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Chronic alnespirone-induced desensitization of somatodendritic 5-HT_{1A} autoreceptors in the rat dorsal raphe nucleus

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Abstract

The effects of long-term (7, 14 or 21 days) administration of the 5-HT_{1A} receptor agonist alnespirone [5 mg/(kg day), i.p.] on the binding characteristics of 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors, and the functional status of 5-HT_{1A} autoreceptors were assessed using biochemical and electrophysiological approaches in rats. Whatever the treatment duration, the specific binding of [^{3}H]8 hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT), [^{3}H]*trans*,4-[(3*Z*)3-(2-dimethylaminoethyl) oxyimino-3(2-fluorophenyl) propen-1-yl] phenol hemifumarate ([^{3}H]SR 46349B), and [^{3}H]S-zacopride to 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors, respectively, were unaltered in all the brain areas examined. In contrast, in vitro electrophysiological recordings performed 24 h after the last injection of alnespirone showed that the potency of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, to depress the firing of serotoninergic neurons in the dorsal raphe nucleus, was significantly reduced after a 21-day treatment with alnespirone. However, no changes were noted after a 7-day or 14-day treatment. These data indicate that desensitization of somatodendritic 5-HT_{1A} autoreceptors is a selective but slowly developing adaptive phenomenon in response to their chronic stimulation in rats.

Keywords: Alnespirone; 5-HT receptor; 5-HT_{1A} autoreceptor; Dorsal raphe nucleus; Nerve impulse flow; Autoradiography

1. Introduction

Among the numerous 5-HT receptors identified to date (Hoyer et al., 1994; Baumgarten and Göthert, 1997), the 5-HT_{1A} receptor is considered as a relevant target for the treatment of psychiatric disorders, notably depression and anxiety (De Vry, 1996). Indeed, potential anxiolytic and/or antidepressant properties have been attributed to full as well as partial 5-HT_{1A} receptor agonists (Keppel Hesselink, 1992; Kurtz, 1992). However, most of these drugs are not completely selective of the 5-HT_{1A} receptor. For instance, the azapirones, such as buspirone, gepirone, ipsapirone and tandospirone bind not only to 5-HT_{1A} receptors (Gozlan et al., 1983; De Vry, 1996) but also to dopaminergic D2 receptors (Cimino et al., 1983; McMillen et al., 1983; Piercey et al., 1994). Furthermore, their principal in vivo metabolite, 1-(2 pyrimidinyl)-piperazine (1-PP), is a potent α2-adrenoceptor antagonist (Bianchi et al., 1988) which is responsible for the activation of noradrenergic neurons in the locus coeruleus of azapirone-treated rats (Engberg, 1989; Sanghera et al., 1990). As an increase in noradrenergic neurotransmission might contribute to anxiety-related behavior (Redmond and Huang, 1979), the in vivo formation of 1-PP is a major drawback for the potential use of azapirones as anxiolytics. Similarly, Martin (1991) provided evidence that 1-PP can counteract the antidepressant-like effect of 5-HT_{1A} receptor stimulation in the learned-helplessness paradigm in rats. Such opposite effects of their common metabolite 1-PP probably explain why the azapirones are generally considered to be less potent than 'classical' anxiolytics and/or antidepressants in relevant tests in animals (De Vry, 1996).

In contrast to azapirones, the methoxy-chroman derivative alnespirone is a potent 5-HT_{1A} receptor agonist (Porsolt et al., 1992; Kidd et al., 1993; Casanovas et al., 1997a; Dugast et al., 1998), the catabolism of which does not result in the production of metabolite(s) acting at α_2-

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adrenoceptors (see Kidd et al., 1993). Recent in vitro membrane binding and autoradiographic studies showed that [^{3}H]alnespirone allows the selective labelling of 5-HT_{1A} receptors in the rat brain (Fabre et al., 1997). Indeed, the 5-HT_{1A} receptor affinity of alnespirone ($K_i = 0.19$ nM) is at least two orders of magnitude higher than its affinity for any other receptor type tested (Kidd et al., 1993).

Accordingly, alnespirone appeared as a good pharmacological tool to assess the effects of chronic selective 5-HT_{1A} receptor stimulation on central 5-HT neurotransmission, in comparison with those found after repeated treatment with the azapirones (Eison and Yocca, 1985; Haskins et al., 1989; Schechter et al., 1990; Dong et al., 1997). For this purpose, rats were treated for 7, 14 or 21 days with alnespirone, and membrane binding, autoradiographic and/or electrophysiological approaches were used to investigate somatodendritic and postsynaptic 5-HT_{1A} receptors, 5-HT_{2A} and 5-HT_3 receptors, in various brain areas.

2. Materials and methods

2.1. Animals

Experiments were performed on young male Sprague–Dawley rats (Centre d'Elevage René Janvier, 53940 Le Genest-Saint-Isle, France) weighing ~100 g at the beginning of the treatment protocols, and ~300 g after the 3-week treatment with alnespirone or saline. Young animals were chosen because their brain tissues are much more resistant than those from adults to the transient anoxic conditions that occur during the preparation of slices for in vitro electrophysiological recording experiments (Di Scenna, 1987).

All the procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (Council directive no. 87–848, October 19, 1987, Ministère de l'Agriculture et de la Fôret, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 0299 to M.H, no. 6181 to E.D. and no. 6269 to L.L).

Animals were maintained under standard laboratory conditions (22 ± 1°C, 60% relative humidity, 12 h light–dark cycle, food and water ad libitum) for 5–10 days before the beginning of treatments.

2.2. Treatments

Alnespirone was dissolved in saline (0.9% NaCl) and injected i.p. (5 mg/kg daily; 9:00 a.m.) during 7, 14 or 21 days. Control rats received saline under the same volume (5 ml/kg, i.p.). Rats were weighed every day, and finally killed by decapitation 24 h after the last injection.

2.3. Methods

2.3.1. Preparation of brain membranes

Immediately after death, the brain was removed and the hippocampus and cortical areas were dissected on ice. Tissues were homogenized in 40 volumes (v/w) of ice-cold 50 mM Tris–HCl, pH 7.4, with a Polytron (type PT10 OD) tissue disrupter. The resulting homogenates were centrifuged at 40,000 × *g* for 20 min at 4°C, and the pellets were washed twice by resuspension in 100 vol of ice-cold buffer, followed by centrifugation. The sedimented material was then resuspended in 40 vol of Tris–HCl and incubated at 37°C for 10 min to allow the dissociation of endogenous 5-HT. Membranes were centrifuged and washed three more times as above, and the final pellet was resuspended in 10 vol of Tris–HCl buffer. Membranes were used for the binding experiments immediately after preparation. Remaining membrane suspensions were aliquoted and frozen at −80°C for subsequent assays.

Protein concentrations were determined according to the method of Lowry et al. (1951), using bovine serum albumin as the standard.

2.3.2. Binding assays

2.3.2.1. 5-HT_{1A} receptors. Aliquots (50 μl corresponding to ~0.25 mg protein) of hippocampal membrane suspensions were incubated at 25°C for 60 min in 0.5 ml (final volume) of 50 mM Tris–HCl, pH 7.4, containing 0.2–6.0 nM [^{3}H]8-hydroxy-2-(di-*n*-propylamino) tetralin ([^{3}H]-OH-DPAT). Nonspecific binding was determined in the presence of 10 μM 5-HT (see Hall et al., 1985, for details).

2.3.2.2. 5-HT_{2A} receptors. Aliquots (50 μl corresponding to ~0.25 mg protein) of membrane suspensions from frontal cortex were incubated at 37°C for 15 min in 0.5 ml (final volume) of 50 mM Tris–HCl, pH 7.7, containing 0.2–5.0 mM [^{3}H]*trans*,4-[(3*Z*)3-(2-dimethylaminoethyl) oxyimino-3(2-fluorophenyl)propen-1-yl]phenol hemifumarate ([^{3}H]SR 46349B, Rinaldi-Carmona et al., 1993). Nonspecific binding was determined in the presence of 1 μM cinanserin to saturate 5-HT_{2A} receptor binding sites (Hoyer et al., 1994).

2.3.2.3. 5-HT_3 receptors. Aliquots (100 μl corresponding to ~0.4–0.5 mg protein) of membrane suspensions from posterior (entorhinal) cortex were incubated at 25°C for 30 min in 0.5 ml (final volume) of 25 mM Tris–HCl, pH 7.4, containing 0.2–4.0 mM [^{3}H]S-zacopride. Nonspecific binding was determined in the presence of 1 μM ondansetron (see Bolaños et al., 1990, for details).

Incubations were stopped by the addition of 3.5 ml ice-cold Tris–HCl buffer and rapid vacuum-filtration using

a Brandel Cell Harvester. GF/B filters were used in all cases, but those for the measurement of [^{3}H]SR 46349B and [^{3}H]S-zacopride specific binding had been presoaked for 30 min in an aqueous dilution of polyethylenimine (0.5% v/v) before filtering samples. After an additional two washes with 3.5 ml ice-cold Tris–HCl, filters were dried and immersed in 4 ml of Aquasol scintillation liquid (New England Nuclear, Boston, MA) for radioactivity counting.

All determinations were done in triplicate. Saturation curves were analysed by computer-assisted non linear regression analysis for the calculation of respective K_d and B_{max} values (Graph Pad software facilities). Values for saline- and alnespirone-treated rats were analyzed by one-way analysis of variance (ANOVA), and in case of significance ($P < 0.05$), the F test for significant treatment was followed by a two tailed t-test to compare the experimental groups with their controls (Snedecor and Cochran, 1967).

2.3.3. Quantitative autoradiography

Immediately after decapitation, the brain was quickly removed and frozen in isopentane cooled at −30°C with dry ice. Brain coronal sections (20 μm thick) were cut at −15°C in a cryostat, mounted on Superfrost Plus slides and kept at −20°C until used.

2.3.3.1. 5-HT$_{1A}$ receptor labelling. Slide-mounted tissue sections were preincubated at room temperature for 30 min in 0.17 M Tris–HCl, pH 7.6. They were then incubated at 20°C for 1 h in the same—but fresh—buffer supplemented with 1.14 nM [^{3}H]8-OH-DPAT. Sections were washed twice for 5 min in Tris–HCl buffer at 4°C, quickly dipped in ice-cold distilled water and dried with cold air. Nonspecific binding was determined on adjacent sections processed under the same conditions except that 10 μM 5-HT was added to the incubation medium. Dried slides were finally apposed to [^{3}H]Hyperfilm (Amersham) with tritium-labeled standards (Amersham) in X-ray cassettes for 5 weeks at 4°C (Laporte et al., 1991).

2.3.3.2. 5-HT$_{2A}$ receptor labelling. Sections were preincubated at room temperature for 15 min in 0.17 M Tris–HCl, pH 7.6, and then incubated at 20°C for 1 h in the same—but fresh—buffer supplemented with 2 nM [^{3}H]SR 46349B (Rinaldi-Carmona et al., 1993). After 2 × 5-min washes in Tris–HCl buffer at 4°C, the sections were quickly dipped in ice-cold distilled water, dried in a stream of cold air, and apposed for 1 month to [^{3}H]Hyperfilm (Amersham). Nonspecific binding was estimated on adjacent sections processed through the same steps except that a saturating concentration (1 μM) of cinanserin was added to the incubation medium.

Autoradiograms were developed in Kodak LX 24 (2 min at 15°C) and quantified using a computerized image analysis system (Biocom). Optical density (O.D.) on the autoradiograms was converted to femtomoles [^{3}H]8-OH-DPAT or [^{3}H]S46349B specifically bound per milligram tissue, with reference to tritium-labelled standards.

2.3.4. In vitro extracellular recording of serotoninergic neurons in the dorsal raphe nucleus

Immediately after death, the brain was rapidly removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 126; KCl, 3.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.3; $CaCl_2$, 2.0; $NaHCO_3$, 25; glucose, 11, and adjusted to pH 7.3–7.4 by continuous bubbling with a mixture of 95% O_2 and 5% CO_2. A block of tissue containing the dorsal raphe nucleus was cut into frontal sections (350 μm thick) using a vibratome (Haj-Dahmane et al., 1991). Slices were kept in ACSF for at least 1 hour at room temperature before their transfer to a recording chamber continuously perfused with oxygenated ACSF (2 ml/min at 34°C).

Extracellular recordings were made using a single-barrel micropipette (filled with 2 M NaCl; impedance: 12–15 MΩ) introduced into the dorsal raphe nucleus (Haj-Dahmane et al., 1991). In all experiments, the otherwise silent serotoninergic neurons were induced to fire by adding 3 μM phenylephrine (α_1-adrenoceptor agonist) to the superfusing ACSF (VanderMaelen and Aghajanian, 1983). When a cell was recorded, it was identified on line as serotoninergic neuron according to the following criteria: biphasic action potentials of 2–3 ms duration, slow (0.5–2.0 Hz) and regular pattern of discharge (VanderMaelen and Aghajanian, 1983).

Baseline activity was recorded for 5–10 min prior to the infusion of the 5-HT$_{1A}$ agonist 8-OH-DPAT (Hamon, 1997) into the chamber via a three-way tap system. Complete exchange of fluids occurred within 2 min of the arrival of a new solution into the chamber. The electric signals were fed into a high-input impedance amplifier, an oscilloscope and an electronic ratemeter triggered by single action potentials. The integrated firing rate was computed and recorded graphically as consecutive 10-s samples. The effects of each concentration of 8-OH-DPAT were evaluated by comparing the mean discharge frequency during the 2 min prior to its addition to the superfusing ACSF and the 2–3 min after the end of drug infusion, when the resulting changes in firing frequency reached their maximal amplitude. Nonlinear regression fitting was carried out using Prism 2.0 (Graph Pad) software facilities for the calculation of EC_{50} values of 8-OH-DPAT.

Electrophysiological data are expressed as percentage of the baseline firing rate ± S.E.M. Data were analyzed by one-way ANOVA. Post-hoc comparisons were made using the Newman–Keuls multiple comparison test. Statistical significance was fixed at $P < 0.05$.

2.4. Chemicals

[^{3}H]8-OH-DPAT (100 Ci/mmol) was from the Service des Molécules Marquées (CEA, 91191 Gif-sur-Yvette,

Table 1
Characteristics of 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptor binding sites in various brain regions after a 7-, 14- or 21-day treatment with alnespirone [5 mg/(kg day) i.p.] in rats

Treatment		5-HT_{1A} receptors [^{3}H]8-OH-DPAT (hippocampus)		5-HT_{2A} receptors [^{3}H]SR 46349B (frontal cortex)		5-HT_3 receptors [^{3}H]S-zacopride (entorhinal cortex)	
		Saline	Alnespirone	Saline	Alnespirone	Saline	Alnespirone
7 days	K_d	0.95 ± 0.13	1.20 ± 0.23	1.13 ± 0.16	1.90 ± 0.15*	0.23 ± 0.14	0.20 ± 0.08
	B_{max}	206.3 ± 25.9	236.6 ± 36.5	293.8 ± 24.1	315.0 ± 30.0	23.0 ± 3.3	25.5 ± 3.0
14 days	K_d	1.36 ± 0.32	1.43 ± 0.48	1.52 ± 0.46	1.90 ± 0.10	0.15 ± 0.03	0.17 ± 0.04
	B_{max}	198.8 ± 23.4	248.4 ± 28.6	335.7 ± 29.3	315.2 ± 29.8	20.4 ± 3.2	20.3 ± 1.8
21 days	K_d	1.18 ± 0.13	1.11 ± 0.27	1.62 ± 0.21	1.63 ± 0.18	0.13 ± 0.03	0.14 ± 0.04
	B_{max}	267.7 ± 21.6	246.5 ± 19.4	326.2 ± 17.1	302.6 ± 23.4	19.0 ± 2.3	22.8 ± 2.7

Rats were killed 24 h after the last injection. 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptor binding sites in hippocampal or cortical membranes were specifically labelled by [^{3}H]8-OH-DPAT, [^{3}H]SR 46349B and [^{3}H]S-zacopride, respectively, as described in Section 2. K_d (in nM) and B_{max} (in fmol/mg protein) values are the means ± S.E.M. of 3–4 independent determinations.
* $P < 0.05$ ($t = 6{,}081$, $df = 4$) as compared to the respective value in saline-treated rats.

France), [^{3}H]SR 46349B (56 Ci/mmol) was from Sanofi-Recherche (34184 Montpellier, France) and [^{3}H]S-zacopride (83 Ci/mmol) was from Delalande-Synthelabo (92500 Rueil-Malmaison, France). Other drugs were: 5-HT-creatinine sulfate (Merck, Darmstadt, Germany), 8-OH-DPAT (Research Biochem. Int., Natick, MA, USA), phenylephrine (Sigma, St Quentin Fallavier, France), alnespirone (S 20499, (+)-4-[*N*-(5-methoxy-chroman-3-yl)-*N*-propylamino] butyl-8-azaspiro-(4,5)-decane-7,9-dione, Servier Laboratories, IRIS, 92415 Courbevoie, France), cinanserin (Bristol–Myers Squibb, Princeton, NJ, USA) and ondansetron (Glaxo Wellcome, Stevenage, UK).

3. Results

3.1. Effects of chronic alnespirone treatment on body weight and behaviour

The daily weighing of rats in the course of chronic treatment indicated that the weight gain was the same in animals treated with alnespirone for up to 21 days and in controls. Thus, after 14 days of treatment, saline-treated animals gained 125.00 ± 2.23 g (mean ± S.E.M., $n = 5$), whereas those receiving alnespirone gained 124.00 ± 3.23 g ($n = 10$) ($P > 0.05$, ns). After 21 days of treatment, these values were 187.50 ± 11.63 g ($n = 5$) and 204.37 ± 7.34 g ($n = 8$), respectively ($P > 0.05$, ns). No differences were noted between alnespirone- and saline-treated rats regarding their behavioural reactions to repeated handling for the injections.

3.2. Effects of chronic alnespirone treatment on the characteristics of 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptor binding sites in membranes from various brain regions

Neither the K_d nor the B_{max} values of 5-HT_{1A} receptors specifically labelled by [^{3}H]8-OH-DPAT in hippocampal membranes were affected 24 h after a 7-, 14- or 21-day treatment with alnespirone (Table 1). Similarly, 5-HT_3 receptor binding sites specifically labelled by [^{3}H]S-zacopride in entorhinal cortex membranes remained unaltered after these treatments. Whatever the duration of alnespirone treatment, the B_{max} value of 5-HT_{2A} receptor binding sites specifically labelled by [^{3}H]SR 46349B in frontal cortex membranes was also unaltered as compared to saline-treated control rats. However, 24 h after the 7-day treatment with alnespirone, but not after the other two treatment conditions, the K_d value of [^{3}H]SR 46349B specific binding sites was significantly higher than in paired-control rats (Table 1).

3.3. Quantitative autoradiography of 5-HT_{1A} and 5-HT_{2A} receptor binding sites after chronic treatment with alnespirone

No changes in the specific labelling by [^{3}H]8-OH-DPAT of 5-HT_{1A} receptors in the dorsal raphe nucleus, the sep-

Table 2
Effects of a 14- or 21-day treatment with alnespirone [5 mg/(kg day) i.p.] on the autoradiographic labelling of 5-HT_{1A} receptor binding sites in various brain regions in rats

Treatment	5-HT_{1A} receptors [^{3}H]8-OH-DPAT specifically bound (fmol/mg tissue)			
	14 days		21 days	
	Saline	Alnespirone	Saline	Alnespirone
Dorsal raphe nucleus	123.8 ± 5.9	132.7 ± 5.0	110.2 ± 4.8	131.4 ± 14.0
Septum	140.3 ± 1.8	140.5 ± 3.6	128.6 ± 9.6	114.0 ± 3.9
Hippocampus				
Dentate gyrus	140.1 ± 3.1	152.8 ± 7.8	149.9 ± 8.2	147.0 ± 6.2
CA1	112.4 ± 5.1	119.6 ± 10.8	108.9 ± 1.2	106.9 ± 4.0

Rats were killed 24 h after the last injection. Coronal brain sections (20 μm thick) were labelled by 1.14 nM [^{3}H]8-OH-DPAT, and apposed to [^{3}H]Hyperfilm to generate autoradiograms. Quantification of specifically bound [^{3}H]8-OH-DPAT was as described in Section 2. Each value is the mean ± S.E.M. of 34–40 determinations (O.D. measurements) in three rats.

Table 3
Effects of a 14- or 21-day treatment with alnespirone [5 mg/(kg day) i.p.] on the autoradiographic labelling of 5-HT_{2A} receptor binding sites in the rat frontal cortex

Treatment	5-HT_{2A} receptors [^{3}H]SR 46349B specifically bound (fmol/mg tissue)			
	14 days		21 days	
	Saline	Alnespirone	Saline	Alnespirone
Frontal cortex	61.99 ± 4.52	73.82 ± 10.40	74.73 ± 2.81	79.74 ± 3.12

Rats were killed 24 h after the last injection. Coronal brain sections (20 μm thick) were labelled by 2 nM [^{3}H]SR 46349B, and apposed to [^{3}H]Hyperfilm to generate autoradiograms. Quantification of specifically bound [^{3}H]S46349B was as described in Section 2. Each value is the mean ± S.E.M. of 23–34 determinations (O.D. measurements) in three rats.

tum and the dentate gyrus and CA_1 area of the hippocampus were noted 24 h after the daily administration of alnespirone for 14 or 21 days (Table 2). Similarly, these

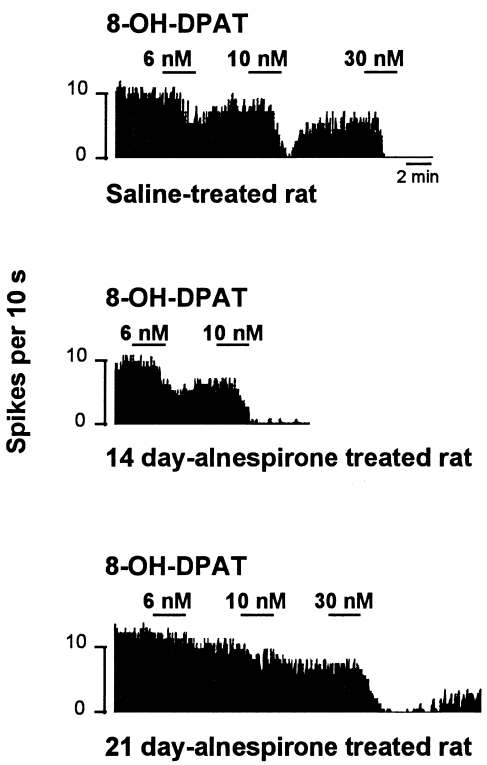

Fig. 1. Effect of 14- or 21-day treatment with alnespirone [5 mg/(kg day) i.p.] on 8-OH-DPAT-induced inhibition of the firing of serotoninergic neurons in the rat dorsal raphe nucleus. Upper record: Integrated firing rate (in spikes per 10 s) histogram of a serotoninergic neuron in the dorsal raphe nucleus from a rat treated with saline for 21 days. The application of 8-OH-DPAT at increasing concentrations (6, 10 and 30 nM, 3 min) dose-dependently suppressed the neuronal discharge. Middle record: Integrated firing rate histogram showing that 8-OH-DPAT (6 and 10 nM, 3 min) was equally potent to reduce the discharge of a serotoninergic neuron after a 14-day treatment with alnespirone as after a 14-day (not shown) or 21-day treatment with saline (upper record). Lower record: A 21-day treatment with alnespirone resulted in a reduced efficacy of 8-OH-DPAT (6, 10 and 30 nM) to inhibit the discharge of a serotoninergic neuron in the dorsal raphe nucleus.

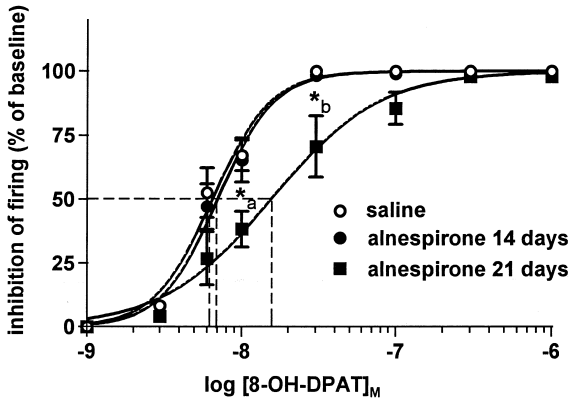

Fig. 2. Concentration-dependent inhibition by 8-OH-DPAT of the firing of serotoninergic neurons in the dorsal raphe nucleus from rats treated with saline or alnespirone for 14 or 21 days. 8-OH-DPAT-induced inhibition is expressed as a percentage of the baseline firing rate. Each point is the mean ± S.E.M. of data obtained from 3–10 individual cells. Data from rats treated with saline for 14 or 21 days did not differ from each other and were pooled for the sake of clarity. EC_{50} values were determined using nonlinear regression fitting (carried out using Prism 2.0, GraphPad, software facilities). (a) ANOVA $F(2,36) = 6.72$, $P < 0.01$; (b) ANOVA $F(2,17) = 5.066$, $P < 0.05$. $*$ $P < 0.05$ as compared to respective values in saline-treated rats (Newman–Keuls' test).

treatments did not significantly alter the specific labelling by [^{3}H]SR 46349B of 5-HT_{2A} receptors in the frontal cortex (Table 3).

3.4. Effects of chronic alnespirone treatment on the inhibitory influence of 8-OH-DPAT on the electrical discharge of serotoninergic neurons in the dorsal raphe nucleus

In the absence of drugs, except 3 μM phenylephrine in the superfusing ACSF, the electrical activity of serotoninergic neurons within the dorsal raphe nucleus from rats that had been chronically treated with alnespirone ($n = 24$) exhibited the same frequency (14-day alnespirone treatment: 13.2 ± 1.4 spikes per 10 s, $n = 17$ cells; 21-day alnespirone treatment: 13.1 ± 1.3 spikes per 10 s, $n = 18$ cells, means ± S.E.M.) as that noted for control animals ($n = 18$) (14- and 21-day saline treatments: 12.3 ± 0.8 spikes per 10 s, $n = 19$ cells, $P > 0.05$, ns).

The addition of 8-OH-DPAT to the superfusing ACSF produced a concentration-dependent inhibition of the discharge of serotoninergic neurons in all treatment groups. However, quantitative differences in the 8-OH-DPAT-evoked response could be observed depending on the duration of alnespirone treatment.

The potency of the 5-HT_{1A} receptor agonist to decrease the electrical activity of serotoninergic neurons in the dorsal raphe nucleus was similar after a 14-day treatment with either saline or alnespirone (Fig. 1). Thus, the dose–response curves of 8-OH-DPAT for both treatment groups were superimposed (Fig. 2). EC_{50} values of 8-OH-DPAT were 6.5 nM (95% confidence interval: 5.6–7.5 nM) and

7.0 nM (95% confidence interval: 6.2–8.0 nM) for saline-treated rats and animals treated with alnespirone, respectively.

In contrast, alnespirone treatment for 21 days significantly reduced the potency of 8-OH-DPAT to inhibit the discharge of serotoninergic neurons in the dorsal raphe nucleus. In particular, at a concentration, 10 nM, high enough to completely suppress the discharge in tissues from rats that had been treated for 14 days with alnespirone or for 14-21 days with saline, 8-OH-DPAT produced only a partial inhibition after a 21-day treatment with alnespirone (Fig. 1). The dose–response curve for the latter treatment was shifted to the right and the EC_{50} value of 8-OH-DPAT was 2.4 times higher in rats that had been treated with alnespirone for 21 days (15.4 nM, 95% confidence interval: 12.3–19.4 nM) than in paired saline-treated rats (6.5 nM) (Fig. 2).

4. Discussion

As expected for a 5-HT_{1A} receptor agonist (Hamon, 1997), long-term treatment with alnespirone induced modifications in the functional properties of somatodendritic 5-HT_{1A} autoreceptors. Indeed, in vitro electrophysiological recordings in the dorsal raphe nucleus revealed a decreased potency of 8-OH-DPAT to inhibit the electrical activity of serotoninergic neurons in brain stem slices from rats that had been treated daily with alnespirone for 3 weeks. Interestingly, no changes in the potency of 8-OH-DPAT were detected after a 14-day treatment with alnespirone, suggesting that functional desensitization of somatodendritic 5-HT_{1A} autoreceptors induced by repeated administration of this exogenous agonist is a slowly developing phenomenon. 5-HT_{1A} autoreceptor desensitization has already been described after repeated treatment with azapirone 5-HT_{1A} receptor agonists such as ipsapirone (Schechter et al., 1990; Dong et al., 1997) or gepirone (Blier and De Montigny, 1987). However, previous studies by our group (Schechter et al., 1990), in agreement with others (Blier and De Montigny, 1987; Dong et al., 1997), showed that only a 2-week treatment, or even less, with the latter drugs was sufficient to trigger 5-HT_{1A} autoreceptor desensitization. Similarly, a decrease in 5-HT_{1A} autoreceptor sensitivity has also been reported to occur in response to sustained overstimulation by extracellular endogenous 5-HT due to repeated administration of selective serotonin reuptake inhibitors for only 2 weeks (Blier and De Montigny, 1983; Blier et al., 1984; Chaput et al., 1986; Jolas et al., 1994) or even a few days (Le Poul et al., 1995).

Although these data show that functional desensitization of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus is a common response to their long-term stimulation by either an exogenous agonist or endogenous 5-HT, differences apparently exist in the duration of treatment required for this phenomenon to occur after the administration of alnespirone versus other (direct or indirect) agonists. One possible explanation of these differences may lie in the nature of interactions between 5-HT_{1A} receptors and alnespirone compared to other agonists. Indeed, Scott et al. (1994) reported that alnespirone does not act as a 'classical' 5-HT_{1A} receptor agonist in vivo, and in vitro binding studies with [^{3}H]alnespirone confirmed that this drug does not interact exactly as the prototypical agonist 8-OH-DPAT with 5-HT_{1A} receptors in rat brain membranes (Fabre et al., 1997). However, continuous infusion of alnespirone [3 mg/(kg day)] by means of s.c. implanted minipump was recently reported to reduce, after only 2 weeks, the inhibitory effect of acutely administered 8-OH-DPAT on 5-HT release in the rat brain (Casanovas et al., 1997b), indicating that, under these conditions, 5-HT_{1A} autoreceptor desensitization could occur as rapidly as with other agonists. Therefore, peculiar pharmacokinetic and/or pharmacodynamic characteristics of alnespirone might also contribute to the slow development of 5-HT_{1A} autoreceptor desensitization after repeated systemic treatment with this drug as compared to other direct or indirect agonists administered under the very same treatment protocol (Schechter et al., 1990; Jolas et al., 1994; Le Poul et al., 1995).

In spite of the clear-cut desensitization of somatodendritic 5-HT_{1A} autoreceptors, no changes in the specific binding of [^{3}H]8-OH-DPAT in the dorsal raphe nucleus, as well as in the other brain areas examined, were observed after a 21-day treatment with alnespirone. These observations agree with most of the relevant data in the literature showing that functional desensitization of 5-HT_{1A} autoreceptors due to chronic treatment with other 5-HT_{1A} receptor agonists such as buspirone (Haskins et al., 1989) and ipsapirone (Schechter et al., 1990) or selective serotonin reuptake inhibitors (Hensler et al., 1991; Le Poul et al., 1995) occurs without any change in the characteristics of 5-HT_{1A} receptor binding sites in the rat brain (but see Welner et al., 1989, and Casanovas et al., 1997b). As already discussed about the effects of the latter drugs, alterations in the coupling of 5-HT_{1A} autoreceptors with G protein(s), possibly due to changes in the tissue concentrations of the latter proteins (Li et al., 1996, 1997), but not in those of the receptors themselves, might account for their functional desensitization (Le Poul et al., 1995).

Interestingly, previous studies with 5-HT_{1A} receptor agonists of the azapirone series showed that chronic treatment with these drugs such as buspirone (Haskins et al., 1989), gepirone (Eison and Yocca, 1985) and ipsapirone (Schechter et al., 1990) down regulates 5-HT_{2A} receptors in the cerebral cortex. In addition, a down regulation of cortical 5-HT_3 receptors has been reported in rats after a 2-week treatment with ipsapirone (Schechter et al., 1990). Because a down regulation of cortical 5-HT_{2A} receptors is regularly observed after chronic treatment with tricyclic antidepressants (Peroutka and Snyder, 1980; Boess and

Martin, 1994), and this phenomenon develops with the same time-course as the therapeutic efficacy of these drugs, Schechter et al. (1990) proposed that the antidepressant properties of the azapirones can be related to their capacity to down regulate cortical 5-HT_{2A} receptors. However, the present study shows that no such regulation occurred after treatment for up to 3 weeks with alnespirone, which also exerts clearcut antidepressant-like properties in rats (Mac Sweeney et al., 1998). Indeed, only a slight decrease in 5-HT_{2A} receptor affinity for the tritiated antagonist [^{3}H]SR 46349B (Rinaldi-Carmona et al., 1993) was noted in the frontal cortex after the first week of treatment with alnespirone (Table 1). This effect could not be ascribed to a direct competitive interaction of alnespirone at 5-HT_{2A} receptors because this would imply that micromolar concentrations of the injected drug (see Kidd et al., 1993) persisted in the membranes used in binding assays. Indeed, no changes in K_d values of [^{3}H]8-OH-DPAT specific binding were found at any time during the 3-week treatment with alnespirone (Table 1), indicating that the drug concentration possibly remaining in membranes used in binding assays did not compete with [^{3}H]8-OH-DPAT binding, i.e., was below the nanomolar range (see Fabre et al., 1997). Furthermore, the change in K_d value of [^{3}H]SR 46349B specific binding was no longer observed after a 2- or 3-week treatment with alnespirone (Table 1). Whether such a transient change in 5-HT_{2A} receptor affinity for [^{3}H]SR 46349B actually reflected functional alterations in 5-HT_{2A} receptors after the first week of treatment with alnespirone is a relevant question to be addressed in future investigations.

In light of the present data concerning 5-HT_{2A} and 5-HT_3 receptors in alnespirone-treated rats, it can be inferred that azapirones-induced changes in these receptors (Eison and Yocca, 1985; Haskins et al., 1989; Schechter et al., 1990) are very probably not related to their antidepressant action, but may reflect the other non-5-HT_{1A} receptor-mediated effects of these drugs (see Section 1). Accordingly, only the selective stimulation of 5-HT_{1A} receptors such as that achieved by alnespirone likely accounts for the antidepressant-like effects of the azapirones. This conclusion agrees with previous inferences from data obtained with the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT (Martin et al., 1990; De Vry, 1996; Mac Sweeney et al., 1998), although some doubts were subsequently raised because of the relatively high affinity of 8-OH-DPAT for 5-HT_7 receptors also (Hoyer et al., 1994).

In addition to exerting antidepressant-like properties, alnespirone, like the azapirones, also exhibits anxiolytic-like properties in relevant tests in animals (Porsolt et al., 1992; Barrett et al., 1994; Curle et al., 1994). Accordingly, it can be inferred that only the stimulation of 5-HT_{1A} receptors by the latter drugs probably accounts for these properties. In line with this interpretation, Jolas et al. (1995) recently provided the clear-cut demonstration that the anxiolytic-like effects of ipsapirone and 8-OH-DPAT results exclusively from the stimulation of somatodendritic 5-HT_{1A} autoreceptors in anterior raphe nuclei.

In conclusion, only a functional desensitization of somatodendritic 5-HT_{1A} autoreceptors, with no changes in 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptor binding sites, were observed as a result of the chronic selective stimulation of 5-HT_{1A} receptors by alnespirone in rats. This suggests that adaptive regulation of 5-HT_{1A} receptors occurs independently of other 5-HT receptor types, in spite of the well-established existence of interactions between the various classes of 5-HT receptors in brain (Kidd et al., 1992; Salmi and Ahlenius, 1998).

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References

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.

Baumgarten, H.G., Göthert, M., 1997. Handbook of Experimental Pharmacology, Vol. 129, Serotoninergic Neurons and 5-HT Receptors in the CNS. Springer, Berlin, 767 p.

Bianchi, G., Caccia, S., 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.

Blier, P., De Montigny, C., 1983. Electrophysiological investigations on the effect of repeated zimelidine administration on serotoninergic neurotransmission in the rat. J. Neurosci. 3, 1270–1278.

Blier, P., De Montigny, C., 1987. Modification of 5-HT neuron properties by sustained administration of the 5-HT_{1A} agonist gepirone: electrophysiologic studies in the rat brain. Synapse 1, 470–480.

Blier, P., De Montigny, C., Tardif, D., 1984. Effects of two antidepressant drugs, mianserin and indalpine, on the serotoninergic system: single-cell studies in the rat. Psychopharmacology 84, 242–249.

Boess, F.G., Martin, J.L., 1994. Molecular biology of 5-HT receptors. Neuropharmacology 33, 275–317.

Bolaños, F.J., Schechter, L.E., Miquel, M.C., Emerit, M.B., Rumigny, J.F., Hamon, M., Gozlan, H., 1990. Common pharmacological and physicochemical properties of 5-HT_3 binding sites in the rat cerebral cortex and NG108-15 clonal cells. Biochem. Pharmacol. 40, 1541–1550.

Casanovas, J.M., Lesourd, M., Artigas, F., 1997a. 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.

Casanovas, J.M., Vilaró, M.T., Mengod, G., Muñoz, C., Lesourd, M., Artigas, F., 1997b. Sensitivity of 5-HT_{1A} receptors in dorsal raphe nucleus after 2-week treatments with alnespirone and 8-OH-DPAT. Soc. Neurosci. Abstr. 23, 970, (385.14).

Chaput, Y., de Montigny, C., Blier, P., 1986. Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 333, 342–348.

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

Curle, P., Mocaër, E., Renard, P., Guardiola, B., 1994. Anxiolytic properties of (+)S 20499, a novel serotonin 5-HT_{1A} full agonist, in the elevated plus-maze and social interaction tests. Drug Dev. Res. 32, 183–190.

De Vry, J., 1996. 5-HT_{1A} receptors in psychopathology and the mechanism of action of clinically effective therapeutic agents. Drug News Perspect. 9, 270–280.

Di Scenna, P., 1987. Method and myth in maintaining brain slices. In: Schurr, A., Teyler, T., Tseng, M. (Eds.), Brain Slices: Fundamentals, Applications and Implications. Karger, Basel, pp. 10–21.

Dong, J.M., De Montigny, C., Blier, P., 1997. Effect of acute and repeated versus sustained administration of the 5-HT_{1A} receptor agonist ipsapirone: electrophysiological studies in the rat hippocampus and dorsal raphe. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 303–311.

Dugast, C., Soulière, F., Schmitt, P., Casanovas, J.M., Fattaccini, C.M., Mocaër, E., Lesourd, M., Renaud, B., Artigas, F., Hamon, M., Chouvet, G., 1998. Is the potent 5-HT_{1A} receptor agonist, alnespirone (S-20499), affecting dopaminergic systems in the rat brain?. Eur. J. Pharmacol. 350, 171–180.

Eison, A.S., Yocca, F.D., 1985. Reduction in cortical 5-HT_2 receptor sensitivity after continuous gepirone treatment. Eur. J. Pharmacol. 111, 389–392.

Engberg, G., 1989. A metabolite of buspirone increases locus coeruleus activity via alpha$_2$-receptor blockade. J. Neural Transm. 76, 91–98.

Fabre, V., Boni, C., Mocaër, E., Lesourd, M., Hamon, M., Laporte, A.M., 1997. [^{3}H]Alnespirone a novel specific radioligand of 5-HT_{1A} receptors in the rat brain. Eur. J. Pharmacol. 337, 297–308.

Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J., Hamon, M., 1983. Identification of presynaptic serotonin autoreceptors using a new ligand ^{3}H-PAT. Nature 305, 140–142.

Haj-Dahmane, S., Hamon, M., Lanfumey, L., 1991. K^+ channel and 5-hydroxytryptamine$_{1A}$ autoreceptor interactions in the rat dorsal raphe nucleus: an in vitro electrophysiological study. Neuroscience 41, 495–505.

Hall, M.D., El Mestikawy, S., Emerit, M.B., Pichat, L., Hamon, M., Gozlan, H., 1985. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino) tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. J. Neurochem. 44, 1685–1696.

Hamon, M., 1997. The main features of central 5-HT_{1A} receptors. In: Baumgarten, H.G., Göthert, M. (Eds), Handbook of Experimental Pharmacology, Vol. 129: Serotoninergic Neurons and 5-HT Receptors in the CNS. Springer, Berlin, pp. 239–268.

Haskins, J.T., Moyer, J.A., Andree, T.H., Muth, E.A., Abou-Gharbia, M., 1989. Preclinical profile of the pyrimidinylpiperazinyl imide compound WY-47,846: a potential anxiolytic. Drug Dev. Res. 18, 29–45.

Hensler, J.G., Kovachich, G.B., Frazer, A., 1991. A quantitative autoradiographic study of serotonin$_{1A}$ receptor regulation. Effect of 5,7-dihydroxytryptamine and antidepressant treatments. Neuropsychopharmacology 4, 131–144.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P.A., 1994. VII. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46, 157–203.

Jolas, T., Haj-Dahmane, S., Kidd, E.J., Langlois, X., Lanfumey, L., Fattaccini, C.M., Vantalon, V., Laporte, A.M., Adrien, J., Gozlan, H., Hamon, M., 1994. Central pre- and postsynaptic 5-HT_{1A} receptors in rats treated chronically with a novel antidepressant, cericlamine. J. Pharmacol. Exp. Ther. 268, 1432–1443.

Jolas, T., Schreiber, R., Laporte, A.M., Chastanet, M., De Vry, J., Glaser, T., Adrien, J., Hamon, M., 1995. Are postsynaptic 5-HT_{1A} receptors involved in the anxiolytic effects of 5-HT_{1A} receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat?. J. Pharmacol. Exp. Ther. 272, 920–929.

Keppel Hesselink, J.M., 1992. Promising anxiolytics? A new class of drugs: the azapirones. In: Stahl, S.M., Gastpar, M., Keppel Hesselink, J.M., Traber, J. (Eds.), Serotonin$_{1A}$ receptors in depression and anxiety. Raven Press, New York, pp. 171–183.

Kidd, E., Laporte, A.M., Langlois, X., Lombard, M.C., Gozlan, H., Hamon, M., 1992. Differential localization and regulation of central 5-HT_{1A} and 5-HT_3 receptors. In: Bradley, P.B., Handley, S.L., Cooper, S.J., Key, B.J., Barnes, N.M., Coote, J.H. (Eds.), Adv. Biosci., Vol. 85: Serotonin, CNS receptors and brain function. Pergamon Press, Oxford, pp. 13–27.

Kidd, E.J., Haj-Dahmane, S., Jolas, T., Lanfumey, L., Fattaccini, C.M., Guardiola-Lemaitre, B., Gozlan, H., Hamon, M., 1993. New methoxy-chroman derivatives, 4[*N*-(5-methoxy-chroman-3-yl)*N*-propylamino]butyl-8-azaspiro-(4,5)-decane-7,9-dione [(±)-S 20244] and its enantiomers, (+)-S 20499 and (−)-S 20500, with potent agonist properties at central 5-hydroxytryptamine$_{1A}$ receptors. J. Pharmacol. Exp. Ther. 264, 863–872.

Kurtz, N., 1992. Efficacy of azapirones in depression. In: Stahl, S.M., Gastpar, M., Keppel Hesselink, J.M., Traber, J. (Eds.), Serotonin$_{1A}$ receptors in depression and anxiety. Raven Press, New York, pp. 163–170.

Laporte, A.M., Schechter, L.E., Bolaños, F.J., Vergé, D., Hamon, M., Gozlan, H., 1991. [^{3}H]5-Methyl-urapidil labels 5-HT_{1A} receptors and Fα_1-adrenoceptors in the rat CNS. In vitro binding and autoradiographic studies. Eur. J. Pharmacol. 198, 59–67.

Le Poul, E., Laaris, N., Doucet, E., Laporte, A.M., Hamon, M., Lanfumey, L., 1995. Early desensitization of somato-dendritic 5-HT_{1A} autoreceptors in rats treated with fluoxetine or paroxetine. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 141–148.

Li, Q., Muma, N.A., Van de Kar, L.D., 1996. Chronic fluoxetine induces a gradual desensitization of 5-HT_{1A} receptors: Reductions in hypothalamic and midbrain Gi and Go proteins and in neuroendocrine responses to a 5-HT_{1A} agonist. J. Pharmacol. Exp. Ther. 279, 1035–1042.

Li, Q., Muma, N.A., Battaglia, G., Van de Kar, L.D., 1997. A desensitization of hypothalamic 5-HT_{1A} receptors by repeated injections of paroxetine: reduction in the levels of Gi and Go proteins and neuroendocrine responses, but not in the density of 5-HT_{1A} receptors. J. Pharmacol. Exp. Ther. 282, 1581–1590.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Mac Sweeney, C.P., Lesourd, M., Gandon, J.M., 1998. Antidepressant-like effects of alnespirone (S 20499) in the learned helplessness test in rats. Eur. J. Pharmacol. 345, 133–137.

Martin, P., 1991. 1-(2-Pyrimidinyl)-piperazine may alter the effects of the 5-HT_{1A} agonists in the learned helplessness paradigm in rats. Psychopharmacology 104, 275–278.

Martin, P., Beninger, R.J., Hamon, M., Puech, A.J., 1990. Anti-depressant-like action of 8-OH-DPAT, a 5-HT_{1A} agonist, in the learned helplessness paradigm: evidence for a postsynaptic mechanism. Behav. Brain Res. 38, 135–144.

McMillen, B., Mathews, 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.

Peroutka, S.J., Snyder, S.H., 1980. Long term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science 210, 88–90.

Piercey, M.F., Smith, M.W., Lumragan, 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.

Porsolt, R., Lenègre, A., Caignard, D., 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.

Redmond, D.E., Huang, Y.H., 1979. New evidence for a locus coeruleus norepinephrine connection with anxiety. Life Sci. 25, 2149–2162.

Rinaldi-Carmona, M., Congy, C., Pointeau, P., Vidal, H., Brelière, J.C., Le Fur, G., 1993. Identification of binding sites for SR 46349B, a 5-hydroxytryptamine$_2$ receptor antagonist, in rodent brain. Life Sci. 54, 119–127.

Salmi, P., Ahlenius, S.B., 1998. Evidence for functional interactions between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in rat thermoregulatory mechanisms. Pharmacol. Toxicol. 82, 122–127.

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

Schechter, L.E., Bolaños, F.J., Gozlan, H., Lanfumey, L., Haj-Dahmane, S., Laporte, A.M., Fattaccini, C.M., Hamon, M., 1990. Alterations of central serotoninergic 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.

Snedecor, G.W., Cochran, W.G., 1967. Statistical Methods, Iowa State College Press, Ames.

VanderMaelen, C.P., Aghajanian, G.K., 1983. Electrophysiological and pharmacological characterization of serotoninergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. Brain Res. 289, 109–119.

Welner, S.A., De Montigny, C., Desroches, J., Desjardins, P., Suranyi-Cadotte, B.E., 1989. Autoradiographic quantification of serotonin$_{1A}$ receptors in rat brain following antidepressant drug treatment. Synapse 4, 347–352.