background, our previous and present results of an increased DA output in the mPFC by adjunctive Ida treatment to Rac, as well as to APDs lacking appreciable $\alpha_2$ receptor affinity, may indeed suggest pro-cognitive properties of such combined treatment. The mechanisms by which adjunctive Ida treatment increases DA output selectively in the mPFC, in the presence of a low DA D$_2$ receptor blockade, are not fully understood. Ida is a highly selective $\alpha_2$ adrenoceptor antagonist, and it is unlikely that other pharmacological properties of Ida, such as for example an action on imidazoline receptors (Molderings et al., 1998), should play a role in the presently observed biochemical effects, as these receptors, although abundant subcortically, are much less frequently expressed in cortical areas (Ruggiero et al., 1998). The observed increase in prefrontal DA following adjunctive Ida treatment might be secondary to an increase in cortical norepinephrine (NE) release (Gresch et al., 1995) following local presynaptic $\alpha_2$ adrenoceptor blockade by Ida on noradrenergic terminals (Hertel et al., 1999b), thus competing with DA for the NE transporter which is used also by DA (Carboni et al., 1990). In addition, Clz, due to its $\alpha_2$ adrenoceptor blocking property, has been reported to co-release NE and DA from noradrenergic terminals in the occipital cortex (Devoto et al., 2005), suggesting that this event does occur in the PFC as well. If so, this could presumably also happen when Ida, as in the present study, is given as an adjunct to other APDs thus creating a Clz-like pharmacological property profile.

Not surprisingly, adjunctive Ida treatment reversed catalepsy induced by the higher (0.1 mg/kg) dose of Hal in the present study, thus confirming previous observations in other laboratories (cf. Introduction). While in the study by Hertel et al. (1999a) Ida did not significantly reverse Rac-induced catalepsy, there was, however, in that study a clear-cut anti-cataleptic tendency by Ida, in particular at the peak (60 min) time of Rac-induced catalepsy. In apparent discrepancy regarding time-course of action, Hal (0.025 mg/kg) produced significant catalepsy 2 h after administration while having no effects on CAR after the 90-min observation time. However, time-course of action for pharmacological effects on CAR vs. catalepsy are different rather than parallel. Thus, effects on
CAR usually have an earlier onset (around 20 min after drug administration), whereas effects on catalepsy usually peak later (around 1–2 h after drug administration). One of the reasons for this difference might be that effects on CAR mainly reflect a preferential modulation of the DA mesolimbic pathway (Wadenberg et al., 1990), while catalepsy is an indication of decreased dopaminergic activity mainly in the nigrostriatal pathway.

Finally, Clz was recently reported to be superior against suicidality in schizophrenia and schizoaffective disorder (cf. Introduction). Given the fact that $\alpha_2$ adrenoceptor antagonism is a major mechanism of action of antidepressants with blockade of $\alpha_2$ autoreceptors and heteroreceptors on nerve terminals, causing an increased release of both NE and serotonin (see Svensson, 2000), this property might well contribute to Clz’s efficacy against suicidality. Thus, adjunctive Ida to APD treatment may also possibly contribute to reduce suicidality in schizophrenia. It should be noted in this context, however, that in animal models of, for example, neuroleptic-induced depression, the significance of $\alpha_2$ adrenoceptor blockade is less clear (Montgomery et al., 2003).

Our data, showing an enhanced suppression of CAR in rats to a level that would predict sufficient clinical antipsychotic activity, paralleled by a substantial increase in DA output in the mPFC, as well as anti-cataleptic effects, by adjunctive Ida treatment to low doses of APDs, themselves lacking appreciable affinity for the $\alpha_2$ adrenoceptor, confirm and extend our previously published observations on the effects of adjunctive Ida treatment to the selective DA $D_2$ antagonist Rac on CAR and prefrontal DA output. This lends further experimental support for the significance of $\alpha_2$ adrenoceptor blockade in APD efficacy, as well as for a favourable EPS profile, similarly to Clz.

Taken together with recently obtained data from our laboratory, showing that combined treatment with Ida + Rac, similarly to Clz, fully reversed impairment of working memory induced by the NMDA receptor antagonist MK-801 in rats, the present results also suggest that adjunctive Ida treatment to APDs lacking appreciable affinity for the $\alpha_2$ adrenoceptor may not only provide sufficient antipsychotic efficacy at a lower DA $D_2$ receptor occupancy, but might also allow for improved efficacy against some aspects of cognitive impairment in schizophrenia.

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Statement of Interest
None.

References


