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Similar pharmacological properties of 8-OH-DPAT and alnespirone (S 20499) at dopamine receptors: comparison with buspirone

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Abstract

Alnespirone (S 20499) has previously been described as a potential anxiolytic drug that acts by stimulation of 5-HT_{1A} receptors. Some data suggest that alnespirone might also be a weak dopamine D_2 receptor agonist: it displays moderate affinity for dopamine D_2 receptors in vitro and it inhibits prolactin release and induces yawning in rats. In order to test for possible interactions of alnespirone with dopamine receptors in vivo, we studied the changes of in vivo striatal [^{3}H]SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) and [^{3}H]raclopride binding following the injection of a tracer dose of either tritiated ligand (4 μCi) in mice treated with increasing doses of alnespirone (5, 10, 20 and 40 mg/kg, i.p.) and, in the same animals, the changes in the levels of dopamine, 5-hydroxytryptamine (5-HT) and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA). These changes were compared with those produced by increasing doses of the reference 5-HT_{1A} receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin, 0.25, 1 and 4 mg/kg, i.p.) or buspirone (5 and 20 mg/kg, i.p.). Decreased in vivo striatal [^{3}H]SCH 23390 specific binding was observed in mice treated with 5, 10 and 40 mg/kg alnespirone. In contrast, increased in vivo striatal [^{3}H]raclopride specific binding was observed in mice treated with 5 and 20 mg/kg alnespirone. In these animals, the striatal 5-HIAA/5-HT ratio was decreased by 5 to 40 mg/kg alnespirone, whereas the striatal HVA/DA ratio was unaffected at all tested doses of alnespirone. Similarly, 8-OH-DPAT decreased specific in vivo striatal [^{3}H]SCH 23390 binding at 0.25, 1 and 4 mg/kg, and increased in vivo specific striatal [^{3}H]raclopride binding at 1 and 4 mg/kg. In the same animals, all tested doses of 8-OH-DPAT decreased the striatal 5-HIAA/5-HT ratio but did not modify the striatal HVA/dopamine ratio. Buspirone (5 and 20 mg/kg) completely inhibited in vivo specific striatal [^{3}H]raclopride binding and increased the striatal HVA/DA ratio but did not modify the striatal 5-HIAA/5-HT ratio, whereas apomorphine (3 mg/kg) decreased both in vivo specific striatal [^{3}H]SCH 23390 and [^{3}H]raclopride binding as well as the striatal HVA/DA and 5-HIAA/5-HT ratios. Finally, increasing doses of alnespirone or 8-OH-DPAT weakly increased sniffing induced by apomorphine (0.75 mg/kg, s.c.) in mice and decreased grooming induced by the dopamine D_1 receptor agonist SK&F 39393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol, 1.87 mg/kg, s.c.), whereas buspirone decreased both apomorphine-induced sniffing and SK&F 39393-induced grooming. These results indicate that alnespirone and 8-OH-DPAT have a similar profile and do not seem to interact directly with dopamine receptors. The results also suggest that the stimulation of 5-HT_{1A} receptors by either alnespirone or 8-OH-DPAT modulates the availability of striatal [^{3}H]SCH 23390 and [^{3}H]raclopride binding sites and possibly the functioning of striatal dopamine D_1 and D_2 receptors in opposite directions.

Keywords: Alnespirone; 5-HT_{1A} receptor agonist; [^{3}H]SCH 23390 binding, in vivo; [^{3}H]Raclopride binding, in vivo; Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Striatum; (Mouse); Behaviour

1. Introduction

Several lines of evidence indicate that drugs acting as agonists at 5-HT_{1A} receptors ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT, and azapirones such as buspirone, gepirone and ipsapirone) exert potent anxiolytic-like effects in rodents (Traber and Glaser, 1987) and that 5-HT_{1A} receptors may be a target for anxiolytic drugs. Alnespirone (S 20499) displays high affinity (K_i = 0.19 nM) for 5-HT_{1A} receptors and acts as a 5-HT_{1A} receptor agonist in various experimental models (Kidd et al., 1993).

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Like other azapirones, alnespirone also has moderate affinity for dopamine D_2 receptors ($K_i = 20$ nM; Kidd et al., 1993). However, in spite of this fact, alnespirone does not affect the turnover rate of dopamine (Dugast et al., 1998), unlike buspirone, gepirone, and ipsapirone (Hamon et al., 1988). The local application of alnespirone by reverse microdialysis into the dorsal striatum does not affect the dopamine output (Dugast et al., 1998). Nevertheless, alnespirone displays weak dopamine D_2 receptor agonist activity: it induces yawning behaviour in rats pretreated with tertatolol (Simon et al., 1992) and inhibits prolactin release in rats (Levy et al., 1995). Unlike the other azapirones, alnespirone is not metabolized to 1-pyrimidyl piperazine, the main metabolite of buspirone which also displays dopamine D_2 receptor antagonist activity and shows an anxiogenic effect.

Looking for additional evidence of agonist activity of alnespirone at dopamine receptors, we compared the effects of 8-OH-DPAT, the reference 5-HT_{1A} receptor agonist (Hjorth et al., 1982), alnespirone and buspirone on the in vivo binding of [^{3}H]SCH 23390 and [^{3}H]raclopride in mice. In the same mice, i.e., at the same doses of 8-OH-DPAT, alnespirone and buspirone administered under the same conditions, we measured the striatal levels of dopamine, 5-hydroxytryptamine (5-HT) and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA). We also compared the effects of 8-OH-DPAT, alnespirone and buspirone on certain spontaneous behaviours and on stereotyped behaviours of mice induced by apomorphine, the reference subtype non-selective dopamine receptor agonist (Ernst, 1967), or SK&F 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol), the reference dopamine D_1 receptor agonist (Setler et al., 1978).

Several reports indicate that, at the doses used in the present study, the duration of the behavioural and biochemical effects induced by 8-OH-DPAT extends over the observation period of the present study (Gudelsky et al., 1986; Aulakh et al., 1988; Hjorth, 1991; Kreiss and Lucki, 1992; Nomikos et al., 1991; Routledge et al., 1993; Protais et al., 1995).

We show here that, unlike buspirone, 8-OH-DPAT and alnespirone do not act directly either on dopamine transmission or at dopamine receptors, but nevertheless induce opposite changes in the in vivo binding of [^{3}H]SCH 23390 and [^{3}H]raclopride and in grooming and sniffing behaviours.

2. Materials and methods

2.1. Animals

All experiments were performed on male Swiss albino mice (CD1, Charles River, France) weighing 28 ± 3 g. Animals were housed in groups of 15 per cage ($L = 38$ cm, $W = 24$ cm, $H = 18$ cm) and maintained under standard laboratory conditions (22 ± 1°C, 12-h light–dark cycle with lights on at 8 AM, food and water ad libitum) for at least 7 days before use. The animals were separated and placed in individual cages ($L = 20$ cm, $W = 10$ cm, $H = 10$ cm) without food and water 30 min before the start of the experiments. The experiments were carried out between 9 AM and 7 PM in a diffusely illuminated room maintained at 22 ± 1°C.

2.2. In vivo binding

[^{3}H]SCH 23390 and [^{3}H]raclopride were injected in a tail vein at a tracer dose (4 μCi diluted in 0.2 ml of saline), 20 min after drug injection and 20 min before the animals were killed. Immediately after death, the striatum, the olfactory bulbs and the cerebellum were dissected on ice and homogenized by sonication (TC4C sonotrode, range 1) in ice-cold saline (1 ml for striatum and olfactory bulbs, 4 ml for cerebellum). The radioactivity of a 400-μl sample of each homogenate was directly counted in a minivial containing 4 ml Biodegradable Counting Scintillant (BCS) scintillation liquid (Amersham, Les Ulis, France). Protein was measured on a 10-μl sample of each homogenate according to the method of Bradford (1976), using bovine serum albumin (Sigma, L'Isle d'Abeau Chesnes, France) as control, and the results are expressed in disintegrations per minute per milligram protein. Specific binding was calculated as the radioactivity retained in striatum or olfactory bulbs (disintegrations per minute per milligram protein) minus the radioactivity retained in cerebellum (disintegrations per minute per milligram protein).

2.3. Determination of tissue levels of dopamine, DOPAC, HVA, 5-HT and 5-HIAA

Immediately after sonication, a 500-μl sample of each striatal homogenate prepared for in vivo binding experiments was mixed with the same volume of an ice-cold solution containing (final concentrations) trichloroacetic acid (5%), EDTA (0.025%), and Na metabisulfite (0.05%). The diluted homogenate was centrifuged (15 000 × *g* for 20 min at 4°C) and the supernatant was preserved. Aliquots were then analysed directly by high-performance liquid chromatography (HPLC Waters, Millford, MA, USA; Wisp sample processor, pump model 510, Maxima 820 Chromatography Workstation) with a CLINREP 'catecholamines in plasma' column (Recipe, München, Deutschland) and electrochemical detection (Waters 460, potential = +0.70 V). The mobile phase consisted of 50 mM Na acetate, 20 mM citric acid, 3.75 mM octane sulfonic acid, 5.9 mM dibutylamine and 7% methanol; pH was adjusted to 3.5 with acetic acid.

2.4. Behavioural experiments

The mice were placed in individual cylindrical cages (12 cm diameter, 14 cm height) with a wall of vertical metal bars (2 mm diameter, 1 cm apart) and smooth ends. After 5 min, the behaviour of each animal was observed every 2 min (approximately during 4–5 s since 24–28 mice were tested simultaneously) by a single observer (Protais et al., 1994). Climbing behaviour was scored as reported by Marçais et al. (1978): mice that had all four paws on the floor were scored 0, mice that stood upright and gripped the vertical bars with their forepaws were scored 1, and mice that gripped the vertical bars with all four paws were scored 2. Sniffing, licking (repeated protrusions of the tongue) and gnawing (repeated biting of the cage bars) were also assessed: mice were scored 0 if they did not show such behaviour, 1 if they showed it with low intensity or in a discontinuous manner, and 2 if they showed such behaviour continuously with high intensity. Grooming was assessed as reported by Vasse and Protais (1988) by attributing, at each observation, a score of 1 to mice that displayed the behaviour and a score of 0 to mice that did not. The scores attributed to each animal during the observation period (60 min) were added and the individual scores were averaged for each group.

2.5. Drugs

[^{3}H]SCH 23390 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride, 85.5 Ci/mmol) and [^{3}H]raclopride (86.5 Ci/mmol) came from NEN, Paris, France. 8-OH-DPAT ((±)-8-hydroxy-2-(di-*n*-propylamino)tetralin) hydrobromide (R.B.I., Bioblock, Illkirch, France), buspirone hydrochloride (RBI) and WAY 100135 (*N-tert*-butyl-3-(4-(2-methoxyphenyl) piperazine-1-yl)-2-phenylpropionamide) hydrochloride (Wyeth Research, Taplow, UK) were dissolved in saline. Apomorphine hydrochloride (Sigma) was dissolved in saline containing 0.1% ascorbic acid to prevent oxidation. SK&F 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol) hydrochloride (RBI) and alnespirone (IRIS, Courbevoie, France) were dissolved in distilled water.

2.6. Statistical analysis

An analysis of variance (ANOVA) followed by Student's *t*-test was applied to evaluate the significance of the results obtained.

3. Results

3.1. Effects of increasing doses of 8-OH-DPAT, alnespirone, buspirone and apomorphine on the in vivo binding of [^{3}H]SCH 23390 and [^{3}H]raclopride

Injection of mice with a tracer dose of [^{3}H]SCH 23390, 20 min before death, led to a pronounced retention of radioactivity in the striatum and to a lower retention in the cerebellum and olfactory bulbs. For the mice treated with vehicle from all experiments ($n = 34$), the retention of radioactivity was 3733 ± 136 dpm/mg protein in striatum, 456 ± 24 dpm/mg protein in olfactory bulbs and 399 ± 22 dpm/mg protein in cerebellum. Therefore, strong specific [^{3}H]SCH 23390 binding was measured in the striatum and not in the olfactory bulbs. The non-specific binding of

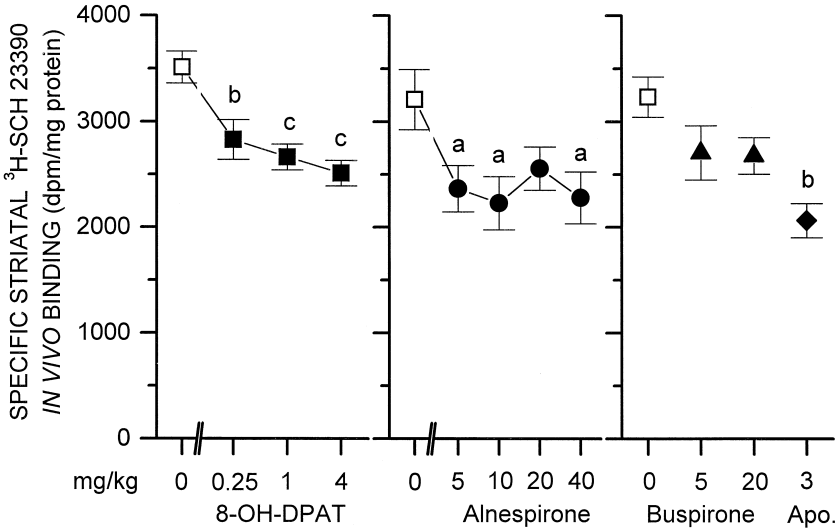

Fig. 1. Effects of 8-OH-DPAT, alnespirone, buspirone and apomorphine on the in vivo specific striatal binding of [^{3}H]SCH 23390 in mice. Mice were injected i.p. with increasing doses of 8-OH-DPAT, alnespirone, buspirone or apomorphine (APO) 20 min before the i.v. injection of [^{3}H]SCH 23390 (4 μCi, 0.2 ml in saline) and 40 min before death. Means ± S.E.M. of data obtained from 12 mice in the experiments with 8-OH-DPAT, from 11 mice in the experiments with alnespirone, and from five mice in the experiments with buspirone and apomorphine. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ when compared to the respective control values.

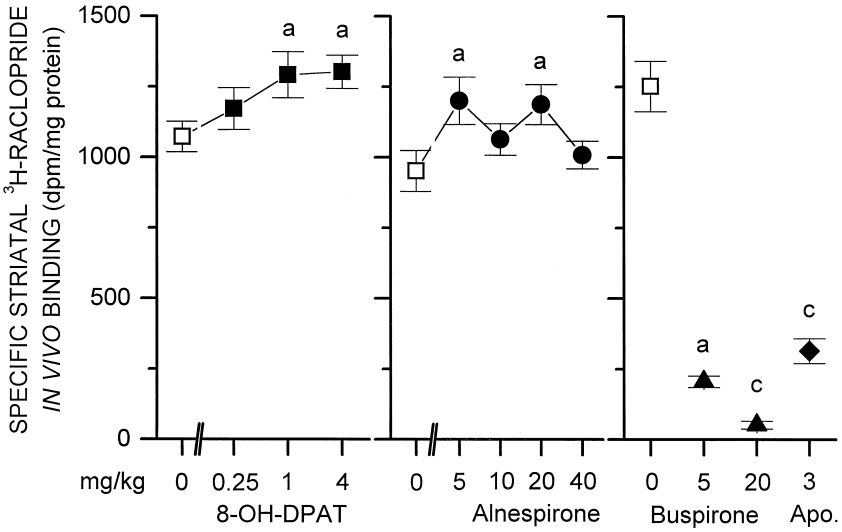

Fig. 2. Effects of 8-OH-DPAT, alnespirone, buspirone and apomorphine on the in vivo specific striatal binding of [^{3}H]raclopride in mice. Mice were injected i.p. with increasing doses of 8-OH-DPAT, alnespirone, buspirone or apomorphine (APO) 20 min before the i.v. injection of [^{3}H]raclopride (4 μCi, 0.2 ml in saline) and 40 min before death. Means ± S.E.M. of data obtained from 12 mice in the experiments with 8-OH-DPAT and alnespirone, and from 5 mice in the experiments with buspirone and apomorphine. [a] $P < 0.05$; [c] $P < 0.001$ when compared to the respective control values.

[^{3}H]SCH 23390 in the cerebellum and olfactory bulbs was not modified by 8-OH-DPAT, alnespirone, buspirone or apomorphine injected 20 min before [^{3}H]SCH 23390. 8-OH-DPAT (0.25, 1 and 4 mg/kg), alnespirone (5, 10 and 40 mg/kg) and apomorphine (3 mg/kg) significantly reduced the specific binding of [^{3}H]SCH 23390 in the striatum, whereas this decrease was not significant with buspirone (Fig. 1).

In mice injected with a tracer dose of [^{3}H]raclopride, 20 min before death, the retention of radioactivity measured in the striatum and olfactory bulbs was higher than that measured in the cerebellum. For the mice treated with vehicle, for all experiments ($n = 37$), the retention of radioactivity was 1246 ± 45 dpm/mg protein in striatum, 332 ± 17 dpm/mg protein in olfactory bulbs and 183 ± 9 dpm/mg protein in cerebellum. Therefore, specific [^{3}H]raclopride binding could be demonstrated in the striatum and olfactory bulbs. The nonspecific binding of [^{3}H]raclopride in the cerebellum was not modified by 8-OH-DPAT, alnespirone, buspirone and apomorphine. 8-OH-DPAT (1 and 4 mg/kg) and alnespirone (5 and 40 mg/kg) significantly increased the specific binding of [^{3}H]raclopride in the striatum (Fig. 2). Significantly increased specific [^{3}H]raclopride binding was also measured in the olfactory bulbs in mice pretreated with 1 mg/kg 8-OH-DPAT or 5 mg/kg alnespirone (data not shown). In contrast, buspirone (5 and 20 mg/kg) and apomorphine (3 mg/kg) decreased or suppressed the specific binding of [^{3}H]raclopride in the striatum (Fig. 2) and olfactory bulbs (data not shown).

Moreover, (+)-WAY 100135, a 5-HT$_{1A}$ receptor antagonist, administered 60 min before death at a dose of 10 mg/kg (s.c.), modified neither the nonspecific binding of [^{3}H]SCH 23390 or [^{3}H]raclopride in the cerebellum nor the

Table 1
Effects of pretreatment with (+)-WAY 100135 on the specific striatal in vivo binding of [^{3}H]SCH 23390 and [^{3}H]raclopride in mice

	Specific striatal in vivo binding (dpm/mg protein) of	
	[^{3}H]SCH 23390	[^{3}H]raclopride
Solvent + solvent	2657 ± 164	1057 ± 95
(+)-WAY 100135 + solvent	2701 ± 210	911 ± 110
Solvent + 8-OH-DPAT	1961 ± 170	1209 ± 93
(+)-WAY 100135 + 8-OH-DPAT	2134 ± 88	857 ± 107[a]
Solvent + alnespirone	1757 ± 145	1207 ± 108
(+)-WAY 100135 + alnespirone	1846 + 86	910 ± 91[a]

(+)WAY 100135 (10 mg/kg, s.c.) was injected 20 min before the administration of 8-OH-DPAT (0.5 mg/kg, i.p.) or alnespirone (5 mg/kg, i.p.) and 40 min before the i.v. injection of [^{3}H]SCH 23390 or [^{3}H]raclopride.
Means ± S.E.M. for eight mice per group.
[a] $P < 0.05$ as compared to respective mice pretreated with solvent instead of (+)-WAY 100135.

Table 2
Effects of 8-OH-DPAT, alnespirone, buspirone and apomorphine on the striatal levels of DA, 5-HT and their metabolites in mice

	DA	DOPAC	HVA	5-HT	5-HIAA
Controls ($n = 25$)	800.2 ± 35.8	68.8 ± 3.3	59.6 ± 3.1	43.1 ± 2.7	20.1 ± 1.2
8-OH-DPAT 0.25 mg/kg ($n = 24$)	793.3 ± 47.1	67.2 ± 3.4	57.4 ± 3.8	43.8 ± 2.8	17.2 ± 1.2[a]
8-OH-DPAT 1 mg/kg ($n = 24$)	787.2 ± 32.8	64.6 ± 3.3	58.0 ± 4.1	44.5 ± 2.5	14.7 ± 0.9[c]
8-OH-DPAT 4 mg/kg ($n = 24$)	808.7 ± 35.7	73.3 ± 4.5	54.5 ± 2.8	47.0 ± 3.1	14.3 ± 0.7[c]
Controls ($n = 23$)	873.5 ± 44.9	60.3 ± 2.7	61.4 ± 5.0	62.7 ± 4.2	28.6 ± 2.3
Alnespirone 5 mg/kg ($n = 23$)	954.9 ± 57.6	67.9 ± 4.0	63.8 ± 5.9	64.6 ± 5.1	22.1 ± 2.3[a]
Alnespirone 10 mg/kg ($n = 23$)	940.9 ± 51.2	66.7 ± 3.3	61.9 ± 4.6	68.0 ± 5.4	21.0 ± 1.9[a]
Alnespirone 20 mg/kg ($n = 23$)	935.6 ± 54.3	71.3 ± 3.1	58.6 ± 3.8	67.5 ± 5.3	20.4 ± 2.2[b]
Alnespirone 40 mg/kg ($n = 23$)	977.2 ± 61.3	69.7 ± 3.0	61.3 ± 5.2	68.7 ± 5.3	21.4 ± 2.0[a]
Controls ($n = 10$)	1047.0 ± 48.3	55.0 ± 5.0	106.7 ± 12.1	82.1 ± 5.8	31.7 ± 1.9
Buspirone 5 mg/kg ($n = 10$)	812.4 ± 32.3[c]	233.0 ± 21.9[c]	297.7 ± 24.1[c]	71.7 ± 5.5	29.9 ± 2.0
Buspirone 20 mg/kg ($n = 10$)	748.3 ± 29.6[c]	255.8 ± 15.1[c]	283.3 ± 16.6[c]	70.9 ± 2.4	28.4 ± 1.3
Apomorphine 3 mg/kg ($n = 10$)	1060.4 ± 49.9	71.1 ± 0.7[a]	38.8 ± 5.7[b]	98.7 ± 7.3[a]	29.9 ± 2.0

The levels of DA, DOPAC, HVA, 5-HT and 5-HIAA, all in pmol/mg protein, were measured in the same animals as those used for the determination of in vivo [^{3}H]SCH 23390 and [^{3}H]raclopride binding.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ when compared to levels in respective controls.
n = Number of animals.

specific binding in the striatum. (+)-WAY 100135 was unable to reverse the decreased specific striatal binding of [^{3}H]SCH 23390 induced by 0.5 mg/kg 8-OH-DPAT or 5 mg/kg alnespirone. In contrast, at the same dosage, (+)-WAY 100135 was able to reverse the increased specific striatal binding of [^{3}H]raclopride (Table 1).

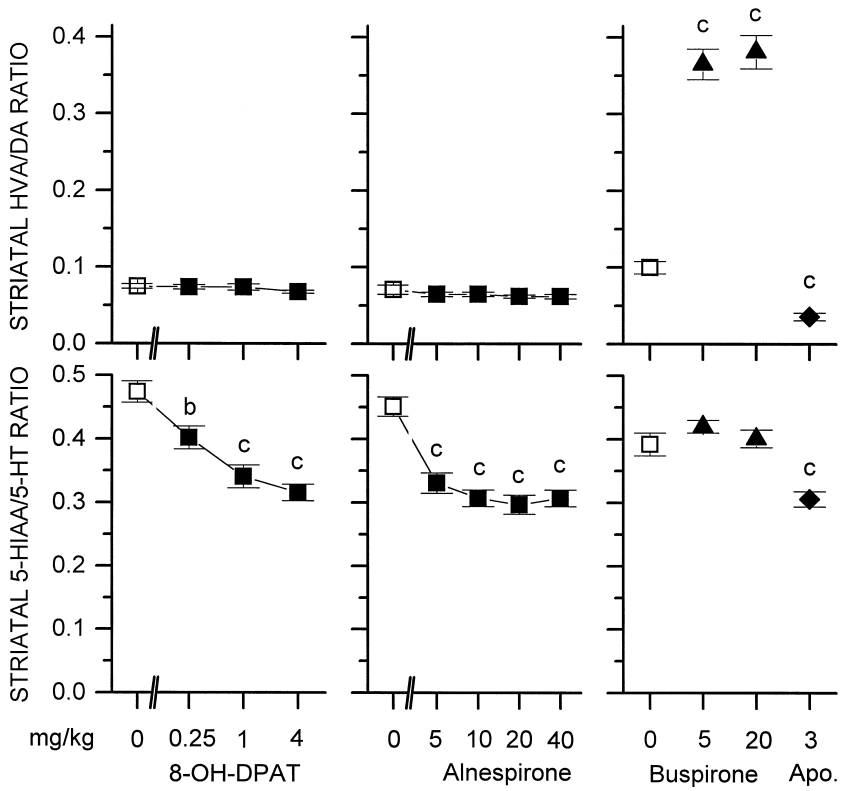

Fig. 3. Effects of 8-OH-DPAT, alnespirone, buspirone and apomorphine on the HVA/DA and 5-HIAA/5-HT ratios from mice striatum. The levels of DA, HVA, 5-HT and 5-HIAA were measured in the striatum of the same mice as those receiving i.v. the tracer dose of either [^{3}H]SCH 23390 or [^{3}H]raclopride, i.e., 40 min after the injection of increasing doses of 8-OH-DPAT, alnespirone, buspirone or apomorphine (APO). [b] $P < 0.01$; [c] $P < 0.001$ when compared to the respective control values.

3.2. Effects of increasing doses of 8-OH-DPAT, alnespirone and buspirone on striatal levels of dopamine, 5-HT and their metabolites

Since striatal levels of dopamine, DOPAC, HVA, 5-HT and 5-HIAA analysed separately for mice injected with [^{3}H]SCH 23390 or [^{3}H]raclopride were similar, the pooled data are presented in Table 2.

Increasing doses of 8-OH-DPAT and alnespirone did not modify the striatal levels of dopamine, DOPAC and HVA, and did not affect dopamine release, as evidenced by the unchanged HVA/DA ratio (Fig. 3). Furthermore, although striatal levels of 5-HT were not modified by increasing doses of 8-OH-DPAT and alnespirone, 5-HIAA levels were decreased by even the lowest tested doses of 8-OH-DPAT (0.25 mg/kg) and alnespirone (5 mg/kg). This led to decreased 5-HT turnover, as revealed by the lowered 5-HIAA/5-HT ratio (Fig. 3).

In contrast, buspirone, at the two tested doses, increased dopamine turnover by decreasing striatal dopamine levels and increasing DOPAC and HVA levels, whereas it did not significantly modify 5-HT and 5-HIAA levels.

Finally, decreased dopamine turnover, corresponding to decreased levels of DOPAC and HVA, and increased 5-HT turnover, corresponding to enhanced 5-HT levels, were measured in mice treated with 3 mg/kg apomorphine (Fig. 3).

3.3. Effects of increasing doses of 8-OH-DPAT, alnespirone and buspirone on some behaviours observed spontaneously or induced by apomorphine or SK&F 38393 in mice

Among the behaviours displayed spontaneously by mice or induced by apomorphine or SK&F 38393, apomorphine-induced sniffing was increased in mice pretreated with 4 mg/kg 8-OH-DPAT and with alnespirone at doses of 10 mg/kg and higher, whereas spontaneous sniffing and sniffing induced in mice treated with SK&F 38393 were unchanged (Fig. 4). In contrast, antagonism of apomorphine-induced sniffing was observed in mice pretreated with buspirone. Spontaneous sniffing was also decreased by buspirone at 20 mg/kg.

Spontaneous grooming as well as grooming induced by SK&F 38393 was dose dependently inhibited by increas-

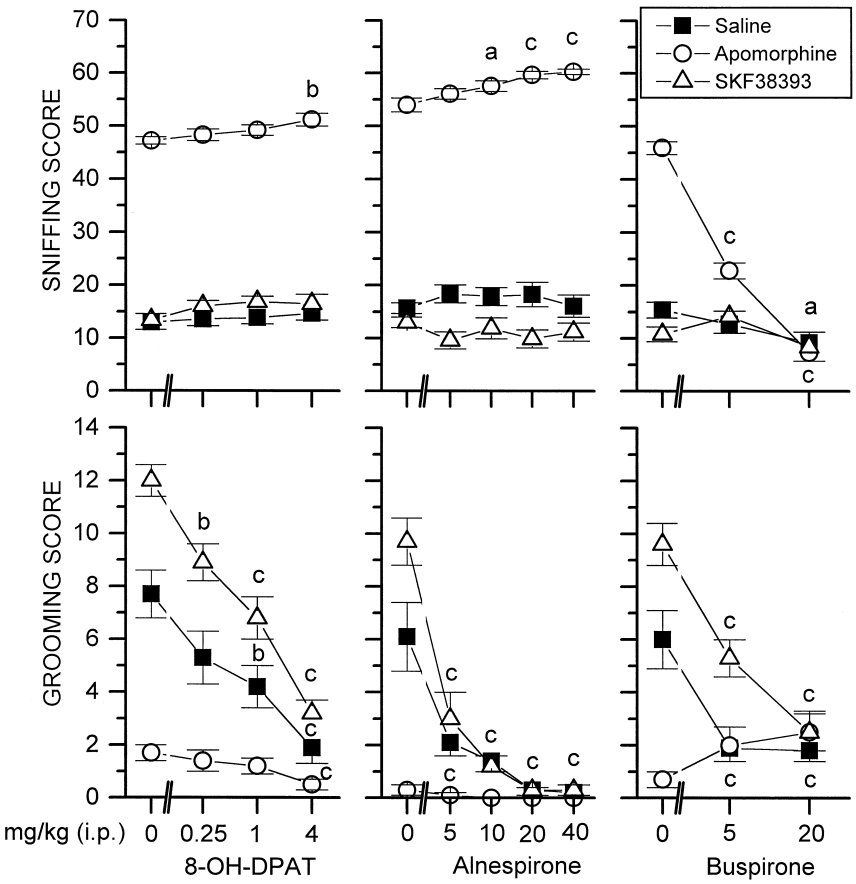

Fig. 4. Effects of 8-OH-DPAT, alnespirone and buspirone on sniffing and grooming behaviour, spontaneous or induced by apomorphine or SK&F 38393. Mice were injected i.p. with increasing doses of 8-OH-DPAT, alnespirone or buspirone 30 min before the s.c. administration of saline, apomorphine (0.75 mg/kg) or SK&F 38393 (1.87 mg/kg). The observation period started 5 min after the s.c. injection and lasted for 60 min. Means ± S.E.M. from 10–18 mice per group. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ as compared to respective mice treated with solvent instead of 8-OH-DPAT, alnespirone or buspirone.

ing doses of 8-OH-DPAT, alnespirone and buspirone. In apomorphine-treated mice, in which grooming was almost abolished, a slight reappearance of this behaviour was observed in mice treated with buspirone, but this effect was not significant (Fig. 4).

Except for antagonism of apomorphine-induced climbing by buspirone (data not shown), all other behaviours investigated in these experiments were not modified by 8-OH-DPAT, alnespirone or buspirone (data not shown).

4. Discussion

The decreased 5-HIAA levels and 5-HIAA/5-HT ratios measured under our experimental conditions in mice confirm that alnespirone displays 5-HT_{1A} receptor agonist activity, at least at somatodendritic autoreceptors, the stimulation of which is known to reduce the turnover rate of 5-HT within the projection areas of serotoninergic neurons (Hjorth and Magnusson, 1988; Hamon et al., 1988). The 5-HT_{1A} receptor agonist activity of alnespirone has already been demonstrated in rats by the displacement of [^{3}H]-8-OH-DPAT binding to postsynaptic 5-HT_{1A} receptors in the hippocampus, by the inhibition of forskolin-activated adenylate cyclase in the rat hippocampus, by the inhibition of the firing of serotoninergic neurons in the dorsal raphe nucleus, and by the decrease of 5-HT turnover in brainstem, hippocampus, striatum and cerebral cortex (Kidd et al., 1993). Furthermore, at doses ranging from 10 to 100 mg/kg (s.c.), alnespirone has been described to inhibit the in vivo binding of [^{3}H]WAY 100635, a 5-HT_{1A} receptor antagonist (Fletcher et al., 1993), in hippocampus, cerebral cortex and striatum of mice (Laporte et al., 1994). Thus, alnespirone displays the same pharmacological properties at 5-HT_{1A} receptors as 8-OH-DPAT, the reference 5-HT_{1A} receptor agonist (Hjorth et al., 1982).

In accordance with the results obtained by Dugast et al. (1998) for rats injected with alnespirone, and like 8-OH-DPAT, alnespirone did not affect the turnover rate of dopamine in the mouse striatum (Table 2, Fig. 3). These data indicate that alnespirone does not show any activity at dopamine D_2 receptors, since dopamine D_2 receptor agonists decrease, and dopamine D_2 receptor antagonists increase the turnover rate of dopamine in mouse striatum (Martres et al., 1977). These data appear difficult to reconcile with the fact that alnespirone displays moderate dopamine D_2 receptor affinity ($K_i = 20$ nM; Kidd et al., 1993). It would seem that alnespirone binds, in vitro, to dopamine D_2 receptors, but is unable, in vivo, either to stimulate these receptors or to block their stimulation.

In spite of this lack of dopaminergic effect, alnespirone decreases the in vivo binding of [^{3}H]SCH 23390, a selective dopamine D_1 receptor ligand (Billard et al., 1984; Hess et al., 1986; Andersen, 1988), and increases the in vivo binding of [^{3}H]raclopride, a selective dopamine D_2 receptor ligand (Köhler et al., 1985; Andersen, 1988), in mouse striatum. These variations were of relatively small magnitude since they never represented more than 20–25% of the specific binding of these selective tritiated ligands in the striatum. It seems unlikely that these variations could be due to changes in the permeability of the blood-brain barrier to the tritiated ligands or to changes in vascular tone induced by 8-OH-DPAT (Parsons et al., 1991) and alnespirone since they were in an opposite direction for [^{3}H]SCH 23390 and [^{3}H]raclopride. These changes in the in vivo binding of [^{3}H]SCH 23390 and [^{3}H]raclopride induced by alnespirone seem to be consequential to the strong stimulation of 5-HT_{1A} receptors, since they occurred at the same doses as those which decreased the turnover rate of 5-HT and since they were in the same direction and of roughly the same intensity as those induced by 8-OH-DPAT, the reference 5-HT_{1A} receptor agonist (Hjorth et al., 1982). This interpretation is strengthened by the fact that increased binding of [^{3}H]raclopride was also observed in olfactory bulbs of mice pretreated with 1 mg/kg 8-OH-DPAT and 5 mg/kg alnespirone. Furthermore, 8-OH-DPAT and alnespirone induced these changes in specific in vivo [^{3}H]SCH 23390 and [^{3}H]raclopride binding at doses similar to those inhibiting the in vivo binding of [^{3}H]WAY 100635 in mouse striatum (Laporte et al., 1994). This similarity of the doses suggests that the changes in [^{3}H]SCH 23390 and [^{3}H]raclopride binding are probably the consequence of the stimulation of postsynaptic 5-HT_{1A} receptors. However, this interpretation of the role of 5-HT_{1A} receptors could be disputed when one considers the results of experiments in which the effects of the simultaneous administration of (+)-WAY 100135 and 8-OH-DPAT or alnespirone were assessed on the in vivo binding of [^{3}H]SCH 23390 and [^{3}H]raclopride in mouse striatum. In these experiments, whereas (+)-WAY 100135 antagonized 8-OH-DPAT- and alnespirone-induced increases in the specific binding of [^{3}H]raclopride, it was unable to reverse 8-OH-DPAT- and alnespirone-induced decreases in the specific binding of [^{3}H]SCH 23390. This weak efficacy of (+)-WAY 100135 to antagonize 8-OH-DPAT- and alnespirone-induced changes in specific binding might be due to the relatively low magnitude of the observed effects, to a strong stimulation of 5-HT_{1A} receptors by 8-OH-DPAT and alnespirone or to the only moderate efficacy of (+)-WAY 100135 as a 5-HT_{1A} receptor antagonist (Assié and Koek, 1996).

Functional consequences of the changes in the specific in vivo binding of selective tritiated ligands at striatal dopamine D_1 and D_2 receptors appeared to be detectable when we studied the behaviour induced by apomorphine, the reference dopamine receptor agonist (Ernst, 1967), and by SK&F 38393, the reference dopamine D_1 receptor agonist (Setler et al., 1978), of mice pretreated with increasing doses of 8-OH-DPAT or alnespirone. In fact, the decrease in the grooming score observed in mice pretreated with 8-OH-DPAT or alnespirone is in agreement with the reduced in vivo binding of [^{3}H]SCH 23390, since

grooming results from the stimulation of dopamine D_1 receptors (Vasse and Protais, 1988). Similarly, the increase in the sniffing score observed in mice pretreated by 8-OH-DPAT and alnespirone agrees with the increased in vivo binding of [^{3}H]raclopride, since sniffing results preferentially from the stimulation of dopamine D_2 receptors (Vasse et al., 1988). Such opposite changes in grooming and sniffing have already been observed in mice pretreated with 8-OH-DPAT (Protais et al., 1994). Our data do not allow us to determine whether the observed effects on the specific striatal binding of [^{3}H]SCH 23390 and [^{3}H]raclopride and on the behaviours induced by apomorphine and SK&F 38393 are the consequences of a direct interaction between striatal 5-HT_{1A} receptors and dopamine receptors or of the involvement of striatal interneurons or of the involvement of large neuronal pathways ending in the striatum. It could be of interest to study the interaction of a 5-HT_{1A} receptor antagonist such as WAY 100635 (Laporte et al., 1994) with 8-OH-DPAT and alnespirone on the changes in the behavioural effects described.

From our study, 8-OH-DPAT and alnespirone appear to act very differently from buspirone. Indeed, in agreement with previous work (Riblet et al., 1982; Wood et al., 1983; McMillen et al., 1983; McMillen, 1985), buspirone displayed, at the tested doses, marked effects on dopaminergic transmission (inhibition of the specific binding of [^{3}H]raclopride, enhancement of striatal DOPAC and HVA levels, antagonism of apomorphine-induced sniffing and climbing) which were able to mask its effects on 5-HT neurons (lack of change of the striatal 5-HIAA/5-HT ratio).

In conclusion, under our experimental conditions and in agreement with the results of Dugast et al. (1998), alnespirone seems to be devoid of direct activity on dopaminergic transmission, in spite of its in vitro dopamine D_2 receptor affinity. However, it appears that its 5-HT_{1A} receptor agonist activity, similar to that of 8-OH-DPAT, has repercussions on the apparent accessibility and functioning of dopamine D_1 and D_2 receptors.

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References

Andersen, P.H., 1988. Comparison of the pharmacological characteristics of [^{3}H]raclopride and [^{3}H]SCH 23390 to dopamine receptors in vivo in mouse brain. Eur. J. Pharmacol. 146, 113–120.

Assié, M.-B., Koek, W., 1996. Effects of 5-HT_{1A} receptor antagonists on hippocampal 5-HT levels: (*S*)-WAY 100135, but not WAY 100635, has partial agonist properties. Eur. J. Pharmacol. 304, 15–21.

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.

Billard, W., Ruperto, V., Crosby, Y., Iorio, L.C., Barnett, A., 1984. Characterization of the binding of ^{3}H-SCH 23390, a selective D_1 receptor agonist ligand, in rat striatum. Life Sci. 35, 1885–1893.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem. 72, 248–250.

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 receptor agonist alnespirone (S 20499) affecting dopaminergic systems in the rat brain? Eur. J. Pharmacol., in press.

Ernst, A., 1967. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. Psychopharmacologia 48, 7–10.

Fletcher, A., Bill, D.J., Bill, S.J., Cliffe, I.A., Dover, G.M., Fortser, E.A., Hskins, J.T., Jones, D., Mansell, H.L., Reilly, Y., 1993. WAY 100135: a novel, selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. Eur. J. Pharmacol. 237, 283–291.

Gudelsky, G.A., Koenig, J.L., Meltzer, H.Y., 1986. Thermoregulatory responses to serotonin (5-HT) receptor stimulation in the rat. Neuropharmacology 25, 1307–1313.

Hamon, M., Fattaccini, C.M., Adrien, J., Gallissot, M.C., Martin, P., Gozlan, H., 1988. Alterations of central 5-HT 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.

Hess, E.J., Battaglia, G., Norman, A.B., Iorio, L.C., Creese, I., 1986. Guanine nucleotide regulation of agonist interaction at [^{3}H]SCH 23390-labelled D_1 dopamine receptors in rat striatum. Eur. J. Pharmacol. 121, 31–39.

Hjorth, S., 1991. Single-dose of 8-OH-DPAT pretreatment does not induce tachyphylaxis to the 5-HT release-reducing effect of 5-HT_{1A} autoreceptor agonists. Eur. J. Pharmacol. 199, 237–242.

Hjorth, S., Magnusson, T., 1988. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 338, 463–471.

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

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-HT_{1A} receptors. J. Pharmacol. Exp. Ther. 264, 863–872.

Köhler, C., Hall, H., Ögren, S.E., Gawell, L., 1985. Specific in vitro and in vivo binding of [^{3}H]raclopride. Biochem. Pharmacol. 34, 2251–2259.

Kreiss, D.S., Lucki, I., 1992. Desensitization of 5-HT_{1A} autoreceptors by chronic administration of 8-OH-DPAT. Neuropharmacology 31, 1073–1078.

Laporte, A.-M., Lima, L., Gozlan, H., Hamon, M., 1994. Selective in vivo labelling of brain 5-HT1A receptors by [^{3}H]WAY 100635 in the mouse. Eur. J. Pharmacol. 271, 505–514.

Levy, A.D., Li, Q., Cabrera, T.M., Gustafson, M., Van de Kar, L.D., 1995. Neuroendocrine profile of S 20499, a potential anxiolytic drug with serotonin 5-HT_{1A} agonist actions. Eur. J. Pharmacol. 274, 141–149.

Marçais, H., Protais, P., Costentin, J., Schwartz, J.C., 1978. A gradual score to evaluate the climbing behaviour elicited by apomorphine in mice. Psychopharmacology 56, 233–234.

Martres, M.-P., Costentin, J., Baudry, M., Marçais, H., Protais, P.,

Schwartz, J.-C., 1977. Long term changes in the sensitivity of pre- and postsynaptic dopamine receptors in mouse striatum evidenced by behavioural and biochemical approaches. Brain Res. 136, 319–337.

McMillen, B.A., 1985. Comparative chronic effects of buspirone or neuroleptics on rat brain dopaminergic neurotransmission. J. Neural Transm. 64, 1–12.

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.

Nomikos, G.G., Arborelius, L., Svensson, T.H., 1991. The novel 5-HT_{1A} receptor antagonist (*S*)-UH-301 prevents (*R*)-8-OH-DPAT-induced decrease in interstitial concentrations of serotonin in the rat hippocampus. Eur. J. Pharmacol. 216, 373–380.

Parsons, A.A., Motevalian, M., Whalley, E.T., 1991. Contractile effects of 8-OH-hydroxy-2-(di-*n*-propylamino)tetralin and flesinoxan in human isolated basilar artery. Eur. J. Pharmacol. 202, 17–23.

Protais, P., Chagraoui, A., Arbaoui, J., Mocaër, E., 1994. Dopamine receptor antagonist properties of S 14506, 8-OH-DPAT, raclopride and clozapine in rodents. Eur. J. Pharmacol. 271, 167–177.

Protais, P., Windsor, M., Mocaër, E., Comoy, E., 1995. Post-synaptic 5-HT_{1A} involvement in yawning and penile erections induced by apomorphine, physostigmine and mCPP in rats. Psychopharmacology 120, 376–383.

Riblet, L.A., Taylor, D.P., Eison, M.S., Stanton, H.C., 1982. Pharmacology and neurochemistry of buspirone. J. Clin. Psychiatry 43, 11–16.

Routledge, C., Gurling, J., Wright, I.K., Dourish, C.T., 1993. Neurochemical profile of the selective and silent 5-HT_{1A} receptor antagonist WAY 100135: an in vivo microdialysis study. Eur. J. Pharmacol. 239, 195–202.

Setler, P.E., Sarau, H.M., Zirckle, C.I., Saunders, H.J., 1978. The central effects of a novel dopamine agonist. Eur. J. Pharmacol. 50, 419–430.

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, 47–51.

Traber, J., Glaser, T., 1987. The 5-HT_{1A} related anxiolytics. Trends Pharmacol. Sci. 8, 432–435.

Vasse, M., Protais, P., 1988. Increased grooming behaviour is induced by apomorphine in mice treated with discriminant benzamide derivatives. Eur. J. Pharmacol. 16, 1–11.

Vasse, M., Chagraoui, A., Protais, P., 1988. Climbing and stereotyped behaviours in mice require the stimulation of D_1 dopamine receptors. Eur. J. Pharmacol. 148, 221–229.

Wood, P.L., Nair, N.P.V., Lal, S., Etienne, P., 1983. Buspirone: a potential atypical neuroleptic. Life Sci. 33, 269–273.