Reprinted from Neuropsychiatric Disease and Treatment 2005, 1(3) 253–260 ORIGINAL RESEARCH

Adjunctive treatment with mianserin enhances effects of raclopride on cortical dopamine output and, in parallel, its antipsychotic-like effect

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Abstract: Clinical studies indicate that adjunctive treatment with the antidepressant drug mianserin, a 5-hydroxytryptamine (5-HT)$_{2A/C}$ receptor antagonist and an α_2- and α_1-adrenoceptor antagonist, may enhance the effect of conventional antipsychotic drugs in schizophrenia, in particular on negative symptoms such as withdrawal retardation, akathisia, and some aspects of cognitive impairment. Here, we have examined the effect of mianserin in combination with the selective dopamine (DA) $D_{2/3}$ receptor antagonist raclopride on conditioned avoidance response (CAR), a preclinical test of antipsychotic efficacy with high predictive validity; catalepsy, a preclinical test of extrapyramidal side effect liability; and DA output in the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAC), respectively. Mianserin (5 mg/kg intraperitoneal) significantly enhanced the suppressant effect of a low dose of raclopride (0.1 mg/kg subcutaneous) on CAR without any increase in catalepsy. Administration of raclopride to rats pretreated with mianserin resulted in a large enhancement of DA output in the mPFC and, at the same time, a small but significant reduction in the raclopride-induced DA output in the NAC. These experimental results indicate that adjunctive treatment with mianserin to a typical D_2 antagonist generates an atypical antipsychotic profile.
Keywords: adjunctive mianserin, typical antipsychotic drugs, dopamine, prefrontal cortex, atypicality

Introduction

The clinical effect of conventional (typical) antipsychotic drugs (APDs) has been largely ascribed to their dopamine (DA) D_2 receptor blocking action (Carlsson 1988). However, although these drugs, eg, haloperidol, are usually effective against positive symptoms, they show less efficacy against negative and cognitive symptoms (Carpenter 1996; Volavka et al 1996; Leucht et al 1999). Clinically effective doses of typical APDs generate about 70%–85% D_2 receptor occupancy in brain, but a D_2 receptor occupancy above 80% has been associated with a relatively high risk of extrapyramidal side effects (EPS) (Farde et al 1992). In contrast, some, but not all, of the so-called second generation (atypical) APDs may be clinically effective at lower D_2 occupancy in brain, with clozapine around 45% (Nordstrom 1995), and are associated with a significantly reduced, and in the case of clozapine essentially abolished, EPS liability (Safferman et al 1991). Yet, clozapine shows superior efficacy in treatment-resistant schizophrenia, and moreover, several atypical APDs have been claimed to show improved efficacy against negative and cognitive symptoms (Meltzer 1995; Davis et al 2003). Clozapine possesses a complex pharmacology, acting as an antagonist not only at several DA receptors (ie, D_2, D_3, and D_4 receptors) but also at,

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for example, 5-HT_{2A} and 5-HT_{2C} receptors, α_1- and α_2-adrenoceptors, and muscarinic and histaminergic (H_1) receptors. Moreover, clozapine may function as a partial agonist at D_1 and 5-HT_{1A} receptors (Schotte et al 1996), raising the possibility that several receptor affinities may contribute to the atypical APD profile (Svensson 2003). By contrast, the alternative D_2 receptor "fast-off" hypothesis of Kapur and Seeman (2001) applies only to clozapine and quetiapine and is inconsistent with the "slow" off-rate of most atypical APDs (Meltzer et al 2003).

Previous clinical augmentation trials involving conventional APDs may help elucidate this issue. Thus, the addition of ritanserin, a 5-$HT_{2A/C}$ antagonist, was found to enhance the antipsychotic effect of typical APDs, in particular negative symptoms (Gelders et al 1986, Gelders 1989). Other clinical studies have shown that adjunctive treatment with idazoxan, a selective α_2-adrenoceptor antagonist, can enhance the efficacy of conventional APDs in treatment-resistant schizophrenia (Litman 1993; Litman et al 1996), including both positive and negative symptoms and total Brief Psychiatric Rating Scale score. Moreover, mianserin, a clinically effective antidepressant drug, which acts as an antagonist at 5-$HT_{2A/C}$ receptors, α_2- and α_1-adrenoceptors, and muscarinic and histaminergic (H_1) receptors (Pinder 1991), has also been found to enhance the clinical effect of typical APDs, particularly concerning negative symptoms such as withdrawal retardation, akathisia, and some aspects of cognitive dysfunction (Itil et al 1974; Mizuki et al 1990, 1992; Poyurovsky 1999; Grinshpoon et al 2000; Shiloh et al 2002; Poyurovsky et al 2003). This generates a clozapine-like clinical effect (Lindstrom 2000) as well as a combined receptor binding profile relatively similar to that of clozapine (cf Pinder 1991; Bymaster et al 1996).

Several experimental studies may contribute to clarify the neurobiological mechanisms of particular importance in this respect. Thus, clozapine as well as other atypicals have, in contrast to typical APDs, been found to preferentially enhance DA outflow in the prefrontal cortex (Imperato and Angelucci 1989; Moghaddam and Bunney 1990; Nomikos et al 1994; Kuroki et al 1999; Westerink et al 2001). As previous experimental results using both a neurodevelopmental model and the phencyclidine model of schizophrenia inter alia have indicated an impaired prefrontal DA projection in this disease (see Weinberger and Lipska 1995; Svensson et al 1995; Egan and Weinberger 1997; Egan et al 2001), the selective augmentation of prefrontal DA output by clozapine and other atypicals may allow for restoration of a pathophysiological deficit in schizophrenia, tentatively related to cognitive and/or negative symptoms (see Svensson 2003). Both the addition of ritanserin and idazoxan to the selective $D_{2/3}$ antagonist, raclopride, produced an enhanced suppression of the conditioned avoidance response (CAR) in rodents, ie, an antipsychotic-like effect, without any concomitant increase in catalepsy (Wadenberg et al 1996; Hertel et al 1999), as well as a preferential enhancement of prefrontal DA output not seen with the $D_{2/3}$ antagonist alone (Andersson et al 1995; Svensson et al 1995; Hertel et al 1999). Since subsequent behavioral (Wadenberg et al 1998) and biochemical (Liégeois et al 2002) studies using the selective 5-HT_{2A} receptor antagonist M100907 produced similar results, whereas 5-HT_{2C} receptor blockage activates both cortical and subcortical DA projections (Di Matteo et al 2000; Gobert et al 2000), the preferential enhancement of prefrontal DA efflux by atypicals may be associated with blockade of 5-HT_{2A} rather than 5-HT_{2C} receptors. Thus, clinical as well as preclinical, correlative behavioral, and biochemical studies indicate that an enhanced effect of typical D_2 antagonists in schizophrenia can be obtained by adding 5-HT_{2A}- and/or α_2-receptor blockage and that this effect is indeed associated with enhanced prefrontal DA output.

Here, we have experimentally investigated the effect of combining raclopride and mianserin, which when given alone increases DA output both in the medial prefrontal cortex (mPFC; Tanda et al 1996) and in the nucleus accumbens (NAC; Di Matteo et al 2000) on DA efflux in both terminal areas of the mesocorticolimbic system, as well as by correlative behavioral studies using the CAR paradigm and additional assessment of catalepsy scores.

Materials and methods

Animals

Adult male BK1:WR (Wistar) rats weighing 300–390 g (CAR), 270–310 g (catalepsy), or 230–390 g (microdialysis) were used in all experiments. Animals arrived at least five days prior to experimental use and were housed (4 per cage [Makrolon IV]) in the animal facility under standard laboratory conditions with a 12-h light/dark cycle. Rats designated for microdialysis had lights on at 6.00 am, whereas animals designated for behavioral experiments were subjected to a reversed light/dark cycle, ie, lights off at 6.00 am. All experiments were performed between 8.00 am and 6.00 pm. Food and water were available ad lib. All experiments were approved by, and conducted in accordance with, the local Animal Ethics Committee (Stockholms Norra och Södra Försöksdjursetiska Kommittéer) (permit numbers N216/00, N11/00).

Conditioned avoidance response

A shuttle-box (530 mm × 250 mm × 225 mm) divided into two compartments by a partition was used. The rats were free to move from one compartment to the other via an opening (75 mm × 75 mm) in the partition. Upon presentation of the conditioned stimulus (CS), 80 dB white noise (White Noise Generator, Lafayette 1501, Lafayette, IN, USA), the rat had 10 s to avoid the unconditioned stimulus (USC), an intermittent electric shock in the grid floor of approximately 0.2 mA (intershock interval 2.5 s, shock duration 0.5 s), by moving into the opposite compartment. The following behavioral variables were recorded: (1) avoidance (response to CS within 10 s); (2) escape (response to CS + UCS); (3) escape failure (if the rat was unable to respond to the shock within 50 s the trial was terminated). The animals were trained for 5 consecutive days, and were adapted to the shuttlebox 5 min before the training session started. Each training session consisted of 20 trials randomly distributed over 15 min. All subsequent pre-tests and experimental test sessions consisted of 10 trials randomly distributed over 7.5 min. Experimental manipulations were always preceded by a pre-test. The same animals were tested repeatedly according to a change-over design (Li 1964) serving as their own controls. Only rats that showed at least 90% avoidance on the last day of training were included in the experiments.

Catalepsy

Animals were placed on an inclined (60°) grid and, excluding the first 30 s, the time the rat remained in the same position was measured for a maximum of 2.5 min. The catalepsy was scored from 0–5 according to the time (square root transformation) the animal remained immobile (min): 0 = 0–0.08, 1 = 0.09–0.35, 2 = 0.36–0.80, 3 = 0.81–1.42, 4 = 1.43–2.24, 5 = ≥ 2.25 min; ie, if the rat remained immobile for ≥ 2.25 min it was scored 5, etc (Ahlenius and Hillegaart 1986).

Microdialysis

The probe implantation and dialysis procedure, as well as the biochemical analyses, were similar to those previously described (Hertel et al 1996). Anesthetized male BKl:WR (Wistar) rats (B&K Universal, Sollentuna, Sweden; sodium pentobarbital, 60 mg/kg, intraperitoneal [IP]) were 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 semipermeable membrane (AN69 Hospal) with an active surface length of 4 and 2.25 mm for mPFC and NAC, respectively. Dialysis experiments were conducted approximately 48 h after surgery in freely moving rats. The dialysis probe was perfused with a physiological perfusion solution (147 mmol/L sodium chloride, 3.0 mmol/L potassium chloride, 1.3 mmol/L calcium chloride, 1.0 mmol/L magnesium chloride, and 1.0 mmol/L sodium phosphate, pH 7.4) at a rate of 2.5 µL/min set by a microinfusion pump (Harvard Apparatus, Holliston, MA). Online quantification of DA in the dialysate was accomplished by high pressure liquid chromatography coupled to electrochemical detection. The detection limit for DA was approximately 0.2 fmol/min. The placement of the probe was later verified in slices stained with neutral red.

Drugs

Mianserin (Sigma-Aldrich, Stockholm, Sweden) and raclopride tartrate (Astra Zeneca, Södertälje, Sweden) were dissolved in saline. Mianserin was subsequently adjusted to pH ~ 6 (at a higher pH the drug precipitated) and raclopride was adjusted to physiological pH (7.0–7.3). Mianserin or saline was administered IP (1.0 mL/kg) and raclopride or saline was administered subcutaneously (SC) (1.0 mL/kg). The dose of mianserin was chosen based on previous studies with mianserin in microdialysis (Tanda et al 1996; Di Matteo et al 2000). The doses of raclopride used (0.05–0.1 mg/kg SC) were chosen based on earlier experiments, where no or only moderate effects on CAR (Hertel et al 1999) and low, subtherapeutic (≤65%) D_2 receptor occupancy (Wadenberg, Kapur, et al 2000) have been observed. During microdialysis, administration of drugs or vehicle was performed after stable outflow (< 10% variation) of DA was obtained.

Statistics

Behavioral experiments. Statistical evaluation was performed by means of the Friedman two-way analysis of variance (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).

Microdialysis. Data were calculated as percent changes of basal DA output over time. Baseline (= 100%) was defined as the average of the last two (mPFC) or four (NAC) preinjection values. Data were statistically evaluated using two-way (treatment × time) ANOVA for repeated measures followed by the Newman-Keuls test for multiple comparisons.

Results

Conditioned avoidance response

Raclopride (0.05 or 0.1 mg/kg SC) produced a statistically significant suppression of CAR 20 minutes after administration, but not at later observation times (90 and 240 minutes). Pretreatment with mianserin (5 mg/kg IP 30 min before) produced a significant enhancement of the suppression of CAR induced by raclopride (0.1 mg/kg) compared with animals treated with raclopride alone. Mianserin by itself had no effect on CAR. No significant effects were observed at later observation times, and no escape failures were recorded under any treatment condition; ie, a decrease in avoidance responses was always accompanied by a corresponding increase in escape responses (Figure 1).

Catalepsy

Raclopride (0.1 mg/kg SC) alone, or in combination with mianserin (5 mg/kg IP), did not produce any significant catalepsy at any of the observation times. The administration of a higher dose of raclopride (1.0 mg/kg SC) resulted in a cataleptic response that peaked at 60 minutes after administration. At this time point and dose of raclopride, pretreatment with mianserin (5 mg/kg IP – 30 min) caused a consistent, albeit not significant, decrease in catalepsy scores compared with animals treated with raclopride alone (Figure 2).

Microdialysis

The mean baseline concentration (fmol/min ± SEM) of DA in the mPFC and the NAC was 0.44 ± 0.09 (n = 24) and 4.41 ± 0.61 (n = 24) fmol/min, respectively, (data not corrected for in vitro dialysis probe recovery). Administration of mianserin (5 mg/kg IP) alone, or in combination with raclopride (0.1 mg/kg SC), resulted in a significant increase in DA output in the mPFC, which was significantly larger in the combination-treatment group (Figure 3a). Administration of raclopride (0.1 mg/kg SC) alone had no significant effect on mPFC DA output but resulted in a significant increase in DA output in the NAC. Pretreatment with mianserin (5 mg/kg IP) caused a small, but significant reduction of the effect of raclopride (0.1 mg/kg SC) on NAC DA output. Administration of saline or mianserin (5 mg/kg IP) alone had no effect on NAC DA output (Figure 3b).

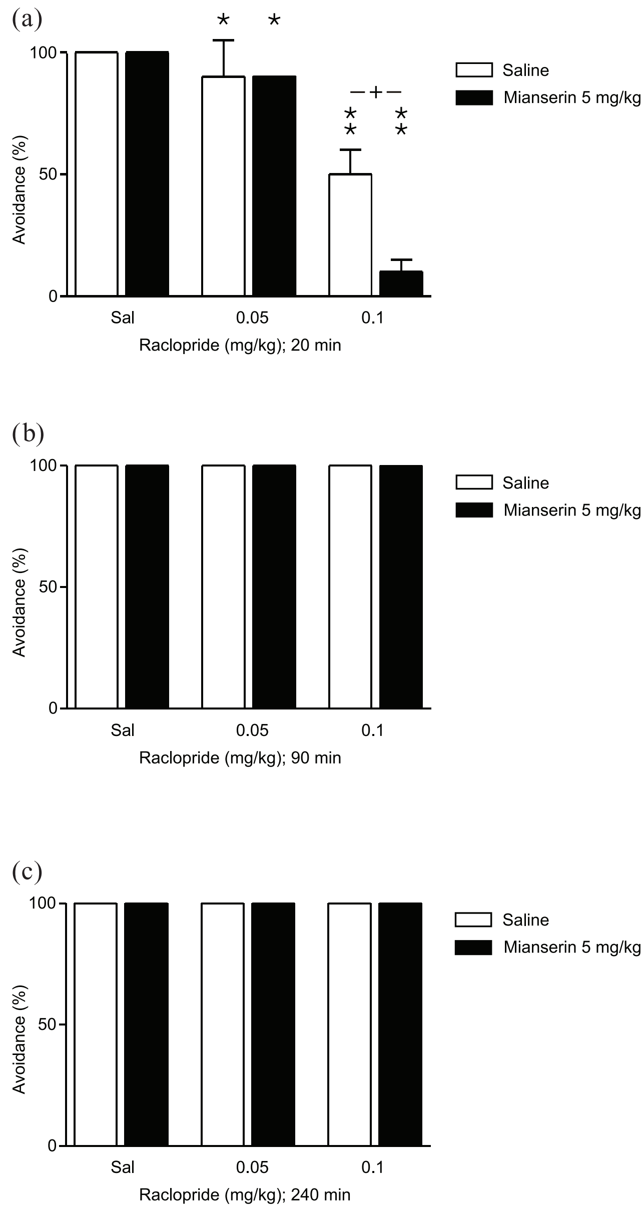

Figure 1 Effects of saline or mianserin (5 mg/kg IP) pre-treatment (30 min) on saline – or raclopride (0.05 and 0.1 mg/kg SC) – induced effects in the conditioned avoidance response test (a) 20 min, (b) 90 min, and (c) 240 min after saline or raclopride administration. Each bar represents the median avoidance % (± semi-interquartile range; n = 9 in all groups). Statistical evaluation was performed by means of the Friedman two-way analysis of variance (ANOVA), followed by the Wilcoxon matched-pairs signed ranks test. ANOVA Chi^2 (df = 5) = 35.5, $p < 0.001$. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with respective control (saline/saline or mianserin/saline). $^{+}p < 0.05$ for comparisons between saline/raclopride and mianserin/raclopride treatment group.

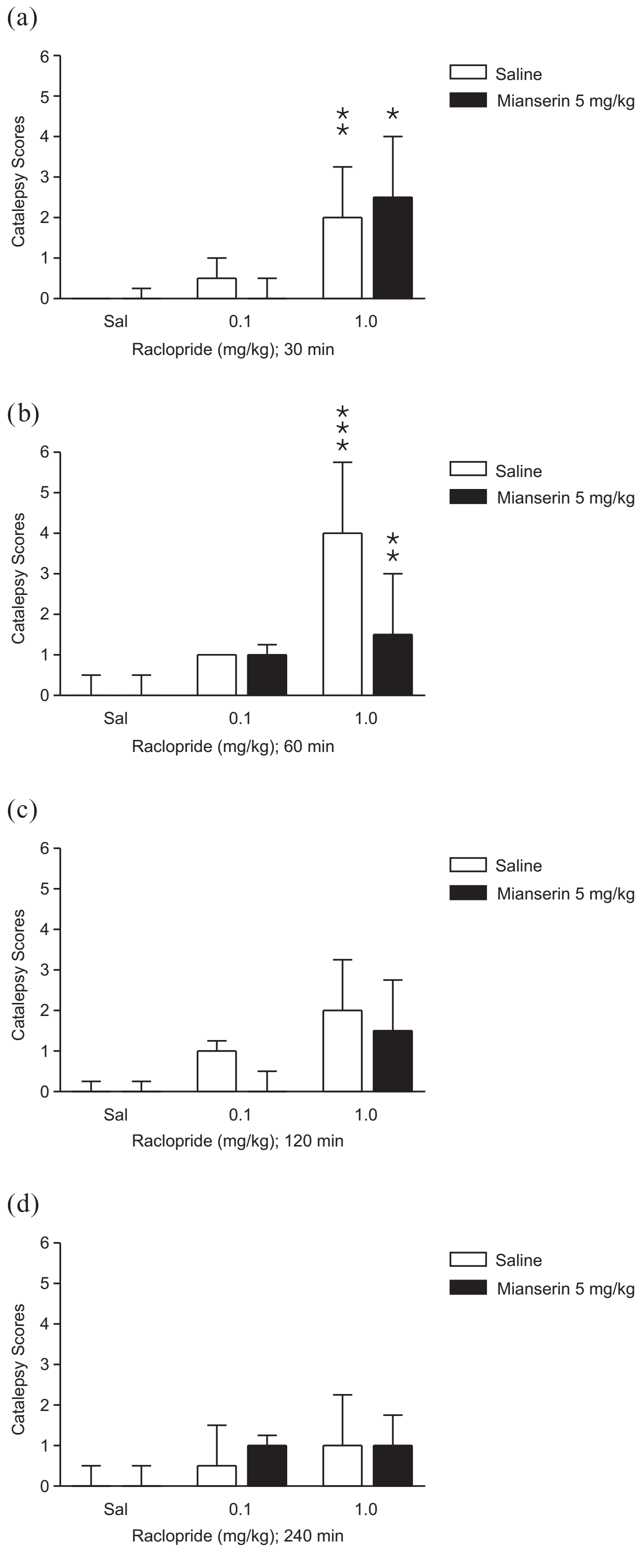

Figure 2 Effects of saline or mianserin (5 mg/kg, IP) pre-treatment (30 min) on saline – or raclopride (0.1 and 1.0 mg/kg SC) – induced effects on catalepsy (a) 30 min, (b) 60 min, (c) 120 min, and (d) 240 min after saline or raclopride administration. Each bar represents the median catalepsy score (± semi-interquartile range; n = 8 in all groups). Statistical evaluation was performed by means of the Kruskal-Wallis one way analysis of variance (ANOVA), followed by the Mann-Whitney U-test. ANOVA Chi^2 (df = 5) = 11.75, $p > 0.05$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with respective control (saline/saline or mianserin/saline).

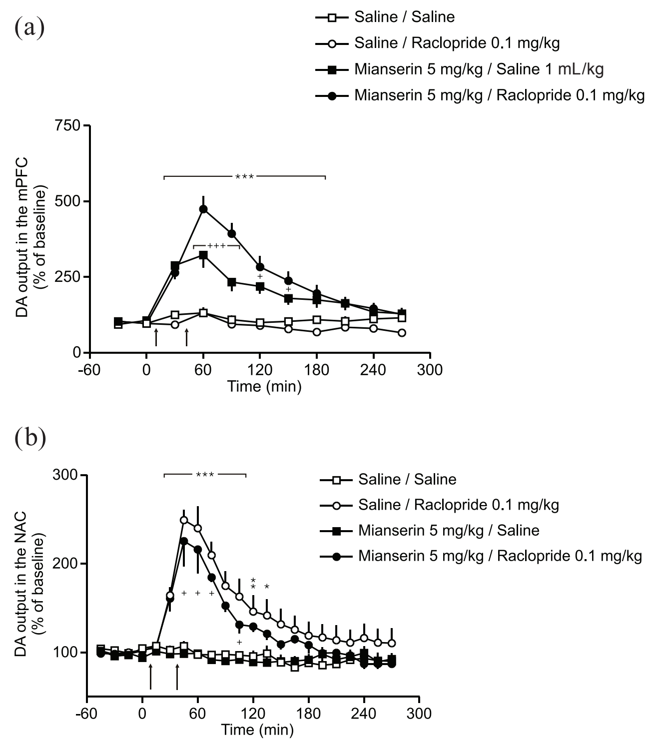

Figure 3 Effects of saline or raclopride (0.1 mg/kg SC) administration on dopamine (DA) output in (a) the medial prefrontal cortex and (b) the nucleus accumbens (NAC) in animals pretreated with saline or mianserin (5 mg/kg IP). Arrows indicate time injections. Each point represents the mean percent (± SEM) change from baseline (n = 6 in all groups). Data were analyzed using two-way (treatment × time) analysis of variance (ANOVA) followed by the Newman-Keuls test for multiple comparisons. (a) $F(\text{treatment}; 3, 19) = 13.99$, $p < 0.001$; $F(\text{time}; 10, 190) = 53.68$, $p<0.001$; and $F(\text{treatment} \times \text{time}; 30, 190) = 16.31$, $p < 0.001$. (b) $F(\text{treatment}; 3, 20) = 10.11$, $p > 0.001$; $F(\text{time}; 21, 420) = 37.22$, $p < 0.001$; and $F(\text{treatment} \times \text{time}; 63, 420) = 11.49$, $p < 0.001$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for difference from saline/saline treatment group. $^{+}p < 0.05$, $^{+++}p < 0.001$ for comparisons between saline raclopride and mianserin/raclopride treatment group.

Discussion

The major finding of the present study is that the addition of mianserin to the selective $D_{2/3}$ receptor antagonist raclopride causes a significant enhancement of the raclopride-induced suppression of CAR without increasing catalepsy scores, providing experimental evidence for an enhanced antipsychotic efficacy of the drug combination compared with raclopride alone, yet without any increased EPS liability. The relatively low doses of raclopride used, that did not cause an effective antipsychotic-like effect when given alone has been observed to generate a central D_2 occupancy of about 50%–65% (Wadenberg, Kapur, et al 2000). Notably, about 80% D_2 occupancy is necessary to produce a suppression of the CAR of such magnitude that it would predict sufficient antipsychotic activity, although at this level of D_2 occupancy catalepsy also begins to occur (Wadenberg, Kapur, et al 2000). Thus, the adjunctive treatment with mianserin allowed for a sufficient antipsychotic-like effect of raclopride even at a dose level of raclopride that by itself was insufficient, but also clearly below its EPS dose-range, ie, generating an atypical antipsychotic profile. Since both treatments with the selective 5-HT_{2A} receptor antagonist M100907 and with the selective α_2-adrenoceptor antagonist idazoxan have been found to potentiate the suppression of CAR by raclopride, blockage of either one of these receptors, or both, by mianserin may be causally related to the enhanced antipsychotic-like effect.

Our results also demonstrate that the addition of mianserin to the selective $D_{2/3}$ receptor antagonist, raclopride, induced a preferential increase in prefrontal DA output, in contrast to the effect of the $D_{2/3}$ antagonist alone but analogous to the effect of clozapine and, to a varying degree, most atypical APDs (cf *Introduction*), including aripiprazole (Li et al 2004). As previously mentioned, this biochemical effect may to a significant extent be related to the 5-HT_{2A} receptor antagonistic effect of mianserin. However, given the fact that a similar, and even more potent, enhancement of the raclopride-induced prefrontal DA efflux has been observed by adjunctive treatment with idazoxan (Hertel et al 1999), which also enhances the effect of typical APDs in treatment-resistant schizophrenia (cf *Introduction*), the potent α_2-receptor antagonistic property of mianserin may well be equally important in this regard. Indeed, previous experimental evidence (Hertel et al 1999) indicates that an increased prefrontal DA output per se may be causally related to enhanced antipsychotic-like effect as assessed by the CAR test. A recent study indicates that addition of mianserin to typical APDs improves performance in neurocognitive tests compared with the effect of typical APDs alone (Poyurovsky et al 2003). Such a pro-cognitive effect, if confirmed by other studies, may clearly be related to an enhanced cortical DA output. Notably, this effect of adjunctive mianserin may not be confined to the prefrontal cortex alone, since recently mianserin, in full analogy with clozapine, was shown to increase dialysate levels of DA also in the parietal and occipital cortex (Valentini et al 2004). A potential cognitive enhancing effect of adding mianserin to conventional D_2 antagonists seems important as the degree of cognitive impairment largely determines treatment outcome in schizophrenia, especially social functioning (Green 1996; Harvey 1998).

In contrast to ritanserin or idazoxan, the addition of mianserin to raclopride also caused a slight, but still significant suppression of the raclopride-induced increase in DA outflow in the subcortical brain region studied; ie, NAC, an effect that in itself is secondary to the D_2 receptor blockade (see Carlsson 1988). Previous studies show that both the α_1-adrenoceptor antagonist prazosin and the selective 5-HT_{2A} receptor antagonist M100907 inhibit the D_2 receptor antagonist-induced increase in accumbal DA release (Andersson et al 1995; Liégeois et al 2002) and, moreover, that prazosin (Wadenberg, Hertel, et al 2000) in similarity with M100907 (cf *Introduction*) also enhances the CAR suppression induced by D_2 antagonists. Thus, both the 5-HT_{2A} receptor antagonistic action and the α_1-adrenoceptor antagonistic effect of mianserin may, in principle, contribute to the slight inhibition of the raclopride-induced accumbal DA release observed in the present study and, by inference, to the suppression of the CAR. Such a mechanism may also have bearing on the overall anti-psychotic efficacy of combining mianserin and typical D_2 receptor antagonists in schizophrenia, albeit not necessarily related to relief of negative symptoms. This notion gains further support from both preclinical and clinical observations. Thus, both prazosin (Mathé et al 1996) and M100907 (Schmidt and Fadayel 1995) act to suppress evoked, but not basal DA release in the NAC of experimental animals, and clinical studies indicate that schizophrenic patients display an enhanced evoked, but not basal subcortical DA release (Laruelle et al 1996; Breier et al 1997).

In conclusion, several pharmacological properties of mianserin, namely blockade of 5-HT_{2A} receptors, α_2- as well as α_1-adrenoceptors may contribute to its clinical effect when added to typical D_2 receptor antagonists in the treatment of schizophrenia, which appears to include not only an effect on negative symptoms, but also a reduction in dysphoria, anxiety, akathisia, and potentially cognitive dysfunction. Yet, the sedative effect of mianserin may, as in the case of clozapine, largely be correlated with its histamine H_1 receptor antagonistic action, which may contribute to weight gain. Several of the new, second generation APDs are relatively expensive in comparison with conventional APDs, as pointed out by Lindstrom (2000). Combining typical APDs with mianserin might thus represent a more affordable treatment strategy in some areas of the world. In addition, by using a combination of mianserin and typical D_2 antagonists one serious side-effect of clozapine, namely agranulocytosis, can be avoided. Tentatively, also mirtazapine, which has a receptor affinity profile similar to mianserin, may be used for the same purpose (Berk et al 2001).

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