British Journal of Pharmacology (1997) 122, 1639–1646

Risperidone inhibits 5-hydroxytryptaminergic neuronal activity in the dorsal raphe nucleus by local release of 5-hydroxytryptamine

Peter Hertel, George G. Nomikos & [1]Torgny H. Svensson

Department of Physiology and Pharmacology, Division of Pharmacology, Karolinska Institutet, S-171 77 Stockholm, Sweden

1. The effects of risperidone on brain 5-hydroxytryptamine (5-HT) neuronal functions were investigated and compared with other antipsychotic drugs and selective receptor antagonists by use of single cell recording and microdialysis in the dorsal raphe nucleus (DRN).

2 Administration of risperidone (25–400 μg kg^{-1}, i.v.) dose-dependently decreased 5-HT cell firing in the DRN, similar to the antipsychotic drug clozapine (0.25–4.0 mg kg^{-1}, i.v.), the putative antipsychotic drug amperozide (0.5–8.0 mg kg^{-1}, i.v.) and the selective α_1-adrenoceptor antagonist prazosin (50–400 μg kg^{-1}, i.v.).

3 The selective α_2-adrenoceptor antagonist idazoxan (10–80 μg kg^{-1}, i.v.), in contrast, increased the firing rate of 5-HT neurones in the DRN, whereas the D_2 and 5-HT_{2A} receptor antagonists raclopride (25–200 μg kg^{-1}, i.v.) and MDL 100,907 (50–400 μg kg^{-1}, i.v.), respectively, were without effect. Thus, the α_1-adrenoceptor antagonistic action of the antipsychotic drugs might, at least partly, cause the decrease in DRN 5-HT cell firing.

4 Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY 100,635 (5.0 μg kg^{-1}, i.v.), a drug previously shown to antagonize effectively the inhibition of 5-HT cells induced by risperidone, failed to prevent the prazosin-induced decrease in 5-HT cell firing. This finding argues against the notion that α_1-adrenoceptor antagonism is the sole mechanism underlying the inhibitory effect of risperidone on the DRN cells.

5 The inhibitory effect of risperidone on 5-HT cell firing in the DRN was significantly attenuated in rats pretreated with the 5-HT depletor PCPA (*p*-chlorophenylalanine; 300 mg kg^{-1}, i.p., day^{-1} for 3 consecutive days) in comparison with drug naive animals.

6 Administration of risperidone (2.0 mg kg^{-1}, s.c.) significantly enhanced 5-HT output in the DRN.

7 Consequently, the reduction in 5-HT cell firing by risperidone appears to be related to increased availability of 5-HT in the somatodendritic region of the neurones leading to an enhanced 5-HT_{1A} autoreceptor activation and, in turn, to inhibition of firing, and is probably only to a minor extent caused by its α_1-adrenoceptor antagonistic action.

Keywords: Neuroleptic drugs; spontaneous firing; 5-hydroxytryptamine; dorsal raphe nucleus; electrophysiology; microdialysis

Introduction

The antipsychotic drug risperidone has in several clinical studies been found to be effective against positive as well as negative symptoms of schizophrenia, while displaying a relatively low incidence of extrapyramidal side effects (EPS; Mesotten *et al*., 1989; Borison *et al*., 1992; Chouinard *et al*., 1993; Davis & Janicak, 1996). Preclinical studies have revealed that risperidone exhibits affinity for a variety of central receptors including 5-hydroxytryptamine (5-$HT)_{2A}$ receptors, dopamine D_2 receptors, α_1- and α_2-adrenoceptors and, similar to the atypical antipsychotic drug clozapine, risperidone yields a high ratio of 5-HT_{2A} to D_2 receptor blocking activity (Leysen *et al*., 1988; Schotte *et al*., 1996; Ashby & Wang, 1996).

We have previously shown that risperidone as well as amperozide, a putative antipsychotic drug also characterized by high affinity for 5-HT_{2A} compared to D_2 receptors (Svartengren & Simonsson, 1990; Axelsson *et al*., 1991; Björk *et al*., 1992), potently increase 5-HT output in the frontal cortex (FC; Hertel *et al*., 1996; 1997). Moreover, we have found that risperidone inhibits the spontaneous firing of 5-HT cells in the dorsal raphe nucleus (DRN; Hertel *et al*., 1997), a major origin for the 5-hydroxytryptaminergic innervation of the FC (see Jacobs & Azmitia, 1992). This effect of risperidone was largely antagonized by blockade of 5-HT_{1A} receptors (Hertel *et al*., 1997), which are known to act as autoreceptors in the somatodendritic region of the 5-HT cells negatively controlling their physiological activity (see Aghajanian, 1995).

Although the precise mechanism underlying this effect could not conclusively be derived from our previous experiments, it was suggested that the decreased firing rate induced by risperidone may be secondary to increased availability of 5-HT in the DRN. However, in view of the facilitatory influence of the noradrenergic system on 5-HT cell firing, it could not be ruled out that the inhibitory effect of risperidone on 5-HT neuronal activity is due to blockade of excitatory α_1-adrenoceptors within the DRN (Svensson *et al*., 1975; Baraban & Aghajanian, 1980; Schotte *et al*., 1996). Moreover, although the decrease of 5-HT cell firing in the DRN induced by risperidone was antagonized by pretreatment with a 5-HT_{1A} receptor antagonist, this effect might still be explained by physiological antagonism.

Consequently, the present study was undertaken to characterize further the effects of risperidone on the firing activity of 5-HT cells and to unravel its underlying mechanism(s). To this end, we compared the effects of risperidone with those obtained with other antipsychotic drugs and various selective receptor antagonists on 5-HT cell firing and analysed the ability of a 5-HT_{1A} receptor antagonist to prevent the decrease in 5-HT neuronal firing in the DRN induced by an α_1-adrenoceptor antagonist, by means of *in vivo* single cell recordings. The importance of endogenous 5-HT for the inhibitory effect

[1] Author for correspondence.

of risperidone on 5-HT cell firing in the DRN was investigated in animals depleted of 5-HT by repeated pretreatment with the 5-HT synthesis inhibitor PCPA (*p*-chlorophenylalanine). In addition, the effects of risperidone on 5-HT output in the DRN were directly studied by means of microdialysis in freely moving animals.

Methods

Animals

Male Sprague-Dawley or Wistar rats (Bantin and Kingman Universal AB, Sollentuna, Sweden) weighing 275–400 g were used for electrophysiological and microdialysis experiments, respectively. Animals were housed under standard laboratory conditions on a 12 h light/dark cycle (lights on at 06 h 00 min) and allowed free access to food and water.

Single cell recordings

Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.). Additional doses were given when needed to maintain surgical anaesthesia 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 (i.v.) 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 & Watson, 1986). Recording electrodes were pulled in a Narishige vertical puller from glass capillaries (outer diameter: 1.5 mm, inner diameter: 1.17 mm; Clark Electromedical Instruments) and filled with 2% Pontamine Sky Blue in 2 M 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 with a David Kopf hydraulic microdrive and the presumed 5-HT neurones 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 neurones in the DRN (Aghajanian *et al*., 1978; Vandermaelen & Aghajanian, 1983). The selective 5-HT_{1A} receptor agonist (**R**)-8-OH-DPAT was administered towards the end of some, randomly selected experiments, in order pharmacologically to ascertain further that the cells were, indeed, 5-hydroxytryptaminergic neurones. 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.

Upon completion of the experiments, animals were killed by an overdose of anaesthetic and their brains preserved in 10% formalin in 25% sucrose. Each brain was sliced on a microtome (50 μm), stained with neutral red and examined under the microscope. All recording sites included in this study were located within the DRN (plates 48–50 in the atlas of Paxinos and Watson, 1986). 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 analysed by the CED Spike2 program.

Drugs were administered intravenously in exponentially increasing doses at 3.0 min intervals. These drug injections were preceded (3.0 min) by either a control (the appropriate drug vehicle) or, in some cases, by a WAY 100,635 injection. In some experiments, rats were pretreated with PCPA at a dose of 300 mg kg^{-1} day^{-1}, i.p., for 3 consecutive days, which has been shown to cause virtually complete and long-lasting depletion of 5-HT in brain tissue (Koe & Weissman, 1966), and tested approximately 24 h after the last injection.

Microdialysis

Concentric dialysis probes were stereotaxically implanted with a lateral angle of 30° under barbiturate anaesthesia (Mebumal, 60 mg kg^{-1}, i.p.) in the DRN. The coordinates (in mm) were: AP = −7.8, ML = ±3.0 and DV = −7.8 relative to bregma and dural surface (Paxinos & Watson, 1986). The dorsoventral coordinate corresponds to the actual descent of the probe along a line inclined 30°. Dialysis occurred through a semipermeable membrane (copolymer of acrylonitrile and sodium methallyl sulfonate, i.d. = 0.24 mm, 40,000 Da, AN69 Hospal), having an active surface length of 1.5 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 & Di Chiara, 1989). Following 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 (mM): NaCl 147, KCl 3.0, $CaCl_2$ 1.3, $MgCl_2$ 1.0 and sodium phosphate 1 (pH 7.4) at a rate of 2.5 μl min^{-1} 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) programme to inject automatically samples every 30 min.

Concentrations of 5-HT and 5-HIAA were determined by high-performance liquid chromatography (h.p.l.c.) with electrochemical detection. 5-HT and 5-HIAA in the dialysate were separated by reversed-phase liquid chromatography (150 × 4.6 mm, Nucleosil 3 μm, C18) with a mobile phase consisting of 0.055 M sodium acetate, 0.7 mM octanesulphonic acid, 0.01 mM Na_2EDTA and 19% methanol (pH = 4.0, adjusted with glacial acetic acid). The mobile phase was delivered by an h.p.l.c. pump (LKB 2150) at 0.8 ml min^{-1}. A precolumn (50 × 3 mm, Nucleosil 5 μm, C18) was placed between the h.p.l.c. pump and the injection loop. Electrochemical detection was accomplished by a coulometric detector (Coulochem II, model 5200, ESA) with a conditioning cell (5021) and an analytical cell (5011). 5-HT and 5-HIAA were detected and quantified by sequential oxidation of the eluent (coulometric electrode = +0.35 V; amperometric electrode = +0.45 V). Chromatograms were recorded on a dual pen chart-recorder (Kipp & Zonen, BD41).

Subcutaneous injections, given in the neck region at volume of 1.0 ml kg^{-1}, were performed after a stable (<10% variation) outflow of 5-HT and 5-HIAA was established. Control animals received injections with the appropriate drug vehicle. Upon completion of the experiments, the animals were killed by an overdose of anaesthetic and their brains preserved in 10% formalin in 25% sucrose. Each brain was sliced on a microtome (50 μm), stained with neutral red, and examined under microscope for probe placement. Only rats with probes verified to be located in the DRN (plates 48–50 in the atlas of Paxinos and Watson, 1986) were included in data analysis.

Drugs

In single cell recording experiments, risperidone (Janssen Pharmaceutica), clozapine (Sandoz), MDL 100,907 (**R**-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol; Marion Merrell Dow Inc.), prazosin (Pfizer Inc.) were dissolved in saline (0.9% NaCl) with the addition of a minimal amount of acetic acid; pH was thereafter adjusted to 6.5–7.0 with sodium hydroxide. Amperozide (*N*-ethyl-4-[4′-4′-bis(p-fluorophenyl)butyl]-1-piperazinecarboximide; Pharmacia AB), raclopride (Astra Arcus AB), idazoxan (Research Biochemicals International), WAY 100,635 (*N*-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-*N*-(2-pyridinyl)cyclohexane carboxamide trihydroxychloride; Wyeth Research), (**R**)-8-OH-DPAT ((+)-(**R**)-8-hydroxy-2-(dipropylamino)-tetralin hydro-

xychloride; synthesized at the Department of Organic Pharmaceutical Chemistry, Uppsala Universitet, Uppsala, Sweden) and PCPA (*p*-chlorophenylalanine methyl ester; Sigma) were dissolved in saline. In microdialysis experiments, risperidone was dissolved in 5.5% glucose solution with the additon of a minimal amount of acetic acid.

Data analysis

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 interval at maximal drug effect at each dose. Baseline values from all animals assigned to various experimental groups were evaluated by one (treatment)-way analysis of variance (ANOVA). Data were also calculated and presented as % changes of baseline values, defined as 100%. Mean % change of baseline ± s.e.mean was calculated for each drug dose within the different treatment groups. Data were analysed statistically by *t* test for dependent samples to evaluate effects within each treatment group and *t* test for independent samples to evaluate effects between the various treatment groups. A *P* value less than 0.05 was considered significant.

Basal dialysate concentration data were statistically evaluated by *t* test for independent samples for comparisons between treatment groups. A *P* value less than 0.05 was considered significant. Dialysis data were also calculated and presented as % 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 ± s.e.mean were calculated for each sample across the rats in all groups. The % change of basal outflow (last baseline plus all posttreatment samples) were analysed by two (time × treatment)-way ANOVA for repeated measures, followed by the Newman-Keuls test for multiple comparisons with a criterion of $P<0.05$ to be considered significant. All data were statistically evaluated by using the CSS : Statistica (Statsoft) program.

Results

Effects of risperidone, clozapine and amperozide on firing activity of 5-HT cells in the DRN

There was no significant difference in baseline firing rate of DRN-5-HT cells between the various treatment groups. The overall mean basal firing rate (± s.e.mean; $n=80$) was 1.10 ± 0.06 spikes s^{-1}. Administration of increasing doses of risperidone (25–400 $\mu g\ kg^{-1}$, i.v.), clozapine (0.25–4.0 mg kg^{-1}, i.v.) and amperozide (0.5–8.0 mg kg^{-1}, i.v.) dose-dependently inhibited the spontaneous firing of 5-HT neurones in the DRN (Figure 1a–c). Statistical analysis indicated that risperidone, clozapine and amperozide at the dose-range 50–400 $\mu g\ kg^{-1}$, i.v., 0.25–2.0 mg kg^{-1}, i.v. and 1.0–8.0 mg kg^{-1}, i.v., respectively, significantly decreased firing rate compared to their respective control value ($P<0.05$–0.001).

Effects of MDL 100,907, raclopride, prazosin and idazoxan on firing activity of 5-HT cells in the DRN

Administration of increasing doses of both MDL 100,907 (50–400 $\mu g\ kg^{-1}$, i.v.) and raclopride (25–200 $\mu g\ kg^{-1}$, i.v.) exerted only minor effects on the firing activity of 5-HT neurones in the DRN (Figure 2a,b). Data analysis failed to reveal any statistically significant effects within the dose-intervals used. In contrast, prazosin (50–400 $\mu g\ kg^{-1}$, i.v.) inhibited, whereas idazoxan (10–80 $\mu g\ kg^{-1}$, i.v.) modestly increased the spontaneous firing of 5-HT neurones in the DRN (Figure 2c,d). Statistical analysis indicated that the prazosin-induced decrease in firing activity was statistically significant compared to control injection within the 200–400 $\mu g\ kg^{-1}$, i.v. dose interval ($P<0.05$–0.01). Statistical evaluation of the effects of idazoxan indicated that the 20 $\mu g\ kg^{-1}$, i.v., dose significantly increased the firing activity of 5-HT neurones in the DRN ($P<0.05$).

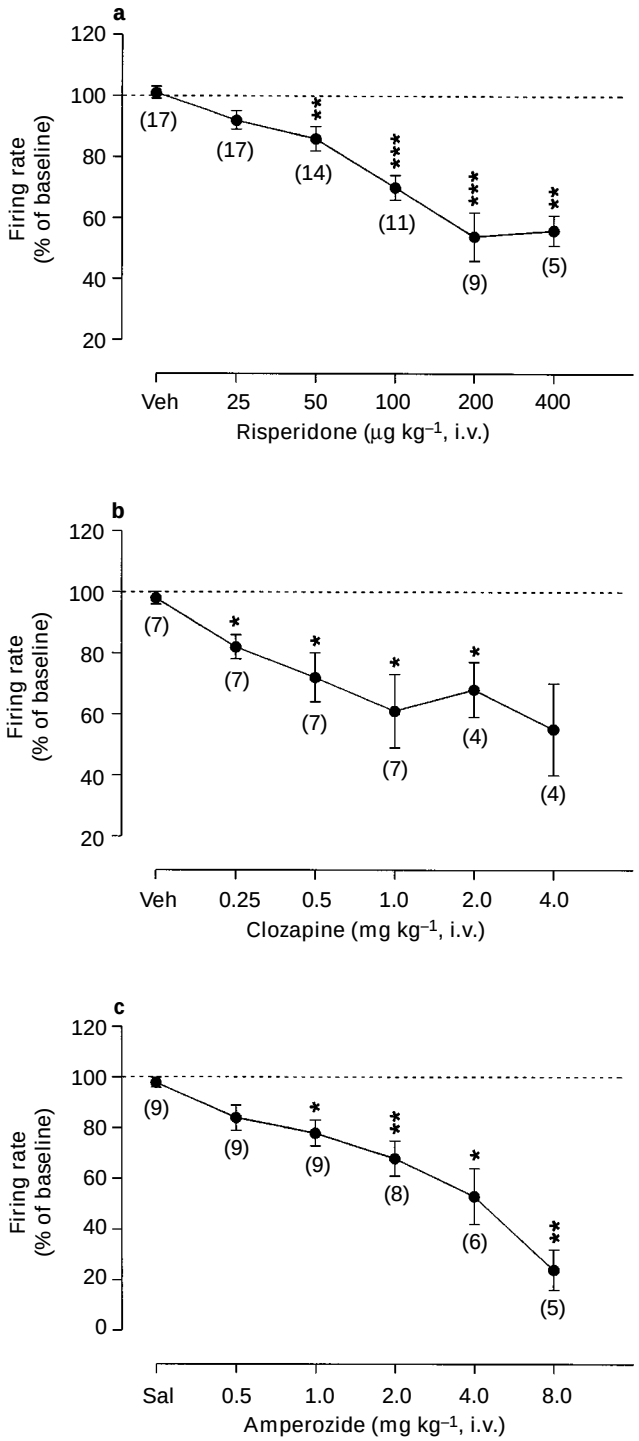

Figure 1 Effects of cumulative doses of (a) risperidone (25–400 $\mu g\ kg^{-1}$, i.v.; 3 min intervals), (b) clozapine (0.25–4.0 mg kg^{-1}, i.v.; 3 min intervals) and (c) amperozide (0.5–8.0 mg kg^{-1}, i.v.; 3 min intervals) on the spontaneous firing rate of presumed 5-HT neurones in the DRN (number of cells in parentheses). Drug injections were preceded (3.0 min) by control injection of vehicle (Veh) or saline (Sal). The mean basal firing rate (± s.e.mean) in the risperidone, clozapine and amperozide treatment group were 1.09 ± 0.09, 1.56 ± 0.29 and 0.90 ± 0.13, respectively. Each point represents the mean percentage of baseline and vertical lines show s.e.mean. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to control.

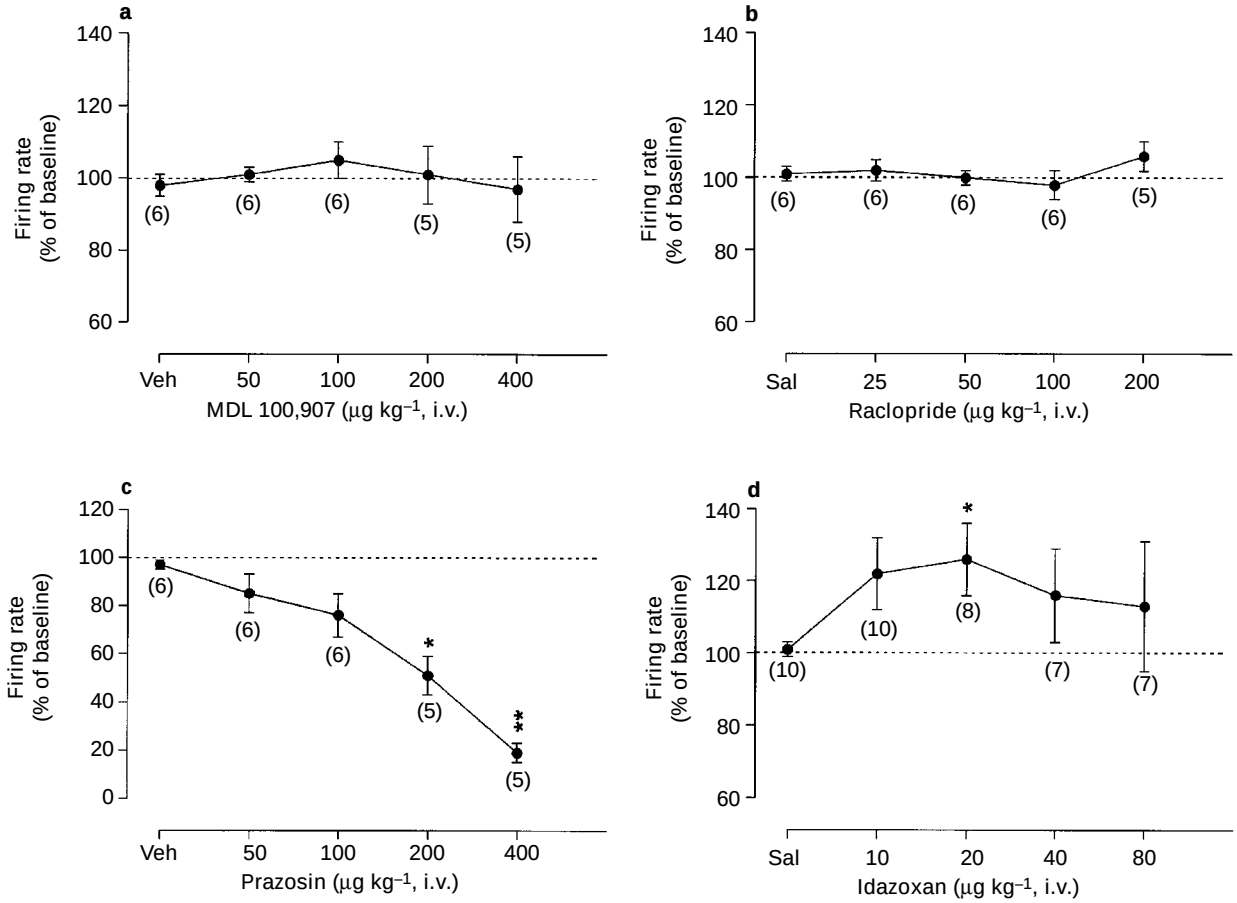

Figure 2 Effects of cumulative doses of (a) MDL 100,907 (50–400 μg kg^{-1}, i.v.; 3 min intervals), (b) raclopride (25–200 μg kg^{-1}, i.v.; 3 min intervals), (c) prazosin (50–400 μg kg^{-1}, i.v.; 3 min intervals) and (d) idazoxan (10–80 μg kg^{-1}, i.v.; 3 min intervals) on the spontaneous firing rate of presumed 5-HT neurones in the DRN (number of cells in parentheses). Drug injections were preceded (3.0 min) by control injection of vehicle (Veh) or saline (Sal). The mean basal firing rate ($\pm$s.e.mean) in the MDL 100,907, raclopride, prazosin and idazoxan treatment group were 0.74 ± 0.14, 1.21 ± 0.17, 0.83 ± 0.16 and 1.03 ± 0.22, respectively. Each point represents the mean percentage of baseline and vertical lines show s.e.mean. *$P<0.05$, **$P<0.01$ compared to control.

Effects of prazosin administered in combination with WAY 100,635 on the firing activity of 5-HT cells in the DRN

We have previously shown that the dose of WAY 100,635 used (5.0 μg kg^{-1}, i.v.), monitored for an extended time period (21 min), failed to influence significantly 5-HT neuronal activity but effectively blocked 5-HT$_{1A}$ receptors, even 21 min postinjection, in the DRN (Hertel *et al*., 1997). Pretreatment with WAY 100,635 (5.0 μg kg^{-1}, i.v.) failed to influence the prazosin-induced decrease in firing activity of 5-HT cells in the DRN (Figures 3a,b and 4). Statistical analysis indicated that both the effects of prazosin alone and the effects of prazosin after pretreatment with WAY 100,635 (5.0 μg kg^{-1}, i.v.) reached statistical significance within the same dose-interval i.e. 200–400 μg kg^{-1}, i.v. ($P<0.05-0.01$). Moreover, no statistically significant differences between the groups were found.

Effects of risperidone on firing activity of 5-HT cells in animals pretreated with PCPA

In agreement with previous findings, 5-HT depletion by PCPA pretreatment did not significantly affect the basal firing rate of 5-HT cells in the DRN (Wang & Aghajanian, 1978; Chaput *et al*., 1990).

The effects of increasing doses of risperidone (25–400 μg kg^{-1}, i.v.) on the spontaneous firing of 5-HT neurones in the DRN in PCPA-pretreated and drug naive rats are shown in Figures 5a,b and 6. Statistical evaluation of the data revealed that higher doses of risperidone were needed to decrease significantly spontaneous 5-HT cell firing in relation to control values in 5-HT depleted than in drug naive rats, i.e. 200–400 μg kg^{-1}, i.v. ($P<0.01$) compared to 50–400 μg kg^{-1}, i.v. ($P<0.05-0.001$), respectively. Thus, the lowest dose of risperidone tested that significantly inhibited 5-HT cell firing in rats pretreated with PCPA was 200 μg kg^{-1}, i.v., compared to 50 μg kg^{-1}, i.v., in drug naive animals. Moreover, *t* test for independent samples between these groups indicated that the suppression of spontaneous 5-HT cell firing in the DRN induced by risperidone was significantly less pronounced within the dose-range 100–400 μg kg^{-1}, i.v. ($P<0.05-0.01$), in 5-HT-depleted compared to drug naive animals.

Effects of systemic administration of risperidone on extracellular concentrations of 5-HT and 5-HIAA in the DRN

There was no statistically significant difference in basal extracellular concentrations of 5-HT or 5-HIAA in the DRN between the risperidone and the vehicle control group. The mean basal dialysate concentrations (fmol min$^{-1}\pm$s.e.mean; $n=12$) of 5-HT and 5-HIAA were 0.78 ± 0.09 and 1171 ± 102, respectively.

Vehicle injections did not significantly affect 5-HT or 5-HIAA output in the DRN as indicated by the statistical analysis (data not shown). Risperidone (2.0 mg kg^{-1}, s.c.) caused an increase in both extracellular 5-HT and 5-HIAA in the DRN (Figure 7). Statistical analysis of the effects of risperidone on extracellular 5-HT and 5-HIAA concentrations re-

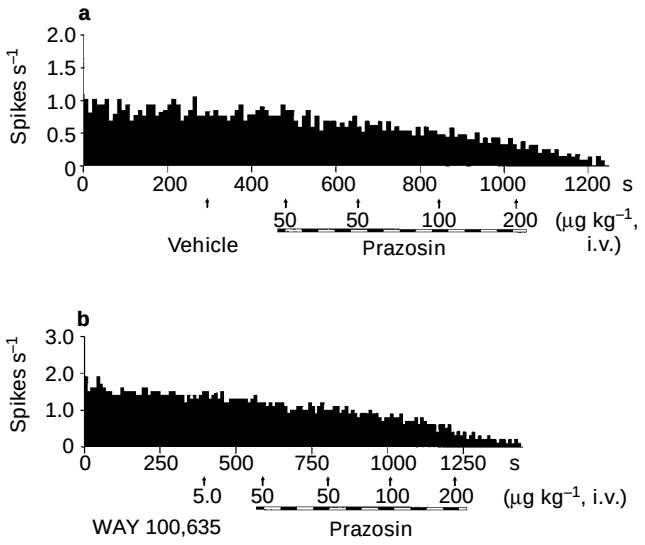

Figure 3 Integrated firing rate histograms of presumed 5-HT neurones in the DRN showing representative effects of prazosin (50–200 μg kg^{-1}, i.v.) after pretreatment with (a) vehicle (0.2 ml, i.v.) or (b) WAY 100,635 (5.0 μg kg^{-1}, i.v.). Arrows indicate time of injection.

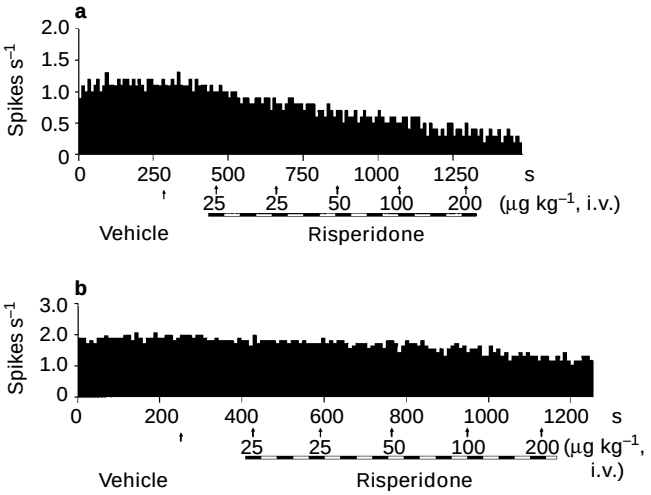

Figure 5 Integrated firing rate histograms of presumed 5-HT neurones in the DRN showing representative effects of risperidone (25–200 μg kg^{-1}, i.v.) in (a) a drug naive rat or (b) in a rat pretreated with PCPA (300 mg kg^{-1}, i.p. for 3 consecutive days). Arrows indicate time of injection.

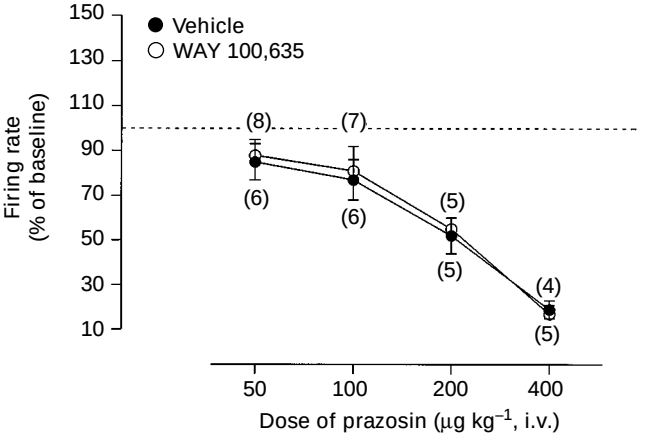

Figure 4 Effects of cumulative doses of prazosin (50–400 μg kg^{-1}, i.v.; 3 min intervals) on the firing rate of presumed 5-HT neurones in the DRN after pretreatment (3 min) with vehicle (0.2 ml, i.v.) or WAY 100,635 (5.0 μg kg^{-1}, i.v.; number of cells in parentheses). The mean basal firing rate ($\pm$s.e.mean) in the prazosin-vehicle and the prazosin-WAY 100,635 treatment group were 0.83 ± 0.16 and 1.25 ± 0.24, respectively. Each point represents the mean percentage of baseline and vertical lines show s.e.mean.

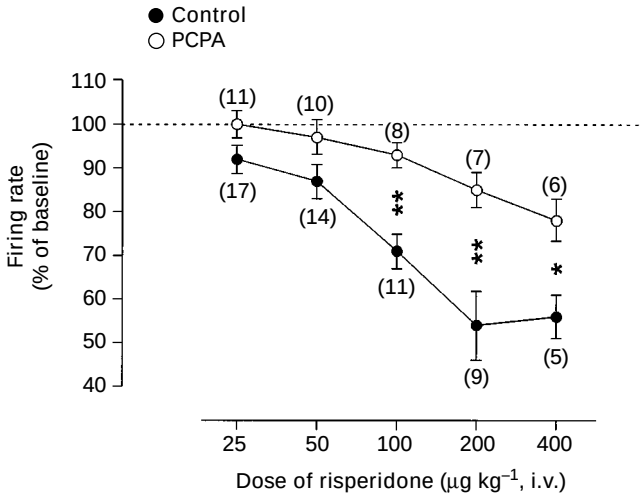

Figure 6 Effects of cumulative doses of risperidone (25–400 μg kg^{-1}, i.v.; 3 min intervals) on the firing rate of presumed 5-HT neurones in the DRN in drug naive rats (control) or in rats pretreated with PCPA (300 mg kg^{-1}, i.p. for 3 consecutive days). Number of cells in parentheses. The mean basal firing rate ($\pm$s.e.mean) in the drug naive and the PCPA pretreated group were 1.09 ± 0.09 and 1.19 ± 0.21, respectively. Each point represents the mean percentage of baseline and vertical lines show s.e.mean. $^*P<0.05$, $^{**}P<0.01$ compared between groups.

vealed, in both cases, a significant overall interaction ($F_{8.80}=7.69$, $P<0.001$ and $F_{8.80}=2.38$, $P<0.05$, respectively). *Post-hoc* analysis showed that risperidone significantly increased 5-HT output throughout the entire sampling period (i.e. 30 to 240 min post-injection) as compared to both baseline and vehicle control values ($P<0.05-0.001$). *Post-hoc* evaluation of the risperidone-induced effects on extracellular 5-HIAA indicated that this treatment significantly elevated extracellular 5-HIAA within the 150 to 240 min and the 120 to 240 min post-injection interval as compared to baseline ($P<0.05-0.01$) and vehicle control values ($P<0.05-0.01$), respectively.

Discussion

The major finding of the present study is that the inhibition of spontaneous firing of 5-HT cells in the rat DRN induced by systemic administration of risperidone is associated with, and probably caused by, increased 5-HT output within the DRN.

Based on the high affinity of risperidone for 5-HT$_{2A}$ receptors and less, but still relatively high, affinity for D$_2$ receptors as shown with both *ex vivo* (Leysen *et al*., 1992; Schotte *et al*., 1996) and *in vivo* (Matsubara *et al*., 1993; Sumiyoshi *et al*., 1994) methods, the doses of risperidone used in the present study were selected to yield a high 5-HT$_{2A}$ receptor occupancy throughout the major part of the dose spectrum and a gradually increasing D$_2$ receptor occupancy. Consequently, both antagonism of 5-HT$_{2A}$ and D$_2$ receptors by risperidone could, theoretically, underlie the observed inhibition of 5-HT cell firing in the DRN. However, this explanation seems less likely since both the highly selective 5-HT$_{2A}$ receptor antagonist MDL 100,907 (Palfreyman *et al*., 1993) and the selective D$_2$/D$_3$ receptor antagonist raclopride (Köhler *et al*., 1985) failed to affect the spontaneous firing of 5-HT cells in the DRN within

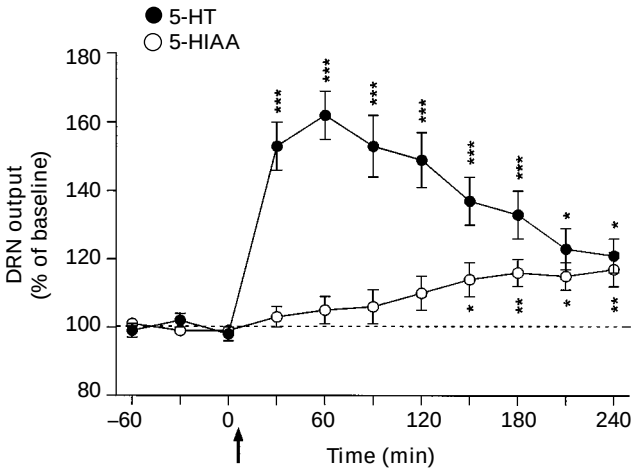

Figure 7 Effects of risperidone (2.0 mg kg^{-1}, s.c.; $n=7$) on extracellular concentrations of 5-HT and 5-HIAA in the DRN. Arrow indicates time of injection. Each point represents the mean percentage of baseline and vertical lines show s.e.mean. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to baseline.

dose intervals which, at least in the higher range, most likely result in maximal occupancy of the respective receptors. These findings, which are in line with previous data (Gallager & Aghajanian, 1976; Cunningham & Lakoski, 1990), suggests that the 5-HT cells in the DRN lack tonic, inhibitory control via either 5-HT$_{2A}$ or D$_2$ receptors. The inhibitory effect of risperidone on DRN cells was mimicked by both clozapine and the purported antipsychotic drug amperozide. Although the mechanisms involved remain to be conclusively determined, it can be inferred (cf above) that neither the D$_2$ nor the 5-HT$_{2A}$ receptor antagonistic properties of clozapine or amperozide account for the inhibition of spontaneous firing of 5-HT cells in the DRN (see Svartengren & Simonsson, 1990; Ashby & Wang, 1996).

By now it is well established that central 5-HT neuronal activity is subjected to a facilitatory noradrenergic input to 5-hydroxytryptaminergic cell-bodies executed via α_1-adrenoceptors. Inhibition of 5-HT cell firing could, accordingly, be achieved either by blockade of α_1-adrenoceptors or by stimulating inhibitory α_2-autoreceptors on noradrenergic terminals in the DRN, resulting in a diminished release of noradrenaline at the 5-HT cell body level (Svensson *et al*., 1975; Baraban & Aghajanian, 1980). It has previously been shown that the degree of suppression of 5-HT cell firing in the DRN induced by clozapine and various other neuroleptics correlates to their α_1-adrenoceptor antagonistic efficacy (Gallager & Aghajanian, 1976). In accordance with these findings, we found in the present study that both clozapine and the selective α_1 antagonist prazosin dose-dependently inhibit, whereas the selective α_2-adrenoceptor blocker idazoxan facilitates, 5-HT cell firing in the DRN (see Freedman & Aghajanian, 1984; Hoffman & Lefkowitz, 1990). Moreover, this effect of clozapine was mimicked by both risperidone and amperozide. Hence, it could be that the inhibitory actions of risperidone and amperozide on 5-HT neuronal activity, in analogy with that of clozapine, are due to their α_1-adrenoceptor antagonistic properties (Svartengren & Simonsson, 1990; Schotte *et al*., 1996).

However, our previous finding that the suppression of 5-HT cell firing elicited by risperidone is largely antagonized by 5-HT$_{1A}$ receptor blockade, indicates that this effect may not entirely be mediated through its α_1-adrenoceptor antagonistic action (Schotte *et al*., 1996; Hertel *et al*., 1997). Indeed, it could be argued that the 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 may be due to physiological, rather than pharmacological antagonism. However, the present data are not compatible with this notion, since the selective 5-HT$_{1A}$ antagonist WAY 100,635 was completely ineffective in counteracting the inhibitory action on 5-HT cell firing of the selective α_1-adrenoceptor antagonist prazosin. This observation is in line with previous biochemical and electrophysiological studies demonstrating that the suppressant effect on central 5-HT output and cell firing by various drugs which block α_1-adrenoceptors is not reversed by 5-HT$_{1A}$ receptor antagonism (Hjorth *et al*., 1995; Assié & Koek, 1996; Gartside *et al*., 1997). Interestingly, the finding by Lejeune *et al*. (1994), that pretreatment with a 5-HT$_{1A}$ receptor antagonist was unable to antagonize the decrease in firing rate induced by clozapine, suggests that risperidone and clozapine may suppress 5-HT cell firing in the DRN by different mechanisms.

Since risperidone exhibits relatively low affinity for 5-HT$_{1A}$ receptors (Schotte *et al*., 1996), the antagonism of risperidone-induced decrease in 5-HT cell firing by blockade of 5-HT$_{1A}$ receptors, known to act as autoreceptors in the somatodendritic region negatively controlling the 5-HT cell firing (Aghajanian, 1995), infers the possibility that risperidone may cause an increased output of 5-HT in the DRN (Hertel *et al*., 1997). Here, we demonstrate that systemically administered risperidone, indeed, increases the extracellular concentrations of both 5-HT and its metabolite 5-HIAA in the DRN. Moreover, we found that the degree of inhibition of 5-HT neuronal firing in the DRN by risperidone is dependent upon the availability of endogenous 5-HT, since previous depletion of 5-HT by PCPA treatment largely abolished this action of the drug. Consequently, the present data derived from electrophysiological and biochemical studies, together with our previous finding that the inhibition of 5-HT cell firing by risperidone can be antagonized by blockade of 5-HT$_{1A}$ receptors, provide strong support for the notion that the risperidone-induced inhibition of 5-HT cell firing in the DRN is, at least partly, secondary to an increased availability of extracellular 5-HT in the DRN. This would result in an increased activation of 5-HT$_{1A}$ autoreceptors and an ensuing reduction in the firing rate of 5-HT neurones (Hertel *et al*., 1997).

Although the suppressant effect of risperidone on 5-HT cell firing was significantly attenuated, it was not completely eliminated in rats pretreated with PCPA. This phenomenon may be explained by several mechanisms or combinations of mechanisms. Since the PCPA pretreatment used does not cause complete depletion of the 5-HT content in brain (Koe & Weissman, 1966), it seems reasonable to assume that risperidone in high doses still increases the availability of remaining 5-HT, which in turn exerts an inhibitory effect on 5-HT cell activity in the DRN. Moreover, since the PCPA pretreatment does not cause any major depletion of brain noradrenaline (Koe & Weissman, 1966), the decrease in 5-HT neuronal firing in 5-HT depleted rats might to some extent also be attributed to the α_1-adrenoceptor antagonistic action of risperidone on 5-HT neurones (see above).

We have previously demonstrated that risperidone dose-dependently increases extracellular concentrations of 5-HT in the FC, a major target area of the 5-HT neurones originating in the DRN (Jacobs & Azmitia, 1992; Hertel *et al*., 1997). Hence, risperidone seems to increase availability of 5-HT both in the terminal and in the somatodendritic region of the central, ascending 5-hydroxytryptaminergic system to the forebrain. The effect of risperidone in the FC has previously been attributed to a local effect in the nerve terminal region involving blockade of α_2-adrenoceptors located on 5-hydroxytryptaminergic terminals (see Starke *et al*., 1989; Maura *et al*., 1992; Hertel *et al*., 1997). However, in view of the opposite action of risperidone and idazoxan on 5-HT cell firing (this study; see Garratt *et al*., 1991), the augmenting action of risperidone on 5-HT levels in the DRN may not necessarily be mediated via α_2-adrenoceptors alone. In contrast, systemic idazoxan may very well increase 5-HT levels in the DRN at least partly through increased, impulse-dependent somatodendritic release of 5-HT (Matos *et al*., 1996).

The observed effects of risperidone on 5-HT neurotransmission are strikingly similar to those produced by 5-HT reuptake inhibitors i.e., increased output of 5-HT and attenuation of 5-HT cell firing in the DRN (Gallager & Aghajanian, 1975; Blier *et al*., 1987; Adell & Artigas, 1991; Invernizzi *et al*., 1992; Arborelius *et al*., 1995). However, risperidone exhibits only low affinity for the 5-HT uptake site (Leysen *et al*., 1992). Also, the augmenting effect of risperidone on extracellular 5-HIAA in the DRN clearly distinguishes risperidone from 5-HT reuptake inhibitors, which have been shown to decrease 5-HIAA output in this area (Adell & Artigas, 1991). Previous data show that acute administration of selective 5-HT reuptake inhibitors preferentially increases the release of 5-HT in the nerve terminal regions in comparison with the somatodendritic area (Adell & Artigas, 1991). This contrasts with the effect of risperidone which seems to facilitate 5-HT output to approximately the same extent in both DRN and FC (see also Hertel *et al*., 1997). In addition, it is well established that acute administration of high doses of 5-HT reuptake inhibitors virtually abolishes the firing activity of 5-HT cells (e.g. see Gallager & Aghajanian, 1975; Blier *et al*., 1987; Arborelius *et al*., 1995; Hajós *et al*., 1995), in contrast to high doses of risperidone which induce a maximal inhibition of 5-HT cell firing to approximately 50% of basal values (this study). Taken together, these findings strongly argue against 5-HT reuptake inhibition as the mechanism underlying the effects of risperidone on 5-HT cell activity.

The present data obtained with risperidone as well as other results clearly indicate that the firing of 5-HT cells and the 5-HT output in the DRN under certain conditions are uncoupled. In fact, recent data suggest that the release of 5-HT in the rat DRN is under the control of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors and may be largely regulated independently of the 5-HT cell firing activity (Davidson & Stamford, 1995; Pineyro *et al*., 1996). However, whether these receptors are located on 5-hydroxytryptaminergic nerve terminals or cell bodies remains a matter of debate (Davidson & Stamford, 1995). Regardless of the precise localization, risperidone might, via interference with these receptors, increase the output of 5-HT in the DRN. The precise mechanism underlying the facilitatory effect of risperidone on 5-HT output in the DRN is currently under investigation in our laboratory.

Several clinical trials have indicated that drugs which enhance availability of 5-HT in brain, i.e. selective 5-HT reuptake inhibitors or α_2-adrenoceptor antagonists, in conjunction with treatment with neuroleptics is associated with significant amelioration of negative symptoms in schizophrenia (Goldman & Janecek, 1990; Silver & 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; Davis & Janicak, 1996) may, at least partly, be related to its capacity to augment 5-hydroxytryptaminergic neurotransmission in some central 5-HT mediated synapses.

In conclusion, the present studies suggest that the inhibition of spontaneous firing of 5-HT cells in the rat DRN induced by systemic administration of risperidone is secondary to its ability to increase somatodendritic availability of 5-HT and essentially not a consequence of α_1-adrenoceptor antagonism.

This work was supported by grants from the Swedish Medical Research Council (projects 4747 and 11026), Karolinska Institutet, Fredrik och Ingrid Thurings Stiftelse, AB LEOs i Helsingborg Stiftelse för Forskning, Janssen Pharmaceutica N.V., Beerse, and Astra Arcus AB, Södertälje. MDL 100,907 and WAY 100,635 were generous gifts from Marion Merrell Dow Inc. and Wyeth Research, respectively. The excellent technical assistance of Mrs Anna Malmerfelt and Mr Martin Svensson is gratefully acknowledged. We also thank Dr Josée E. Leysen, Janssen Research Foundation, for valuable advice and discussions throughout this work.

References

ADELL, A. & ARTIGAS, F. (1991). Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **343,** 237–244.

AGHAJANIAN, G.K. (1995). Electrophysiology of serotonin receptor subtypes and signal transduction pathways. In *Psychopharmacology: the Fourth Generation of Progress*. ed. Bloom, F.E. & Kupfer, D.J. pp. 451–460. New York: Raven Press.

AGHAJANIAN, G.K., WANG, R.Y. & BARABAN, J. (1978). Serotonergic and non-seretonergic neurons of the dorsal raphe: reciprocal changes in firing induced by peripheral nerve stimulation. *Brain Res.*, **153,** 169–175.

ARBORELIUS, L., NOMIKOS, G.G., GRILLNER, P., HERTEL, P., BACKLUND HÖÖK, B., HACKSELL, U. & SVENSSON, T.H. (1995). 5-HT_{1A} receptor antagonists increase the activity of serotonergic cells in the dorsal raphe nucleus in rats treated acutely or chronically with citalopram. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **352,** 157–165.

ASHBY, C.R. & WANG, R.Y. (1996). Pharmacological actions of the atypical antipsychotic drug clozapine: a review. *Synapse*, **24,** 349–394.

ASSIÉ, M.-B. & KOEK, W. (1996). Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-hydroxytryptamine levels: (S)-WAY100135, but not WAY100635, has partial agonist properties. *Eur. J. Pharmacol.*, **304,** 15–21.

AXELSSON, R., NILSSON, A., CHRISTENSSON, E. & BJÖRK, A. (1991). Effects of amperozide in schizophrenia. *Psychopharmacology*, **104,** 287–292.

BARABAN, J.M. & AGHAJANIAN, G.K. (1980). Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonist. *Neuropharmacology*, **19,** 355–363.

BJÖRK, A., BERGMAN, I. & GUSTAVSSON, G. (1992). Amperozide in treatment of schizophrenic patients. A preliminary report. In *Novel Antipsychotic Drugs*. ed. Meltzer, H.Y. pp. 47–57. New York: Raven.

BLIER, P., DE MONTIGNY, C., & CHAPUT, Y. (1987). Modifications of the serotonin system by antidepressant treatment: Implications for the therapeutic response in major depression. *J. Clin. Psychopharmacol.*, **7,** S24–S35.

BORISON, R.L., PATHIRAJA, P.A., DIAMOND, B.I. & MEIBACH, R.C. (1992). Risperidone: clinical safety and efficacy in schizophrenia. *Psychopharmacol. Bull.*, **28,** 213–218.

CARBONI, E. & DI CHIARA, G. (1989). Serotonin release estimated by transcortical dialysis in freely moving animals. *Neuroscience*, **32,** 637–645.

CHAPUT, Y., LESIEUR, P. & DE MONTIGNY, C. (1990). Effects of short-term serotonin depletion on the efficacy of serotonin neurotransmission: Electrophysiological studies in the rat central nervous system. *Synapse*, **6,** 328–337.

CHOUINARD, G., JONES, B., REMINGTON, G., BLOOM, D., ADDINGTON, D., MACEWAN, G.W., LABELLE, A., BEAUCLAIR, L. & ARNOTT, W. (1993). A Canadian multicenter placebo-controlled study of fixed doses of risperidone and haloperidol in the treatment of chronic schizophrenic patients. *J. Clin. Psychopharmacol.*, **13,** 25–40.

CUNNINGHAM, K.A. & LAKOSKI, J.M. (1990). The interaction of cocaine with serotonin dorsal raphe neurons: single-unit extracellular recording studies. *Neuropsychopharmacology*, **3,** 41–50.

DAVIDSON, C. & STAMFORD, J.A. (1995). Evidence that 5-hydroxytryptamine release in the rat dorsal raphé nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. *Br. J. Pharmacol.*, **114,** 1107–1109.

DAVIS, J.M. & JANICAK, P.G. (1996). Risperidone: A new, novel (and better?) antipsychotic. *Psychiat. Ann.*, **26,** 78–87.

FREEDMAN, J.E. & AGHAJANIAN, G.K. (1984). Idazoxan (RX 781094) selectively antagonizes α_2-adrenoceptors on rat central neurons. *Eur. J. Pharmacol.*, **105,** 265–272.

GALLAGER, D.W. & AGHAJANIAN, G.K. (1975). Effects of chloripramine and lysergic acid diethylamide on efflux of precursor-formed ^{3}H-serotonin: Correlations with serotonergic impulse flow. *J. Pharmacol. Exp. Ther.*, **193,** 785 – 795.

GALLAGER, D.W. & AGHAJANIAN, G.K. (1976). Effect of antipsychotic drugs on the firing of dorsal raphe cells. I. Role of adrenergic system. *Eur. J. Pharmacol.*, **39,** 341 – 355.

GARRATT, J.C., CRESPI, F., MASON, R. & MARSDEN, C.A. (1991). Effects of idazoxan on dorsal raphe 5-hydroxytryptamine neuronal function. *Eur. J. Pharmacol.*, **193,** 87 – 93.

GARTSIDE, S.E., UMBERS, V. & SHARP, T. (1997). Inhibition of 5-HT cell firing in the DRN by non-selective 5-HT reuptake inhibitors: Studies on the role of 5-HT$_{1A}$ autoreceptors and noradrenergic mechanisms. *Psychopharmacology*, **130,** 261 – 268.

GOFF, D.C., MIDHA, K.K., SARID-SEGAL, O., HUBBARD, J.W. & AMICO, E. (1995). A placebo-controlled trial of fluoxetine added to neuroleptic in patients with schizophrenia. *Psychopharmacology*, **117,** 417 – 423.

GOLDMAN, M.B. & JANECEK, H.M. (1990). Adjunctive fluoxetine improves global function in chronic schizophrenia. *J. Neuropsychiatr.*, **2,** 429 – 431.

HAJÓS, M., GARTSIDE, S.E. & SHARP, T. (1995). Inhibition of median and dorsal raphe neurones following administration of the selective serotonin reuptake inhibitor paroxetine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **351,** 624 – 629.

HERTEL, P., NOMIKOS, G.G., IURLO, M. & SVENSSON, T.H. (1996). Risperidone: regional effects in vivo on release and metabolism of dopamine and serotonin in the rat brain. *Psychopharmacology*, **124,** 74 – 86.

HERTEL, P., NOMIKOS, G.G., SCHILSTRÖM, B., ARBORELIUS, L. & SVENSSON, T.H. (1997). Risperidone dose-dependently increases extracellular concentrations of serotonin in the rat frontal cortex: role of α_2 adrenoceptor antagonism. *Neuropsychopharmacology*, **17,** 44 – 55.

HJORTH, S., BENGTSSON, H.J., MILANO, S., LUNDBERG, J.F. & SHARP, T. (1995). Studies on the role of 5-HT$_{1A}$ autoreceptors and α_1-adrenoceptors in the inhibition of 5-HT release-1. BMY7378 and prazosin. *Neuropharmacology*, **34,** 615 – 620.

HOFFMAN, B.B. & LEFKOWITZ, R.J. (1990). Adrenergic receptor antagonists. In *Goodman and Gilmans The Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Rall, T.W., Nies, A.S. & Taylor, P. 8th Ed. pp. 221 – 243. New York: Pergamon Press.

INVERNIZZI, R., BELLI, S. & SAMANI, R. (1992). Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe nuclei prevents the drug's effect in the frontal cortex. *Brain Res.*, **584,** 322 – 324.

JACOBS, B.L. & AZMITIA, E.C. (1992). Structure and function of the brain serotonin system. *Physiol. Rev.*, **72,** 165 – 229.

KANE, J., HONIGFELD, G., SINGER, J. & MELTZER, H.Y. (1988). Clozapine for the treatment-resistant schizophrenic. *Arch. Gen. Psychiatry*, **45,** 789 – 796.

KOE, B.K. & WEISSMAN, A. (1966). *p*-Chlorophenylalanine: A specific depletor of brain serotonin. *J. Pharmacol. Exp. Ther.*, **154,** 499 – 516.

KÖHLER, C., HALL, H., ÖGREN, S.-O. & GAWELL, L. (1985). Specific in vitro and in vivo binding of ^{3}H-raclopride. A potent substituted benzamide drug with high affinity for dopamine D-2 receptors in the brain. *Biochem. Pharmacol.*, **34,** 2251 – 2259.

LEJEUNE, F., AUDINOT, V., GOBERT, A., RIVET, J.M., SPEDDING, M. & MILLAN, M.J. (1994). Clozapine inhibits serotonergic transmission by an action at α_1-adrenoceptors not at 5-HT$_{1A}$ receptors. *Eur. J. Pharmacol.*, **260,** 79 – 83.

LEYSEN, J.E., GOMMEREN, W., EENS, A., DE CHAFFOY DE COURCELLES, D., STOOF, J.C. & JANSSEN, P.A.J. (1988). Biochemical profile of risperidone, a new antipsychotic. *J. Pharmacol. Exp. Ther.*, **247,** 661 – 670.

LEYSEN, J.E., JANSSEN, P.F.M., GOMMEREN, W., WYNANTS, J., PAUWELS, P.J. & JANSSEN, P.A.J. (1992). In vitro and in vivo receptor binding and effects on monoamine turnover in rat brain regions of the novel antipsychotics risperidone and ocaperidone. *Mol. Pharmacol.*, **41,** 494 – 508.

LITMAN, R.E., SU, T.P., POTTER, W.Z., HONG, W.W. & PICKAR, D. (1996). Idazoxan and response to typical neuroleptics in treatment-resistant schizophrenia: comparison with the atypical neuroleptic, clozapine. *Br. J. Psychiatry*, **168,** 571 – 579.

MATOS, F.F., URBAN, C. & YOCCA, F.D. (1996). Serotonin (5-HT) release in the dorsal raphé and ventral hippocampus: raphé control of somatodendritic and terminal 5-HT release. *J. Neural. Transm.*, **103,** 173 – 190.

MATSUBARA, S., MATSUBARA, R., KUSUMI, I., KOYAMA, T. & YAMASHITA, I. (1993). Dopamine D_1, D_2 and serotonin$_2$ receptor occupation by typical and atypical antipsychotic drugs in vivo. *J. Pharmacol. Exp. Ther.*, **265,** 498 – 508.

MAURA, G., BONANNO, G. & RAITERI, M. (1992). Presynaptic α_2-adrenoceptors mediating inhibition of noradrenaline and 5-hydroxytryptamine release in rat cerebral cortex: further characterization of different α_2-adrenoceptor subtypes. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **345,** 410 – 416.

MESOTTEN, F., SUY, E., PIETQUIN, M., BURTON, P. & HEYLEN, S. (1989). Therapeutic effect and safety of increasing dose of risperidone (R64744) in psychotic patients. *Psychopharmacology*, **99,** 445 – 449.

PALFREYMAN, M.G., SCHMIDT, C.J., SORENSEN, S.M., DUDLEY, M.W., KEHNE, J.H., MOSER, P., GITTOS, M.W. & CARR, A.A. (1993). Electrophysiological, biochemical and behavioral evidence for 5-HT$_2$ and 5-HT$_3$ mediated control of dopaminergic function. *Psychopharmacology*, **112,** 60 – 67.

PAXINOS, G. & WATSON, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. 2nd Ed. Syndey: Academic Press.

PINEYRO, G., DE MONTIGNY, C., WEISS, M. & BLIER, P. (1996). Autoregulatory properties of dorsal raphe-5HT neurons: possible role of electrotonic coupling and 5-HT$_{1D}$ receptors in the rat brain. *Synapse*, **22,** 54 – 62.

SCHOTTE, A., JANSSEN, P.F.M., GOMMEREN, W., LUYTEN, W.H.M.L., VAN GOMPEL, P., LESAGE, A.S., DE LOORE, K. & LEYSEN, J.E. (1996). Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology*, **124,** 57 – 73.

SILVER, H. & NASSAR, A. (1992). Fluvoxamine improves negative symptoms in treated chronic schizophrenia: an add-on double-blind, placebo-controlled study. *Biol. Psychiatry*, **31,** 698 – 704.

SPINA, E., DE DOMENICO, P., RUELLO, C., LONGOBARDO, N., GITTO, C., ANCIONE, M., DI ROSA, A.E. & CAPUTI, A.P. (1994). Adjunctive fluoxetine in the treatment of negative symptoms in chronic schizophrenic patients. *Int. Clin. Psychopharmacol.*, **9,** 281 – 285.

STARKE, K., GÖTHERT, M. & KILBINGER, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev.*, **69,** 864 – 989.

SUMIYOSHI, T., KIDO, H., SAKAMOTO, H., URASAKI, K., SUZUKI, K., YAMAGUCHI, N., MORI, H., SHIBA, K. & YOKOGAWA, K. (1994). In vivo dopamine-D_2 and serotonin-5-HT$_2$ receptor binding study of risperidone and haloperidol. *Pharmacol. Biochem. Behav.*, **47,** 553 – 557.

SVARTENGREN, J. & SIMONSSON, P. (1990). Receptor binding properties of amperozide. *Pharmacol. Toxicol.*, **66,** 8 – 11.

SVENSSON, T.H., BUNNEY, B.S. & AGHAJANIAN, G.K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the α-adrenergic agonist clonidine. *Brain Res.*, **92,** 291 – 306.

VANDERMAELEN, C.P. & AGHAJANIAN, G.K. (1983). Electrophysiological and pharmacological characterization of serotonergic dorsal raphe neurons recorded extracellulary in rat brain slices. *Brain Res.*, **289,** 109 – 119.

WANG, R.Y. & AGHAJANIAN, G. (1978). Collateral inhibition of serotonergic neurones in the rat dorsal raphe nucleus: Pharmacological evidence. *Neuropharmacology*, **17,** 819 – 825.

(Received September 15, 1997
Accepted September 19, 1997)