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# Is the potent 5-$HT_{1A}$ receptor agonist, alnespirone (S-20499), affecting dopaminergic systems in the rat brain?

Christophe Dugast [a], Fabienne Soulière [a], Patricia Schmitt [a], Josep M. Casanovas [b], Claude-Michelle Fattaccini [c], Elizabeth Mocaër [d], Monique Lesourd [d], Bernard Renaud [a], Francesc Artigas [b], Michel Hamon [c], Guy Chouvet [a,*]

[a] *Laboratoire de Neuropharmacologie et Neurochimie, INSERM CJF 95-06, Université Claude Bernard-Lyon-1, 8 avenue Rockefeller, 69373 Lyon Cedex 08, France*

[b] *Department of Neurochemistry, Instituto de Investigaciones Biomédicas de Barcelona, CSIC, Jordi Girona 18–26, 08034 Barcelona, Spain*

[c] *Laboratoire de NeuroPsychoPharmacologie, INSERM U288, Faculté de Médecine Pitié-Salpêtrière, 91 boulevard de l'Hôpital, 75634 Paris Cedex 13, France*

[d] *Institut de Recherches Internationales SERVIER, 6 place des Pléiades, 92415 Courbevoie Cedex, France*

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**Abstract**

The effects of the new methoxy-chroman 5-$HT_{1A}$ receptor agonist, alnespirone (S-20499), on the dopamine systems in the rat brain were assessed in vivo by means of electrophysiological and neurochemical techniques. Cumulative doses of alnespirone (0.032–4.1 mg $kg^{-1}$, i.v.) did not modify the spontaneous firing rate of dopamine neurons in the substantia nigra as well as in the ventral tegmental area. The local application of alnespirone (0.1–10 $\mu$M) by reverse microdialysis into the dorsal striatum did not affect the dopamine output but induced a moderate, although dose-independent, increase of 5-HT (5-hydroxytryptamine, serotonin) concentrations in the dialysate. As expected of a 5-$HT_{1A}$ receptor agonist, intraperitoneal (i.p.) administration of alnespirone at 2–32 mg $kg^{-1}$ markedly decreased 5-HT turnover in the striatum. Parallel measurements of dopamine turnover showed that alnespirone exerted no effect except at the highest dose (32 mg $kg^{-1}$, i.p.) for which a significant increase was observed. Interestingly, both alnespirone-induced reduction in 5-HT turnover and increase in dopamine turnover could be prevented by pretreatment with the selective 5-$HT_{1A}$ receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]–*N*-(2-pyridinyl)cyclohexane carboxamide). Altogether, these data indicate that alnespirone does not exert any direct influence on central dopamine systems. The enhanced dopamine turnover due to alnespirone at high dose appeared to result from 5-$HT_{1A}$ receptor stimulation, further supporting the idea that this receptor type may play a key role in 5-HT-dopamine interactions in brain. 

*Keywords:* Alnespirone (S-20499); Single unit recording; Microdialysis, in vivo; Dopamine release; 5-HT (5-hydroxytryptamine, serotonin) release; DOPA (3,4-dihydroxyphenylalanin); DOPAC (3,4-dihydroxyphenylacetic acid); HVA (homovanillic acid); 5-HTP (5-hydroxytryptophan)

## 1. Introduction

Full as well as partial agonists at central serotonin 5-$HT_{1A}$ receptors have been shown to be clinically effective anxiolytics and/or antidepressants (Glitz and Pohl, 1991; Deakin, 1993). However, most available 5-$HT_{1A}$ receptor agonists are not completely selective of this receptor type and frequently affect other neurotransmitter systems in addition to serotonergic neurotransmission. In particular, 5-$HT_{1A}$ receptor agonists of the azapirone series such as buspirone, gepirone and ipsapirone also affect catecholamine systems. Indeed, buspirone also acts as an antagonist at dopamine receptors (Cimino et al., 1983; McMillen et al., 1983; Van Wijngaarden et al., 1990; Piercey et al., 1994) and this action accounts for its marked stimulatory effect on dopamine turnover in the rat brain (Hamon et al., 1988; Mansbach et al., 1988). Although less potent than buspirone at dopamine receptors, ipsapirone has also been shown to exert some positive influence on the turnover of both dopamine (Hamon et al., 1988; Schechter et al., 1990) and noradrenaline (Gleeson

* Corresponding author. Tel.: +33-4-7877-7559; fax: +33-4-78-77-7209.

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et al., 1989) in the rat brain. Direct investigations on the electrical activity of noradrenergic neurons and noradrenaline release confirmed that 5-$HT_{1A}$ agonists of the azapirone series markedly increase these parameters (Broderick and Piercey, 1991; Done and Sharp, 1993) through the action of their common metabolite, 1-pyrimidinyl-piperazine (1-PP), which exhibits potent $\alpha_2$ adrenoceptor antagonist properties (Sanghera et al., 1983, 1990; Bianchi et al., 1988; Engberg, 1989; Gobbi et al., 1990). On the other hand, the prototypical 5-$HT_{1A}$ receptor agonist of the tetralin family, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), which does not produce 1-pyrimidinyl-piperazine, reduces 5-HT synthesis and release as expected (Arvidsson et al., 1981; Hjorth et al., 1982; Arvidsson et al., 1986; Sharp et al., 1989) but affects also midbrain dopamine systems (Ahlenius et al., 1989; Arborelius et al., 1993).

The new aminochroman derivative alnespirone (S-20499) has been characterized as a full and selective agonist at 5-$HT_{1A}$ receptors (Porsolt et al., 1992; Kidd et al., 1993; Barrett et al., 1994). It binds with high affinity to 5-$HT_{1A}$ receptors, inhibits the forskolin-activated adenylate cyclase in hippocampal homogenates, dose-dependently reduces the spontaneous firing of serotonin neurons in the dorsal raphe nucleus in vivo and in vitro, and causes a marked reduction in the rate of 5-HT turnover (Kidd et al., 1993). Consistent with its full agonistic properties at somatodendritic 5-$HT_{1A}$ autoreceptors, alnespirone induces a profound and long-lasting reduction of 5-HT release in several brain areas (Casanovas et al., 1997), comparable to that observed after the administration of other 5-$HT_{1A}$ receptor agonists such as 8-OH-DPAT and ipsapirone (Casanovas and Artigas, 1996). Surprisingly, although alnespirone displays a 100-fold higher affinity for 5-$HT_{1A}$ than for dopamine $D_2$ receptors, some dopaminergic properties of this agent have been recently suggested in studies on conscious male rats (Simon et al., 1992; Levy et al., 1995). Whether alnespirone actually exerts direct and/or indirect effects on central dopamine systems has been thoroughly investigated using electrophysiological and neurochemical methods in adult rats.

## 2. Materials and methods

### 2.1. Animals

All experiments were performed on male albino rats (Sprague–Dawley, IFFA CREDO, Lyon, France) weighing 250–320 g. Animals were kept in a controlled environment (12 h light–dark cycle, $22 \pm 2$°C room temperature and 60% relative humidity). Food and water were provided ad libitum. All animals were housed and cared according to the National Institute of Health 'Guide for the Care and Use of Laboratory Animals' (NIH Publication 80-23), European Union regulations (O.J. of E.C. L358/1 18/12/1986) and legal authorization from the French Ministry of Agriculture (no. 03-505).

### 2.2. Electrophysiological procedures

Animals were anesthetized with chloral hydrate (400 mg $kg^{-1}$, i.p.) and the femoral vein was catheterized for continuous administration of the anesthetic agent throughout the experiment (120 mg $kg^{-1}$ $h^{-1}$) and/or subsequent drug injections. A tracheal cannula was inserted to facilitate spontaneous respiration. Body temperature was maintained at 37°C with a feedback-controlled heating pad, and heart rate was continuously monitored. The animal was mounted in a stereotaxic frame and an incision was made to expose the dorsal skull surface. A craniotomy was drilled over the coordinates for the substantia nigra zona compacta or the ventral tegmental area according to the atlas of Paxinos and Watson (1986): 5.0–6.3 mm posterior to bregma, 1.5–2.2 and 0.5–1.2 mm lateral to the midline, respectively.

Extracellular single-cell recording electrodes were obtained from fiber-filled glass micropipettes (GC 150-F10, Clark Instruments) broken to 2–3 $\mu$m external tip diameter and filled with sodium acetate solution (0.5 M, pH 7.4) containing 2% (w/v) pontamine sky blue (typical impedance 5–20 M$\Omega$ at 10 Hz). Extracellular electrical activity was amplified (Grass P16 amplifier), filtered (band-pass 0.3–3 kHz), displayed (a.c. and d.c. traces, Tektronix oscilloscopes) and monitored via an audiomonitor (CEMI, Lyon, France). Spikes were used for data only if they were superimposing impulses of a single neuron of at least three times the noise level. They were discriminated with a voltage level detector (CEMI, Lyon, France) and fed to a Epson AX3 computer via a 1401 interface and Spike2 software (Cambridge Electronic Design, UK). Ratemeter records were generated on-line on a chart recorder (Kipp-Zonen, 1–5 s integration time) and the time of occurrence of discriminator output pulses was stored for later off-line analysis. For technical reasons, only the mean firing rate of single cells has been considered in this study, irrespective of their firing pattern.

Typical dopamine neurons were found 7.3–7.9 mm from the brain surface in the substantia nigra and 7.7–8.4 mm in the ventral tegmental area. Cells were identified as dopaminergic on the basis of well established electrophysiological characteristics (Wang, 1981; Grace and Bunney, 1983; see also Section 3). Only one dopamine cell was pharmacologically studied in each animal. Negative current (10 $\mu$A for 10 min, pulsed at 0.1 Hz) was used after the completion of each experiment to deposit pontamine sky blue from the tip of the recording pipette. After euthanasia, the brain was removed and frozen in 2-methylbutane ($-15$°C) and recording sites were examined on 25-$\mu$m thick coronal sections stained with cresyl violet. All of the cells included in this study were histologically localized within the substantia nigra or the ventral tegmental area.

### 2.3. Microdialysis procedures

Animals were anesthetized with sodium pentobarbital (60 mg $kg^{-1}$, i.p.) and placed in a stereotaxic frame. Concentric dialysis probes made as previously described (Adell and Artigas, 1991) with membrane exposed to the brain tissue of 4 mm length (O.D. = 0.25 mm) were implanted in the dorsal striatum at the following coordinates: 0.2 mm anterior and 3.0 mm lateral to bregma, 8.0 mm dorso-ventral (Paxinos and Watson, 1986). Animals were allowed to recover from surgery and anesthesia for approximately 20 h. Probes were then perfused at 0.25 $\mu$l $min^{-1}$ with artificial cerebrospinal fluid (CSF) (125 mM NaCl, 2.5 mM KCl, 1.26 mM $CaCl_2$ and 1.18 mM $MgCl_2$) containing citalopram (1.0 $\mu$M). Sample collection started 60 min after the beginning of perfusion. Usually 4–5 fractions were collected to obtain basal values before administration of drugs by reverse dialysis. Successive 20 min (5 $\mu$l) dialysate samples were collected. At the end of the experiments, rats were sacrificed by a pentobarbital injection. The correct location of the microdialysis probes was checked by visual inspection after infusing methylene blue and cutting the brain at the appropriate level.

Serotonin and dopamine were concurrently analyzed in dialysate samples by a high-performance liquid chromatography (HPLC) method previously described (Ferré et al., 1994). The composition of HPLC eluent was as follows: 0.15 M $NaH_2PO_4$, 0.46 mM octyl sodium sulphate, 0.5 mM EDTA (pH 2.8 adjusted with phosphoric acid) and 21% methanol. Serotonin and dopamine were separated on a 3-$\mu$m ODS column (7.5 × 0.46 cm; Beckman, CA, USA) and were detected amperometrically with a Hewlett-Packard 1049 detector. The detection limit was of 0.5–1 fmol for both amines, whose retention times were in the range of 3–5 min.

### 2.4. Biochemical procedures

Animals were treated with alnespirone (2–32 mg $kg^{-1}$, i.p.) or the solvent (normal saline). After 30 min, the aromatic L-amino acid decarboxylase inhibitor, NSD-1015 (3-hydroxybenzylhydrazine) (100 mg $kg^{-1}$, i.p.), was injected (Carlsson et al., 1972). In other experiments, animals were pretreated with WAY-100635 (2 mg $kg^{-1}$, i.p.) 20 min before receiving alnespirone (32 mg $kg^{-1}$, i.p.). After 20 min has elapsed following this second injection, NSD-1015 (100 mg $kg^{-1}$, i.p.) was administered. In all cases, animals were decapitated 30 min after NSD-1015 treatment. Their brains were dissected (Glowinski and Iversen, 1966) and striata were weighed and homogenized in five volumes (v/w) of 0.1 M perchloric acid. Tissue samples were centrifuged and the pH of the supernatant was adjusted to 7.6 with 2 M potassium phosphate buffer. The precipitate was eliminated by recentrifugation and supernatant aliquots (10–20 $\mu$l) were injected directly into a HPLC column (Ultrasphere IP) protected by a precolumn (Brownlee). The mobile phase was a mixture of 70 mM $K_2HPO_4$, 2 mM triethylamine, 1.5 mM octanesulfonic acid, 0.1 mM EDTA and methanol (11% by volume), adjusted to pH 3.1 with citric acid (Hamon et al., 1988; Schechter et al., 1990). Elution of DOPA (3,4-dihydroxyphenylalanin), 5-HTP (5-hydroxytryptophan), DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid) was followed by electrochemical detection at a potential of 0.65 V (Hamon et al., 1988).

### 2.5. Drugs and reagents

Apomorphine hydrochloride (Sigma, France) and haloperidol (Haldol, Janssen, Beerse, Belgium) were dissolved in physiological saline. These drugs were given i.v. via the femoral catheter in a volume of 1–2 ml $kg^{-1}$. Quinpirole and citalopram were provided by RBI (Natick, MA, USA) and Lundbeck (Copenhagen, Denmark), respectively. They were dissolved in the artificial CSF used for the microdialysis in vivo. WAY-100635 (Wyeth, Taplow, UK) and NSD-1015 (Aldrich, France) were dissolved in physiological saline and given i.p. Alnespirone was kindly provided by the Institut de Recherches Internationales Servier (Paris, France). In electrophysiological experiments, alnespirone was dissolved in physiological saline and given i.v. at exponentially increasing doses at 2-min intervals, to cover the range of 0.032–4.1 mg $kg^{-1}$. In microdialysis experiments, alnespirone and quinpirole were dissolved in artificial CSF at three different concentrations: 0.1, 1.0 and 10 $\mu$M, which were sequentially administered during the collection of 4 fractions (80 min) each. In biochemical experiments, alnespirone was dissolved in physiological saline and injected i.p.

### 2.6. Data analysis and statistics

Analysis of the firing rate of dopamine neurons before and after drug administration was performed off-line with the Spike2 and Statview softwares, by quantifying the mean firing rate in spikes per second (spikes $s^{-1}$) over two 60-s periods, the first one immediately prior to the first injection of the drug (baseline control) and the second immediately after the administration of each cumulative dose. Microdialysis data are given as absolute concentration values (fmol of 5-HT or dopamine per sample). To facilitate the comparison of drug effects, data are presented as percentages of baseline values. These have been calculated as the individual means of four predrug values. In biochemical experiments, metabolite levels were calculated by comparing areas under the sample curves with those obtained with standard compounds using an integrator (Shimadzu CR3A). Data are given as absolute values of tissue contents expressed as $\mu$g $g^{-1}$. All data are expressed as means ± S.E.M. Statistical comparisons were

made using either analysis of variance (ANOVA) with repeated measures, one-way ANOVA with replicate and paired or unpaired Student's *t*-test. A two-tailed *P*-value of less than 0.05 was considered significant.

## 3. Results

### *3.1. In vivo electrophysiological results*

Extracellular single unit activity of all recorded neurons fulfilled the usual identification criteria of dopamine neurons (Wang, 1981; Grace and Bunney, 1983; Akaoka et al., 1992; White, 1996): (1) positive–negative spike waveform on the d.c. trace, often with a notch in the ascending part; (2) long spike duration (bi- or triphasic wave of 3–5 ms duration on the a.c. trace); (3) slow firing rate (around 5 spikes $s^{-1}$, range 0.7–10.4) with regular or bursting firing pattern; (4) low pitch sound on the audiomonitor and (5) stereotaxic depth and 'postmortem' histological location in substantia nigra or ventral tegmental area.

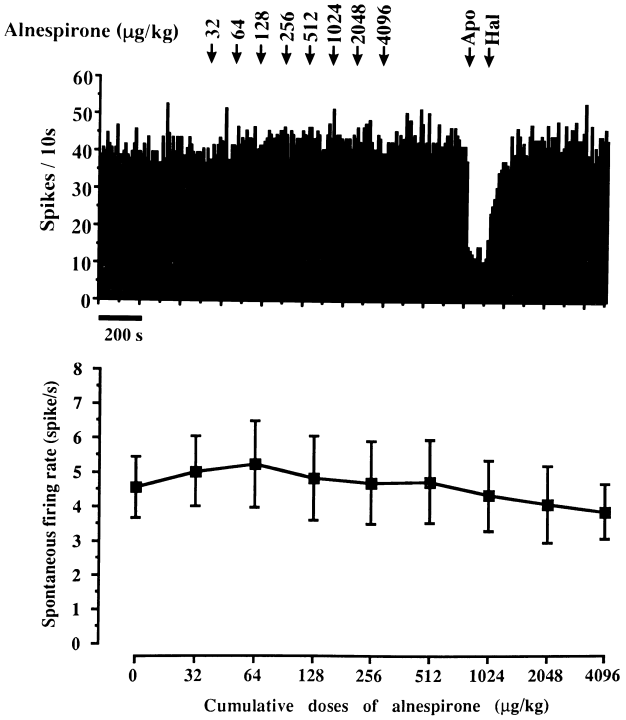


Fig. 1. Effects of cumulative doses of alnespirone on the spontaneous firing rate of dopamine neurons within the substantia nigra. Upper panel: Representative cumulative rate histogram. Arrowheads denote time of drug injections. After the last injection of alnespirone, a single dose of apomorphine (Apo, 50 $\mu$g $kg^{-1}$) and haloperidol (Hal, 100 $\mu$g $kg^{-1}$) was injected i.v. Alnespirone did not affect the spontaneous firing rate of this dopamine neuron on which apomorphine induced a large and immediate decrease in the firing rate easily reversed to baseline by haloperidol. Lower panel: Cumulative dose–response curve. Each point represents the mean ± S.E.M. firing rates of dopamine neurons ($n = 6$) computed over 60 s after each injected dose. Whatever the dose, alnespirone exerted no statistically significant effects on the averaged firing rate of these neurons.

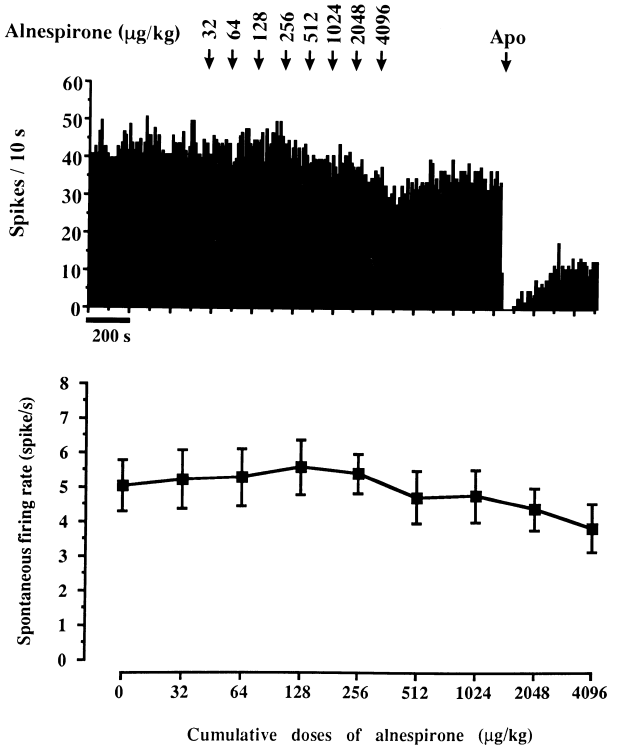


Fig. 2. Effects of cumulative doses of alnespirone on the spontaneous firing rate of dopamine neurons within the ventral tegmental area. Upper panel: Representative cumulative rate histogram. Arrowheads denote time of drug injections. After the last injection of alnespirone, a single dose of apomorphine (Apo, 50 $\mu$g $kg^{-1}$) was injected i.v. Under alnespirone, this neuron exhibited a similar firing rate than that observed before the first injection. Apomorphine administration produced a complete and immediate inhibition of the discharge of this dopamine neuron followed by a slow recovery. Lower panel: Dose–response curve. Each point represents the mean ± S.E.M. firing rates of dopamine neurons ($n = 6$) computed over 60 s after each injected dose. Whatever the dose, alnespirone produced no statistically significant modifications of the averaged firing rate of these neurons.

Mean spontaneous firing rates of substantia nigra and ventral tegmental area dopamine neurons ($4.54 \pm 0.87$ spikes $s^{-1}$, $n = 6$, and $5.07 \pm 0.74$ spikes $s^{-1}$, $n = 6$, respectively) were similar to rates reported in previous studies using chloral hydrate anesthesia (Grace and Bunney, 1983; Akaoka et al., 1992; White, 1996). The administration of cumulative doses of alnespirone from 0.032 to 4.1 mg $kg^{-1}$ i.v. did not significantly affect (ANOVA, $P > 0.05$) the firing rate of dopamine neurons in the substantia nigra (Fig. 1) as well as in the ventral tegmental area (Fig. 2). Although a trend towards a weak inhibition with the highest doses of alnespirone was noted, especially in the latter area, there was no statistically significant difference in the mean spontaneous firing rate just prior to the treatment (see above) and the rate observed after administration of 4.1 mg $kg^{-1}$ of the drug ($3.85 \pm 0.81$

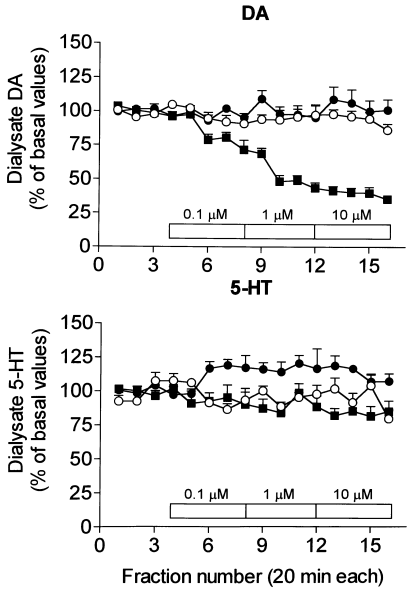


Fig. 3. Effects of alnespirone and quinpirole on the output of dopamine (DA, upper panel) and serotonin (5-HT, lower panel) in the dorsal striatum of rats. The local infusion of the dopamine D2 receptor agonist quinpirole markedly reduced the output of dopamine but did not affect the 5-HT output (filled squares, $n = 6$). The perfusion of alnespirone (filled circles, $n = 6$) elicited a slight increment of 5-HT output which was independent of the dose. This drug was ineffective on the dopamine output. Open bars show the periods of infusion of the indicated doses of both compounds. Open circles represent the dialysate dopamine and 5-HT output in control rats ($n = 4$) perfused with artificial CSF during the same period.

spikes $s^{-1}$ for substantia nigra and $3.87 \pm 0.70$ spikes $s^{-1}$ for ventral tegmental area; Student's *t*-test for paired values, $P > 0.05$). The lack of effect of alnespirone contrasted with the immediate inhibitory action of a low dose of the dopamine receptor agonist apomorphine (50 $\mu$g $kg^{-1}$, i.v.) on the same neurons, which could be reversed by administration of the dopamine receptor antagonist, haloperidol (100 $\mu$g $kg^{-1}$, i.v.; Fig. 1, upper panel).

### 3.2. Microdialysis experiments

Basal concentrations of 5-HT and dopamine in dialysates from the dorsal striatum were $22.7 \pm 3.2$ and $51.3 \pm 5.8$ fmol/5 $\mu$l, respectively ($n = 16$). No significant differences were found in baseline 5-HT and dopamine concentrations among the different groups (control, alnespirone, quinpirole).

The local application of quinpirole (0.1–10 $\mu$M) by reverse dialysis dose-dependently reduced striatal dopamine output (Fig. 3, upper panel). Due to the low flow rate used in microdialysis experiments, maximal reductions in dopamine levels were observed at the end of perfusion periods corresponding to each dose (fractions numbers 8, 12 and 16 in Fig. 3). The final dopamine outputs were 65%, 56% and 28% of the baseline value at 0.1, 1.0 and 10 $\mu$M quinpirole, respectively ($n = 6$; $P < 0.001$, repeated measures ANOVA). In contrast, neither the perfusion of artificial CSF nor that of alnespirone (0.1–10 $\mu$M) altered significantly the dopamine output (Fig. 3, upper panel).

Consistent with previous results (Ferré et al., 1994), the local infusion of quinpirole did not modify the striatal 5-HT output (Fig. 3, lower panel). In contrast, alnespirone induced a 10–15% increment in the dialysate concentrations of 5-HT ($n = 6$; $P < 0.05$, repeated measures ANOVA). However, this effect was independent of the drug concentration used (Fig. 3, lower panel).

### 3.3. Biochemical experiments

5-HTP accumulation in the striatum of NSD-1015-treated rats was markedly reduced by alnespirone pretreatment (Fig. 4). This effect reached significance already at the lowest dose tested (2 mg $kg^{-1}$, i.p.) and was maximum ($-40\%$, $P < 0.05$) at doses $\geq 4.0$ mg $kg^{-1}$ i.p. In contrast, DOPA accumulation was unaffected by alnespirone treatment up to the dose of 8 mg $kg^{-1}$ i.p. At the highest dose tested, 32 mg $kg^{-1}$ i.p., the drug produced a significant increase ($+64\%$, $P < 0.05$) in striatal DOPA levels (Fig. 4).

Pretreatment with the $5\text{-HT}_{1A}$ receptor antagonist, WAY-100635 (2 mg $kg^{-1}$, i.p.), prevented the effects of alnespirone (32 mg $kg^{-1}$, i.p.) on both 5-HTP and DOPA accumulation (Fig. 5). On its own, WAY-100635 produced

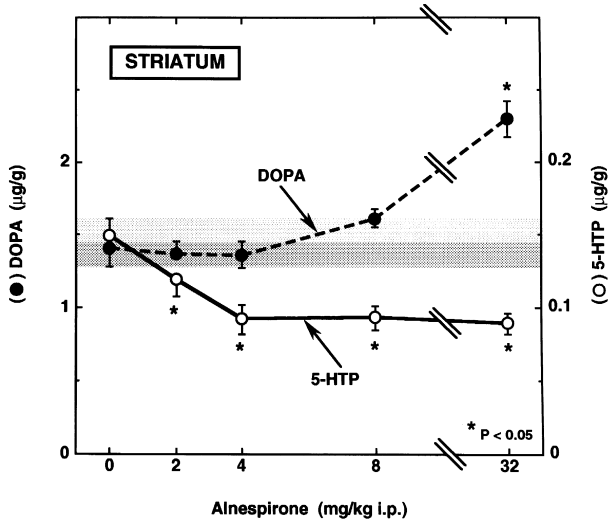


Fig. 4. Dose–response curves showing the effects of alnespirone treatment on the accumulation of 5-HTP and DOPA in the striatum of NSD-1015-treated rats. Rats received exponentially increasing doses of alnespirone (abscissae) or saline. Twenty minutes after this treatment, each rat was injected with NSD-1015 (100 mg $kg^{-1}$, i.p.). Animals were then killed by decapitation 30 min after the latter treatment. Levels of DOPA and 5-HTP were then measured in the striatum by HPLC coupled to electrochemical detection. Values are the means ± S.E.M. of six rats for each injected dose. $* P < 0.05$ as compared to control values (in saline treated rats, shaded area).

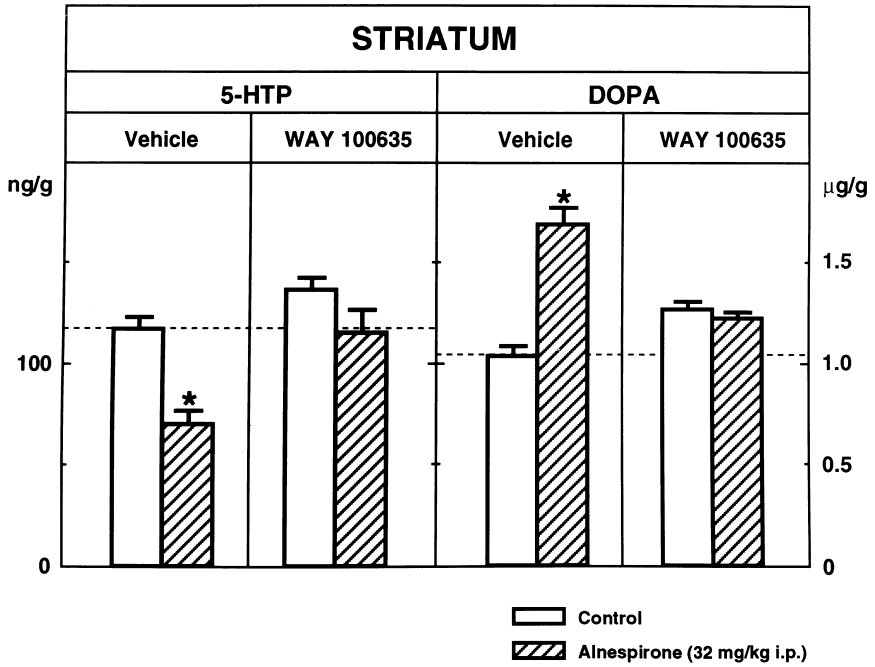


Fig. 5. Prevention by WAY-100635 of the effects of alnespirone on the accumulation of 5-HTP and DOPA in the striatum of NSD-1015-treated rats. Rats were treated with WAY-100635 (2 mg $kg^{-1}$, i.p.) or saline, then 20 min later, with alnespirone (32 mg $kg^{-1}$, i.p.) or saline, and finally, after a further period of 20 min, with NSD-1015 (100 mg $kg^{-1}$, i.p.). Animals were killed by decapitation 30 min after the last injection. 5-HTP and DOPA levels in the striatum were measured by HPLC coupled to electrochemical detection. Values are the means ± S.E.M. of six rats. $* P < 0.05$ as compared to control values (in saline-treated rats).

a slight but significant increase (+16–20%, $P < 0.05$) in the striatal accumulation of the two monoamine precursors.

Measurements of dopamine metabolites also indicated that the highest dose of alnespirone (32 mg $kg^{-1}$, i.p.) produced significant increases in the striatal levels of DOPAC (+40%, $P < 0.05$) and HVA (+39%, $P < 0.05$) in NSD-1015-treated rats (Table 1). In contrast, dopamine levels were unchanged, and none of the other doses tested affected the striatal contents of the catecholamine and its metabolites (data not shown). Pretreatment with WAY-100635 (2 mg $kg^{-1}$, i.p.) prevented the alnespirone effects on DOPAC levels. The possible prevention by WAY-100635 of alnespirone-induced increase in HVA levels could not be assessed because the former drug produced, on its own, a significant elevation of the striatal concentrations of this metabolite (Table 1).

Table 1
Effects of treatment with alnespirone and/or WAY-100635 on the levels of DOPAC and HVA in the striatum of NSD-1015-treated rats

| Drugs | DOPAC ($\mu g\ g^{-1}$) | HVA ($\mu g\ g^{-1}$) |
|---|---|---|
| Saline | 0.148 ± 0.010 | 0.417 ± 0.025 |
| Alnespirone (32 mg $kg^{-1}$) | 0.207 ± 0.024[a] | 0.581 ± 0.038[a] |
| WAY-100635 (2 mg $kg^{-1}$) | 0.160 ± 0.012[NS] | 0.574 ± 0.019[a] |
| WAY-100635 + alnespirone | 0.162 ± 0.013[NS] | 0.536 ± 0.019[a] |

Rats were treated with WAY-100635 (2 mg $kg^{-1}$, i.p.) or saline, then 20 min later, with alnespirone (32 mg $kg^{-1}$, i.p.) or saline, and finally, after a further period of 20 min, with NSD-1015 (100 mg $kg^{-1}$, i.p.). Animals were killed by decapitation 30 min after the last injection. DOPAC and HVA levels in the striatum were measured by HPLC coupled to electrochemical detection. Values are the means ± S.E.M. of six rats for each treatment.

[a] $P < 0.05$ as compared to control values (saline-treated rats).

NS: not significant.

## 4. Discussion

The present electrophysiological and microdialysis results indicate that at doses used to elicit anxiolytic and/or antidepressant-like effects (Porsolt et al., 1992; Barrett et al., 1994), alnespirone is devoid of significant effects on both the electrical activity of dopamine neurons and the striatal release of dopamine in rats.

All cell recorded in this study fulfilled the expected electrophysiological characteristics of dopamine neurons according to the classical identification criteria of these neurons previously described by Grace and Bunney (1983) and Wang (1981). First, at their expected location, the a.c. spike waveform was typically triphasic with a low sound pitch on the audiomonitor; second, their firing rate was regular or irregular with bursts of action potentials (around 5 spikes $s^{-1}$ on average), and in the range of those of dopamine neurons under the same anesthetic agent; third, administration of apomorphine, at a dose acting preferentially at dopamine $D_2$ autoreceptors, produced the well-described inhibitory effect on their firing rate; fourth, histological control of the recording sites confirmed that they

were all located within the substantia nigra or the ventral tegmental area.

The lack of change in the firing rate of dopamine neurons in the substantia nigra and ventral tegmental area, even at the highest dose used (4 mg $kg^{-1}$, i.v.), suggests that alnespirone does not interact with dopamine autoreceptors located at the somatodendritic region of midbrain dopamine neurons. In fact, alnespirone did not significantly reduce their spontaneous activity, as it should be expected from a dopamine $D_2$ receptor agonist. Moreover, alnespirone did not prevent the inhibitory action of subsequent apomorphine administration on the same neurons (see Figs. 1 and 2). This confirms the dopaminergic nature of the recorded neurons and indicates the lack of significant dopaminergic antagonistic properties of alnespirone. In contrast, alnespirone completely suppressed the firing of serotonin neurons within the dorsal raphe nucleus in vivo after the administration of a cumulative i.v. dose of 10 $\mu$g $kg^{-1}$ as well as in vitro (Kidd et al., 1993). Thus, the present data indicate that, even at doses at least two orders of magnitude higher than those sufficient to elicit substantial changes in serotonergic transmission in brain, alnespirone exerts no influence on midbrain dopamine neurons.

Microdialysis experiments showed that, unlike the dopamine receptor agonist quinpirole, alnespirone did not reduce the dopamine output in a region rich in nerve terminals, such as the striatum, in freely moving rats. It is well established that the inhibitory effect of quinpirole on the striatal dopamine output is due to its interaction with presynaptic dopamine $D_2$ autoreceptors (Imperato and Di Chiara, 1988; Reid et al., 1990; Ferré et al., 1994). Thus, the unchanged striatal dopamine output during the local application of alnespirone is fully consistent with the above electrophysiological data indicating the lack of agonist or antagonist action of alnespirone at dopamine $D_2$ autoreceptors. Furthermore, since the local application of the selective dopamine D1 receptor agonist SKF 38393 in striatum by reverse dialysis locally reduced dopamine release (Ferré et al., 1994), an effect likely involving dopamine D1 receptors in striatonigral GABAergic neurones, the present data also enable to exclude an interaction of alnespirone with striatal dopamine D1 receptors.

In vivo microdialysis experiments also showed that the application of alnespirone directly into the striatum exerted no inhibitory influence on the local release of 5-HT, in line with well established data on the lack of effect of 5-$HT_{1A}$ receptor ligands on the presynaptic negative feed-back control of the amine release from axon terminals (Hoyer and Middlemiss, 1989). Indeed, this mechanism is selectively triggered by the stimulation of (presynaptic) 5-$HT_{1B}$ autoreceptors (Engel et al., 1986), for which alnespirone has very low affinity (Kidd et al., 1993). To date, no obvious explanation can be given to the slight increase in 5-HT outflow associated with the intrastriatal application of alnespirone, especially because this effect exhibited no concentration-dependency.

The absence of significant changes of these electrophysiological and neurochemical indexes of dopamine systems after the administration of alnespirone is in good agreement with previous biochemical studies supporting the lack of dopamine $D_2$ receptor agonist properties of this 5-$HT_{1A}$ receptor agonist (Kidd et al., 1993). However, some supposedly dopaminergic-like actions have been reported in relevant experimental paradigms. Thus, like apomorphine, alnespirone induced hypothermia in mice, and this effect could be prevented by haloperidol (Simon et al., 1992). However, in contrast to apomorphine, alnespirone did not produce other typical responses to dopamine $D_2$ receptor stimulation such as yawning and penile erection (Simon et al., 1992). On the other hand, because haloperidol pretreatment could antagonize the increased motor activity induced by a very large dose of alnespirone (75 mg $kg^{-1}$) in reserpinized rats, this effect was ascribed to dopamine receptor activation by the latter drug (Scott et al., 1994). The possible involvement of dopamine $D_2$ receptors, rather than 5-$HT_{1A}$ receptors, in some of the endocrine effects of alnespirone has also been recently suggested (Levy et al., 1995) because of the inability of the non-selective 5-$HT_{1A}$ antagonists, (−)pindolol and spiperone to fully prevent these effects. However, further investigations have to be performed in order to reach a definitive conclusion. Indeed, Levy et al. used (−)pindolol at a rather low dose, 0.3 mg $kg^{-1}$, i.p., which might have been insufficient to block completely the effects of potent 5-$HT_{1A}$ receptor agonists such as alnespirone at 5-$HT_{1A}$ receptors (Aulakh et al., 1988; Scott et al., 1994).

Based on some of the behavioural and endocrine data mentioned above, it cannot be excluded that alnespirone can affect dopamine neurons, but in view of the present biochemical results, this should take place at doses much higher than those for the activation of 5-$HT_{1A}$ receptors. For instance, the $ED_{50}$ of alnespirone to reduce striatal 5-HT release as a result of somato-dendritic 5-$HT_{1A}$ autoreceptor stimulation in vivo is as low as 0.19 mg $kg^{-1}$ s.c. in the awake rat (Casanovas et al., 1997), whereas we presently found that doses of the drug higher than 8.0 mg $kg^{-1}$ i.p., at least, have to be administered to enhance dopamine turnover in the rat striatum. Thus, only the highest dose of alnespirone tested, 32 mg $kg^{-1}$ i.p. was found to increase the rate of DOPA accumulation in the striatum of NSD-1015-treated rats. In addition, this dose also produced significant elevations in the striatal levels of the dopamine metabolites, DOPAC and HVA (although their synthesis was globally reduced because of monoamine oxidase blockade by NSD-1015 administered to both alnespirone-treated and control rats, see the work of Carlsson et al., 1972). Interestingly, pretreatment by the potent and selective 5-$HT_{1A}$ receptor antagonist WAY-100635 (Fletcher et al., 1996) prevented the increase in dopamine turnover due to alnespirone, indicating that the latter effect indirectly resulted from the stimulation of 5-$HT_{1A}$ receptors by this drug. On its own, WAY-100635 was found to

slightly but significantly increase 5-HT turnover in the striatum probably through the blockade of the negative influence of somatodendritic 5-$HT_{1A}$ autoreceptor stimulation by endogenous 5-HT (Fletcher et al., 1996). In addition, WAY-100635 also increased striatal dopamine turnover but a possible link between this effect and the change in 5-HT turnover has yet to be established.

Previous studies have shown that the stimulation of 5-$HT_{1A}$ receptors by other 5-$HT_{1A}$ agonists such as buspirone, gepirone, or ipsapirone is associated with an increase in dopamine turnover (Cimino et al., 1983; Hamon et al., 1988). Since dopamine neurons, in particular in the substantia nigra, do not express 5-$HT_{1A}$ receptors (Miquel et al., 1991; Pompeiano et al., 1992), this activation might result from the inhibition of serotonergic systems by the 5-$HT_{1A}$ agonists acting at somatodendritic 5-$HT_{1A}$ autoreceptors. Thus, the indirect stimulatory action of alnespirone on dopamine systems might derive from its ability to reduce the serotonergic tone in midbrain and forebrain structures, as a result of the suppression of 5-HT release. Indeed, serotonergic agents are known to affect dopamine cell firing and release (Blandina et al., 1989; Chen et al., 1991; Prisco et al., 1992; Benloucif et al., 1993; Parsons and Justice, 1993; Prisco et al., 1994). In particular, the tetralin derivatives (*R*)-8-OH-DPAT and (*S*)-UH-301, agonist and antagonist respectively at 5-$HT_{1A}$ receptors, differentially modulate the activity of midbrain dopamine neurons (Ahlenius et al., 1989; Arborelius et al., 1993). 8-OH-DPAT increased dose-dependently the firing rate and percentage of burst firing of ventral tegmental area neurons at doses selective for the stimulation of 5-$HT_{1A}$ receptors (2–32 $\mu$g $kg^{-1}$, i.v.). In contrast, high doses (0.25–1.0 mg $kg^{-1}$, i.v.) of this compound reduced firing rate and percentage of burst firing of these neurons, and these effects could be prevented by prior administration of the dopamine D2 receptor antagonist raclopride (Arborelius et al., 1993). Interestingly, we observed a similar non significant trend with alnespirone in our studies. However, because burst firing analysis was technically not feasible, it is difficult to ascertain whether alnespirone may exert a similar effect on the firing pattern of dopamine neurons.

Taken together, these results indicate that alnespirone does not affect dopamine systems at doses which induce anxiolytic and/or antidepressant-like effects in rats. Even at higher doses, for which a clear increase in dopamine turnover was observed, this effect appeared to result exclusively from the selective activation of 5-$HT_{1A}$ receptors by this drug.

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## References

Adell, A., Artigas, F., 1991. Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. An in vivo microdialysis study. Naunyn-Schmiedeberg's Arch. Pharmacol. 343, 237–244.

Ahlenius, S., Hillegaart, V., Wijkström, A., 1989. Evidence for selective inhibition of limbic forebrain dopamine synthesis by 8-OH-DPAT in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 339, 551–556.

Akaoka, H., Charlety, P., Saunier, C.F., Buda, M., Chouvet, G., 1992. Inhibition of nigral dopamine neurons by systemic and local apomorphine: possible contribution of dendritic autoreceptors. Neuroscience 49, 879–891.

Arborelius, L., Chergui, K., Murase, S., Nomikos, G., Höök, B.B., Chouvet, G., Hacksell, U., Svensson, T.H., 1993. The 5-$HT_{1A}$ receptor selective ligands, (*R*)-8-OH-DPAT and (*S*)-UH-301 differentially affect the activity of midbrain dopamine neurons. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 353–362.

Arvidsson, L.E., Hacksell, U., Nilsson, J.L.G., Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D., Wikström, H., 1981. 8-Hydroxy-2-(di-*n*-propylamino) tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. J. Med. Chem. 24, 921–923.

Arvidsson, L.E., Hacksell, U., Glennon, R.A., 1986. Recent advances in central 5-hydroxytryptamine receptor agonists and antagonists. Prog. Drug Res. 30, 365–471.

Aulakh, C.S., Wozniak, K.M., Haas, M., Hill, J.L., Zohar, J., Murphy, D.L., 1988. Food intake, neuroendocrine and temperature effects of 8-OH-DPAT in the rat. Eur. J. Pharmacol. 146, 253–259.

Barrett, J.E., Gamble, E.H., Zhang, L., Guardiola-Lemaître, B., 1994. Anticonflict and discriminative stimulus effects in the pigeon of a new methoxy-chroman 5-$HT_{1A}$ agonist, (+)S 20244 and its enantiomers (+)S 20499 and (−)S 20500. Psychopharmacology 116, 73–78.

Benloucif, S., Keegan, M.J., Galloway, M.P., 1993. Serotonin-facilitated dopamine release in vivo: pharmacological characterization. J. Pharmacol. Exp. Ther. 265, 373–377.

Bianchi, G., Caccia, S., Della Vedova, F., Garattini, S., 1988. The alpha-2 adrenoceptor antagonist activity of ipsapirone and gepirone is mediated by their common metabolite 1-(2-pyrimidinyl)-piperazine (PmP). Eur. J. Pharmacol. 151, 365–371.

Blandina, P., Goldfarb, J., Craddock-Royal, B., Green, J.P., 1989. Release of endogenous dopamine by stimulation of 5-hydroxytryptamine$_3$ receptors in rat striatum. J. Pharmacol. Exp. Ther. 251, 803–809.

Broderick, P.A., Piercey, M.F., 1991. 5-$HT_{1A}$ agonists uncouple noradrenergic somatodendritic impulse flow and terminal release. Brain Res. Bull. 27, 693–696.

Carlsson, A., Davis, J.N., Kehr, W., Lindqvist, M., Atack, C.V., 1972. Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo, using an inhibitor of the aromatic amino acid decarboxylase. Naunyn-Schmiedeberg's Arch. Pharmacol. 275, 153–168.

Casanovas, J.M., Artigas, F., 1996. Differential effects of ipsapirone on 5-HT release in the dorsal and median raphe neuronal pathways. J. Neurochem. 67, 1945–1952.

Casanovas, J.M., Lesourd, M., Artigas, F., 1997. The effect of the selective 5-$HT_{1A}$ agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. Br. J. Pharmacol. 122, 733–741.

Chen, J., Van Praag, H.M., Gardner, E.L., 1991. Activation of 5-$HT_3$ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res. 543, 354–357.

Cimino, M., Ponzio, F., Achilli, G., Vantini, G., Perego, C., Algeri, S., Garattini, S., 1983. Dopaminergic effects of buspirone, a novel anxiolytic agent. Biochem. Pharmacol. 32, 1069–1074.

Deakin, J.F.W., 1993. A review of clinical efficacy of 5-HT(1A) agonists in anxiety and depression. J. Psychopharmacol. 7, 283–289.

Done, C.J.G., Sharp, T., 1993. Biochemical evidence for the regulation of central noradrenergic activity by 5-HT(1A) and 5-HT(2) receptors: microdialysis studies in the awake and anesthetized rat. Neuropharmacology 33, 411–421.

Engberg, G., 1989. A metabolite of buspirone increases locus coeruleus activity via alpha2-receptor blockade. J. Neural Transm. 76, 91–98.

Engel, G., Göthert, M., Hoyer, D., Schlicker, E., Hillenbrand, K., 1986. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-$HT_{1B}$ binding sites. Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 1–7.

Ferré, S., Cortès, R., Artigas, F., 1994. Dopaminergic regulation of the serotonergic raphe-striatal pathway: microdialysis studies in freely moving rats. J. Neurosci. 14, 4839–4846.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K.J., Critchley, D.J.P., Childs, K.J., Middlefell, V.C., Lanfumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-$HT_{1A}$ receptor antagonist. Behav. Brain Res. 73, 337–353.

Gleeson, S., Ahlers, S.T., Mansbach, R.S., Foust, J.M., Barrett, J.E., 1989. Behavioral studies with anxiolytic drugs: VI. Effects on punished responding of drugs interacting with serotonin receptor subtypes. J. Pharmacol. Exp. Ther. 250, 809–817.

Glitz, D.A., Pohl, R., 1991. 5-$HT_{1A}$ partial agonists—what is their future?. Drugs 41, 11–18.

Glowinski, J., Iversen, L., 1966. Regional studies of catecholamines in the rat brain. I. Disposition of $^3$H-norepinephrine, $^3$H-dopamine and $^3$H-dopa in various regions of the brain. J. Neurochem. 13, 655–669.

Gobbi, M., Frittoli, E., Mennini, T., 1990. Antagonist properties of 1-(2-pyrimidinyl)-piperazine at presynaptic alpha-2 receptors in the rat brain. Eur. J. Pharmacol. 180, 183–186.

Grace, A.A., Bunney, B.S., 1983. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons: 1. Identification and characterization. Neuroscience 10, 301–315.

Hamon, M., Fattaccini, C.M., Adrien, J., Gallissot, M.C., Martin, P., Gozlan, H., 1988. Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine$_{1A}$ agonists with potential anxiolytic properties. J. Pharmacol. Exp. Ther. 246, 745–752.

Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D., Wikström, H., Arvidsson, L.E., Hacksell, U., Nilsson, J.L.G., 1982. 8-hydroxy-2-(di-*n*-propylamino) tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. J. Neural Transm. 55, 169–188.

Hoyer, D., Middlemiss, D.N., 1989. Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. Trends Pharmacol. Sci. 10, 131–132.

Imperato, A., Di Chiara, G., 1988. Effects of locally applied D-1 and D-2 receptor agonists and antagonists: studies with brain dialysis. Eur. J. Pharmacol. 156, 385–393.

Kidd, E.J., Haj-Dahmane, S., Jolas, T., Lanfumey, L., Fattaccini, C.M., Guardiola-Lemaître, B., Gozlan, H., Hamon, M., 1993. New methoxy-chroman derivatives, 4(*N*-(5-methoxy-chroman-3-yl)–*N*-propylamino)butyl-8-azapiro-(4-5)-decane-7,9-dione((±)-S20244) and its enantiomers (+)-S20499 and (−)-S20500, with potent agonist properties at central 5-hydroxytryptamine$_{1A}$ receptors. J. Pharmacol. Exp. Ther. 264, 863.

Levy, A.D., Li, Q., Gustafson, M., Van de Kar, L.D., 1995. Neuroendocrine profile of the potential anxiolytic drug S-20499. Eur. J. Pharmacol. 274, 141–149.

Mansbach, R.S., Harrod, C., Hoffmann, S.M., Nader, M.A., Lei, Z., Witkin, J.M., Barrett, J.E., 1988. Behavioral studies with anxiolytic drugs: V. Behavioral and in vivo neurochemical analyses in pigeons of drugs that increase punished responding. J. Pharmacol. Exp. Ther. 246, 114–120.

McMillen, B.A., Matthews, R.T., Sanghera, M.K., Shepard, P.D., German, D.C., 1983. Dopamine receptor antagonism by the novel anti-anxiety drug buspirone. J. Neurosci. 3, 733–738.

Miquel, M.C., Doucet, E., Boni, C., El Mestikawy, S., Matthiessen, L., Daval, G., Verge, D., Hamon, M., 1991. Central serotonin$_{1A}$ receptors: respective distributions of encoding mRNA, receptor protein and binding sites by in situ hybridization histochemistry, radioimmunohistochemistry and autoradiographic mapping in the rat brain. Neurochem. Int. 19, 453–465.

Parsons, L.H., Justice, J.B., 1993. Perfusate serotonin increases extracellular dopamine in the nucleus accumbens as measured by in vivo microdialysis. Brain Res. 606, 195–199.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd edn. Academic Press, Sydney.

Piercey, M.F., Smith, M.W., Lum-Ragan, J.T., 1994. Excitation of noradrenergic cell firing by 5-hydroxytryptamine(1A) agonists correlates with dopamine antagonist properties. J. Pharmacol. Exp. Ther. 268, 1297–1303.

Pompeiano, M., Palacios, J.M., Mengod, G., 1992. Distribution and cellular localization of mRNA coding for 5-$HT_{1A}$ receptor in the rat brain: correlation with receptor binding. J. Neurosci. 12, 440–453.

Porsolt, R.D., Lenègre, A., Caignard, D.H., Pfeiffer, B., Mocaër, E., Guardiola-Lemaître, B., 1992. Psychopharmacological profile of a new chroman derivative with 5-hydroxytryptamine$_{1A}$ agonist properties: S 20499 (+). Drug Dev. Res. 27, 389–402.

Prisco, S., Pessia, M., Ceci, A., Borsini, F., Esposito, E., 1992. Chronic treatment with DAU 6215, a new 5-$HT_3$ receptor antagonist, causes a selective decrease in the number of spontaneously active dopaminergic neurons in the rat ventral tegmental area. Eur. J. Pharmacol. 214, 13–19.

Prisco, S., Pagannone, S., Esposito, E., 1994. Serotonin–dopamine interaction in the ventral tegmental area: an electrophysiological study in vivo. J. Pharmacol. Exp. Ther. 271, 83–90.

Reid, M.S., O'Connor, W.T., Herrera-Marschitz, M., Ungerstedt, U., 1990. The effects of intranigral GABA and dynorphin A injections on striatal dopamine and GABA release: evidence that dopamine provides inhibitory regulation of striatal GABA neurons via D2 receptors. Brain Res. 519, 255–260.

Sanghera, M.K., McMillen, B.A., German, D.C., 1983. Buspirone, a non benzodiazepine anxiolytic, increases locus coeruleus noradrenergic neuronal activity. Eur. J. Pharmacol. 86, 107–110.

Sanghera, M.K., Coke, J.A., Williams, H., McMillen, B.A., 1990. Ipsapirone and 1-(2-pyrymidinyl)-piperazine increase rat locus coeruleus noradrenergic activity. Brain. Res. Bull. 24, 17–22.

Schechter, L.E., Bolanos, F., Gozlan, H., Lanfumey, L., Haj-Dahmane, S., Laporte, A.M., Fattaccini, C.M., Hamon, M., 1990. Alterations of central serotonergic and dopaminergic neurotransmission in rats chronically treated with ipsapirone: biochemical and electrophysiological studies. J. Pharmacol. Exp. Ther. 255, 1335–1347.

Scott, P.A., Chou, J.M., Tang, H., Frazer, A., 1994. Differential induction of 5-$HT_{1A}$-mediated responses in vivo by three chemically dissimilar 5-$HT_{1A}$ agonists. J. Pharmacol. Exp. Ther. 270, 198–208.

Sharp, T., Bramwell, S.R., Grahame-Smith, D.G., 1989. 5-$HT_1$ agonists reduce 5-hydroxytryptamine release in rat hippocampus in vivo as determined by brain microdialysis. Br. J. Pharmacol. 96, 283–290.

Simon, P., Guardiola, B., Bizot-Espiard, J., Schiavi, P., Costentin, J.,

1992. 5-$HT_{1A}$ receptor agonists prevent in rats the yawning and penile erections induced by direct dopamine agonists. Psychopharmacology 108, 47–50.

Van Wijngaarden, I.V., Tulp, M.T.H.M., Soudijn, W., 1990. The concept of selectivity in 5-HT receptor research. Eur. J. Pharmacol. 188, 301–312.

Wang, R.Y., 1981. Dopaminergic neurons in the rat ventral tegmental area: I. Identification and characterization. Brain Res. Rev. 3, 123–140.

White, F.J., 1996. Synaptic regulation of mesocorticolimbic dopamine neurons. Annu. Rev. Neurosci. 19, 405–436.