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Involvement of presynaptic 5-HT_{1A} and benzodiazepine receptors in the anticonflict activity of 5-HT_{1A} receptor antagonists

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Abstract

In the present paper, we examined the effect of lesions of 5-hydroxytryptamine (5-HT) neurons, produced by p-chloroamphetamine (*p*-CA; 2×10 mg/kg), and the influence of flumazenil (Ro 15-1788, 10 mg/kg), a benzodiazepine receptor antagonist, on the anticonflict activity of *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635) and *trans*-1-(2-methoxyphenyl)-4-[4-succinimidocyclohexyl]piperazine (MP 349), pre- and postsynaptic 5-HT_{1A} receptor antagonists, and 1-(2-methoxyphenyl)-4-(4-succinimidobutyl)piperazine (MM 77), a postsynaptic 5-HT_{1A} receptor antagonist, in the Vogel conflict drinking test in rats. Diazepam was used as a reference compound. WAY 100635 (0.5–1 mg/kg), MP 349 (0.25–0.5 mg/kg), MM 77 (0.03–0.25 mg/kg) and diazepam (2.5–5 mg/kg) significantly increased the number of shocks accepted during experimental sessions in the conflict drinking test. In *p*-chloroamphetamine-pretreated rats, neither WAY 100635 (1 mg/kg) nor MP 349 (0.25 mg/kg) induced an anticonflict effect, whereas MM 77 (0.06 mg/kg) did produce it. Flumazenil fully blocked the anticonflict effects of WAY 100635 (1 mg/kg) and diazepam (5 mg/kg), and it partly but significantly reduced the anticonflict effects of MP 349 (0.25 mg/kg) and MM 77 (0.06 mg/kg). *p*-Chloroamphetamine and flumazenil alone were inactive in the conflict drinking test. The present results suggest that, first, the anticonflict effect of the 5-HT_{1A} receptor antagonists, WAY 100635 and MP 349, but not MM 77, is linked to the presynaptically located 5-HT_{1A} receptors, and second, benzodiazepine receptors are indirectly involved in such effects of WAY 100635, MP 349 and MM 77, due maybe to a possible interaction between the 5-HT and the γ-aminobutyric acid (GABA)/benzodiazepine systems.

Keywords: 5-HT_{1A} receptor antagonist; *p*-Chloroamphetamine; Flumazenil; Conflict drinking test; (Rat)

1. Introduction

It is well established that the central serotoninergic (5-hydroxytryptamine, 5-HT) system has been involved in the pathophysiology and treatment of anxiety (see Barnes and Sharp, 1999). Despite long-lasting intense research, the precise role of 5-HT_{1A} receptors in the mechanism of action of ligands of these receptors as novel or potential anxiolytic drugs has not been fully elucidated. The anxiolytic-like effect produced by 5-HT_{1A} receptor partial agonists, e.g., buspirone and homologous compounds, seems to be related to the stimulation of 5-HT_{1A} receptors, because it was blocked by 5-HT_{1A} receptor ligands with a predominantly antagonistic action (e.g., 1-(2-methoxyphenyl)-4-[4-(2-phtalimido)butyl]-piperazine, NAN-190), or by a silent 5-HT_{1A} receptor antagonist (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide, WAY 100635) (Cervo et al., 2000a,b; Chojnacka-Wójcik and Przegaliński, 1991; Hascoët et al., 1994; Tsui et al., 2001). On the other hand, the relative contribution of pre- and/or postsynaptic 5-HT_{1A} receptors in the anxiolytic effects of 5-HT_{1A} receptor agonists has not been explicitly determined (for reviews, see De Vry, 1995; Handley, 1995; López-Rubalcava, 1996). It can be inferred from all these findings that 5-HT_{1A} receptor partial agonists exert their anxiolytic effects upon stimulation of somatodendric and/or postsynaptic receptors. However, regarding the anxiolytic-like effects of 5-HT_{1A} receptor agonists observed after their local administration into brain regions rich in postsynaptic 5-HT_{1A} receptors, a possibility of ventricular diffusion from the injection site to other structures, including the dorsal raphe nucleus, should also be considered (Jolas et al., 1995; Schreiber and De Vry, 1993). Furthermore, it has also been hypothesized that the anxiolytic effects of 5-HT_{1A} agonists stem from their interaction with postsynaptic 5-HT_{1A} recep-

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tors involved in the postulated negative feedback loop to the dorsal raphe nuclei (Blier and De Montigny, 1987; Ceci et al., 1994); however, the findings presented by Jolas et al. (1995) do not corroborate the latter hypothesis.

Recently, it has been proposed that 5-HT_{1A} receptor antagonists may exert beneficial effects in anxiety (see Levine and Potter, 1999; Schechter and Kelly, 1997). Several authors have shown that 5-HT_{1A} receptor antagonists, including the silent antagonist WAY 100635, exert anxiolytic-like activity in rats and mice (Cao and Rodgers, 1997, 1998; Fletcher et al., 1996; Griebel et al., 1999, 2000; Joordens et al., 1998; Moreau et al., 1992; Rodgers and Cole, 1994; Sanchez, 1996). Our recent findings also have revealed remarkably consistent, anxiolytic-like effects of a novel pre- and postsynaptic 5-HT_{1A} receptor antagonist *trans*-1-(2-methoxy-phenyl)-4-[4-succinimidocyclohexyl]-piperazine (MP 349) in a conflict drinking test in rats, a plus-maze test in rats and four-plate test in mice (Paluchowska et al., 2002; Wesołowska et al., 2003). Compound MP 349 is a conformationally restricted analog of 1-(2-methoxyphenyl)-4-[(4-succinimido)-butyl]piperazine (MM 77), a postsynaptic 5-HT_{1A} receptor antagonist (Mokrosz et al., 1994), whose potential anxiolytic activity in rats was described by Griebel et al., (1999, 2000). Moreover, the anxiolytic-like effect of WAY 100635 in mice was observed after its microinfusions into the median raphe nucleus (Canto-de-Souza et al., 2002) and ventral hippocampus (Nunes-de-Souza et al., 2002). The latter findings suggest that pre- and postsynaptic 5-HT_{1A} receptors may play some role in the anxiolytic-like effect of WAY 100635 in the plus-maze test in mice. On the other hand, a number of studies with 5-HT_{1A} receptor antagonists reported lack of anxiolytic-like effects in animals (Cervo and Samanin, 1995; Charrier et al., 1994; Collinson and Dawson, 1997; File et al., 1996; Kennett et al., 1998; King et al., 1997; Moreau et al., 1992; Przegaliński et al., 1995).

Irrespective of difficulties in establishing the neuroanatomical mechanism of anxiolytic effects of 5-HT_{1A} ligands, it is noteworthy that 5-HT_{1A} receptors appear to function at sites that can remotely modulate the effect of other neurotransmitters, such as, e.g., γ-aminobutyric acid (GABA). A possible interaction between the GABA/benzodiazepine and the 5-HT systems has been indicated by several anatomical (e.g., Bowery et al., 1987; Nanopoulos et al., 1982), neurophysiological (e.g., Celada et al., 2001, 2002; Gervasoni et al., 2000; Varga et al., 2001) and biochemical data (e.g., Bagdy et al., 2000; Nishikawa and Scatton, 1985; Söderpalm et al., 1997). Moreover, the results reported by López-Rubalcava et al. (1992) and Fernández-Guasti and López-Rubalcava (1998) showed that the anxiolytic-like effect of a 5-HT_{1A} receptor agonist, or 5-HT_{1A} receptor partial agonists in mice, was inhibited by the benzodiazepine receptor antagonist flumazenil (Ro 15-1788), which suggests that the $GABA_A$/benzodiazepine complex may be indirectly involved in the anxiolytic-like effect of 5-HT_{1A} receptor ligands.

In the present study, we examined the anxiolytic-like effect of 5-HT_{1A} receptor antagonists WAY 100635 (Assié and Koek 1996; Fletcher et al., 1996), MP 349 (Paluchowska et al., 2002; Wesołowska et al., 2003) and MM 77 (Mokrosz et al., 1994) in the punished drinking test (Vogel test) in rats with the lesions of central 5-HT neurons induced by *p*-chloroamphetamine (*p*-CA). Moreover, the effect of the benzodiazepine receptor antagonist flumazenil on the anticonflict activity of those 5-HT_{1A} receptor ligands in rats was also studied.

The tested compounds WAY 100635 and MP 349 display high affinity for 5-HT_{1A} receptors (IC_{50}=1.35 nM and K_i=15 nM, respectively), but only low to moderate affinities for 5-HT_{2A}, dopamine D1 or D2, benzodiazepine receptors and α_1-adrenoceptors, and they have demonstrated antagonistic activity at both pre- and postsynaptic 5-HT_{1A} receptor models (Assié and Koek 1996; Fletcher et al., 1996; Paluchowska et al., 2002; Wesołowska et al., 2003). Unlike these compounds, MM 77 is a potent ligand of 5-HT_{1A} receptors and α_1-adrenoceptors (K_i=6 and 12 nM, respectively) and showed features of postsynaptic 5-HT_{1A} receptor antagonist (Mokrosz et al., 1994), while its potential presynaptic 5-HT_{1A} receptor activity has not been established (Mokrosz et al., 1994; Wesołowska et al., 2002).

2. Methods

2.1. Animals and housing

The experiments were carried out on male Wistar rats (220–250 g). The animals were kept at an ambient temperature of 20±1 °C and had free access to food (standard laboratory pellets) and water before the experiments. Each experimental group consisted of 6–10 naive animals per drug dose. All the experiments were conducted in the light phase on a natural light/dark cycle (November to May), between 9 AM and 2 PM. The animals were used only once in each test. All the injections were given in a volume of 2 ml/kg. The experiments were performed by an observer blind to the treatment. All the experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland.

2.2. Drugs

Flumazenil (Hoffman-La Roche, Basel, Switzerland), *p*-chloroamphetamine hydrochloride (Sigma-Aldrich, Poznań, Poland) and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635; synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences) were used as aqueous solutions. MM 77 and MP 349 (both being synthesized by Dr. M.H. Paluchowska, Institute of Pharmacology, Polish Academy of Sciences) and diazepam (PZF Polfa, Poznań, Poland) were suspended in a 1% aqueous solution of

Table 1
Effects of WAY 100635, MP 349, MM 77 and diazepam in the punished drinking conflict test in rats[a]

Treatment and dose (mg/kg)		*n*	Number of accepted shocks/5 min (mean±S.E.M.)
Vehicle	0	7	7.3±1.1
WAY 100635	0.1	7	15.9±4.8
	0.5	7	26.1±6.7*
	1	8	37.5±5.7**
	2	8	12.7±3.7
			$F(4,32)=7.021$
			$P<0.001$
Vehicle	0	7	10.7±1.5
MP 349	0.125	7	17.3±2.9
	0.25	8	34.6±7.5**
	0.5	8	28.0±3.7*
			$F(3,26)=4.404$
			$P<0.05$
Vehicle	0	7	6.9±0.9
MM 77	0.01	7	13.3±4.4
	0.03	9	18.6±5.1*
	0.06	7	42.0±7.9**
	0.125	8	58.5±3.5**
	0.25	8	45.5±5.6**
			$F(5,40)=27.980$
			$P<0.001$
Vehicle	0	8	7.7±1.1
Diazepam	2.5	7	23.5±3.0*
	5	8	40.4±5.1**
			$F(2,20)=41.708$
			$P<0.001$

[a] WAY 100635 and diazepam were injected 30 min, while MP 349 and MM 77 60 min, before the test.
* $P<0.05$ vs. respective vehicle.
** $P<0.01$ vs. respective vehicle.

Tween 80. Flumazenil, *p*-chloroamphetamine, MM 77, MP 349 and diazepam were given intraperitoneally (i.p.), WAY 100635 was injected subcutaneously (s.c.). WAY 100635, flumazenil and diazepam were injected 30 min before the test, while MP 349 and MM 77 were injected 60 min before the test. *p*-Chloroamphetamine was given 9 and 8 days before the test.

2.3. Conflict drinking test (Vogel test)

A modification of the method of Vogel et al. (1971), described below, was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a Plexiglas box (27×27×50 cm), equipped with a grid floor of stainless-steel bars and a drinking bottle containing tap water. After the adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for another 10-min adaptation period, during which they had free access to the drinking bottle. Afterwards, they were allowed a 30-min free-drinking session in their home cage. After another 24-h water deprivation period, the rats were placed again in the test chamber and were allowed to drink for 30 s. Immediately afterwards, their drinking attempts were punished with an electric shock (0.5 mA). The impulses were released every 2 s (timed from the moment when a preceding shock was delivered) between the grid floor and the spout of the drinking bottle. Each shock lasted 1 s, and if a rat was drinking when an impulse was released, it received a shock. The number of shocks accepted throughout a 5-min experimental session was recorded.

2.4. Biochemical determinations

5-Hydroxytryptamine and 5-hydroxyindoleacetic acid (5-HIAA) were determined in the hippocampus by the high-pressure liquid chromatography (HPLC) method as described previously (Przegaliński et al., 1990).

2.5. Statistical analysis

All the data are expressed as the mean±S.E.M.; they were evaluated by a one-way or two-way analysis of variance (ANOVA), followed by Dunnett's test (when only one drug was given), or by the Newman–Keuls test (when two drugs were used).

3. Results

As shown in Table 1, WAY 100635 (0.5–1 mg/kg), MP 349 (0.25–0.5 mg/kg) and MM 77 (0.03–0.25 mg/kg)

Table 2
Effect of *p*-CA on the anticonflict action of WAY 100635, MP 349 and MM 77 in the punished drinking conflict test in rats[a]

Treatment and dose (mg/kg)	*n*	Number of accepted shocks/5 min (mean±S.E.M.)
Vehicle+vehicle	7	8.3±1.2
p-CA+vehicle	7	12.1±1.5
vehicle+WAY 100635 (1)	10	28.1±2.7*
p-CA+WAY 100635 (1)	9	10.7±1.8**
		$F(1,29)=23.815$
		$P<0.001$
Vehicle+vehicle	7	8.1±1.1
Vehicle+MP 349 (0.25)	7	31.7±4.6*
p-CA+MP 349 (0.25)	8	8.1±3.7***
		$F(1,25)=31.296$
		$P<0.001$
Vehicle+vehicle	7	8.1±1.5
Vehicle+MM 77 (0.06)	8	30.0±3.3*
p-CA+MM 77 (0.06)	6	36.0±5.4*
		$F(1,24)=1.443$
		n.s.

p-CA (2×10 mg/kg) was given 9 and 8 days before the test. WAY 100635 was injected 30 min, while MP 349 and MM 77 60 min, before the test.
* $P<0.01$ vs. respective vehicle+vehicle.
** $P<0.01$ vs. vehicle+WAY 100635.
*** $P<0.01$ vs. vehicle+MP 349.

significantly increased the number of shocks accepted in the conflict drinking test; however, their dose–response curves were bell-shaped, and the maximum effects (an increase by +414, +223 and +748%, respectively) were observed after administration of WAY 100635 (1 mg/kg), MP 349 (0.25 mg/kg) and MM 77 (0.125 mg/kg). Diazepam (2.5–5 mg/kg), used as a reference drug, produced a dose dependent anticonflict effect; it significantly increased (+205 and +425%, respectively) the number of the shocks accepted. The depletion of 5-HT with *p*-chloroamphetamine (2×10 mg/kg) abolished the anticonflict action of WAY 100635 (1 mg/kg) and MP 349 (0.25 mg/kg) but did not modify the effect of MM 77 (0.06 mg/kg) (Table 2). *p*-Chloroamphetamine (2×10 mg/kg) given alone did not affect the punished responding of rats (Table 2).

p-Chloroamphetamine (2×10 mg/kg) reduced the hippocampal concentrations of 5-HT and 5-HIAA by about 84% and 87%, respectively (absolute values: controls, 450±36 and 310±14 ng/g, respectively; *p*-chloroamphetamine-treated: 72±7 and 40±4 ng/g, respectively, $P<0.01$; $N=7$ rats per group).

The benzodiazepine receptor antagonist flumazenil (10 mg/kg) significantly reduced the anticonflict activity of WAY 100635 (1 mg/kg), MP 349 (0.25 mg/kg) and MM 77 (0.06 mg/kg) by 79%, 73% and 64%, respectively; the effect of diazepam (5 mg/kg) was completely abolished by flumazenil (10 mg/kg) (Table 3). Flumazenil (10 mg/kg) given alone did not change the punished responding of rats (Table 3).

Table 3
Effect of flumazenil on the anticonflict action of WAY 100635, MP 349, MM 77 and diazepam in the punished drinking conflict test in rats[a]

Treatment and dose (mg/kg)	*n*	Number of accepted shocks/5 min (mean±S.E.M.)
Vehicle+vehicle	7	10.1±2.3
Vehicle+flumazenil	7	8.5±0.9
WAY 100635 (1)+vehicle	7	37.1±6.3*
WAY 100635 (1)+flumazenil	8	13.5±2.3**
		$F(1,24)=9.665$
		$P<0.01$
Vehicle+vehicle	7	9.0±1.4
MP 349 (0.25)+vehicle	7	36.6±4.8*
MP 349 (0.25)+flumazenil	7	16.4±3.2***
		$F(1,24)=9.357$
		$P<0.01$
Vehicle+vehicle	7	8.4±1.5
MM 77 (0.06)+vehicle	9	35.8±5.1*
MM 77 (0.06)+flumazenil	7	18.3±3.9****
		$F(1,25)=3.014$
		n.s.
Vehicle+vehicle	8	7.7±1.1
Diazepam (5)+vehicle	8	40.4±5.1*
Diazepam (5)+flumazenil	8	8.5±1.8*****
		$F(1,27)=81.233$
		$P<0.001$

WAY 100635 and diazepam were injected at 2 min, while MP 349 and MM 77 30 min prior to flumazenil (10 mg/kg); flumazenil was given 30 min before test.

* $P<0.01$ vs. respective vehicle+vehicle.

** $P<0.01$ vs. WAY 100635+vehicle.

*** $P<0.01$ vs. MP 349.

**** $P<0.01$ vs. MM 77+vehicle.

***** $P<0.01$ vs. diazepam+vehicle.

4. Discussion

In agreement with some earlier data (Griebel et al., 2000; Wesołowska et al., 2003), the 5-HT_{1A} antagonists WAY 100635, MP 349 and MM 77 induced a clear-cut anticonflict effect, as evaluated by an increase in the number of shocks accepted in the punished drinking test in rats. In the present experiment and that of Griebel et al. (2000), the anticonflict effects of WAY 100635 and MP 349 were observed in the range of doses that inhibited the 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)-induced reduction of 5-HT release in terminal fields of 5-HT neurones (Assié and Koek, 1996, 2000; Wesołowska et al., 2003). However, these doses were higher than those that blocked other in vivo functional effects of 8-OH-DPAT such as, e.g., the behavioral syndrome and lower lip retraction in rats, or the hypothermia in mice (Fletcher et al., 1996; Paluchowska et al., 2002). Although WAY 100635 exhibits only moderate affinity for α_1-adrenoceptors (Fletcher et al., 1996), its main metabolite, shows high affinity for these binding sites in vitro (Pike et al., 1996). Thus, the behaviourally non-selective, high-dose effect of WAY 100635 may be mediated via an action at α_1-adrenoceptors. However, this α_1-adrenoceptor activity of WAY 100635 does not seem to be important to its anxiolytic-like effect, because the α_1-adrenoceptor antagonist prazosin is apparently inactive in the Vogel conflict test in rats (Chojnacka-Wójcik and Przegaliński, 1991). Compound MM 77 exhibits anticonflict activity at doses ca. 150-fold lower than those counteracting the effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT in functional tests (Mokrosz et al., 1994).

The main finding of the present study was that in rats whose 5-HT neurones were destroyed by prior administration of *p*-chloroamphetamine (which reduced hippocampal concentrations of 5-HT and 5-HIAA by ca. 85%), the anticonflict effect of WAY 100635 and MP 349, but not of MM 77, was completely abolished. These results indicate that, in contrast to WAY 100635 and MP 349, the anxiolytic-like activity of MM 77 does not seem to depend on the integrity of presynaptic 5-HT_{1A} receptors. However, while WAY 100635 (Fletcher et al., 1996) and MP 349 (Paluchowska et al., 2002; Wesołowska et al., 2003) have been classified as pre- and postsynaptic 5-HT_{1A} antagonists, the functional profile of MM 77 (a 5-HT_{1A} and α_1-adrenoceptor ligand) has not been fully established. The latter compound has been described by our team workers as a postsynaptic 5-HT_{1A} receptor antagonist (it inhibited behavioral syndrome and lower lip retraction induced in rats by 8-OH-DPAT), but

its action at presynaptic 5-HT_{1A} receptor remains controversial (Mokrosz et al., 1994). Indeed, MM 77 shared with 5-HT_{1A} receptor agonists or partial agonists, the capacity to induce hypothermia in mice, an effect claimed to be mediated through 5-HT_{1A} somatodendritic receptors (Martin and Heal, 1991). However, MM 77-induced hypothermia in mice does not seem to result from the stimulation of presynaptic 5-HT_{1A} receptors because it was not reversed by WAY 100635 (Wesłowska et al., 2002).

The present finding that anticonflict effect of WAY 100635 is mediated by presynaptic 5-HT_{1A} receptors is in line with the results reported by Canto-de-Souza et al. (2002) who found that infusions of WAY 100635 into the median raphe nucleus reduced the anxiety-like behavior of mice in the elevated plus-maze. On the other hand, it should be stressed that the same authors (Nunes-de-Souza et al., 2002) observed an anxiolytic-like effect of WAY 100635 in the mouse plus-maze test when the compound was injected locally into the ventral hippocampus. This finding suggests that the blockade of postsynaptic 5-HT_{1A} receptors may also produce antianxiety effects; it cannot be excluded that the latter mechanism accounts for the anticonflict effect of MM 77. However, such a concept of the role of postsynaptic 5-HT_{1A} receptors in anxiety is contrary to results showing that the anxiolytic-like activity of 5-HT_{1A} partial agonists and 8-OH-DPAT might result from a stimulation of 5-HT_{1A} receptors located postsynaptically (Chojnacka-Wójcik and Przegaliński, 1991; Jolas et al., 1995; López-Rubalcava, 1996; Menard and Treit, 1999; Przegaliński et al., 1992; Stefański et al., 1993). In the light of the latter data, it may be speculated that the blockade by WAY 100635 or MP 349 of presynaptic 5-HT_{1A} receptors can disinhibit 5-HT release at postsynaptic targets, and that the anxiolytic-like response may result stem from the activation of postsynaptic 5-HT_{1A} or other 5-HT receptors. Some results of electrophysiological data are consistent with such a possibility, since it was demonstrated that WAY 100635 increased serotonergic neuronal activity probably by blocking 5-HT_{1A} autoreceptors (Corradetti et al., 1996; Fornal et al., 1996; Mundey et al., 1996). Such an effect should lead to an elevated 5-HT concentration in terminal field synapses, which would consequently stimulate receptors to produce an anxiolytic-like effect. However, the majority of the published microdialysis studies do not support the above suggestion, because they have failed to observe an expected increase in extracellular 5-HT concentration following administration of 5-HT_{1A} receptor antagonists, including WAY 100635 and MP 349 (Celada et al., 2001; Gartside et al., 1995; Invernizzi et al., 1996; Romero et al., 1996; Wesołowska et al., 2003). Only a few studies have reported that selective 5-HT_{1A} receptor antagonists (WAY 100635 and *S*-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin) can enhance extracellular 5-HT release in some regions of rat brain (Gurling et al., 1994; Arborelius et al., 1996). In another in vivo study, WAY 100635 produced ear twitches in guinea pigs and the head twitches in mice, which were antagonized by 5-HT_{2A} antagonists (Darmani, 1998; Mundey et al., 1996); moreover, it was suggested that those responses produced by WAY 100635 were induced indirectly via disinhibition of the endogenous serotonergic inhibitory tone operating on the somatodendric pulse-modulating 5-HT_{1A} autoreceptors.

An intriguing question is whether the anxiolytic-like effects of 5-HT_{1A} receptor ligands, including 5-HT_{1A} receptor antagonists, depend exclusively on serotonergic mechanisms. Indeed, some data suggest possible interactions between GABA-ergic and serotoninergic systems in the mediation of anxiolytic-like effects of 5-HT_{1A} agonists or partial agonists (Fernández-Guasti and López-Rubalcava, 1998; López-Rubalcava et al., 1992). The present study demonstrated that the anxiolytic-like effects of WAY 100635, MP 349 and MM 77 were blocked or reduced by the benzodiazepine receptor antagonist flumazenil at a dose (10 mg/kg), which has been reported to antagonize various effects of diazepam and/or other benzodiazepines (Boast et al., 1983; File et al., 1985; Löscher and Hönack, 1994), including anxiolytic ones (Liliequist and Engel, 1984; present study). Because neither WAY 100635 (Fletcher et al., 1996) nor MP 349 (Wesołowska et al., 2003) nor MM 77 (Paluchowska, unpublished data) exhibits affinity for $GABA_A$ and benzodiazepine receptors, this observation suggests that their anxiolytic-like effect results from some functional interaction between the 5-HT and the GABA/benzodiazepine systems. In fact, such a conclusion seems to be supported by several neuroanatomical and functional studies. For example, the dorsal raphe nucleus, i.e., a structure rich in 5-HT_{1A} receptors, is characterized by a large density of GABA-ergic neurons (Gervasoni et al., 2000; Nanopoulos et al., 1982). Moreover, Celada et al. (2001) have recently reported that the activity of dorsal raphe nucleus 5-HT neurones is potently regulated by the prefrontal cortex, and that presynaptic and postsynaptic 5-HT_{1A} receptors, as well as GABA and glutamate ionotropic receptors, are involved in this control. Also, Bagdy et al. (2000) concluded that a reciprocal influence exists between serotonergic projection neurons and GABA-ergic interneurons or afferents in the raphe nuclei, and that this interaction may be mediated by 5-$HT_{1A/B}$ and $GABA_{A/B}$ receptors. Finally, it has also been reported that in vivo administration of an "anxiolytic" dose of 8-OH-DPAT doubled the K_d value for the in vivo binding of [^{3}H]-flunitrazepam to rat cortical membranes and enhanced the GABA-stimulated $^{36}Cl^-$ influx in rat corticohippocampal synaptoneurosomes (Söderpalm et al., 1997). However, the above-quoted data do not show how the anxiolytic-like effects of 5-HT_{1A} ligands develop as a result of an interaction between the 5-HT and GABA/benzodiazepine systems. It seems that López-Rubalcava's (1992) hypothesis about a putative indirect action of 5-HT_{1A} receptor antagonists—via an activation of the 5-HT neuro-transmission in terminal areas—on 5-HT_{1A} (or other 5-HT) receptors located postsynaptically on $GABA_A$ neurons has been corroborated by the results of the present experiment. Howewer, further studies are necessary to

explain how such an interaction may occur. Importantly, benzodiazepine receptors seem to be involved in the anxiolytic-like activity not only of 5-HT_{1A} receptor ligands (Fernández-Guasti and López-Rubalcava, 1998; López-Rubalcava et al., 1992; present study) but also of other potential non-benzodiazepine anxiolytics. Thus, the anxiolytic-like effects of *N*-methyl-D-aspartate and $glycine_B$ receptor ligands were found to be counteracted by flumazenil in the conflict drinking test (Kłodzińska and Chojnacka-Wójcik, 2000; Przegaliński et al., 2000). Because *N*-methyl-D-aspartate or $glycine_B$ ligands have no affinity for GABA/benzodiazepine receptors, the above effects have been interpreted in terms of an interaction beetwen *N*-methyl-D-aspartate and GABA/benzodiazepine systems (Kłodzińska and Chojnacka-Wójcik, 2000; Przegaliński et al., 2000).

Summing up, our results show that, first, the anxiolytic-like effects of the pre- and postsynaptic 5-HT_{1A} antagonists MP 349 and WAY 100635 (but not of the postsynaptic antagonist MM 77) are linked to the presynaptically located 5-HT_{1A} receptors, and second, further support the hypothesis that the anxiolytic-like effects of 5-HT_{1A} receptor ligands, including the 5-HT_{1A} receptor antagonists, WAY 100635, MP 349 and MM 77, may result from an indirect activation of the $GABA_A$/benzodiazepine receptor complex.

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