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Risperidone Dose-Dependently Increases Extracellular Concentrations of Serotonin in the Rat Frontal Cortex: Role of α_2-Adrenoceptor Antagonism

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We have previously shown that risperidone, an antipsychotic drug with high affinity for 5-hydroxytryptamine (5-HT)$_{2A}$ and dopamine (DA)$_2$ receptors, as well as for α_1- and α_2-adrenoceptors, enhances 5-HT metabolism selectively in the rat frontal cortex (FC). To further study the influence of risperidone on central 5-HT systems, we compared its effects on dialysate 5-HT in the FC, as assessed by microdialysis, with those obtained with other antipsychotic drugs, i.e., clozapine, haloperidol, and amperozide, as well as with the selective α_2- or 5-HT$_{2A}$ receptor antagonists idazoxan or MDL 100,907, respectively. The underlying mechanism for risperidone's effect on 5-HT output in the FC was also investigated using single-cell recording in the dorsal raphe nucleus (DRN). Administration of risperidone (0.2, 0.6, and 2.0 mg/kg, SC) dose-dependently increased 5-HT levels in the FC. This stimulatory action was mimicked by amperozide (10 mg/kg, SC) and, to some extent, by idazoxan (0.25 mg/kg, SC). In contrast, clozapine (10 mg/kg, SC), haloperidol (2.0 mg/kg, SC), and MDL 100,907 (1.0 mg/kg, SC) exerted only minor effects on 5-HT output in brain. Local administration of risperidone or idazoxan (1.0–1000 μmol/L) in the FC dose-dependently increased dialysate levels of 5-HT in this region. On the other hand, risperidone 25-800 μg/kg, IV) dose-dependently decreased the firing rate of 5-HT cells in the DRN, an effect that was largely antagonized by pretreatment with the selective 5-HT$_{1A}$ receptor antagonist WAY 100,635 (5.0 μg/kg, IV). These results indicate that the risperidone-increased 5-HT output in the FC may be related to its α_2-adrenoceptor antagonistic action, a property shared with both amperozide and idazoxan, and that this action probably is executed at the nerve terminal level. The inhibition of 5-HT cell firing by risperidone is probably secondary to increased 5-HT availability, e.g., in the DRN, since it could be antagonized by a 5-HT$_{1A}$ receptor antagonist. The enhanced 5-HT output in the FC by risperidone may be of particular relevance for the treatment of schizophrenia when associated with depression and in schizoaffective disorder. ***[Neuropsychopharmacology 17:44–55, 1997]*** *© 1997 American College of Neuropsychopharmacology*

KEY WORDS: *Neuroleptic drugs; Serotonin; Frontal cortex; Dorsal raphe nucleus; Microdialysis; Electrophysiology*

The antipsychotic effect of neuroleptic drugs is generally thought to be related to their interference with central dopamine (DA)-mediated neurotransmission, primarily through antagonism of DA-D$_2$ receptors in limbic structures. However, a high degree of neurolep-

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tic-induced DA-D_2 receptor antagonism is not always sufficient or necessary to obtain a therapeutic action covering the whole spectrum of symptoms in schizophrenia (Farde et al. 1988; Coppens et al. 1991). Also, the compliance with traditional neuroleptic drugs is limited by the appearance of extrapyramidal side effects (EPS), which have been found to be related to a high degree of occupancy at DA-D_2 receptors in the basal ganglia (Seeman et al. 1976; Seeman 1980; Van Wielinck and Leysen 1983; Farde et al. 1992). Therefore, considerable attention has been focused on the putative implication of other neurotransmitter systems and receptors in the pathophysiology and pharmacotherapy of schizophrenia.

The importance of serotonin (5-HT) in the pharmacology of schizophrenia was underlined by some pioneering clinical studies in the 1980s indicating that ritanserin, a selective 5-HT_2 receptor antagonist (Leysen et al. 1985), in combination with classical DA-D_2 receptor blocking neuroleptic drugs can enhance the therapeutic action and attenuate EPS liability of such agents (Bersani et al. 1986; Gelders et al. 1986; Gelders 1989). Also, the potent serotonin 5-HT_2 receptor antagonistic property of clozapine, an antipsychotic drug classified as atypical based on its superior efficacy in treatment-resistant schizophrenia and a very low incidence of EPS (Claghorn et al. 1987; Kane et al. 1988), have increased the recent interest in the role of 5-HT in schizophrenia, as well as in the mechanism of action of antipsychotic drugs. Although the underlying mechanism(s) responsible for clozapine's antipsychotic action at present still remains unclear, it has been hypothesized that concurrent 5-HT_2 and DA-D_2 receptor antagonism may contribute to its atypical antipsychotic profile (Meltzer et al. 1989; Deutch et al. 1991; Meltzer and Nash 1991). Clearly, such findings and hypotheses have spurred efforts to develop novel antipsychotic drugs with combined antiserotonergic and antidopaminergic properties. This strategy has led to the development of risperidone and several other compounds, characterized by a high affinity for the 5-HT_{2A} receptor relative to the DA-D_2 receptor (Janssen et al. 1988; Leysen et al. 1988; Gerlach and Peacock 1995). By now risperidone, in double-blind, placebo-controlled clinical trials, has been shown to display an effective antipsychotic action, improving both positive and negative symptoms, while displaying low propensity to induce EPS (Kane et al. 1988; Borison et al. 1992; Chouinard et al. 1993).

Given the purported role of 5-HT in schizophrenia and in the mechanism of action of antipsychotic drugs (see above), surprisingly few preclinical studies have examined neuroleptic-induced effects on regional 5-HT transmission in brain. We have previously shown that a high dose of risperidone increases the metabolism and the extracellular concentrations of DA, as well as of 5-HT in cortical regions (Hertel et al. 1996). The present study sought to further characterize the effects of risperidone on 5-HT output and metabolism of 5-HT and DA in the FC of the rat and to unravel the underlying mechanisms for the stimulatory action of risperidone on cortical dialysate 5-HT. The effects of risperidone were compared with those obtained with other established antipsychotic drugs, i.e., clozapine and haloperidol, as well as, with the putative antipsychotic drug amperozide. The stimulatory effects of risperidone on cortical 5-HT output were also compared with those of MDL 100,907, a highly selective 5-HT_{2A} receptor antagonist (Palfreyman et al. 1993), and idazoxan, a selective α_2-adrenoceptor antagonist (e.g., Freedman and Aghajanian 1984). The tentative site of risperidone's action was investigated by means of local drug administration. In addition, the influence of risperidone on 5-HT cell firing in the dorsal raphe nucleus (DRN), a major origin for the serotonergic innervation of the FC (Moore et al. 1978; McQuade and Sharp 1995), was assessed.

METHODS

Animals

Male albino rats weighing 275–350 g (BK Universal, Sollentuna, Sweden), BK1:WR or BK1:SD in case of microdialysis or electrophysiological experiments, respectively, were used in all experiments. Animals were housed under standard laboratory conditions on a 12-h light/dark cycle (lights on 6:00 A.M.) and allowed free access to food and water. The present study was approved by the Ethical Committee of Northern Stockholm, Sweden (permit no. N18/94 and N19/94).

Drugs

In microdialysis experiments, risperidone (Janssen), clozapine (Sandoz), haloperidol (Sigma), and MDL 100,907 (Marrell Dow) were dissolved in 5.5% glucose solution with the addition of a minimal amount of acetic acid. Amperozide hydrochloride (Pharmacia AB) and idazoxan (RBI) were dissolved in saline (0.9% NaCl). Risperidone, idazoxan, and tetrodotoxin (TTX; Sigma) were also dissolved in perfusion solution for local infusion via the dialysis probe. Control animals received injections with the appropriate drug vehicle. In single-cell recording experiments, risperidone was dissolved in saline with the addition of a minimal amount of acetic acid; pH was thereafter adjusted to 6.5–7.0 with sodium hydroxide. WAY 100,635 (Wyeth Research) and (R)-8-OH-DPAT (synthesized at the Department of Organic Pharmaceutical Chemistry, Uppsala Universitet, Uppsala, Sweden) were dissolved in saline. Control injections were performed with the vehicle used for risperidone administration.

Microdialysis

Experiments were performed with transcortical dialysis probes (Carboni and Di Chiara 1989; Nomikos et al. 1992). In brief, probes were stereotaxically implanted under barbiturate anesthesia (Mebumal, 60 mg/kg, IP) in the FC. The coordinates (in mm) were: AP = 1.7 and DV = −1.7 relative to bregma and skull surface (Paxinos and Watson 1986). Dialysis occurred through a semipermeable membrane (copolymer of acrylonitrile and sodium methallyl sulfonate, ID = 0.24 mm, 40,000 Da, AN69 Hospal), with an active surface length of 8.0 mm. The in vitro recovery of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) across this type of membrane has previously been estimated to be approximately 40% (Carboni and Di Chiara 1989), whereas the in vitro recovery of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) has been reported to be approximately 15% (Nomikos et al. 1992). After surgery, the animals were housed individually in plastic cages (32 × 35 × 50 cm) and given free access to food and water.

Dialysis experiments were conducted approximately 48 h after surgery during the daylight period in freely moving rats. The dialysis probes were perfused with a physiological solution containing 147 mmol/L NaCl, 3.0 mmol/L KCl, 1.3 mmol/L $CaCl_2$, 1.0 mmol/L $MgCl_2$, and 1.0 mmol/L sodium phosphate (pH 7.4) at a rate of 2.5 μl/min set by a microperfusion pump (Harvard Apparatus). The dialysate was loaded directly into a 100-μl sample loop of the injector (Valco) which was controlled, via a PE Nelson 900 interface (Perkin Elmer), by the Turbochrom 4.1 (Perkin Elmer) program to automatically inject samples every 30 min.

Concentrations of 5-HT, 5-HIAA, DOPAC and HVA were determined by high-performance liquid chromatography (HPLC) with electrochemical detection as previously described (Hertel et al. 1996). 5-HT and 5-HIAA in the dialysate were separated by reversed-phase liquid chromatography (150 × 4.6 mm, Nucleosil 5 μmol/L, C18) with a mobile phase consisting of 0.055 mol/L sodium acetate with 0.3 mmol/L octanesulfonic acid, 0.01 mmol/L Na_2EDTA, and 11% methanol (pH = 4.1, adjusted with glacial acetic acid). The mobile phase was delivered by an HPLC pump (LKB 2150) at 0.8 ml/min. A precolumn (50 × 3 mm, Nucleosil 5 μmol/L, C18) was placed between the HPLC pump and the injection loop. Electrochemical detection was accomplished using a coulometric detector (Coulochem II, model 5200, ESA) with a conditioning cell (5021) and an analytical cell (5011 or 5014). 5-HT, 5-HIAA, DOPAC, and HVA were detected and quantified by sequential oxidation of the eluent (coulometric electrode = 0.3 V; amperometric electrode = 0.35 V). Chromatograms were recorded on a dual pen chart-recorder (Kipp & Zonen, BD41).

Subcutaneous (SC) injections, given in the neck region at volume of 1.0 ml/kg, or local drug perfusions were performed after a stable (<10% variation) outflow of 5-HT and metabolites was established. Upon completion of the experiments, the animals were killed with an overdose of anesthetic, and their brains were preserved in 10% formalin in 25% sucrose. Each brain was sliced on a microtome (50 μmol/L), stained with neutral red and examined under microscope for probe placement (Figure 1*A*). Only rats with probes verified to be located

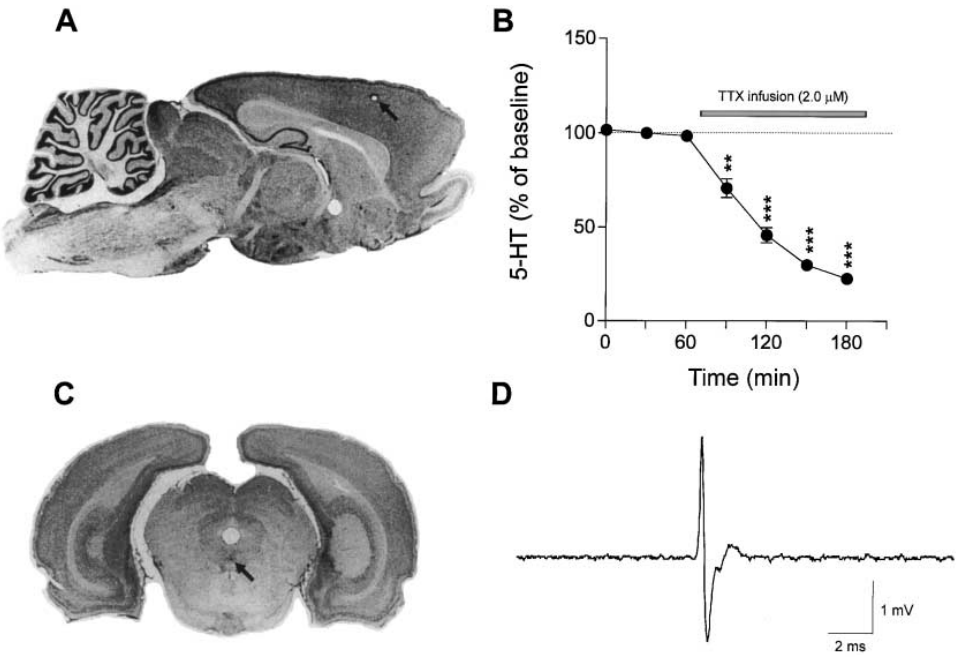

Figure 1. Verification of dialysis probe and electrode placement (**A**) Typical placement (*arrow*) of transversal dialysis probe in the FC. (**B**) Effects of local, cortical infusion of TTX (2.0 μmol/L) on extracellular 5-HT concentrations in the FC (n = 7). Bar indicates time (120 min) of drug infusion; ** = $p < .01$, *** = $p < .001$ compared to the last baseline sample. (**C**) Typical placement (*arrow*) of recording electrode in the DRN. (**D**) Oscilloscope trace of a typical extracellularly recorded, presumed 5-HT neuron from the DRN.

in the FC (plates 10–13 in the atlas of Paxinos and Watson) were included in data analysis.

Single-Cell Recordings

Rats were anesthetized with chloral hydrate (400 mg/kg, IP) with additional doses given when needed to maintain surgical anesthesia throughout the experiment. Rectal temperature was kept at 37–38°C by means of an electrical heating pad. A tracheal cannula and a jugular vein catheter for intravenous (IV) administration of drugs were inserted before the rat was mounted in a stereotaxic frame (David Kopf). The skull was exposed and a hole was drilled above the DRN, i.e., 1.0 ± 0.2 mm anterior to the interaural line and 0.0 ± 0.1 mm lateral to the midline (Paxinos and Watson 1986). Recording electrodes were pulled in a Narishige vertical puller from glass capillaries (OD: 1.5 mm, ID: 1.17 mm; Clark Electromedical Instruments) and filled with 2% Pontamine Sky Blue in 2 mmol/L NaCl. The tip of the electrodes were broken under microscope, yielding an impedance of 2.0–4.0 mΩ at 135 Hz in vitro. The electrode was lowered into the brain using a David Kopf hydraulic microdrive and the presumed 5-HT neurons were found 5.0–6.0 mm beneath the brain surface. Experiments were only performed on cells displaying electrophysiological characteristics corresponding to those previously described for 5-HT neurons in the DRN (see Figure 1*D*; Aghajanian et al. 1978; Vandermaelen and Aghajanian 1983). Recordings were made from one cell in each animal, and at the end of each experiment a negative current of 5 μA was passed for 8 min through the electrode to mark the recording site with dye. The animals were killed by an overdose of anesthetic, and their brains were preserved in 10% formalin in 25% sucrose. Each brain was sliced on a microtome (50 μmol/L), stained with neutral red, and examined under microscope. All recording sites included in this study were located within the DRN (plates 48–50 in the atlas of Paxinos and Watson; see Figure 1*C*). Extracellular action potentials were amplified, discriminated, and monitored on an oscilloscope and an audiomonitor. Discriminated spikes were fed, via a CED 1401 interface (Cambridge Electronics Design), into an AST Bravo LC 4/66d computer and the action potentials were collected and analyzed by the CED Spike2 program.

Risperidone was administered IV in exponentially increasing doses at 3.0-min intervals. WAY 100,635 or vehicle was administered IV 3.0 min before risperidone.

Data Analysis

Dialysis data were calculated as percent changes of dialysate basal concentrations, 100% being defined as the average of the last three preinjection values. All subsequent measures were related to these values, and mean percentages were calculated for each sample across the rats in all the groups. Basal, absolute values of 5-HT and metabolites were evaluated by one-way (treatment) analysis of variance (ANOVA). The percent change of basal outflow (last baseline plus all posttreatment samples) were analyzed by two (treatment x time)-way ANOVA for repeated measures, followed by the Neuman-Keuls test for multiple comparisons with a criterion of $p < .05$ to be considered significant.

In electrophysiological experiments, the drug effects were assessed by comparisons of the mean discharge rate during 1.5 min immediately preceding drug injection (baseline value) to the mean discharge rate during the same time period at maximal drug effect at each dose. Data were calculated and presented as percent changes of baseline values, defined as 100%. Mean percentages were calculated for each sample across the rats in all groups. Data were analyzed statistically by *t*-test for dependent samples to compare effects within treatment groups and *t*-test for independent samples to compare effects between treatment groups. A *p*-value less than .05 was considered significant. All data were statistically evaluated by using the CSS:Statistica (Statsoft) program.

Verification of Probe and Electrode Placements (Figure 1)

Figures 1A and 1C show photomicrographs of a sagittal section of the rat brain with a typical probe placement in the FC and a coronal section with a typical electrode placement in the DRN, respectively. Cortical infusion of TTX (2.0 μmol/L) through the dialysis probe (Figure 1*B*) significantly lowered the dialysate concentrations of 5-HT from the FC (time effect: $F_{4,24} = 133$, $p < .001$). The decrease was apparent during the entire TTX infusion period ($p < .01$), starting already at the first 30-min sample. This indicates that the measured 5-HT in the dialysate from the FC was of neuronal origin, in agreement with previous results (Carboni and Di Chiara 1989). Figure 1*D* shows an oscilloscope trace of an extracellularly recorded typical, presumed 5-HT neuron from the DRN of an anesthetized rat.

RESULTS

Effects of Systemic Administration of Risperidone on Extracellular Concentrations of 5-HT, 5-HIAA, DOPAC and HVA (Figures 2 and 3)

No statistically significant differences in basal dialysate concentrations of 5-HT and the metabolites were found between groups that were subsequently subjected to various treatments. The overall mean basal concentrations (fmol/min ± SEM, $n = 72$) of 5-HT, 5-HIAA,

DOPAC, and HVA were 0.49 ± 0.036, 444.1 ± 23, 43.9 ± 4.1, and 173.3 ± 14, respectively. Vehicle injections did not significantly affect extracellular concentrations of 5-HT, 5-HIAA, DOPAC, or HVA in the FC (Figures 2 and 3).

Risperidone caused a dose-dependent increase in 5-HT output in the FC. Statistical analysis of the effects of risperidone (0.2, 0.6 and 2.0 mg/kg, SC) on 5-HT concentrations (Figure 2), revealed a significant overall interaction ($F_{24,152} = 2.35$, $p < .001$). Post-hoc analysis showed that the highest dose of risperidone significantly increased 5-HT concentrations within the 30- to 210-min postinjection interval as compared to both baseline and control group ($p < .01$–$.001$), whereas the middle dose caused a significant increase during the 60-min interval as compared to its baseline ($p < .05$). The lowest dose of risperidone and control injections failed to significantly influence 5-HT levels. The highest dose of risperidone caused a significant larger increase in 5-HT concentrations as compared to both the middle and lowest dose of the drug during the 30- to 90-min ($p < .01$–$.001$) and during the 60- to 210-min ($p < .05$–$.001$) posttreatment period, respectively. No differences were indicated between the middle and lowest dose of risperidone.

Risperidone administration enhanced 5-HIAA levels in the FC. Statistical analysis of the effects of risperidone on 5-HIAA concentrations (Figure 3*A*), revealed a significant overall interaction ($F_{24,152} = 6.72$, $p < .001$). The highest dose of risperidone elevated 5-HIAA concentrations during the 150- to 240-min postinjection interval in comparison to its baseline and to the control group ($p < .001$). Both the middle and lowest dose of risperidone significantly increased 5-HIAA levels compared to baseline and the control values starting from the 120-min postinjection interval and lasting throughout the entire sampling period ($p < .01$–$.001$). The highest dose of risperidone caused a significantly larger increase in 5-HIAA concentrations compared to both the middle and lowest dose of the drug during the 210- to 240-min posttreatment period ($p < .01$–$.001$). No differences were indicated between the middle and lowest dose of risperidone. Risperidone significantly increased both DOPAC and HVA extracellular concentrations (overall interactions: $F_{24,152} = 12.1$, $p < .001$ and $F_{24,152} = 46.1$, $p < .001$, respectively, Figures 3*B*–*C*). Post-hoc

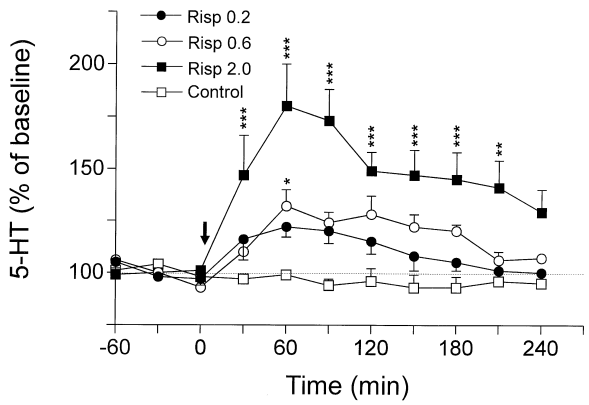

Figure 2. Effects of risperidone (Risp: 0.2, 0.6 or 2.0 mg/kg, SC; $n = 6$, 6, or 5, respectively) or control injections (1.0 ml/kg, SC; $n = 6$) on 5-HT extracellular concentrations in the FC. *Arrow* indicates time of injection. * = $p < .05$, ** = $p < .01$, *** = $p < .001$ compared to the last baseline sample.

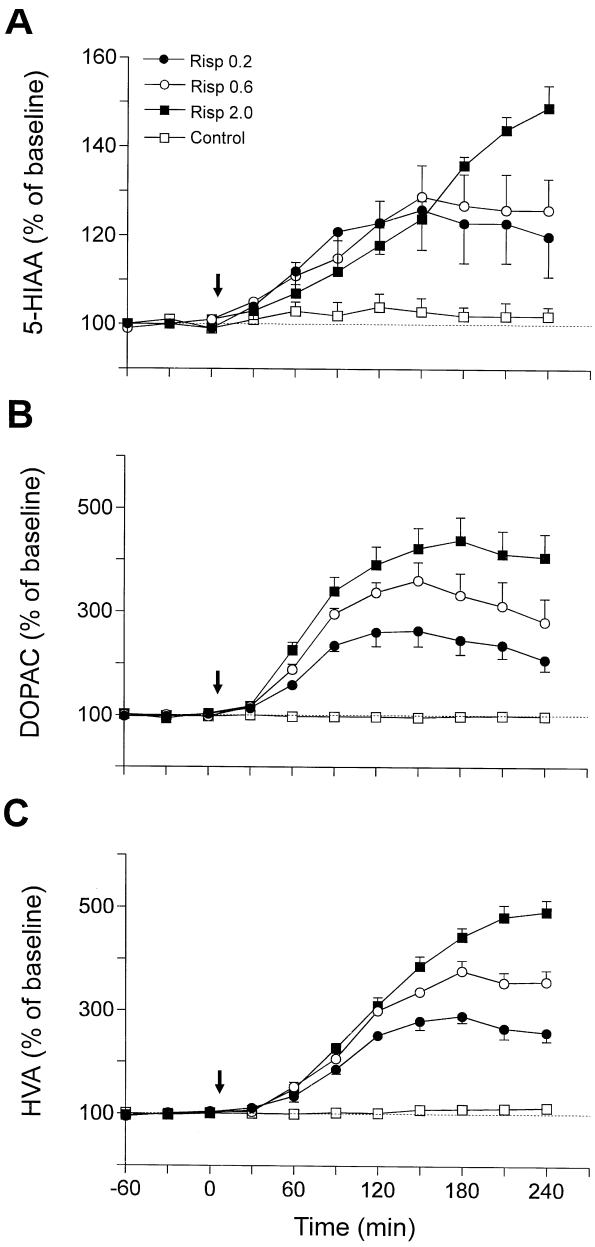

Figure 3. Effects of risperidone (Risp; 0.2, 0.6 or 2.0 mg/kg, SC; $n = 6$, 6, or 5, respectively) or control injections (1.0 ml/kg, SC; $n = 6$) on extracellular concentrations of (**A**) 5-HIAA, (**B**) DOPAC, and (**C**) HVA in the FC. *Arrows* indicate time of injection. Results of the statistical evaluation of these data are, for reasons of clarity, only presented in the result section.

analysis indicated that all doses of risperidone significantly increased DOPAC ($p < .05$–$.001$), as well as HVA ($p < .001$) concentrations during the 60- to 240-min postinjection interval compared to both the baseline and control group. The highest dose of risperidone caused a significantly larger increase in DOPAC concentrations compared to both the middle and lowest dose of the drug during the 180- to 240-min ($p < .01$–$.001$) and during the 60- to 240-min ($p < .05$–$.001$) posttreatment period, respectively. Also, the post-hoc analysis indicated significant differences between the middle and lowest dose of risperidone during the 120- to 150-min posttreatment interval ($p < .05$–$.01$). Significant differences between the effects of the highest and both the middle and lowest dose of risperidone on HVA concentrations were revealed during the 180- to the 240-min and during the 120- to 240-min posttreatment period ($p < .05$–$.001$), respectively. The middle dose of risperidone caused a significant larger effect on HVA concentrations as compared to the lowest dose of the drug during the 120- to 240-min posttreatment interval.

Effects of Systemic Administration of Clozapine, Amperozide, Haloperidol, Idazoxan, or MDL 100,907 on Extracellular Concentrations of 5-HT, 5-HIAA, DOPAC, and HVA (Figures 4 and 5)

Neither saline nor vehicle injections significantly affected the levels of 5-HT, 5-HIAA, DOPAC or HVA in the FC and no difference between the saline and the vehicle group was indicated by statistical analysis. Thus, these groups were merged and treated as one control group.

Amperozide (10 mg/kg, SC) idazoxan (0.25 mg/kg, SC) and haloperidol (2.0 mg/kg, SC) increased, clozapine (10 mg/kg, SC) decreased, whereas MDL 100,907 (1.0 mg/kg, SC) was without apparent effect on dialysate

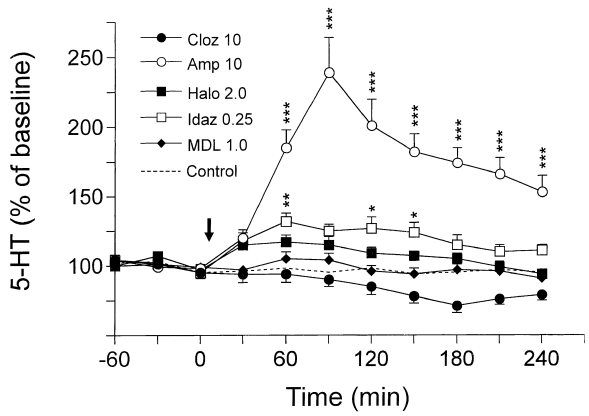

Figure 4. Effects of clozapine (Cloz; 10 mg/kg, SC; $n = 6$), amperozide (Amp; 10 mg/kg, SC; $n = 5$), haloperidol (Halo; 2.0 mg/kg, SC; $n = 6$), idazoxan (Idaz; 0.25 mg/kg, SC; $n = 5$), MDL 100,907 (MDL; 1.0 mg/kg, SC; $n = 5$) or control injections (1.0 ml/kg, SC; $n = 9$) on 5-HT extracellular concentrations in the FC. For reasons of clarity, the SEMs for the control group were not included. *Arrow* indicates time of injection. * = $p < .05$, ** = $p < .01$, *** = $p < .001$ compared to the last baseline sample.

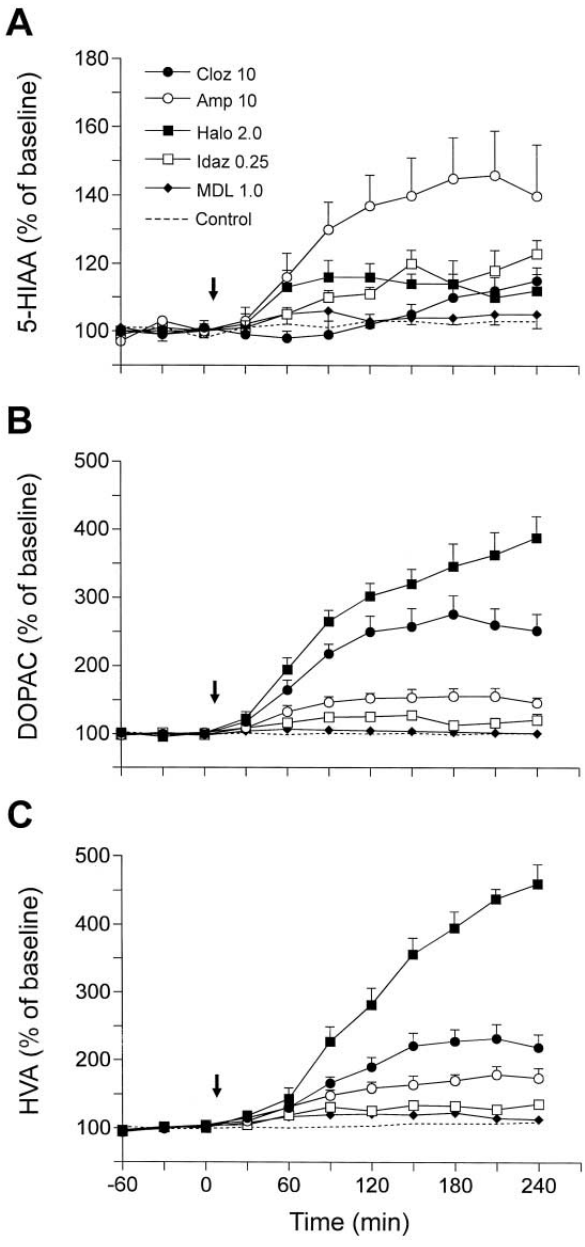

Figure 5. Effects of clozapine (Cloz; 10 mg/kg, SC; $n = 6$), amperozide (Amp; 10 mg/kg, SC; $n = 5$), haloperidol (Halo; 2.0 mg/kg, SC; $n = 6$), idazoxan (Idaz; 0.25 mg/kg, SC; $n = 5$), MDL 100,907 (MDL; 1.0 mg/kg, SC; $n = 5$) or control injections (1.0 ml/kg, SC; $n = 9$) on extracellular concentrations of (**A**) 5-HIAA, (**B**) DOPAC, and (**C**) HVA in the FC. *Arrows* indicate time of injection. Results of the statistical evaluation of these data are, for reasons of clarity, only presented in the result section.

concentrations of 5-HT in the FC (Figure 4). Statistical evaluation of these data revealed a significant overall interaction ($F_{40,240} = 9.75$, $p < .001$). However, post-hoc analysis indicated that only amperozide and idazoxan significantly elevated 5-HT concentrations. Amperozide significantly increased 5-HT levels in relation to baseline and control values starting within the 60-min postinjection interval and lasting throughout the whole sampling period ($p < .001$), whereas 5-HT concentrations after idazoxan administration were significantly increased within the 60-min and 120- to 150-min postinjection intervals as compared to baseline and controls ($p < .05$–.01).

A significant overall interaction ($F_{40,240} = 4.63$, $p < .001$) was indicated by statistical analysis when evaluating the temporal effects of these drugs on 5-HIAA concentrations (Figure 5*A*). In similarity with the effects on 5-HT, only amperozide and idazoxan administration significantly affected 5-HIAA levels as indicated by the post-hoc analysis. Amperozide increased 5-HIAA levels starting within the 60-min postinjection interval and the levels remained significantly elevated throughout the whole sampling period in comparison to the baseline and controls ($p < .001$), whereas the 5-HIAA concentrations after idazoxan administration were significantly increased within the 150-min and 210- to 240-min postinjection intervals as compared to baseline ($p < .05$–.001) and during the 240-min postinjection period as compared to control values ($p < .01$).

Statistical evaluation of the temporal effects on DOPAC and HVA concentrations (Figures 5*B–C*), revealed significant overall interactions ($F_{40,240} = 24.3$, $p < .001$ and $F_{40,240} = 47.0$, $p < .001$, respectively). Post-hoc analysis indicated that clozapine, haloperidol, and amperozide significantly increased both DOPAC and HVA, whereas idazoxan and MDL 100,907 had no effect. The effects of clozapine and haloperidol on DOPAC were significantly different from baseline and controls during the 60- to the 240-min postinjection interval ($p < .001$) and during the 120- to 240-min postinjection interval after amperozide treatment ($p < .05$–.01). The effects of clozapine, haloperidol, and amperozide on HVA reached statistical significance within the 90- to the 240-min postinjection interval as compared to both baseline and control values ($p < .05$–.001).

Effects of Intracortical Infusion of Risperidone or Idazoxan on 5-HT Extracellular Concentrations (Figure 6)

Infusion of risperidone or idazoxan by reverse dialysis (1.0-1000 μmol/L) dose-dependently increased 5-HT levels in the FC (Figure 6). Statistical analysis indicated a significant dose effect ($F_{11,110} = 33.7$, $p < .001$). The increase in 5-HT concentrations became statistically apparent after the 100 μmol/L dose of either risperidone or idazoxan ($p < .05$–.001). After the discontinuation of risperidone or idazoxan infusion, 5-HT concentrations remained significantly elevated for 30 or 60 min, respectively. No statistically significant differences between these groups were indicated. Intracortical infusion of risperidone or idazoxan did not significantly affect 5-HIAA, DOPAC, or HVA concentrations (data not shown).

Effects of Risperidone Administered Alone or in Combination with WAY 100,635 on the Activity of 5-HT Cells in the DRN (Figures 7 and 8)

There was no significant difference in baseline firing rate of DRN-5-HT cells between the different treatment groups. The overall mean basal firing rate was 1.19 ± 0.09 spikes/s ($n = 43$). Administration of increasing doses of risperidone (25-800 μg/kg, IV) dose-dependently inhibited the spontaneous firing of 5-HT neurons in the DRN (Figures 7 and 8). Statistical analysis indicated that risperidone at the dose-range 50-800 μg/kg IV significantly decreased firing rate compared to vehicle control ($p < .05$–.001). In the animals pretreated with WAY 100,635 (5.0 μg/kg, IV), which by itself did not significantly affect firing rate expressed either as absolute values or as percent of baseline compared to control (see below), risperidone failed to significantly suppress the firing rate of 5-HT DRN cells. In addition, there was a significant difference between the groups at the 100 and 200 μg/kg IV, dose of risperidone ($p < .05$–.01).

In separate control experiments, the effect of WAY 100,635 (5.0 μg/kg, IV) was monitored for a period of 21 min after drug injection, i.e., the time used for a com-

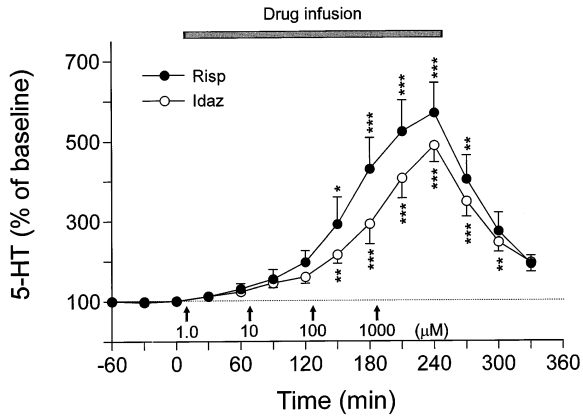

Figure 6. Effects of cortical infusion of risperidone (Risp; 1.0, 10, 100, and 1000 μmol/L; 60 min each concentration; $n = 5$) or idazoxan (Idaz; 1.0, 10, 100, and 1000 μmol/L; 60 min each concentration; $n = 7$) on 5-HT extracellular concentrations in the FC. *Arrows* and *bar* indicate the start of drug infusion and the whole drug infusion period, respectively. * = $p < .05$, ** = $p < .01$, *** = $p < .001$ compared to the last baseline sample.

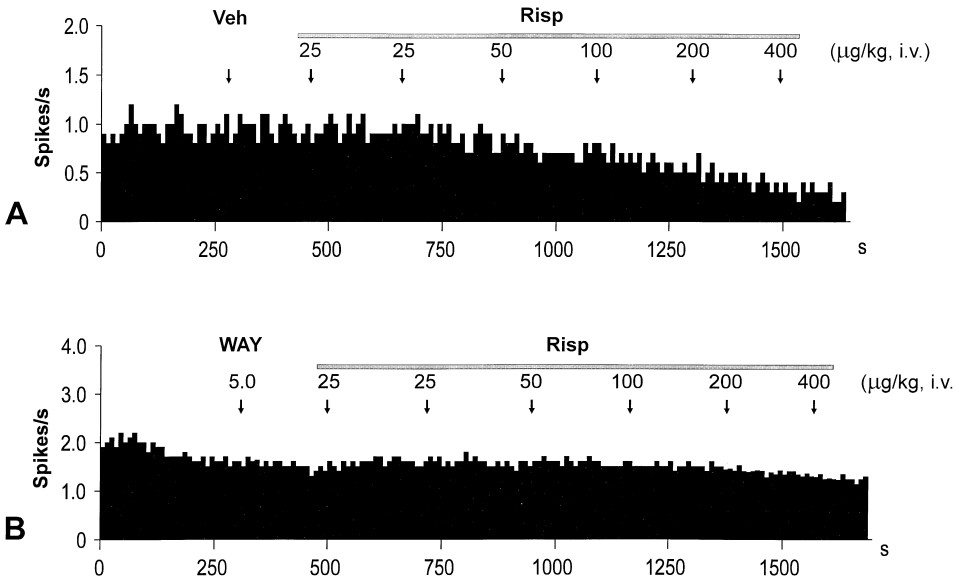

Figure 7. Integrated firing rate histograms of presumed 5-HT neurons in the DRN showing representative effects of risperidone (Risp; 25-400 μg/kg, IV) after pretreatment with (**A**) vehicle (Veh; 0.2 ml) or (**B**) WAY 100,635 (WAY; 5.0 μg/kg, IV). *Arrows* indicate time of injection.

plete risperidone dose-response experiment, and the mean firing rate was measured at 3.0-min intervals. Statistical analysis revealed that the effect of this dose of WAY 100,635 on 5-HT neuronal activity, i.e., a slight increase, represented as absolute firing rate or calculated as percent of baseline, failed to reach statistical significance (data not shown, $n = 9$). The selective 5-HT_{1A} receptor agonist (R)-8-OH-DPAT was administered at the end of some of these control experiments to verify that the used dose of WAY 100,635 effectively blocked 5-HT_{1A} receptors. Indeed, a much higher dose of (R)-8-OH-DPAT (1.6 μg/kg, IV) was required to inhibit 5-HT cell firing in the DRN by 50% after pretreatment with WAY 100,635 than has previously been reported in drug-naive rats, i.e., 0.4 μg/kg, IV (Arborelius et al. 1994), indicating that the used dose of WAY 100,635 effectively blocked 5-HT_{1A} receptors, even 21-min postinjection. In addition, a similar dose of WAY 100,635 has previously been shown to antagonize the inhibitory effect of (R)-8-OH-DPAT on 5-HT cell firing in the DRN (Fletcher et al. 1996).

DISCUSSION

The major finding of the present study is that acute, systemic, or local administration of risperidone dose-dependently increases dialysate concentrations of 5-HT in the rat FC, an effect which is probably related to its α_2-adrenoceptor antagonistic action and seems to be executed at the nerve terminal level.

Risperidone is characterized by a high affinity for 5-HT_{2A} receptors with less, but still relatively high, affinity for DA-D_2 receptors (Janssen et al. 1988; Leysen et al. 1988). The doses of risperidone used in the present study were selected on the basis of receptor occupancy data, which were obtained with ex vivo autoradiography (Leysen et al. 1992, 1993; Schotte et al. 1993), in vivo receptor binding (Sumiyoshi et al. 1994), or with the EEDQ-induced receptor inactivation method (Matsubara et al. 1993), to give a high 5-HT_{2A} receptor occupancy throughout the whole dose spectrum and a gradually increasing DA-D_2 receptor occupancy. According to the calculated ED_{50}-values, the occupancy of 5-HT_{2A} receptors in the present study can be estimated to be high (about 70%) after administration of the lowest dose (0.2 mg/kg, SC) of risperidone with a concurrent DA-D_2 receptor occupancy less than 50%. The highest

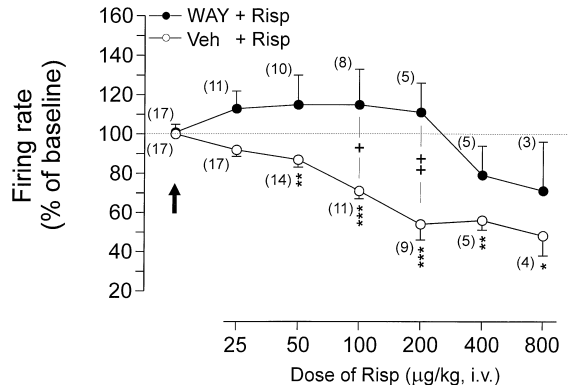

Figure 8. Effects of cumulative doses of risperidone (Risp; 25-800 μg/kg, IV; 3-min intervals) on the firing rate of presumed DRN 5-HT neurons after pretreatment (3 min) with vehicle (Veh; 0.2 ml, IV; number of cells in parentheses) or WAY 100,635 (WAY; 5.0 μg/kg, IV; number of cells in parentheses). *Arrow* indicates the first sample after injection of either vehicle or WAY 100,635. * = $p < .05$, ** = $p < .01$, *** = $p < .001$ compared to vehicle. $^{+}$ = $p < .05$, $^{++}$ = $p < .01$ compared between treatment groups.

dose (2.0 mg/kg, SC) of risperidone probably results in a nearly maximal 5-HT_{2A} receptor occupancy (about 90%) but also a high (around 70%) occupancy of DA-D_2 receptors. Thus, the antagonism of DA-D_2 receptors of risperidone could, theoretically, underlie the increase in dialysate concentrations of 5-HT, based on the apparent positive correlation between occupation of DA-D_2 receptors and cortical 5-HT output. However, this notion seems less likely since haloperidol, in a dose which probably results in a near to maximum DA-D_2 receptor occupancy (Leysen et al. 1992; Matsubara et al. 1993; Sumiyoshi et al. 1994) failed to significantly influence extracellular concentrations of 5-HT in the FC. Our finding that haloperidol exerts only minor effects on 5-HT output in brain is in agreement with the results of a recent study, in which the effect of haloperidol on extracellular levels of 5-HT in another terminal region, i.e., the nucleus accumbens, was investigated (Ferré and Artigas 1995). These authors showed that neither systemic nor local administration of haloperidol significantly affects the extracellular levels of 5-HT in the nucleus accumbens. A tentative contribution of risperidone's 5-HT_{2A} receptor antagonistic property to its facilitatory effect on central dialysate 5-HT is also highly unlikely for the following reasons: first, a high occupancy of 5-HT_{2A} receptors is probably achieved already at the lowest dose of risperidone, which failed to affect 5-HT levels. Second, a presumed, nearly full occupancy of 5-HT_{2A} receptors, achieved by either clozapine (Sumiyoshi et al. 1995) or by the highly selective 5-HT_{2A} receptor antagonist MDL 100,907 (Palfreyman et al. 1993), was not associated with an increase in extracellular levels of 5-HT. In fact, clozapine decreased brain 5-HT output in accordance with previous data (Ferré and Artigas 1995). Taken together, it appears that neither the DA-D_2 nor the 5-HT_{2A} receptor antagonistic properties of risperidone can account for the increase in dialysate 5-HT in the FC.

Interestingly, risperidone, besides its high affinity for 5-HT_{2A} and DA-D_2 receptors, has also been shown to exhibit considerable affinity for both central α_1- and α_2-adrenoceptors in vivo. Thus, the ED_{50}-values of risperidone for occupying DA-D_2, 5-HT_{2A}, α_1- and α_2-receptors 2 h after SC injections have been reported to be 1.2, 0.062, 1.6, and 3.7 mg/kg, respectively (Schotte et al. 1996). It is now well established that central 5-HT neurons are subjected to noradrenergic control, which is executed both at the cell body and the nerve terminal levels. Blockade of α_2-adrenoceptors may, accordingly, facilitate 5-HT neurotransmission at two levels: first, by enhanced release of NA at the 5-HT cell body level, which results in an increase in firing rate of 5-HT cells via enhanced stimulation of α_1-adrenoceptors (Svensson et al. 1975; Baraban and Aghajanian 1980; Vandermaelen and Aghajanian 1983), and second, by a diminished inhibitory noradrenergic influence via release controlling α_2-heteroreceptors located on 5-HT terminals (Göthert et al. 1981; Starke et al. 1989; Maura et al. 1992). In accordance with this notion, we found that the α_2-adrenoceptor antagonist idazoxan (Doxey et al. 1983), administered systemically or locally, increases dialysate 5-HT in the FC, in line with previous biochemical observations (Garratt et al. 1991). Interestingly, both risperidone and the putative antipsychotic drug amperozide, which also was found to enhance cortical output of 5-HT, have been reported to exhibit α_2-adrenoceptor antagonistic action (Waters et al. 1989; Svartengren and Simonsson 1990; Herberg et al. 1995; Schotte et al. 1996). Thus, the increase in extracellular concentrations of 5-HT observed after risperidone, amperozide, and idazoxan could be attributed to α_2-adrenoceptor blockade, because this mechanism appears as a common denominator for these drugs at the doses used.

Systemic administration of risperidone decreased the firing rate of 5-HT cells in the DRN. Because the dialysate concentration of 5-HT in the FC was, thus, not correlated to an increase in the firing rate of 5-HT DRN neurons, our observation indicates, indirectly, that the nerve terminal region is the site of action for risperidone's 5-HT output facilitating effect. The mechanism responsible for the suppression of DRN 5-HT cell firing by risperidone cannot be conclusively derived from our experiments. However, in view of the neurochemical profile of risperidone and the serotonergic afferent control of the DRN (see above) it seems likely that risperidone's α_1-adrenoceptor antagonistic action may play a significant role. It has previously been shown that the degree of suppression of 5-HT cell firing in the DRN induced by various neuroleptics correlates to their α_1-adrenoceptor antagonistic efficacy (Gallager and Aghajanian 1976). However, our finding that the risperidone-induced decrease in firing rate could largely be antagonized by 5-HT_{1A} receptor blockade suggests an involvement also of other mechanisms. Indeed, it could be argued that the observed suppression of 5-HT cell firing by risperidone is related to an α_1-adrenoceptor antagonistic action and that the blockade of this effect by a 5-HT_{1A} receptor antagonist is due to physiological antagonism. This explanation is, however, not compatible with the findings of Lejeune et al. (1994) who showed that 5-HT_{1A} receptor antagonists failed to block α_1-adrenoceptor antagonist-induced suppression of 5-HT cell firing in the DRN. Because risperidone exhibits relatively low affinity for 5-HT_{1A} receptors (Leysen et al. 1992) and given that these receptors are known to act as autoreceptors in the DRN, negatively regulating nerve impulse flow (see Aghajanian 1995), the antagonism of the risperidone-induced decrease in cell firing by autoreceptor blockade infers that risperidone may cause an increased availability of extracellular 5-HT also in the DRN. In support of this notion, we have in preliminary studies found

that both systemic and local raphe administration of risperidone increases extracellular concentrations of 5-HT in the DRN. The mechanism responsible for this facilitatory effect of risperidone on 5-HT output in the DRN is currently under investigation.

In accordance with our previous findings, the present study revealed that both risperidone and amperozide markedly enhanced the extracellular concentration of 5-HIAA in the FC (Hertel et al. 1996). In line with other findings, we also found that idazoxan significantly increased the extracellular levels of 5-HIAA at certain time intervals (Garratt et al. 1991). Thus, there seems to exist a positive correlation between the effects of the drugs on central 5-HT and 5-HIAA levels. However, differences in the effects of 5-HT cell firing between risperidone and idazoxan clearly suggest different sites of action of these drugs. Previous studies have shown that administration of idazoxan increases the firing rate of 5-HT neurons in the DRN, an effect which is temporally correlated with increases in both 5-HT and 5-HIAA levels (Freedman and Aghajanian 1984; Garratt et al. 1991). In contrast, the risperidone-induced increase in 5-HIAA levels was associated with decreased firing of 5-HT neurons. Thus, although the precise mechanism involved in risperidone's effect on 5-HIAA remains to be elucidated, our data support a local action at the nerve terminal level. Indeed, α_2-heteroreceptors located in the cortex have been found to modulate the synthesis of 5-HT, and such receptors may also provide the sites for a local mechanism of action of these drugs on central 5-HT turnover (Esteban et al. 1996). In consonance with previous studies, all compounds that induce some degree of DA-D_2 receptor blockade, i.e., risperidone, haloperidol, clozapine, and amperozide, were found to increase the levels of DOPAC and HVA in the FC (Hernandez and Hoebel 1989; Hertel et al. 1996). Furthermore, the degree of increase in DA metabolites levels was largely correlated to the assumed level of DA-D_2 receptor blockade. Accordingly, the two compounds that seem to be without any DA-D_2 receptor blocking properties, i.e., idazoxan and MDL 100,907, failed to affect DA metabolism in the FC. Similar results have previously been reported for MDL 100,907 (Schmidt and Fadayel 1995).

A putative contribution of α_2-adrenoceptor antagonism to the therapeutic efficacy of antipsychotic drugs has previously been discussed. In fact, it has been suggested that blockade of α_2-adrenoceptors may be essential for the favourable therapeutic properties of risperidone (Leysen et al. 1988; Nutt 1994). This notion is in line with some clinical studies demonstrating that administration of the α_2-adrenoceptor antagonist idazoxan augments the therapeutic response of conventional neuroleptic drug therapy (Litman et al. 1996). Also, several clinical trials have indicated that enhanced availability of 5-HT in brain achieved by other drugs than α_2-adrenoceptor antagonists, i.e., selective 5-HT reuptake inhibitors, in conjunction with treatment with neuroleptics is associated with significant amelioration of negative symptoms in schizophrenia (Goldman and Janecek 1990; Silver and Nassar 1992; Spina et al. 1994; Goff et al. 1995; Litman et al. 1996). Consequently, the beneficial actions of risperidone against negative symptoms in schizophrenia (Kane et al. 1988; Borison et al. 1992; Chouinard et al. 1993) may, at least partly, be related to its capability to augment central 5-HT availability.

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