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Antipsychotic drugs classified by their effects on the release of dopamine and noradrenaline in the prefrontal cortex and striatum

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Abstract

Dose–effect curves were established for the effects of the antipsychotic drugs haloperidol, clozapine, olanzapine, risperidone and ziprasidone on extracellular levels of dopamine and noradrenaline in the medial prefrontal cortex, and of dopamine in the striatum. Haloperidol was more effective in stimulating the release of dopamine in the striatum, whereas clozapine was much more effective in the medial prefrontal cortex. The efficacy of risperidone, olanzapine and ziprasidone did not differ for the two brain areas. The benzamides sulpiride and raclopride increased dopamine release in the striatum but did not affect the release of dopamine and noradrenaline in the medial prefrontal cortex. In the presence of dopamine/noradrenaline reuptake inhibitors, the benzamides strongly increased the release of dopamine—but not of noradrenaline—in the medial prefrontal cortex. The 5-HT_2 receptor antagonist *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (MDL100,907) (800 nmol/kg) and the dopamine D_2 receptor antagonist raclopride (2 μmol/kg) displayed a clear synergism in increasing the release of dopamine in the medial prefrontal cortex. No such synergism was seen in the case of noradrenaline. Co-administration of the 5-HT_2 receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine HCl (DOI) (850 nmol/kg) with clozapine (10 μmol/kg) or haloperidol (800 nmol/kg) blocked the increase in dopamine as well as noradrenaline in the medial prefrontal cortex. It is concluded that typical and non-benzamide atypical antipsychotics increase extracellular dopamine in the medial prefrontal cortex via a synergistic interaction by blocking 5-HT_2 as well as dopamine D_2 receptors. The increase in extracellular noradrenaline in the medial prefrontal cortex that was observed after administration of antipsychotics is explained by inhibition of 5-HT_2 receptors and not dopamine D_2 receptors. Finally, the significance of the classification of antipsychotic drugs based on their selective action on the release of dopamine and noradrenaline in the medial prefrontal cortex is discussed. In particular, the position of the benzamides is discussed.

Keywords: Antipsychotic drug; Prefrontal cortex; Striatum

1. Introduction

All antipsychotic drugs increase the release of dopamine in the striatum or mesolimbic-mesocortical areas (e.g. Carlsson and Lindqvist, 1963; Moghaddam and Bunney, 1990; Volonté et al., 1997; Westerink et al., 1998; Kuroki et al., 1999). This effect is often explained by a negative feedback receptor response that is triggered by blockade of dopamine D_2 autoreceptors. The feedback mechanism is proposed to enhance the release of dopamine via two different mechanisms. First it may stimulate the nerve impulse flow activity of dopamine neurons. This effect is exerted directly by dopamine D_2 receptors located on dopaminergic cell bodies, or indirectly via neuronal loops (Bunney et al., 1973; Melis et al., 1999). Secondly, the dopamine release may be mediated by presynaptic autoreceptors localized on dopamine nerve terminals (Di Chiara et al., 1977; Westerink and De Vries, 1989).

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Various authors have emphasized that the so-called "atypical antipsychotics", such as clozapine, amperozide, olanzapine and risperidone stimulate the release of dopamine more potently in mesolimbic-mesocortical innervated brain regions (including the nucleus accumbens shell) and in the medial prefrontal cortex, than in the striatum (Moghaddam and Bunney, 1990; Nomikos et al., 1994; Hertel et al., 1996; Kuroki et al., 1999). It is assumed that this selective action is associated with a lower incidence of extrapyramidal side effects in the clinic. In addition, this enhancement of cortical dopamine release may underlie the efficacy of these drugs against negative symptoms, since the negative symptomatology in schizophrenia has been hypothesized to be associated with a functional impairment of mesocortical dopaminergic transmission (Weinberger and Lipska, 1995).

We have recently shown that a series of antipsychotic agents caused identical increases in extracellular levels of dopamine and noradrenaline in the medial prefrontal cortex (Westerink et al., 1998). This somewhat unexpected observation prompted us to further investigate the mechanism by which antipsychotic drugs affect the release of dopamine and noradrenaline in the medial prefrontal cortex.

Here, we compare the effects of the classical antipsychotic agent haloperidol and the atypical compounds clozapine, olanzapine, risperidone and ziprasidone on the release of noradrenaline and dopamine in the medial prefrontal cortex and on the release of dopamine in the striatum. In addition, the benzamides sulpiride and raclopride were studied.

Experiments were designed to explain the lack of efficacy of the benzamide antipsychotics to increase the release of dopamine and noradrenaline in the medial prefrontal cortex. In this respect, we studied the role of tonic activation of dopamine D_2 receptors by pre-treatment of the rats with dopamine/noradrenaline reuptake inhibitors.

As inhibition of 5-HT_2 receptors play an important role in the pharmacology of atypical antipsychotics (Meltzer, 1995), several experiments were carried out to investigate the role of this serotonin receptor in the observed increase in the release of dopamine and noradrenaline in the medial prefrontal cortex. First, the synergism between inhibition of dopamine D_2 receptors and 5-HT_2 receptors, in increasing the release of dopamine in the medial prefrontal cortex (Andersson et al., 1995), was further investigated by using the selective dopamine D_2 receptor antagonist raclopride and the selective 5-HT_{2A} receptor antagonist *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (MDL100,907). Next, the 5-HT_2 receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine HCl (DOI) was co-administered with clozapine or haloperidol and the effects on extracellular dopamine and noradrenaline in the medial prefrontal cortex were compared.

Finally, the question whether the clinical properties of antipsychotic drugs can be classified based on their selective action on the release of dopamine and noradrenaline in the medial prefrontal cortex is discussed.

2. Materials and methods

2.1. Animals, drug treatment, and doses

Male albino rats of a Wistar-derived strain (275–320 g; Harlan, Zeist, The Netherlands) were used for the experiments. The rats were housed in plastic cages (35 × 35 × 40 cm) with lights on from 7 a.m. until 7 p.m. and had free access to food and water. After probe implantation and during the experiments, the rats were individually housed in a plastic cage (30 × 30 × 30 cm). Experiments were carried out in the light cycle.

The following drugs were used: haloperidol, (±)-sulpiride, *S*(−)-raclopride-tartrate, clozapine, desipramine HCl, nomifensine maleate, (±)-2,5-dimethoxy-4-iodoamphetamine HCl (DOI) (Research Bochemicals International, Natick, MA, USA), olanzapine (kindly donated by Eli Lilly, Indianapolis, USA), risperidone (kindly donated by Janssen Pharmaceuticals, Beerse, Belgium) and ziprasidone (kindly donated by Pfizer, Croton, CT, USA). *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (MDL100,907) was provided by Solvay Pharmaceuticals (Weesp, the Netherlands).

The experiments were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

2.2. Surgery and brain dialysis

Microdialysis was performed with home-made I-shaped cannulas. The dialysis tube was prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (ID: 0.22 mm; OD: 0.31 mm; AN 69, Hospal, Bologna, Italy). Coordinates of probe implantation were medial prefrontal cortex A/P 3.0, L/M 1.2, and V/D −5.0, and striatum A/P 0.7, L/M 1.4, and V/D −7.3, from the bregma and dura, respectively. The exposed tip of the membrane was 4 mm. Probes were implanted under general anesthesia (5 mg/kg midazolam, 50 mg/kg ketamine and 8 mg/kg xylazine).

Microdialysis experiments were carried out 24–48 h after implantation of the probes. An on-line microdialysis approach was used in which the probes were perfused with a Ringer solution at a flow rate of 2.0 μl/min (CMA100 infusion pump, Carnegie, Stockholm, Sweden). Fractions of 15 min were collected. The composition of the Ringer solution was (in mM): NaCl, 140.0; KCl, 4.0; $CaCl_2$, 2.4; and $MgCl_2$, 1.0.

When the experiment was terminated, the rat was given an overdose of chloral hydrate and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μm thick) were made, and dialysis

probe placement was verified according to the atlas of Paxinos and Watson (1982).

2.3. Chemical assays

Noradrenaline and dopamine were quantified by high pressure liquid chromatography with electrochemical detection. A Shimazu pump (LC-10AD) was used in conjunction with an electrochemical detector (ESA, potential first cell: +175 mV; potential second cell: −250 mV). A reverse-phase column (150 × 4.7 mm, Supelco LC18; Belonte, PA) was used. The mobile phase consisted of a mixture of 0.1 M sodium acetate adjusted to pH 4.1 with acetic acid, 1.8 mM octanesulfonic acid, 0.3 mM Na_2EDTA, and 120 ml/l methanol. The flow rate was 1.0 ml/min. The detection limit of the assay was about 2 fmol per sample (on-column).

2.4. Expression of results and statistics

All values given are expressed as percentages of controls ± S.E.M. The average concentration of three stable samples (less than 10% variation) before drug administration was considered as the control and was defined as 100%. A statistical program (Sigmastat 1.0) was used to calculate the statistics. Data were analyzed by a non-parametric repeated measurement one-way analysis of variance on ranks (Friedman's test), followed by the Dunnett's

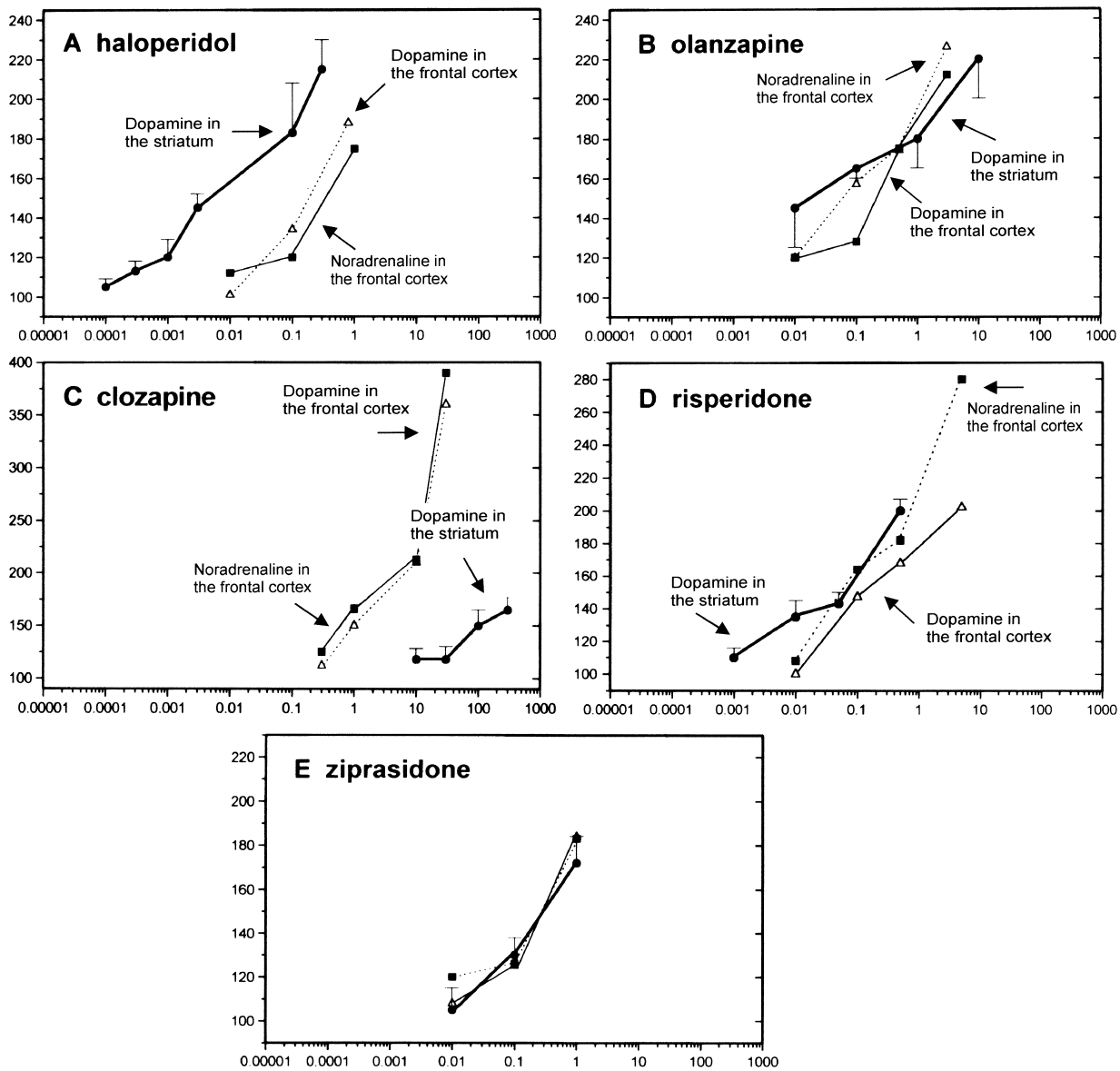

Fig. 1. Dose–effect curves of haloperidol, olanzapine, clozapine, risperidone and ziprasidone on extracellular dopamine levels in the striatum (closed circles) and medial prefrontal cortex (open triangles) and on extracellular noradrenaline levels in the medial prefrontal cortex (closed squares). The *y*-axis indicates the change in transmitter level (% of controls ± S.E.M., $n = 4–5$) 60 min after s.c. administration. Values on the *x*-axis are in μmol/kg.

multiple comparisons test when appropriate. The level of significance was set at $P < 0.05$.

3. Results

3.1. Basal values

The mean basal extracellular concentration (±S.E.M.) in the medial prefrontal cortex was 1.49 ± 0.04 fmol/min for noradrenaline ($n = 50$) and 0.97 ± 0.07 fmol/min for dopamine ($n = 54$). In the presence of nomifensine, it was 6.8 ± 0.24 fmol/min for noradrenaline ($n = 10$) and 4.0 ± 0.60 fmol/min for dopamine ($n = 10$). The mean basal value for dopamine in the striatum was 7.2 ± 0.2 fmol/min ($n = 88$).

3.2. Effect of haloperidol, risperidone, olanzapine, clozapine, and ziprasidone on extracellular levels of dopamine in the striatum, and noradrenaline and dopamine in the medial prefrontal cortex

Haloperidol (0.1, 0.3, 1, 3, 100 and 300 nmol/kg s.c.), olanzapine (10, 100, 1000 and 10,000 nmol/kg s.c.), clozapine (10, 30, 100 and 300 μmol/kg s.c.) risperidone (1, 10, 50 and 500 nmol/kg s.c.) and ziprasidone (10, 100 and 1000 nmol/kg s.c.) were administered and dose–effect curves for extracellular dopamine in the striatum were generated. Results are shown in Fig. 1A–E. For comparison, the dose–effect curves for dopamine and noradrenaline in the medial prefrontal cortex, calculated from a recent study (Westerink et al., 1998), were included.

Vehicle injection caused slight increases in extracellular dopamine and noradrenaline in the medial prefrontal cortex to about 140% of controls, an effect that lasted for about 45 min (data not shown). To circumvent vehicle effects, the dose–effect curves shown in Fig. 1 were based on changes in extracellular levels of dopamine and noradrenaline recorded 60 min after administration of the antipsychotics.

Haloperidol (Fig. 1A) was much more potent in the striatum (on dopamine) than in the medial prefrontal cortex (on dopamine and noradrenaline). The reverse was seen in the case of clozapine (Fig. 1C). The effects of olanzapine, risperidone and ziprasidone on dopamine in the striatum, and on dopamine and noradrenaline in the medial prefrontal cortex were very similar.

The dose–effect curves had a similar slope for both brain areas, with the exception of clozapine, which caused a more pronounced (non-proportional) increase in dopamine and noradrenaline in the medial prefrontal cortex, at 30 μmol/kg.

3.3. Effects of benzamides and haloperidol on extracellular levels of dopamine and noradrenaline in the medial prefrontal cortex and striatum: modification by reuptake inhibition

The dopamine D_2 receptor antagonist sulpiride (58 μmol/kg s.c.) clearly stimulated the release of dopamine

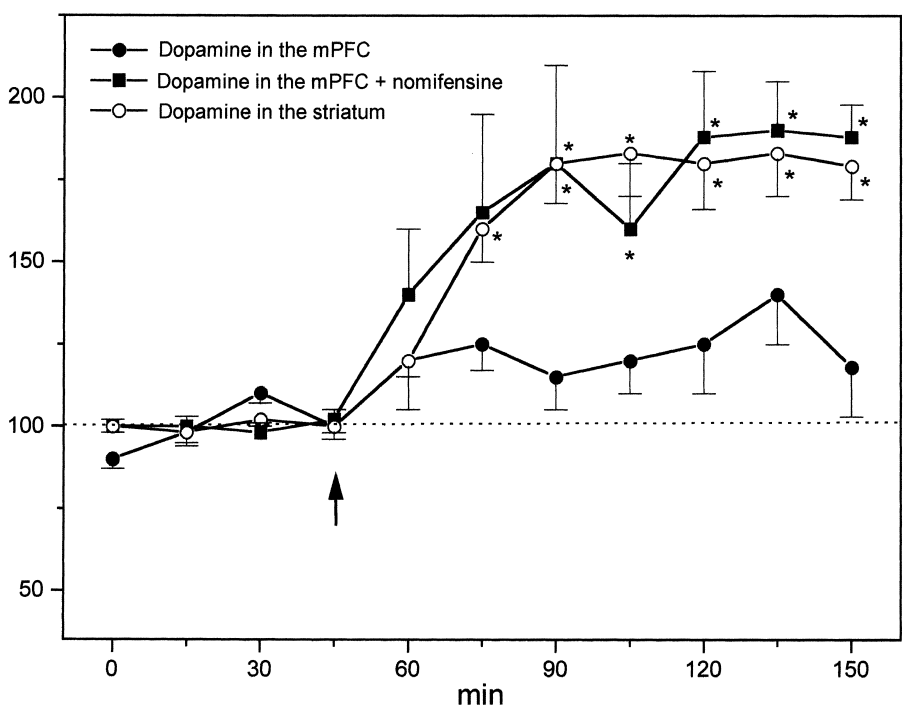

Fig. 2. Effect of sulpiride (58 μmol/kg s.c; arrow) on extracellular dopamine in the medial prefrontal cortex (closed circles) and the striatum (open circles). Experiments were also carried out in the medial prefrontal cortex with nomifensine (5 μmol/l) present in the perfusion fluid (closed squares). On the *y*-axis, the change in neurotransmitter level is expressed as % of control ± S.E.M., $n = 4–5$. $^{*}P < 0.05$ vs. control.

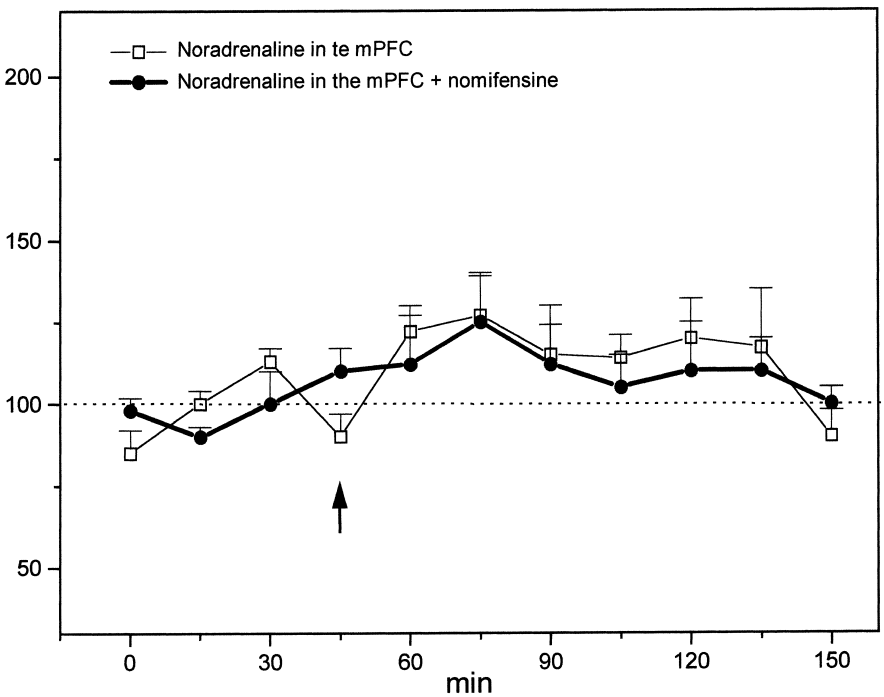

Fig. 3. Effect of sulpiride (58 μmol/kg s.c; arrow) on extracellular noradrenaline levels in the medial prefrontal cortex in the absence (open squares) and presence (closed circles) of nomifensine (5 μmol/l) added to the perfusion fluid. On the *y*-axis, the change in noradrenaline level is expressed as % of control ± S.E.M., $n = 4–5$.

in the striatum to about 200% of controls but the compound was ineffective on dopamine release in the medial prefrontal cortex (Fig. 2). When nomifensine (50 μmol/l) was added to the perfusion fluid, dopamine and noradrenaline levels in dialysates of the medial prefrontal cortex increased about 5-fold. Under these conditions, sulpiride

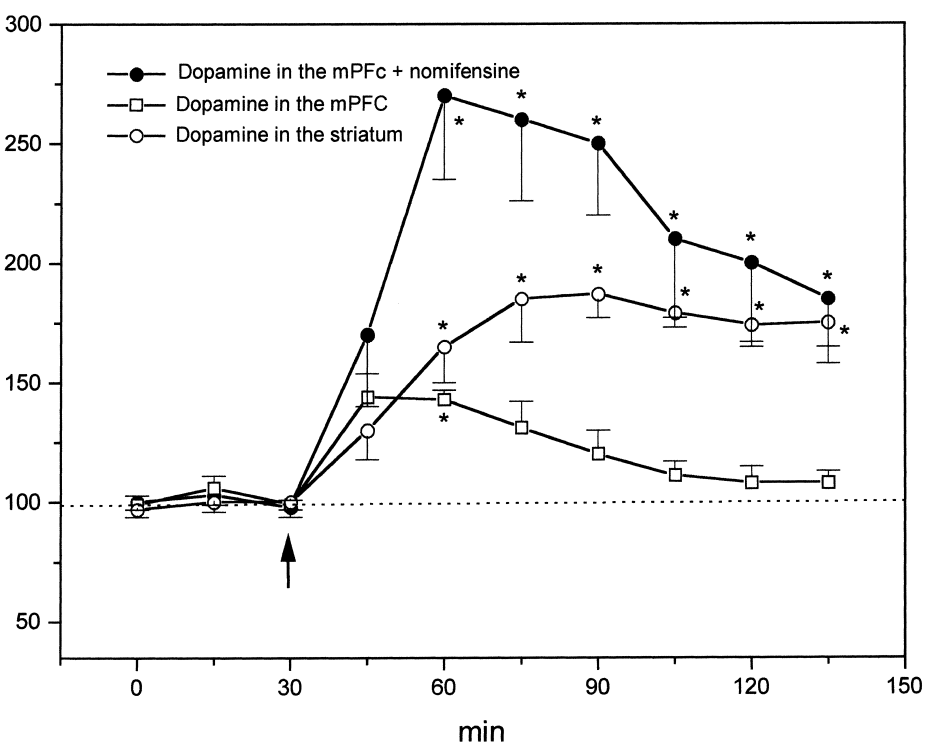

Fig. 4. Effect of raclopride (2 μmol/kg s.c; arrow) on extracellular dopamine levels in the medial prefrontal cortex (open squares) and the striatum (open circles). Experiments were also carried out in with nomifensine (5 μmol/l) present in the perfusion fluid (closed circles). On the *y*-axis, the change in neurotransmitter level is expressed as % of control ± S.E.M., $n = 4–5$. $^{*}P < 0.05$ vs. control.

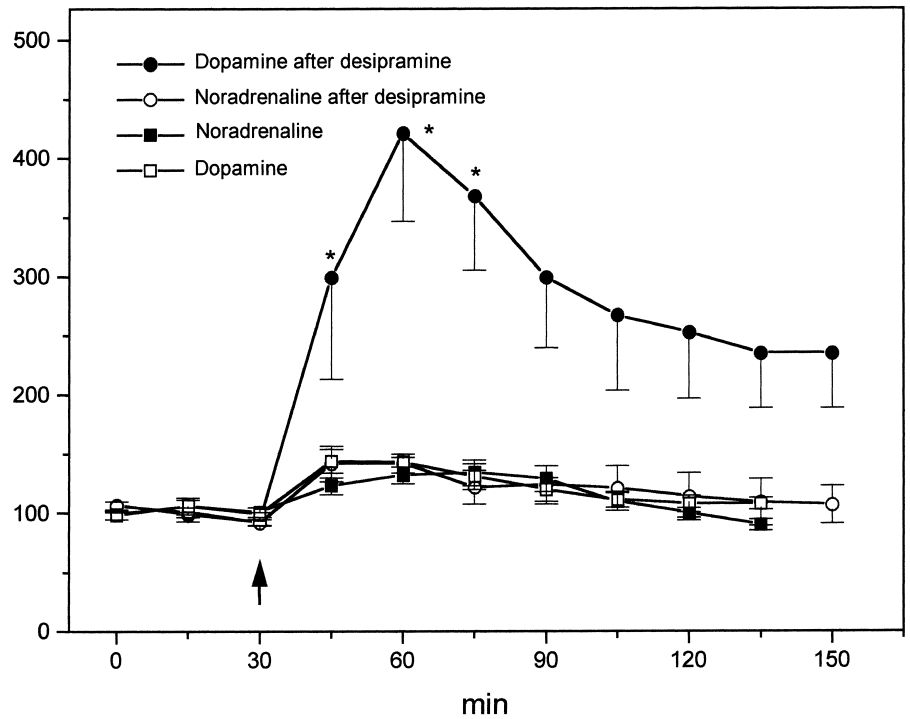

Fig. 5. Effect of raclopride (2 μmol/kg s.c; arrow) on extracellular dopamine (closed circles) and noradrenaline (open circles) levels in the medial prefrontal cortex. Experiments were also carried out after pretreatment with desipramine (10 μmol/kg). Values after desipramine were reset to 100%; open squares: dopamine, closed squares: noradrenaline. On the *y*-axis, the change in neurotransmitter level is expressed as % of control ± S.E.M., $n = 4–5$. $^{*}P < 0.05$ vs. controls.

increased extracellular dopamine to the same extent in the medial prefrontal cortex and in the striatum (without nomifensine).

Neither in the absence of nomifensine nor in its presence did sulpiride modify extracellular levels of noradrenaline in the medial prefrontal cortex (Fig. 3).

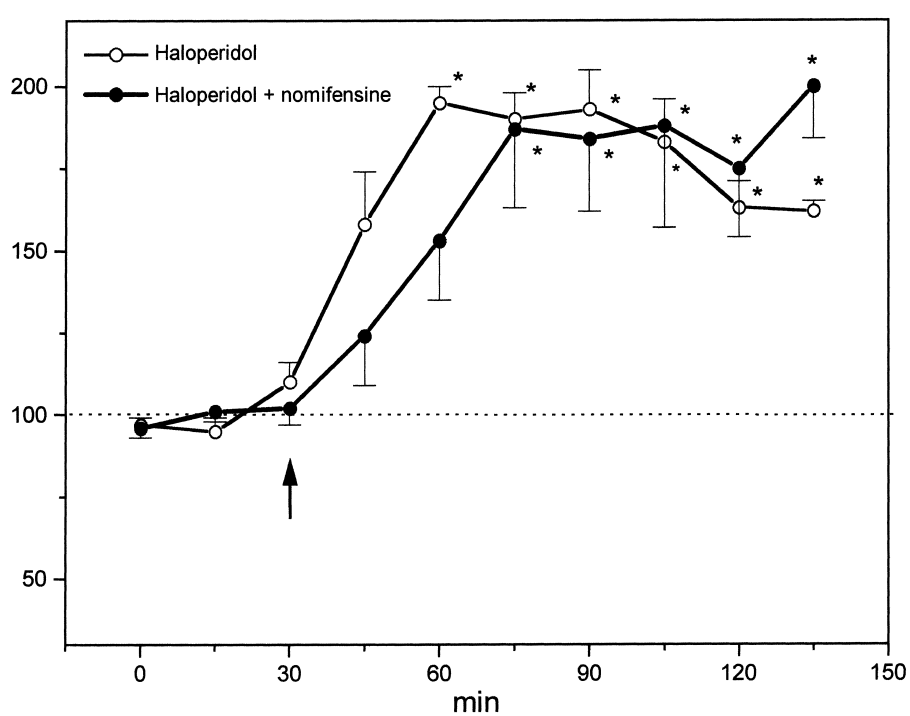

Fig. 6. Effect of haloperidol (800 nmol/kg s.c; arrow) on extracellular dopamine levels in the medial prefrontal cortex in the absence (open circles) and presence (closed circles) of nomifensine (5 μmol/l) added to the perfusion fluid. On the *y*-axis, the change in dopamine level is expressed as % of control ± S.E.M., $n = 4–5$. $^{*}P < 0.05$ vs. controls.

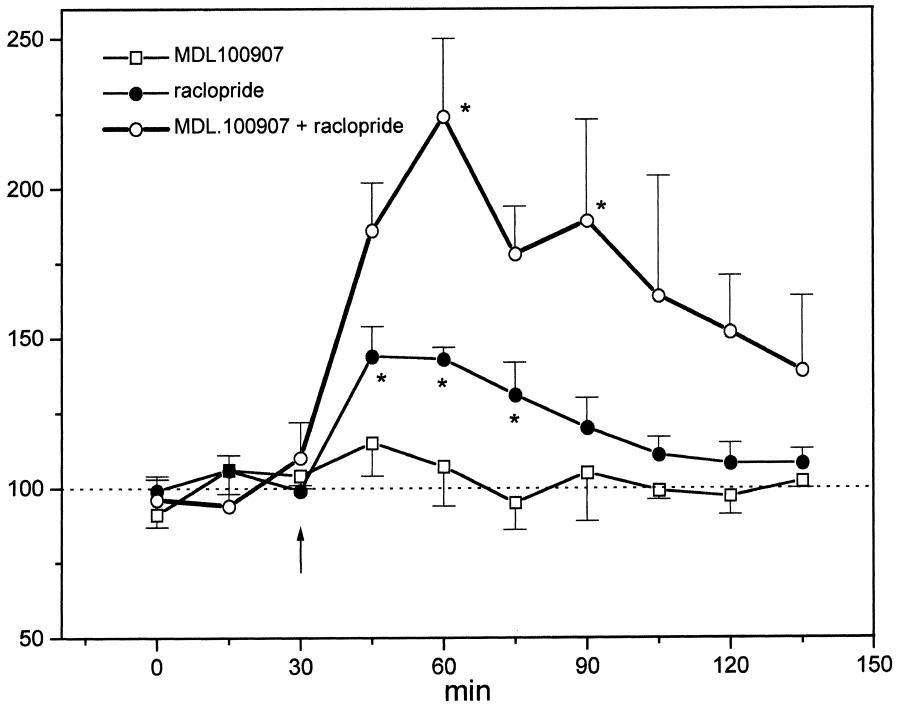

Fig. 7. Effect of raclopride (2 μmol/kg s.c.) (closed circles), MDL100,907 (0.8 μmol/kg s.c.) (open squares) and raclopride (2 μmol/kg s.c.) + MDL100,907 (0.8 μmol/kg s.c.) (open circles) on extracellular levels of dopamine in the medial prefrontal cortex. On the *y*-axis, the change in dopamine levels is expressed as % of control ± S.E.M., $n = 5$. $^{*}P < 0.05$ vs. control.

The dopamine D_2 receptor antagonist raclopride (2 μmol/kg s.c.) stimulated the release of dopamine in the striatum to about 185% of controls, but the compound was ineffective on dopamine release in the medial prefrontal cortex (Fig. 4). When, as in the case of sulpiride, nomifensine (50 μmol/l) was added to the perfusion fluid, dopamine levels in the medial prefrontal cortex rose to about 250% of control levels (Fig. 4).

Desipramine (10 μmol/kg s.c.) induced a long-lasting (> 3 h) increase in extracellular levels of dopamine and

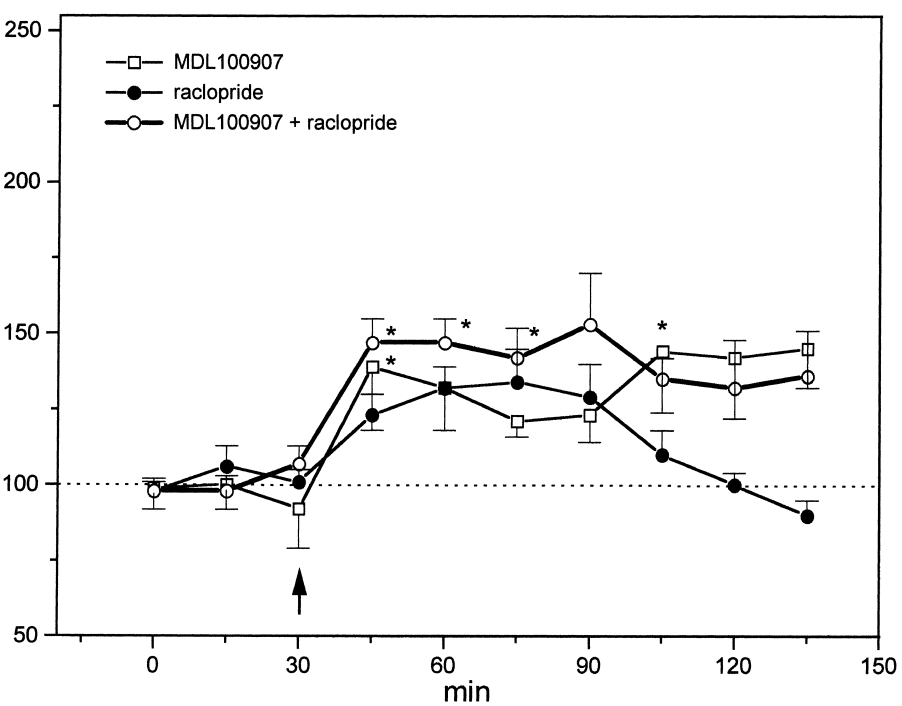

Fig. 8. Effect of raclopride (2 μmol/kg s.c.) (closed circles), MDL100,907 (0.8 μmol/kg s.c.) (open squares) and raclopride (2 μmol/kg s.c.) + MDL100,907 (0.8 μmol/kg s.c.) (open circles) on extracellular levels of noradrenaline in the medial prefrontal cortex. On the *y*-axis, the change in noradrenaline levels is expressed as % of control ± S.E.M., $n = 5$. $^{*}P < 0.05$ vs. control.

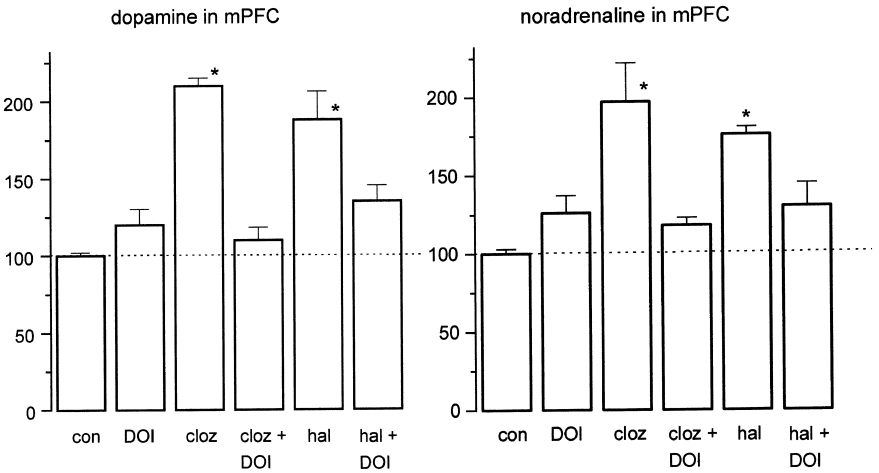

Fig. 9. Effect of combined treatment with DOI (0.85 μmol/kg s.c.) and clozapine (CLOZ, 10 μmol/kg s.c.) or haloperidol (HAL, 800 nmol/kg s.c.) on extracellular levels of dopamine and noradrenaline in the medial prefrontal cortex. Shown are % of basal values (±S.E.M., $n = 5$) 60 min after administration of the compound(s). $^{*}P < 0.05$ vs. control.

noradrenaline in the medial prefrontal cortex. Dopamine and noradrenaline levels rose to 225% and 350% of control levels, respectively. The stimulated levels of dopamine and noradrenaline were reset to 100% values in Fig. 5. Raclopride (2 μmol/kg s.c.) administered 60 min after desipramine induced a long-lasting increase in the release of dopamine in the medial prefrontal cortex to about 420% of control levels. No effects were seen on noradrenaline release in the medial prefrontal cortex (Fig. 5).

Administration of nomifensine to the perfusion fluid had no effect on the response of extracellular dopamine to haloperidol (800 nmol/kg s.c.) in the medial prefrontal cortex (Fig. 6).

3.4. Effect of MDL100,907 and raclopride on extracellular levels of dopamine and noradrenaline in the medial prefrontal cortex

MDL100,907, in a 5-HT_{2A} receptor-specific dose (0.8 μmol/kg), did not affect extracellular dopamine levels in the medial prefrontal cortex. When raclopride was co-administered with MDL100,907 a synergistic stimulation of the release of dopamine (to about 230% of controls) was observed in the medial prefrontal cortex (Fig. 7).

MDL100,907 increased significantly extracellular levels of noradrenaline in the medial prefrontal cortex to about 140% of controls, but no synergistic rise was seen when the compound was co-administered with raclopride (Fig. 8).

3.5. Effects of haloperidol or clozapine on the extracellular levels of noradrenaline and dopamine in the medial prefrontal cortex: effects of pretreatment with the 5-HT_2 receptor agonist DOI

Finally, we evaluated directly the contribution of 5-HT_2 receptors to the effects of antipsychotics by co-administering haloperidol (800 nmol/kg) or clozapine (10 μmol/kg) with the 5-HT_2 receptor agonist DOI (850 nmol/kg). The stimulatory effects of haloperidol or clozapine on extracellular dopamine levels, as well as noradrenaline levels in the medial prefrontal cortex, were fully and long-lastingly suppressed. Fig. 9 shows the percentage of basal levels recorded 60 min after administration of the compound(s).

4. Discussion

The dose–effect curves for the antipsychotics (Fig. 1) showed that haloperidol was most effective in stimulating the release of dopamine in the striatum, whereas clozapine was much more effective in the medial prefrontal cortex. The efficacy of the atypical antipsychotics risperidone, olanzapine and ziprasidone did not differ for the two brain areas. The changes in the release of dopamine in the striatum are in good agreement with the dopamine D_2 receptor-blocking properties of the antipsychotics, of which haloperidol is considered to be the most potent, risperidone and olanzapine, ziprasidone to be moderately potent and clozapine to be the least potent (Leysen et al., 1994). However, the observed changes in dopamine (and noradrenaline) in the medial prefrontal cortex cannot only be explained by the dopamine D_2 receptor-blocking properties of these antipsychotics. Apparently, other receptor interactions contribute to the neurochemical changes that these antipsychotics cause in the medial prefrontal cortex (see below).

In general, the present data confirm and elaborate earlier studies on the effect of antipsychotic drugs on the release of dopamine in the medial prefrontal cortex and striatum (Moghaddam and Bunney, 1990; Hertel et al., 1996; Marcus et al., 1996; Volonté et al., 1997; Li et al., 1998; Kuroki et al., 1999), although our result on the

efficacy of haloperidol in the medial prefrontal cortex is at variance with earlier studies that observed little or no effect of this antipsychotic (Moghaddam and Bunney, 1990; Pehek et al., 1993). The results presented in Fig. 1 are in agreement with the hypothesis that the clinical properties of atypical antipsychotic drugs are due to the selective stimulation of the release of dopamine in the mesocortical dopamine neurons. Haloperidol and clozapine are prototypic drugs in this respect: haloperidol induces frequently extrapyramidal side effects, whereas clozapine is virtually devoid of these side effects. The atypical antipsychotics risperidone, olanzapine and ziprasidone are often characterized as "intermediate" atypical antipsychotics, as—in contrast to clozapine—extrapyramidal side effects are to be expected in the higher dose range (Hertel et al., 1996; Volonté et al., 1997; Arnt and Skarsfeldt, 1998).

4.1. The benzamides

It is emphasized that the above "mesocortical" hypothesis is complicated by the properties of antipsychotic drugs based on the benzamide structure. In accordance in data in the literature (Moghaddam and Bunney, 1990; Andersson et al., 1995; Kuroki et al., 1999), sulpiride and raclopride strongly stimulated dopamine release in the striatum but had little, if any, effect on dopamine release in the medial prefrontal cortex. Raclopride is a strong and specific dopamine $D_{2/3}$ receptor blocking compound and has little clinical potential, but the low-potency benzamides amisulpride and remoxipride (and to a certain extent also sulpiride) are effective antipsychotic compounds that induce relatively few extrapyramidal side effects (Lewander, 1994; Peuskens et al., 1999; Ramaekers et al., 1999). Animal data also support that benzamides can be classified as atypical antipsychotics (Arnt and Skarsfeldt, 1998). In addition, there are an increasing number of clinical studies reporting that amisulpride improves the negative symptoms of schizophrenic patients (Perrault et al., 1997; Danion et al., 1999; Peuskens et al., 1999). Based on clinical and behavioral data, there is little evidence to distinguish these benzamides from the atypical antipsychotic compounds such as olanzapine, risperidone and ziprasidone.

The clinical profile of clozapine and other atypical antipsychotics is often explained by their broad profile of receptor interactions (Roth et al., 1994). However, the clinical properties of the benzamides do not essentially fit with this view because they represent a specific class of receptor-selective agents that predominantly act at dopamine $D_{2/3}$ receptors (of which the dopamine D_2 receptor subtype is believed to control the release of dopamine). The fact that the low-potency benzamides have so much in common with atypical drugs, both in the clinic and in animal experiments, is in our opinion overlooked in the literature.

4.2. Tonic occupation of autoreceptors

An explanation for the lack of efficacy of benzamides to enhance the release of dopamine in the medial prefrontal cortex may be found in the fact that the dopaminergic tone is too low to display a dopamine D_2 autoreceptor response. Similarly, serotonergic and muscarinic autoreceptors antagonists are often silent (De Boer et al. 1990; Routledge et al., 1993). To investigate this matter, we co-administered reuptake inhibitors through the dialysis probe or systemically. The results (Figs. 2–5) clearly indicated that, during conditions of increased levels of extracellular dopamine, the benzamides effectively stimulated the release of this transmitter in the medial prefrontal cortex.

4.3. Noradrenaline in the medial prefrontal cortex

The above data, however, do not explain why non-benzamide antipsychotics increase the release of dopamine in the medial prefrontal cortex without additional reuptake inhibition. As has been suggested earlier (Meltzer, 1995; Kuroki et al., 1999), other receptors than dopamine D_2 receptors are involved. In this respect, it is of interest to discuss the present data on extracellular noradrenaline. Haloperidol, but also the atypical antipsychotic olanzapine, risperidone, ziprasidone and clozapine, induced very similar changes in extracellular levels of dopamine and noradrenaline in the medial prefrontal cortex (Fig. 1; Li et al., 1998; Westerink et al., 1998). The similarity of the effects on the two catecholamines is as yet unexplained, but it is of interest to know whether a common mechanism (e.g. a particular receptor interaction) might explain the observed effects.

The present finding that benzamides—even in the presence of reuptake inhibitors—did not stimulate the release of noradrenaline indicates that dopamine D_2 receptors are not involved in the regulation of cortical noradrenaline release. Moreover, stimulation of the release of noradrenaline by haloperidol or clozapine was also seen in the locus coeruleus and other brain areas such as the hippocampus (data not shown), suggesting that there is no strict co-localization in areas in which dopamine D_2 receptors are present. The effect of haloperidol on the release of dopamine was also not affected by nomifensine infusion (Fig. 6), which suggests that the dopaminergic tone in the medial prefrontal cortex does not contribute to the modulation of the release of dopamine (and noradrenaline) in the medial prefrontal cortex. Synergism with other receptor subtypes might be a mechanism by which antipsychotics overcome the low dopaminergic tone. In this respect, we further investigated the role of the 5-HT_{2A} receptors.

4.4. The role of the 5-HT_2 receptor

A possible candidate receptor subtype that may contribute to the pharmacological effects of atypical antipsy-

chotics is the 5-HT_{2A} receptor (Stockmeier et al., 1993; Leysen et al., 1994; Meltzer, 1995; Schotte et al., 1996; Kuroki et al, 1999). Andersson et al. (1995) described a synergism between inhibition of dopamine D_2 receptors and 5-HT_{2A} receptors in increasing the release of dopamine in the medial prefrontal cortex. We therefore investigated the effect of the selective 5-HT_{2A} receptor antagonist MDL100,907 in combination with the specific dopamine D_2 receptor antagonist raclopride. At a 5-HT_{2A} receptor-specific dose (0.8 μmol/kg), MDL100,907 had no effect on dopamine in the medial prefrontal cortex, but when co-administered with raclopride a clear synergistic increase was seen (Fig. 7). Our data confirm the results of Gobert and Millan (1999) but are at variance with those of Schmidt and Fadayel (1995), who showed that MDL100,907 (but not ritanserin) increased the release of dopamine in the medial prefrontal cortex. Interestingly, the effects of MDL100,907 and raclopride on noradrenaline in the medial prefrontal cortex were very different: the 5-HT_{2A} receptor antagonist alone significantly increased the release of noradrenaline to about 140% of controls, but no synergism was seen when raclopride was co-administered (Fig. 8).

Apparently, antipsychotics are able to increase extracellular noradrenaline concentrations in the medial prefrontal cortex by blockade of the 5-HT_2 receptor only, whereas for effects on dopamine, an interplay between 5-HT_2 and dopamine D_2 receptors is needed. An explanation for this rather complex pharmacological interaction is not available at present.

To further substantiate the role of 5-HT_2 receptors in enhancing the release of dopamine and noradrenaline, we performed a crucial experiment in which the rats were pretreated with the 5-HT_2 receptor agonist DOI. The clear suppression of both the haloperidol and clozapine-induced increase of dopamine as well as noradrenaline in the medial prefrontal cortex (Fig. 9) demonstrates the importance of 5-HT_2 receptor blockade for the neurochemical changes induced by the non-benzamide antipsychotics in the medial prefrontal cortex.

Although it is concluded that 5-HT_2 receptor inhibition plays a major role in the effects of antipsychotics on the release of dopamine and noradrenaline in the medial prefrontal cortex, it is likely that other receptor subtypes contribute as well. It is appreciated that the dose–effect curves for dopamine and noradrenaline in the medial prefrontal cortex (Fig. 1) cannot be explained fully by the potency of the antipsychotics to block 5-HT_{2A} receptors. If the latter were the case, risperidone would have been the most potent compound, and haloperidol the weakest (Leysen et al., 1994). Antagonism at adrenoceptors and histamine receptors (Dringenberg et al., 1998) might have contributed to the observed neurochemical changes. Especially α_1-adrenoceptors are relevant in this respect, as all the presently studied antipsychotics have a high inhibitory affinity for this site. A recent study (Hertel et al., 1999) described the synergism between raclopride and idazoxan in enhancing extracellular cortical dopamine levels, indicating that α_2-adrenoceptors might also modify the release of cortical dopamine. In the case of clozapine, agonism at 5-HT_{1A} receptors might explain the strong additional increase in the release of dopamine and noradrenaline that was seen at the highest dose (30 μmol/kg) (Fig. 1C) (Rollema et al., 1997). In this respect, it is of interest to note that 5-HT_{1A} receptor agonists alone are able to preferentially stimulate the release of dopamine in the prefrontal cortex (Arborelius et al., 1996).

4.5. Do changes in dopamine release in the medial prefrontal cortex prediction atypical antipsychotic activity?

In the search for new drugs, it is important to know whether specific changes in the release of dopamine in the medial prefrontal cortex are predictive of atypical antipsychotic activity. Clozapine indeed stimulates, at low doses, the firing of mesocortical dopamine neurons (Melis et al., 1999). This finding suggests that selective stimulation of dopamine release in the medial prefrontal cortex might predict an atypical antipsychotic drug. However, the present data and earlier evidence (Hertel et al., 1996) are not consistent with this assumption, because the substituted benzamides were found to be practically inactive in increasing dopamine release in the medial prefrontal cortex.

The present study confirmed that various receptor subtypes contribute to the changes that atypical antipsychotic drugs induce in the release of dopamine and noradrenaline in the medial prefrontal cortex. Synergism between the blockade of various receptor subtypes (5-HT_2, 5-HT_{1A}, α_1, α_2, H_1, H_3) or reuptake sites (present paper) in enhancing dopamine release seems to be typical for the medial prefrontal cortex. The fact that atypical antipsychotic drugs, such as clozapine, olanzapine and risperidone, interact with so many receptor subtypes makes it difficult to discern which of the neurochemical effects they induce in the medial prefrontal cortex are of significance for their clinical properties. This difficulty was most pronounced for the medial prefrontal cortex, which suggests that this brain area is not suitable for classifying antipsychotic drugs. Subcortical areas such as the nucleus accumbens shell show equally complex changes after the administration of antipsychotics (Marcus et al., 1996). However, there is increasing evidence that the pharmacologically "pure" substituted benzamides, such as remoxipride and amisulpride, are atypical antipsychotics (defined as inducing few extrapyramidal side effects and improving negative symptoms). These benzamides are expected to have a low dopamine $D_{2/3}$ receptor occupancy. One could speculate from these findings that a low dopamine $D_{2/3}$ receptor occupancy is the only prerequisite for an antipsychotic drug to display atypical properties. In this respect, it is of interest to note that lowering the dose of haloperidol is

reported to improve negative symptoms in schizophrenia (Volavka et al., 1996).

The latter conclusion is only valid if the substituted benzamides are indeed atypical antipsychotics. In this respect, future research comparing the clinical properties of clozapine and amisulpride will be of decisive importance.

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