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# Noradrenaline Reuptake Inhibition Enhances the Antipsychotic-like Effect of Raclopride and Potentiates $D_2$-blockage–induced Dopamine Release in the Medial Prefrontal Cortex of the Rat

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*We have previously observed that addition of an $\alpha_2$-adrenoceptor antagonist to a selective dopamine (DA) $D_2$ receptor antagonist enhances the antipsychotic-like effect of the $D_2$ blocker and also selectively increases DA output in the medial prefrontal cortex (mPFC) in rats. These data also correlate well with previous clinical trials showing augmentation by an equivalent drug combination in schizophrenia. Since the selective noradrenaline reuptake inhibitor reboxetine was found to cause similar effects on the mesolimbocortical DA system as $\alpha_2$-adrenoceptor antagonists, the present study was undertaken to explore whether also reboxetine might augment the effect of the DA $D_2$ receptor antagonist raclopride in the same preclinical model of antipsychotic activity, the conditioned avoidance response (CAR) test. We also investigated the effect of this combination in the catalepsy test for extrapyramidal side effect liability, as well as on DA output in the mPFC and the nucleus accumbens, respectively. Reboxetine (6 mg/kg, i.p.) significantly enhanced the suppressant effect of raclopride (0.1 mg/kg, s.c.) on CAR without affecting catalepsy. Administration of raclopride (0.1 mg/kg, s.c.) to rats pretreated with reboxetine (6 mg/kg, i.p.) resulted in a greatly enhanced effect on DA output in the mPFC but not in the nucleus accumbens when compared with raclopride alone. Consequently, these results suggest that noradrenaline reuptake inhibition may provide means of augmenting the efficacy of classical $D_2$-antagonists in the treatment of schizophrenia, and, in principle, to generate an atypical antipsychotic drug profile.*

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Classical pharmacotheraphy of schizophrenia is based upon blockage of central postsynaptic dopamine (DA) $D_2$ receptors (see Carlsson 1988; Farde et al. 1988). However, while being efficient in ameliorating positive symptoms, most of the typical antipsychotic drugs exhibit only a limited efficacy on, and may even exacerbate, negative symptoms in schizophrenia (Carpenter 1996). In addition, since clinically adequate dosage of



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typical neuroleptics results in levels of $D_2$ receptor occupancy as high as 75 percent, the use of these drugs often induce parkinsonism or other extrapyramidal side effects (EPS), which become manifest at approximately 80–85% $D_2$ receptor occupancy (Farde et al. 1992). In contrast to the clinical profile of typical $D_2$ receptor antagonists, clozapine, which unfortunately may cause serious side effects such as agranulocytosis, is efficacious at considerably lower levels of $D_2$ receptor occupancy (Farde et al. 1992), and rarely induces EPS (see Safferman et al. 1991). The diminished induction of EPS, as well as a purportedly advantageous effect on negative and certain cognitive symptoms in schizophrenia, has led to the definition of clozapine as the prototypical atypical antipsychotic drug (Kane et al. 1988; Meltzer et al. 1989). Theories regarding the mode of action of typical versus atypical antipsychotics have emphasized the frequently potent antagonistic effect of the atypicals on serotonin $(5\text{-HT})_{2A}$ receptors and/or $\alpha_1$-adrenoceptors (Meltzer et al. 1989; see Svensson et al. 1995), as well as their differential effect on regional DA neurotransmission. Thus, whereas classical antipsychotic drugs mainly enhance extracellular DA concentrations in subcortical brain regions, atypical antipsychotics preferentially increase DA, as well as noradrenaline (NA), concentrations in the medial prefrontal cortex (mPFC) of experimental animals (Imperato and Angelucci 1989; Moghaddam and Bunney 1990; Nomikos et al. 1994; Westerink et al. 2001), an effect that to a large extent seems to be mediated locally in the mPFC (Gessa et al. 2000).

Among other properties, clozapine displays considerable affinity also for $\alpha_2$-adrenoceptors (see Ashby and Wang 1996), and we recently reported that addition of an $\alpha_2$-adrenoceptor antagonist to a selective $D_2$ receptor antagonist, results in a markedly enhanced DA output specifically in the mPFC, as well as an enhancement of the $D_2$ receptor blockage induced effect in the conditioned avoidance response (CAR) paradigm, a preclinical test with high predictive validity for clinical antipsychotic effect (Arnt 1982; see Wadenberg and Hicks 1999). Thus, when adding the $\alpha_2$-adrenoceptor antagonist idazoxan to the $D_2$ receptor blocking regimen, an antipsychotic-like effect was obtained in spite of a considerably lower dose of the $D_2$-blocker being used (Hertel et al. 1999a), yet without any concomitant increase in catalepsy, thus indicative of an atypical antipsychotic profile of the drug combination. This notion is also supported by previous clinical data demonstrating an augmenting effect of idazoxan on the therapeutic efficacy of haloperidol (Litman et al. 1996).

Reboxetine is a new selective NA reuptake inhibitor (NRI), clinically used as an antidepressant, which unlike the tricyclic antidepressants shows low affinity for the muscarinic, cholinergic and adrenergic receptor families (Wong et al. 2000). We have recently observed that the effects of reboxetine on the mesolimbocortical DA system in the rat show several similarities to the effects induced by the $\alpha_2$-adrenoceptor antagonist idazoxan (Hertel et al. 1999b, Linnér et al. 2001). Thus, systemic, and local, administration of reboxetine, in similarity with idazoxan, was found to preferentially increase DA release in the mPFC when compared with the nucleus accumbens (NAC). In addition, both $\alpha_2$-adrenoceptor antagonism by idazoxan and NA reuptake inhibition by reboxetine increases burst firing of DA neurons in the ventral tegmental area (Grenhoff and Svensson 1993; Linnér et al. 2001).

Due to the similarities between the effects of $\alpha_2$-adrenoceptor antagonism and NA reuptake inhibition on central dopaminergic systems, the present study was undertaken to evaluate the potential augmentation by reboxetine of the antipsychotic-like effect of the selective $D_2$ receptor antagonist raclopride (Köhler et al. 1985), utilizing the same experimental paradigm as our previous study with idazoxan (Hertel et al. 1999a).

## MATERIALS AND METHODS

### Animals

Adult male BK1:WR (Wistar) rats weighing 300–380 g (CAR), 260–310 g (catalepsy) or 255–360 g (microdialysis), were used in all experiments. Animals arrived at least five days prior to experimental use and were housed (four in each cage (Makrolon IV)) in the animal facility under standard laboratory conditions with a 12 h light/dark cycle. For rats designated for microdialysis lights on was 6:00 A.M., whereas animals designated for behavioral experiments were subjected to a reversed light/dark cycle: lights off at 6:00 A.M. All experiments were performed between 8:00 A.M. and 6:00 P.M. Food and water was available ad lib. All experiments were approved by, and conducted in accordance with, the local Ethical Committee (Stockholms Norra och Södra Försöksdjursetiska Kommittéer).

### Conditioned Avoidance Response

A conventional shuttle-box (530 × 250 × 225 mm), divided into two compartments by a partition, was used. Upon presentation of the conditioned stimulus (CS), 80 dB white noise, the rat had 10 s to move into the opposite compartment in order to avoid the unconditioned stimulus (UCS), an intermittent shock of approximately 0.6 mA in the grid floor (inter-shock interval 2.5 s, shock duration 0.5 s). The following variables were recorded: (1) avoidance (response to CS within 10 s); (2) escape (response to CS + UCS within 50 s); (3) failure (failure to escape within 50 s). Following an adaptation time of five minutes each day, the animals were trained for five consecutive days in a session of 20 trials randomly distributed over 15 min. All experimental sessions con-

sisted of 10 trials randomly distributed over 7.5 min, and were performed 20, 90 and 240 min after the last drug administration. Experimental manipulations were always preceded by a 10 trial pretest session (see also Wadenberg and Ahlenius 1991). Experiments in individual rats were performed every third day.

### 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. Experiments were performed 30, 60, 120 and 240 min after last drug injection. The catalepsy was scored from 0 to 5 according to the time (square root transformation) the animal remained immobile: score 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 (see Ahlenius and Hillegaart 1986).

### Microdialysis

The probe implantation and dialysis procedure as well as the biochemical analyses were similar to those that we have previously described (Hertel et al. 1996). Anesthetized (sodium pentobarbital, 60 mg/kg, intraperitoneally (i.p.)) animals were implanted with a dialysis probe in the mPFC or NAC (AP: +3.0, +1.6 ML: 0.6, 1.4; DV: −5.2, −8.2, respectively, relative to bregma and dural surface; Paxinos and Watson 1998). The coordinates for the mPFC probe results in a placement in the rat prelimbic cortex, which has many similarities to the primate medial prefrontal cortex (see e.g. Preuss 1995), and is thus referred to as the rat mPFC. Dialysis occurred through a semipermeable membrane (AN69 Hospal; cut-off 40,000 Da) with an active surface length of 4 and 2.25 mm for mPFC and NAC, respectively. The outer diameter of the probe (0.3 mm) was considered too large to be specifically implanted in the NAC subregions core or shell. Dialysis experiments were conducted approximately 48 h after surgery in 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 mM sodium phosphate, pH 7.4) at a rate of 2.5 μl/min set by a microinfusion pump (Harvard Apparatus). Samples were collected for 30 and 15 min for the mPFC and the NAC, respectively. On-line quantification of DA and dihydroxyphenylacetic acid (DOPAC) in the dialysate was accomplished by high performance liquid chromatography coupled to electrochemical detection. The detection limit for DA was approximately 0.2 fmol/min. The location of the probes were later verified in slices stained with neutral red.

### Drugs

Reboxetine (Pharmacia Corp., Kalamazoo, MI) and raclopride tartrate (Astra Arcus, Södertälje, Sweden) were dissolved in saline (0.9% NaCl) and subsequently adjusted to physiological pH (7.0 – 7.3). Reboxetine or vehicle was administered i.p. (1.0 ml/kg) and raclopride or vehicle was administered subcutaneously (s.c.; 1.0 ml/kg). The dose of reboxetine used (6 mg/kg, i.p.) was based on earlier experiments, where an effect of reboxetine on both prefrontal DA outflow and electrophysiological activity of midbrain DA neurons in vivo was obtained in the dose interval used, which also generated plasma concentrations of reboxetine grossly of the same magnitude as the plasma levels in humans undergoing chronic treatment (see Linnér et al. 2001). The doses of raclopride used (0.05–0.1 mg/kg, s.c.) were chosen based on earlier experiments, where moderate effects on CAR (Hertel et al. 1999a) as well as subthreshold (<80%) $D_2$-receptor occupancy (Wadenberg et al. 2000) have been observed. During microdialysis, administration of drugs or vehicle was performed after a stable outflow (<10% variation) of DA and DOPAC.

### Statistics

***Behavioral Experiments.*** Statistical evaluation was performed by means of the Friedman 2-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test (CAR) or by means of the Kruskal-Wallis 1-way ANOVA, followed by the Mann-Whitney U-test (Catalepsy; Siegel and Castellan 1988).

***Microdialysis.*** Data were calculated as changes of basal DA and DOPAC output over time or as average percent change of baseline during two hours after last drug injection. Baseline (= 100%) was defined as the average of the last two preinjection values. Data were statistically evaluated using 2-way (Treatment X Area or Treatment X Time) analysis of variance (ANOVA) for repeated measures followed by the Newman-Keuls test for multiple comparisons.

## RESULTS

### Conditioned Avoidance Response

The dopamine $D_2$ receptor antagonist raclopride (0.05 or 0.1 mg/kg, s.c.) produced a statistically significant suppression of CAR at the 20 min observation time, but not at later observation times (90 and 240 min). Pretreatment with the NRI reboxetine (6 mg/kg, i.p. +30 min) produced an enhancement of the raclopride-induced suppression of CAR (20 min after raclopride administration). This effect was statistically significant with the higher dose of raclopride. Reboxetine alone exerted no significant effect on CAR (Figure 1, panel A). No signif-

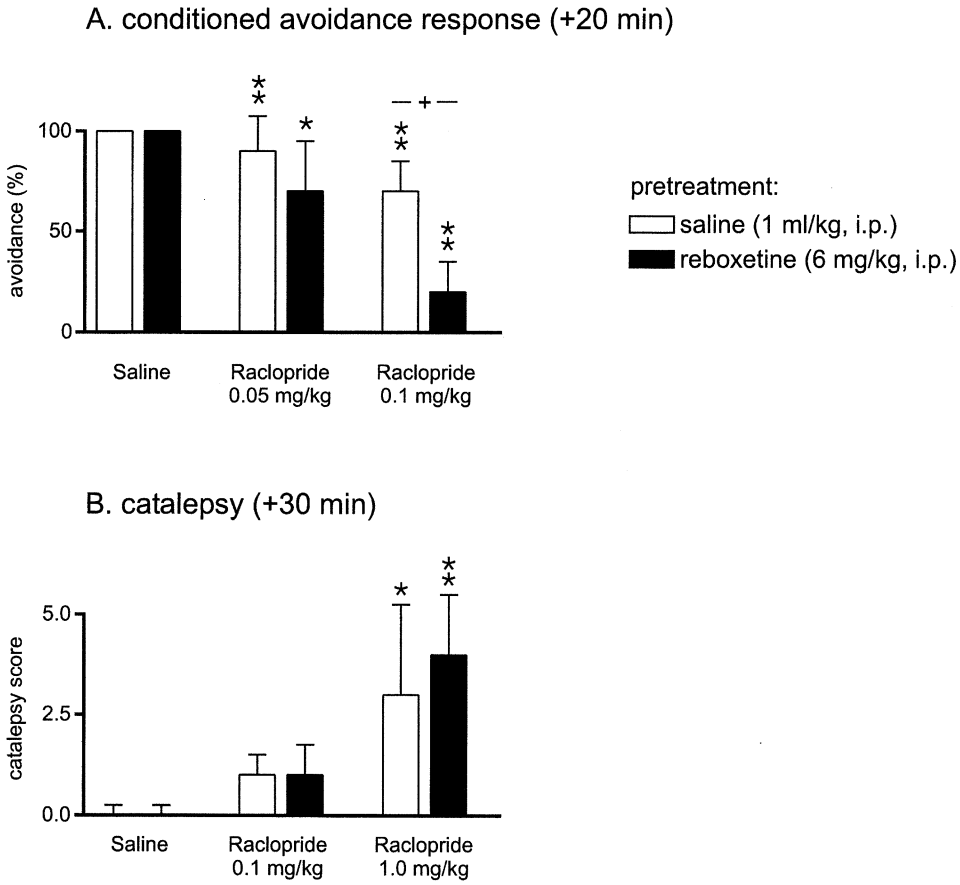


**Figure 1.** Effects of raclopride alone, or in combination with reboxetine (6 mg/kg, i.p.) on: A. Conditioned avoidance response. Each bar represents the median avoidance (± semi-interquartile range; n = 11 in all groups). Statistical evaluation was performed by the Friedman 2-way ANOVA, followed by the Wilcoxon matched-pairs signed-ranks test. ANOVA $\chi^2$ (df = 5) = 39.10, $p < .001$. B. Catalepsy. Each bar represents the median catalepsy score (± semi-interquartile range; n = 8 in all groups). Statistical evaluation was performed by the Kruskal-Wallis 1-way ANOVA, followed by the Mann-Whitney U-test. ANOVA $\chi^2$ (df = 5) = 19.49, $p < .01$. *$p < .05$, **$p < .01$ compared with respective control group (saline/saline or reboxetine/saline). $^{+}p < .05$, $^{+++}p < .001$ for comparisons between saline/raclopride and reboxetine/raclopride treatment group.

icant effects were obtained at later observation times (data not shown), and no escape failures in the CAR test were recorded under any treatment conditions in the CAR test (i.e. a decrease in CAR responding was always accompanied by a corresponding increase in number of escape responses). Thus, escape values are correlated to avoidance as (100% − % avoidance).

### Catalepsy

Raclopride (0.1 mg/kg, s.c.) alone, or in combination with reboxetine (6 mg/kg i.p.), did not produce any significant catalepsy at any of the observation times. The administration of a higher dose of raclopride (1.0 mg/kg, s.c.) resulted in a cataleptic response at the 30 min observation time, which was not significantly affected by pretreatment with reboxetine (6 mg/kg, i.p. +30 min; Figure 1, panel B). The catalepsy produced by the high dose of raclopride, alone or in combination with reboxetine, was still present at the 60 min observation time but not at later observation times (data not shown).

### Microdialysis

The mean baseline concentration (fmol/min ± SEM) of DA or DOPAC in dialysate samples from the mPFC and the NAC was 0.25 ± 0.03 or 28.63 ± 1.87 (n = 24) and 2.31 ± 0.49 or 470.1 ± 59.82 (n = 10) fmol/min, respectively. Data were not corrected for in vitro dialysis probe recovery. Administration of reboxetine (6 mg/kg, i.p.) alone, or in combination with raclopride (0.1 mg/kg, s.c.), resulted in a significant increase in DA output in the mPFC, which was significantly larger in the combination-treatment group (Figure 2, panel A). Administration of saline or raclopride (0.1 mg/kg, s.c.) had small, apparently enhancing, effects on mPFC DA output, which did not reach criterion for statistical significance, however. The enhancing effect of raclopride (0.1 mg/kg, s.c.) on NAC DA levels was not affected by pretreatment with reboxetine (6 mg/kg, i.p.; Figure 3, panel A).

Administration of raclopride (0.1 mg/kg, s.c.) alone, or in combination with reboxetine (6 mg/kg, i.p.), significantly enhanced DOPAC levels in the mPFC (Figure 2, panel B) and the NAC (Figure 3, panel B) to a similar extent in both groups, whereas administration of reboxetine alone (6 mg/kg, i.p.) significantly reduced mPFC DOPAC levels when compared with the control group (Figure 2, panel B).

## DISCUSSION

The major finding of the present study is that the suppressant effect of raclopride in the CAR test, when given in a dose resulting in approximately 65% $D_2$-

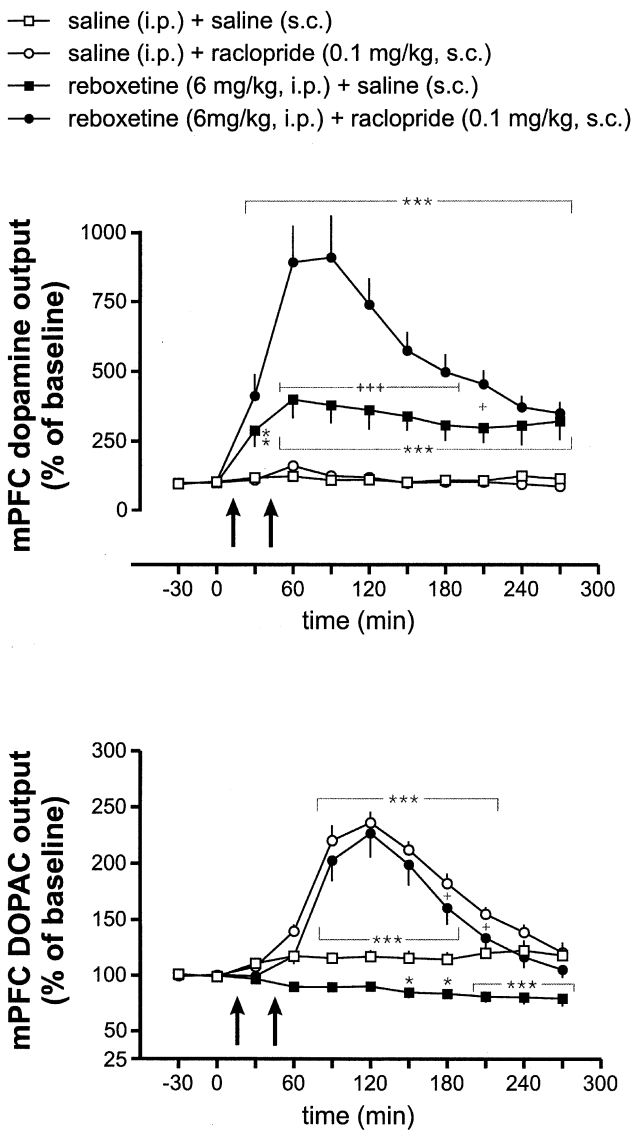


**Figure 2.** Effects of saline or raclopride (0.1 mg/kg, s.c.) administration on (upper panel) dopamine and (lower panel) DOPAC output in the medial prefrontal cortex in animals pretreated with saline or reboxetine (6 mg/mg, i.p.). Arrows indicate time of injections. Each point represents the mean percent (± SEM) change from baseline (n = 6 in all groups). Data was analyzed using 2-way (Treatment X Time) ANOVA followed by the Newman-Keuls test for multiple comparisons. Upper panel. $F(Treatment)_{3,20} = 20.89$, $p < .001$; $F(Time)_{10,200} = 38.67$, $p < .001$ and $F(Treatment\ X\ Time)_{30,200} = 16.97$, $p < .001$. Lower panel. $F(Treatment)_{3,20} = 23.24$, $p < .001$; $F(Time)_{10,200} = 75.88$, $p < .001$ and $F(Treatment\ X\ Time)_{30,200} = 25.94$, $p < .001$. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$ for difference from saline/saline treatment group. $+p < .05$, $+++p < .001$ for comparisons between saline/raclopride and reboxetine/raclopride treatment group.

receptor occupancy in the rat (Wadenberg et al. 2000), was significantly augmented by additional treatment with reboxetine, without any concomitant effect on catalepsy scores (Figure 1). Thus, NA reuptake inhibition and $\alpha_2$-adrenoceptor blockage, two drug regimens that primarily act on, and enhance, central NA neurotransmission (see Dennis et al. 1987), may both serve to enhance the antipsychotic-like effect of $D_2$-receptor blockade, tentatively by means of their principally common effects on mesolimbocortical neurotransmission. Indeed, as observed also with idazoxan, reboxetine, when added to raclopride, strongly and selectively enhanced the DA extracellular concentrations in the mPFC (Figure 2 and Figure 3), an observation that is consistent with previous experiments using other NRIs under similar conditions (Carboni et al. 1990; Yamamoto and Novotney 1998; Westerink et al. 2001). The large increase in prefrontal DA output induced by the drug combination, as well as the almost negligible effect of raclopride alone (Figure 2, panel A), may indicate that the increase in DA output in the mPFC induced by NRIs alone is diminished by concomitant activation of terminal $D_2$ autoreceptors, which under normal conditions may be under poor tonic activation (see also Westerink et al. 2001). This interpretation is also supported by the observed reduction of extracellular DOPAC levels by reboxetine alone (Figure 2, panel B). The enhancing effect of reboxetine on DA output in the mPFC is probably in large part related to the fact that a significant proportion of DA in the prefrontal cortex may be removed from the extracellular space by the NA transporter (Gresch et al. 1995; Yamamoto and Novotney 1998; see Linner et al. 2001), which is blocked by reboxetine. In addition, the enhanced extracellular concentration of NA, produced by reboxetine (Sacchetti et al. 1999), should cause an enhanced occupation of the NA transporter proteins per se, thereby limiting its efficacy in DA reuptake.

Thus, reboxetine, as well as the $\alpha_2$-adrenoceptor antagonist idazoxan and the unselective 5-$HT_{2A}$ receptor antagonist ritanserin, in conjunction with an enhanced preclinical antipsychotic-like effect of raclopride (Figure 1, panel A, Wadenberg et al. 1996; Hertel et al. 1999a), all induced a large increase in DA output in the mPFC, but not in subcortical regions (Figure 3, Andersson et al. 1995; Hertel et al. 1999a) when co-administered with raclopride, hence generating a biochemical profile similar to most so-called atypical antipsychotic drugs (see Westerink et al. 2001). This observation gains further interest in view of clinical reports on the augmentation by the $\alpha_2$-adrenoceptor antagonist idazoxan (Litman et al. 1996), as well as the 5-$HT_{2A}/\alpha_2$-receptor antagonists mianserin (Mizuki et al. 1990; Grinshpoon et al. 2000) and mirtazapine (Berk et al. 2001) of the clinical effect of typical antipsychotics, especially in the treatment of negative symptoms.

Current theories of the underlying pathophysiological mechanisms in schizophrenia suggest a prefrontal hypodopaminergic state, especially in relation to negative and/or cognitive symptoms (Weinberger 1987; Svens-

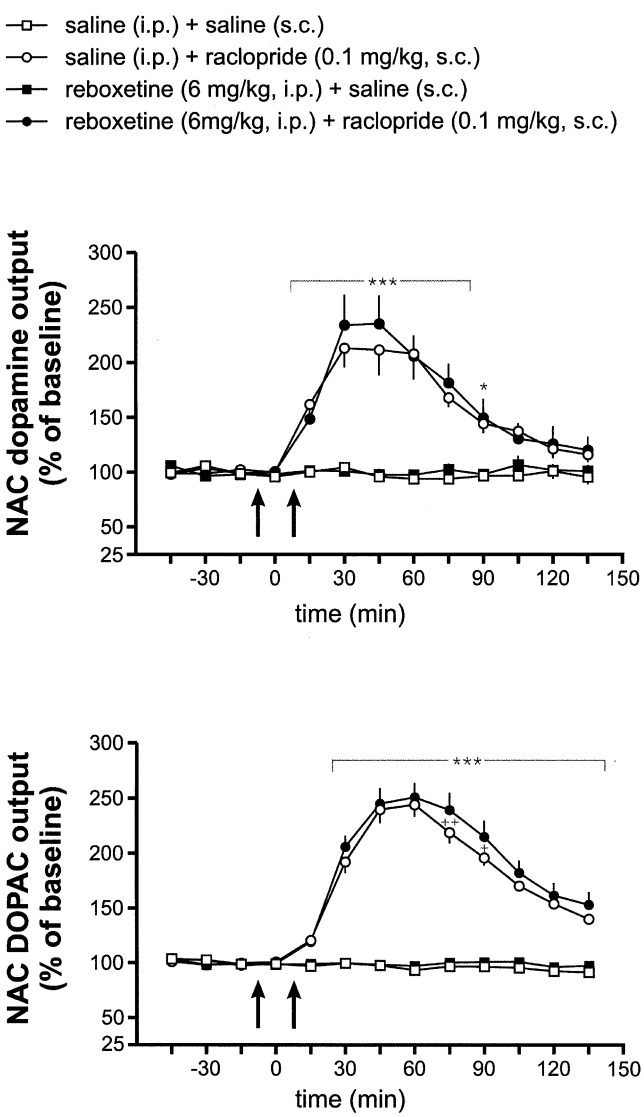


**Figure 3.** Effects of saline or raclopride (0.1 mg/kg, s.c.) administration on (upper panel) dopamine and (lower panel) DOPAC output in the nucleus accumbens in animals pretreated with saline or reboxetine (6 mg/mg, i.p.). Arrows indicate time of injections. Each point represents the mean percent (± SEM) change from baseline (n > 4 in all groups). Data was analyzed using 2-way (Treatment X Time) ANOVA followed by the Newman-Keuls test for multiple comparisons. Upper panel. $F(Treatment)_{3,14} = 14.06$, $p < .001$; $F(Time)_{12,168} = 26.12$, $p < .001$ and $F(Treatment\ X\ Time)_{36,168} = 9.42$, $p < .001$. Lower panel. $F(Treatment)_{3,14} = 67.20$, $p < .001$; $F(Time)_{12,168} = 99.75$, $p < .001$ and $F(Treatment\ X\ Time)_{36,168} = 35.88$, $p < .001$. $^{*}p < .05$, $^{***}p < .001$ for difference from saline/saline treatment group. $+p < .05$, $++p < .01$ for comparisons between saline/raclopride and reboxetine/raclopride treatment group.

son et al. 1993, 1995; Jentsch et al. 1997). Accordingly, the ability of atypical antipsychotics to selectively enhance prefrontal DA output has been suggested to play a role in their advantageous clinical profile. Consequently the present study indicates that adjunctive treatment with NRIs may enhance the antipsychotic effect of classical neuroleptics, tentatively reducing the level of $D_2$-receptor occupancy (i.e. dosage of neuroleptic) required for an antipsychotic effect, resulting in less EPS induction and less cognitive impairment (see Castner et al. 2000). In addition, given the antidepressant effect of NRIs alone, an enhanced therapeutic effect on negative symptoms may also be suggested on this ground. Indeed, the antipsychotic drug zotepine, which appears to increase prefrontal DA extracellular concentrations by concomitant NA reuptake inhibition and $D_2$-receptor blockage (Rowley et al. 2000), seems to display an atypical clinical profile, both with regard to EPS induction as well as effect on negative symptoms (Petit et al. 1996).

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