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Presynaptic 5-HT_{1A} receptors mediate the effect of ipsapirone on punished responding in rats

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Abstract

The effect of ipsapirone, a partial agonist at 5-HT_{1A} receptors, and of diazepam on punished operant responding was studied in rats injected intracerebroventricularly with 150 μg 5,7-dihydroxytryptamine to deplete brain serotonin or pretreated with (*S*)-WAY 100135 (*N*-tert-butyl 3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride), an antagonist at 5-HT_{1A} receptors. 5,7-Dihydroxytryptamine markedly depleted brain serotonin and caused a sustained increase in punished responding with no effect on rates of unpunished responding. Rates of punished responding returned to control values about 2 weeks after 5,7-dihydroxytryptamine. At doses ranging from 2.5 to 10 mg/kg s.c. ipsapirone significantly increased the rates of punished responding in sham-operated rats but had no effect in animals which had received 5,7-dihydroxytryptamine. At 5 and 10 mg/kg ipsapirone reduced unpunished responding similarly in sham-operated and 5,7-dihydroxytryptamine-treated rats. Diazepam 2.5 mg/kg i.p. significantly increased punished responding and reduced rates of unpunished responding similarly in sham-operated and in 5,7-dihydroxytryptamine-treated animals. At 3 and 10 mg/kg (*S*)-WAY 100135 did not modify punished or unpunished responding but at 10 mg/kg it completely antagonized the effect of 5 mg/kg s.c. ipsapirone on unpunished and punished responding. The results suggest that ipsapirone releases behaviour that is suppressed by punishment by stimulating presynaptic 5-HT_{1A} receptors.

Keywords: 5-HT_{1A} receptor; Ipsapirone; (*S*)-WAY 100135; Punishment; Conflict; Anxiolytic activity

1. Introduction

Ipsapirone, a partial agonist at central 5-HT_{1A} receptors (Traber et al., 1984), shows anxiolytic activity in various experimental animal models (Chopin and Briley, 1987) including water-lick (Higgins et al., 1988) and Geller-Seiffer (Sanger, 1992) conflict tests, and also in humans (Borison et al., 1990). The exact mechanism of the anxiolytic activity of ipsapirone is not clear.

Przegalinski et al. (1992) reported that the drug's anticonflict effect in a Vogel-like test was not modified by *p*-chloroamphetamine, which depleted brain serotonin (5-HT), but was blocked by NAN-190, a 5-HT_{1A} receptor antagonist, in animals treated with *p*-chloroamphetamine. On the basis of these findings it was suggested that the anticonflict effect of ipsapirone is mediated by stimulation of postsynaptic 5-HT_{1A} receptors.

This contrasts with various findings suggesting that stimulation of presynaptic 5-HT_{1A} receptors is involved in the anxiolytic-like activity of drugs with high affinity for these receptors. Engel et al. (1984) reported that 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, had an anticonflict effect in the Vogel drinking test but enhanced suppression when administered to rats treated with *p*-chlorophenylalanine to deplete brain 5-HT. This was interpreted as evidence that stimulation of postsynaptic 5-HT_{1A} receptors causes anxiogenic effects. The anxiolytic activity of buspirone and gepirone was abolished in rats in which 5-HT neurons were destroyed by intracerebroventricularly injected 5,7-dihydroxytryptamine (5,7-DHT) (Eison et al., 1986; Carli et al., 1989).

Anxiolytic-like effects were found on administering

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5-HT_{1A} receptor agonists and partial agonists in the raphe nuclei (Higgins et al., 1988; Carli and Samanin, 1988; Carli et al., 1989) and injection of 8-OH-DPAT into the amygdala caused anxiogenic-like effects in a model of punished operant responding (Hodges et al., 1987).

We have recently found that ipsapirone produces a dose-dependent increase in the rates of punished responding in a Geller-Seiffer model in rats (Cervo and Samanin, 1995). We therefore decided to use this model to re-examine the role of presynaptic 5-HT_{1A} receptors in the effect of ipsapirone. In one experiment, we studied the dose-related effect of ipsapirone in animals injected intracerebroventricularly with 5,7-DHT. To prove that stimulation of 5-HT_{1A} receptors was involved, in a second experiment we studied the effect of ipsapirone in animals pretreated with a novel compound, (*S*)-WAY 100135 (*N*-tert-butyl 3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride), which reportedly blocks pre- and post-synaptic 5-HT_{1A} receptors (Fletcher et al., 1993).

2. Materials and methods

Thirty-two male Spague-Dawley rats (CD-COBS, Charles River, Italy) weighing 300–325 g at the beginning of the study were used. They were housed in pairs at constant room temperature (21 ± 1°C) and relative humidity (60%), under a regular light/dark schedule (light: 7.30 a.m.–7.30 p.m.) with water available ad libitum. They received their food (15–20 g of standard laboratory chow) in the early evening, at the conclusion of each weekday and over the weekend. Animals were weighed each training day and their body weights were kept at 85% of those of free-feeding animals for the duration of the experiments.

Testing and training were done between 9.00 a.m. and 2.00 p.m.

2.1. Apparatus

Animals were tested using four standard rodent operant test chambers (Campden Instrument) constructed from heavy-duty aluminium except for the front downward opening door, which was of clear Plexiglas. The floor of the chamber was composed of 16 bars of 0.48-cm diameter stainless steel spaced 0.95 cm apart. The overall dimensions were 34 × 50.8 × 34 cm. Each rat was always exposed to the same chamber.

The chamber contained two stainless-steel levers projecting 1.6 cm from one wall, 5.5 cm from the ground, 11 cm apart. In two chambers only the right lever was presented, in the others the left. Approximately 15 g pressure was required to depress either lever and close the switch. The chamber had five lights, each 2.8 W, 24 V. One was positioned in the middle of the ceiling (the houselight), three on the front panel above the two levers and magazine, respectively, and one in the magazine itself. Reinforcement was provided by 45-mg food pellets (Noyers improved formula A), delivered from a dispenser to the magazine tray, which was accessible to the rat by pushing a hinged panel. The magazine tray was equidistant between the two levers.

The experimental chamber was contained within a sound-attenuating chamber and external noises were masked by an exhaust fan mounted on one side.

When appropriate, a scrambled electric footshock was applied across the floor bars by a Campden shock generator.

Stimulus lights, pellet dispenser and shock generator were controlled by a Paul Fray computer (Cambridge, UK) with Spider software, which also monitored input from the levers.

2.2. Procedure

Animals were trained to press the lever for food reward on a continuous reinforcement schedule (i.e. each lever press resulted in reinforcement). Once this had been mastered, reinforcement was then programmed on a variable interval (VI) 20 s schedule. Under this condition lever pressing was reinforced on average every 20 s but could occur at any time between 5 and 30 s after the last reinforced response. A three-component multiple schedule was then established as follows: (1) Unpunished responding when lever pressing was reinforced according to the VI-20 s schedule. This period was signalled by illumination of the houselight alone. Each reinforced response was also signalled by illumination of the magazine light for 0.5 s. (2) Time-out when no food was given. This period was signalled by darkness. (3) Punished responding (conflict) when lever pressing was again reinforced according to the VI-20 s schedule, but each reinforced response was punished with an electric foot-shock delivered through the grid floor. The shock level was initially set at 0.10 mA for 0.5 s and was increased daily by 0.02 mA until responding during the conflict period was significantly suppressed to less than 10% of the rate of unpunished responding. As the experiment progressed, it was necessary occasionally to raise the shock level for some animals. This period was signalled by illumination of the three lights on the front panel, one above each lever and one centrally over the magazine. Reinforcing responses were also signalled by illumination of the magazine light for 0.5 s.

The three components, each lasting 5 min, were presented twice in the same fixed order for each rat, in a daily session of 30 min/rat. Animals were extensively trained on this schedule until the following criteria

were satisfied: (a) rate of responding during the individual VI-20 s components did not differ by more than 10%; (b) rate of responding during time-out was less than 20% of the rate of responding during the unpunished period; (c) these two criteria were satisfied for 6 days.

For drug testing, only animals showing at least 3 days of stable baseline, meaning responses in individual VI-20 s components did not differ by more than 10%, were used.

2.3. Effect of intracerebral 5,7-dihydroxytryptamine on ipsapirone's effect on suppressed operant responding

Sixteen of the 32 initially trained rats were randomly divided into two groups of eight to receive 5,7-DHT or vehicle. After 4 days of recovery from surgery rats returned to daily training in the operant chambers. Shock levels were never adjusted in 5,7-DHT-treated rats and their controls. Twenty days were necessary for punished responding in 5,7-DHT-lesioned rats to meet the criteria again. At this time the ability of 2.5 mg/kg diazepam or vehicle to modify punished responding was evaluated in sham-operated and 5,7-DHT-lesioned rats. One week later the activity of 0, 2.5, 5.0 and 10.0 mg/kg ipsapirone was assessed in sham-operated and 5,7-DHT-lesioned rats. In both experiments, drugs or their vehicles were administered according to a latin-square design.

2.4. Injection of 5,7-dihydroxytryptamine

5,7-DHT creatinine sulphate (Serva Feinbiochemical, Heidelberg, Germany), 75 μg of free base dissolved in a volume of 10 μl of ascorbic acid 0.1%, or vehicle was infused bilaterally into the right and left lateral ventricles of rats anesthetized with 3 ml/kg equitesin. To protect noradrenaline-containing neurons from the action of 5,7-DHT (Baumgarten et al., 1973), 30 min before the neurotoxin the rats received 25 mg/kg i.p. desipramine, an inhibitor of noradrenaline uptake into the nerve endings (Breese and Cooper, 1975). Animal training re-started 4 days after the neurotoxin.

Twenty-four hours after completion of the behavioural studies, vehicle- and 5,7-DHT-treated rats were killed by decapitation for biochemical assays. 5-HT, noradrenaline and dopamine in the forebrain were determined by high-performance liquid chromatography with electrochemical detection according to Achilli et al. (1985).

One of eight 5,7-DHT-lesioned rats was not included in the analysis of data because brain 5-HT was depleted by less than 50% compared to a mean of 88% in the remaining rats. Dixon's test confirmed that the exclusion was statistically justified.

2.5. Effect of (S)-WAY 100135 on ipsapirone activity

Two groups of eight rats were used to evaluate the ability of 3.0 and 10.0 mg/kg (*S*)-WAY 100135 to modify the effects of 5.0 mg/kg ipsapirone. Drugs and vehicles were administered according to a latin-square design. The four test sessions for each subject were separated by at least 3 days of stable baseline. The doses of (*S*)-WAY 100135 were selected on the basis of the results of Routledge et al. (1993) and our unpublished findings that they effectively block the effect of 100 μg/kg 8-OH-DPAT on extracellular brain concentration of 5-HT.

2.6. Drugs

Ipsapirone (Troponwerke, Germany) was dissolved in 0.9% NaCl, and given subcutaneously (s.c) 30 min before testing. Diazepam (Roche, Basel, Switzerland), dissolved in a mixture of propylene glycol : ethanol : 0.9% sodium chloride (50 : 40 : 10), was given intraperitoneally (i.p.) 30 min before testing. (*S*)-WAY 100135, (*N*-tert-butyl 3-4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropanamide dihydrochloride) (Wyeth Research, Taplow, UK), sonicated in distilled water, was given s.c. 1 h before testing.

2.7. Animal care

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 February 1992) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

2.8. Statistical analysis

In all the experiments, data expressed as rate of responding (times lever pressed/min) were analyzed after square-root transformation because of non-homogeneity of variance ($P < 0.01$, Barlett's test). Student's *t*-test was used to compare rates of responding between sham-operated and 5,7-DHT-lesioned rats before i.c.v. injections.

To evaluate the effects of i.c.v. 5,7-DHT or vehicle (ascorbic acid) on punished and unpunished rates of responding, the number of times the lever was pressed/min was analyzed by two-way analysis of variance (ANOVA) for repeated measurements with i.c.v. injection (5,7-DHT or vehicle) as between-subjects factors and days (after i.c.v. injection) as within-subjects factor. Post-hoc comparisons were made with Tukey's test. To assess diazepam's and ipsapirone's effects in

sham-operated and 5,7-DHT-lesioned rats a two-way ANOVA for repeated measurements was used with i.c.v. injection (5,7-DHT or vehicle) as between-subjects factor and treatments (drug or vehicle) as within-subjects factor. Post-hoc comparisons were made with Tukey's test.

Wilcoxon's test was used to compare the rates of responding within 5,7-DHT-lesioned rats before and after testing with diazepam and ipsapirone vehicles.

The effects of (*S*)-WAY 100135, ipsapirone and their interaction were analyzed by two-way ANOVA and post-hoc comparisons with the appropriate controls by Tukey's test. 5-HT, noradrenaline and dopamine tissue levels in sham-operated and 5,7-DHT-lesioned rats were compared with Student's *t*-test.

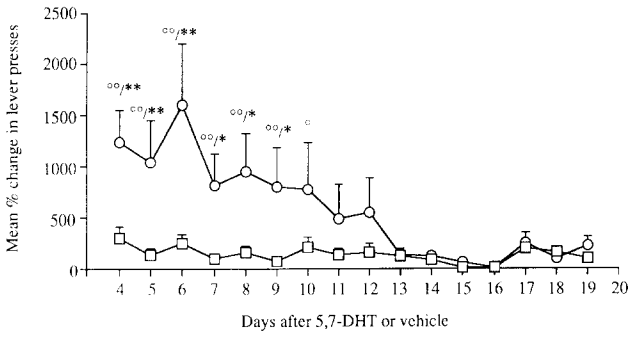

Fig. 1. Effects of 5,7-DHT lesion on punished rates of responding of rats in a modified Geller-Seifter conflict test. Mean percentage increases in lever presses per minute ± S.E.M. compared to pre-surgery baseline, for at least seven rats. (□) Sham-operated, (○) 5,7-DHT-lesioned. Data were analyzed with two-way ANOVA for repeated measurements followed by Tukey's test. See text for further details. $^{\circ}P < 0.05$ and $^{\circ\circ}P < 0.01$ vs. respective pre-surgery baseline, Tukey's test. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. respective sham-operated, Tukey's test.

3. Results

Baseline values for sham-operated and 5,7-DHT-lesioned rats before surgery were respectively: unpunished responding 36.4 ± 3.7 and 32.7 ± 2.0, time-out 5.7 ± 1.5 and 5.5 ± 1.0, conflict 1.2 ± 0.3 and 1.2 ± 0.4 ($P > 0.05$ Student's *t*-test). The rates of unpunished and time-out responding were not modified by the 5,7-DHT lesion at any time after i.c.v. injection (F(lesion) × days(16,208) = 1.0, $P > 0.05$ and F(lesion) × days(16,208) = 1.4, $P > 0.05$ for unpunished and time-out responding respectively). A significant effect was found for the rate of responding during conflict (F(lesion) × days(16,208) = 3.8, $P < 0.01$, F(lesion)(1,13) = 2.0, $P > 0.05$, F(days)(16,208) = 6.1, $P < 0.01$). Post-hoc comparisons indicated that 5,7-DHT-treated rats had a higher rate of responding from day 4 to 10 ($P < 0.01$ from day 4 to day 9 and $P < 0.05$ for day 10, Tukey's test) than at the presurgery baseline level, and from day 4 to 9 ($P < 0.01$, from day 4 to 6 and $P < 0.05$ from day 7 to 9, Tukey's test), compared to sham-operated animals (Fig. 1).

When rates of punished responding of 5,7-DHT-treated rats returned to control values, they tended again to be higher when the rats received the vehicle for diazepam or ipsapirone. These effects, however, never reached statistical significance ($P > 0.05$, Wilcoxon's test).

Table 1 shows the effects of diazepam and ipsapirone in sham-operated and 5,7-DHT-lesioned rats on unpunished responding and conflict; time-out was never modified (data not shown). Control values for the diazepam group were 42.8 ± 5.8 and 42.4 ± 2.0 for unpunished responding and 3.0 ± and 5.3 ± 1.5 for conflict in sham-operated and 5,7-DHT-lesioned rats. Control values for the ipsapirone group were 40.7 ± 6.1 and 48.2 ± 1.8 for unpunished responding and 0.7 ± 0.2 and 6.3 ± 2.7 for conflict in sham-operated and 5,7-DHT-lesioned rats. Analysis of variance indicated that 5,7-DHT did not modify the effects of 2.5 mg diazepam (F(interaction)(1,13) = 0.5, $P > 0.05$) or of 2.5–10 mg

Table 1
Effects of diazepam and ipsapirone on unpunished and punished rates of responding of sham-operated and 5,7-DHT-lesioned rats

Treatment (mg/kg)	Mean % change in lever presses			
	Unpunished responding		Punished responding	
	Sham-operated	5,7-DHT-lesioned	Sham-operated	5,7-DHT-lesioned
Diazepam				
2.5	−21.1 ± 3.1 [b]	−15.6 ± 1.0 [a]	+514.2 ± 51.1 [b]	+403.0 ± 39.3 [b]
Ipsapirone				
2.0	−14.5 ± 3.5	−22.2 ± 1.3	+442.9 ± 128.2 [a]	+63.5 ± 32.0 [c]
5.0	−31.4 ± 10.8 [b]	−34.0 ± 2.7 [b]	+714.3 ± 200.5 [b]	+38.1 ± 16.6 [c]
10.0	−37.6 ± 9.0 [b]	−53.1 ± 5.2 [b]	+871.4 ± 205.0 [b]	+12.7 ± 3.8 [c]

Mean percentage change in lever presses per minute ± S.E.M. compared with vehicle in at least seven rats. Diazepam and ipsapirone were given 30 min before testing. Data were analyzed with two-way ANOVA for repeated measurements followed by Tukey's test. See text for further details. [a] $P < 0.05$ and [b] $P < 0.01$ vs respective vehicle, Tukeys' test. [c] $P < 0.05$ two-way ANOVA for repeated measurement. See Results for further details.

ipsapirone (F(interaction)(3,39) = 1.3, $P > 0.05$) on rates of unpunished responding compared to the respective controls. Post-hoc comparisons showed that diazepam and ipsapirone similarly reduced the rates of unpunished responding in sham-operated and 5,7-DHT-lesioned rats ($P < 0.01$ for diazepam and for 5.0 and 10.0 mg ipsapirone in sham-operated, $P < 0.05$ for diazepam and $P < 0.01$ for ipsapirone 5 and 10 mg in 5,7-DHT-lesioned compared to respective vehicle, Tukey's test).

5,7-DHT did not modify the effect of diazepam on punished responses (F(interaction)(1,13) = 1.1, $P > 0.05$) but significantly reduced the effects of ipsapirone (F(interaction)(3,39) = 2.8, $P < 0.05$) (Table 1). Post-hoc comparisons indicated that diazepam increased the rate of punished responding in sham-operated and 5,7-DHT-lesioned rats ($P < 0.01$ vs. respective vehicle, Tukey's test) while ipsapirone increased the rate of punished responding in sham-operated rats at all doses ($P < 0.05$ for 2.5 mg and $P < 0.01$ for 5.0 and 10.0 mg vs. vehicle, Tukey's test), but not in 5,7-DHT-lesioned rats ($P > 0.05$ compared to vehicle, Tukey's test). Intraventricularly injected 5,7-DHT caused a very marked drop in the levels of 5-HT in the forebrain, from 370 ± 8 ng/g ± S.E.M. for sham-operated to 44 ± 3 ng/g ± S.E.M. for 5,7-DHT-lesioned rats, $P < 0.01$ Student's t-test. Catecholamines were only slightly affected (less then 10%) by 5,7-DHT.

Table 2 shows the effects of (S)-WAY 100135 on the effect of ipsapirone on unpunished and punished responses. Baseline values for rats treated with the vehicles for (S)-WAY 100135 and ipsapirone were respectively: unpunished responding 38.4 ± 3.7 and 36.8 ± 2.3, time-out 5.4 ± 1.7 and 6.5 ± 2.4, conflict 1.1 ± 0.2 and 2.0 ± 0.9 for (S)-WAY 100135 3 and 10 mg/kg. (S)-WAY 100135 dose dependently antagonized the effects of ipsapirone without affecting the rates of responding; the 10 mg dose prevented the effect of ipsapirone (F(interaction for unpunished responding)(1,28) = 5.5, $P < 0.05$; F(interaction for conflict)(1,28) = 5.3, $P < 0.05$) while 3 mg had no effect (F(interaction for unpunished responding)(1,28) = 0.5, $P > 0.05$; F(interaction for conflict)(1,28) = 1.1, $P > 0.05$).

Table 2
Effect of ipsapirone on unpunished and punished rates of responding in rats treated with (S)-WAY 100135.

Treatment	Mean % change in lever presses	
	Unpunished responding	Punished responding
(S)-WAY 3 mg + vehicle	−0.8 ± 0.1	+109.1 ± 33.2
Vehicle + ipsapirone	−34.6 ± 3.0 [b]	+509.1 ± 136.8 [b]
(S)-WAY 3 mg + ipsapirone	−25.3 ± 2.4 [d]	+418.2 ± 88.0 [d]
(S)-WAY 10 mg + vehicle	−13.0 ± 0.9	+117.6 ± 41.3
Vehicle + ipsapirone	−42.4 ± 5.6 [b]	+441.2 ± 139.1 [a]
(S)-WAY 10 mg + ipsapirone	−24.7 ± 2.1 [c]	+188.2 ± 70.6 [c]

Mean percentage change in lever presses per minute ± S.E.M. compared with vehicle in at least seven rats. (S)-WAY 100135 (3 or 10 mg/kg) and ipsapirone (5 mg/kg) or their vehicles were given respectively 60 and 30 min before testing. Data were analyzed with two-way ANOVA followed by Tukey's test. See text for further details. [a] $P < 0.05$, [b] $P < 0.01$ and vs. vehicle + vehicle, Tukey's test. [c] $P < 0.05$ two-way ANOVA. See Results for further details. [d] $P > 0.05$ two-way ANOVA. See Results for further details.

4. Discussion

5,7-DHT-treated rats showed a sustained increase in punished responding but no changes in the rates of unpunished responding. Their rates were significantly higher than those of sham-injected animals from day 4 to day 10 and gradually decreased close to control values about 2 weeks after 5,7-DHT. This agrees with previous findings that procedures reducing 5-HT transmission release responses suppressed by punishment (Stein et al., 1975; Tye et al., 1977, 1979). Adaptive mechanisms such as hypersensitivity of postsynaptic receptors (Nelson et al., 1978; Zemlan et al., 1983) may have contributed to punished responding returning to normal levels in 5,7-DHT-lesioned rats.

At doses from 2.5 to 10 mg/kg ipsapirone significantly increased the rates of punished responding in control animals. It also reduced the rates of unpunished responding at doses (5 and 10 mg/kg) that markedly reduced the locomotor activity of rats (Mittman and Geyer, 1989).

Although 5-HT_{1A} receptor agonists increase feeding (Dourish et al., 1985; Bendotti and Samanin, 1986) and may attenuate responses to nociception (Cervo et al., 1994), it is unlikely that these effects were involved in ipsapirone's ability to increase punished responding. Agonists at 5-HT_{1A} receptors increased food intake in free-feeding rats but reduced it in food-deprived animals (Bendotti and Samanin, 1987). Moreover, an enhanced motivation to eat would have increased unpunished responding whereas 5 and 10 mg/kg ipsapirone significantly reduced it. That antinociceptive activity had contributed to the effect of ipsapirone is excluded by the evidence that morphine has no effect on conflict responding (Geller et al., 1963; Tye et al., 1979).

Intracerebroventricularly injected 5,7-DHT reduced 5-HT by about 90% in rat forebrain and abolished the effect of ipsapirone on punished responding without changing the ipsapirone-induced reduction of unpunished responding rates. The results cannot be explained by a general inability of 5,7-DHT-treated rats to respond to drugs that release punished responses since the effect of diazepam was not modified.

Injection of 5,7-DHT into the nucleus raphe dorsalis to destroy 5-HT neurons attenuated the anti-punishment effect of chlorodiazepoxide injected in the same area but did not modify the effect of systemically administered diazepam (Thiébot et al., 1984). The possibility was considered that 5-HT neurons spared by this lesion, for instance those originating in the nucleus raphe medianus, might mediate the effect of diazepam on punished responding, but the fact that rats with a marked depletion of forebrain 5-HT caused by i.c.v. 5,7-DHT and their controls responded similarly to diazepam argues against this. 5,7-DHT-lesioned rats have been found to be even more sensitive to the anticonflict effect of 0.5 mg/kg diazepam than sham-operated controls (Söderpalm and Engel, 1991). Besides the marked decrease in drop of forebrain 5-HT in 5,7-DHT-treated rats, the fact that the neurotoxin impaired 5-HT transmission was confirmed by the inability of ipsapirone to increase punished responding in lesioned rats. The results suggest that 5-HT-containing neurons are necessary for the effect of ipsapirone on punished responses but not for the effect of systemic diazepam. This is not surprising since the amygdaloid nucleus appears to be a major site of the anti-punishment action of benzodiazepines (Hodges et al., 1987; Shibata et al. 1982).

The results obtained with ipsapirone apparently contrast with those of Przegalinski et al. (1992), who found no changes in the anti-conflict effect of ipsapirone in animals depleted of brain 5-HT by *p*-chloroamphetamine. The reasons for the different results are not completely clear. One explanation is that different neuronal 5-HT subsystems have different sensitivity to the neurotoxic effect of *p*-chloroamphetamine (Mamounas et al., 1991). Since 5-HT projections of the dorsal raphe nucleus are preferentially affected by *p*-chloroamphetamine (Mamounas et al., 1991) and stimulation of 5-HT_{1A} receptors in the raphe medianus also causes anti-conflict effects (Carli et al., 1989), 5-HT neurons spared by *p*-chloroamphetamine may have mediated the effect of ipsapirone in the Przegalinski et al. experiments (1992).

A role of presynaptic 5-HT_{1A} receptors in the anxiolytic effect of ipsapirone is also suggested by one recent study (Schreiber and De Vry, 1993) showing that ipsapirone administered in the dorsal raphe nucleus inhibited shock-induced ultrasonic vocalization. The same authors found a good correlation between the time course of ipsapirone's effects in the ultrasonic vocalization test, inhibition of 5-HT cell firing in the dorsal raphe and reduced 5-HT release in the hippocampus (Sommermeyer et al., 1993).

They also reported (Schreiber and De Vry, 1993) that administration of ipsapirone in the dorsal hippocampus and the amygdala inhibited ultrasonic vocalization and intra-hippocampal 8-OH-D-PAT significantly enhanced punished responding. Although relatively high drug concentrations were effective in regions rich in postsynaptic 5-HT_{1A} receptors and no selective 5-HT_{1A} receptor antagonist was used in these studies, their results suggest that, in addition to presynaptic receptors in the raphe nuclei, postsynaptic 5-HT_{1A} receptors may contribute to the anxiolytic action of ipsapirone and other 5-HT_{1A} receptor ligands in certain tests. Further work will be necessary to clarify the relative roles of pre- and post-synaptic 5-HT_{1A} receptors in the effect of 5-HT_{1A} ligands in different anxiety tests.

(*S*)-WAY 100135, at doses (3 and 10 mg/kg) reported to block pre- and post-synaptic 5-HT_{1A} receptors (Routledge et al., 1993), did not modify punished or unpunished responding, but 10 mg/kg completely blocked the effect of ipsapirone. These results, together with those obtained with 5,7-DHT, certainly suggest that the anti-conflict effect of ipsapirone, as assessed by punished operant responding, is mediated by stimulation of presynaptic 5-HT_{1A} receptors. Since stimulation of presynaptic 5-HT_{1A} receptors reduces 5-HT release in various forebrain regions (Hjorth and Sharp, 1991), the findings are compatible with the suggestion that a reduced availability of 5-HT at postsynaptic receptors releases the responses suppressed by punishment. Reduced stimulation of postsynaptic 5-HT_{1A} receptors is not involved since (*S*)-WAY 100135, a 5-HT_{1A} receptor antagonist reported to have anxiolytic-like effects in the light-dark and elevated plus-maze tests with mice (Rodgers and Cole, 1994), did not modify punished responding. A reduced availability of 5-HT at postsynaptic 5-HT_{2C} receptors may be involved in the effect of ipsapirone since blockade of these receptors appears to have anxiolytic activity in a model of punished operant responding (Kennett et al., 1994; Cervo and Samanin, submitted).

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