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5-HT_{1A} Receptor Full and Partial Agonists and 5-HT_{2C} (But Not 5-HT_3) Receptor Antagonists Increase Rates of Punished Responding in Rats

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CERVO, L. AND R. SAMANIN. *5-HT_{1A} receptor full and partial agonists and 5-HT_{2C} (but not 5-HT_3) receptor antagonists increase rates of punished responding in rats.* PHARMACOL BIOCHEM BEHAV **52**(4) 671–676, 1995.—Drugs with different intrinsic activity at 5-HT_{1A} receptors and antagonists at 5-$HT_{2A/2C}$ and 5-HT_3 receptors were studied for their ability to increase the rates of punished operant responding in rats. Like chlordiazepoxide (5 and 10 mg/kg) and diazepam (1.25 and 2.5 mg/kg), 0.125 mg/kg 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, and 5 and 10 mg/kg ipsapirone, a partial agonist at these receptors, increased the rates of punished responding, whereas (S)-WAY 100135, a 5-HT_{1A} receptor antagonist, had no effect at doses from 1 to 10 mg/kg. 8-OH-DPAT and ipsapirone, like benzodiazepines, significantly reduced unpunished responding. The 5-$HT_{2A/2C}$ receptor antagonists ritanserin (2 mg/kg), mianserin (8 mg/kg), and mesulergine (0.1 mg/kg) significantly increased the rates of punished responding, whereas 0.5–2 mg/kg ketanserin, that has higher affinity for 5-HT_{2A} than 5-HT_{2C} receptors, had no effect. Antagonists, at 5-HT_3 receptors such as ondansetron (0.001–0.1 mg/kg) and tropisetron (0.001–0.1 mg/kg), had no effect on punished or unpunished responding. The results show that agents acting as full or partial agonists at 5-HT_{1A} receptors and blockers of postsynaptic 5-HT_{2C} receptors have anxiolytic-like effects in a model of punished operant responding, whereas antagonists at 5-HT_{1A} and 5-HT_3 receptors have no such effect.

5-HT_{1A} receptors 5-HT_{2C} receptors 5-HT_3 receptors Benzodiazepines Operant conflict Punishment Anxiolytic activity

PUNISHMENT procedures are considered the methods of choice for investigating the effects of anxiolytic agents (34), but recent studies using the Geller-Seifter or Vogel-type models in rats have produced variable results with anxiolytic agents acting on serotonin (5-HT) receptors (3,4,32).

In contrast with a previous report that 8-hydroxy-(di-n-propylamino) tetralin (8-OH-DPAT), a 5-HT_{1A} receptor agonist, produced anticonflict effects in the Vogel drinking test (14) and attenuated punishment-induced suppression of operant responding in rats (23), Sanger (38) found that buspirone and ipsapirone, two partial agonists at 5-HT_{1A} receptors, but not 8-OH-DPAT, increased punished operant responding in rats. Similar results with buspirone and 8-OH-DPAT were reported in a test of conditioned suppression of drinking (6,30).

The different effects of 8-OH-DPAT and buspirone in some tests may be due to their different intrinsic activies on 5-HT_{1A} receptors: buspirone has predominantly agonist and antagonist actions respectively at presynaptic and postsynaptic 5-HT_{1A} receptors, while 8-OH-DPAT is a full agonist at both sites (25). Engel et al. (14) found that 8-OH-DPAT enhanced suppression in a Vogel-like test when administered to rats treated with parachlorophenylalanine to deplete brain 5-HT; this was interpreted as evidence that stimulation of postsynaptic 5-HT_{1A} receptors by 8-OH-DPAT causes anxiogenic effects. Moreover, 8-OH-DPAT-induced stimulation of postsynaptic 5-HT_{1A} receptors causes a motor syndrome (19,40) that might mask the disinhibitory effect of 8-OH-DPAT in some conflict procedures.

It is interesting that Sanger (38) found no effect on punished responding with MDL 73005EF, a partial 5-HT_{1A} receptor agonist that, like 8-OH-DPAT, is more effective than buspirone in inhibiting forskolin-stimulated adenylate cyclase in rat hippocampal membranes (11,17). Together with the fact that anticonflict effects have been reported on administering 8-OH-DPAT and buspirone in the raphe region (6,20), it is likely that agonism at presynaptic 5-HT_{1A} receptors and antagonism on postsynaptic sites is more effective than full agonism on both sites for increasing punished responding.

A chiral phenylpiperazine derivative (S)-WAY 100135 has recently been proposed as a selective 5-HT_{1A} receptor antago-

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nist at pre- and postsynaptic sites (16). Investigation of its effect on punished operant responding could clarify whether stimulation of 5-HT_{1A} receptors is a prerequisite for the anti-conflict effects of agents with high affinity for these sites. It has been recently reported that (S)-WAY 100135 has an anxiolytic-like effect in the murine elevated plus maze (37) but had no effect in a safety signal withdrawal conflict model (10).

In order to establish which compounds with different intrinsic activity at 5-HT_{1A} receptors are identified as anxiolytics, we studied the effects of 8-OH-DPAT, ipsapirone, and (S)-WAY 100135 in a model based on operant responding suppressed by punishment and compared their effects with diazepam and chlordiazepoxide.

It has been suggested that antagonists at 5-HT_3 receptors exert anxiolytic activity in various animal models (2), but they were ineffective in a Vogel (21) or operant (13) conflict test. Since variations in the punishment procedure may influence the effect of potential anxiolytic agents (3,38) we studied the effect of ondansetron and tropisetron, two potent 5-HT_3 receptor antagonists (5,36), to confirm that their effects differ from those of conventional anxiolytic agents in a model of punished operant responding in rats (3).

Recent evidence suggests that 5-HT_{2C} receptor antagonists have anxiolytic-like effects in the rat Geller-Seifter model (28). To get more information on how the present model identifies the anxiolytic-like activity of agents acting on 5-HT receptors, we included in the study various 5-$HT_{2A/2C}$ receptor antagonists to confirm and extend the results of Kennett et al. (28).

To our knowledge, this is the first time that the effects of the three major classes of 5-HT anxiolytics are examined on punished operant responding in the same model. Because it is not clear how valid conflict-type procedures are for the detection of 5-HT anxiolytics in rodents (3), the present study hopes to help clarify which 5-HT compounds produce effects similar to those of the benzodiazepine anxiolytics.

METHOD

Twenty-four male Sprague–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 (lights: 0730–1930 h), with water available at lib. 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 kept at 85% of free-feeding animals for the duration of the experiments. Testing and training were done between 0900 and 1400 h.

Apparatus

Animals were tested using four standard rodent operant test chambers (Campden Instrument Ltd.) constructed from heavy-duty aluminium except for the front downward-opening door, which was 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 house light), 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 the rat could reach 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, scrambled electric foot shock could be applied across the floor bars by a Campden shock generator.

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

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: a) unpunished responding when lever pressing was reinforced according to the VI-20-s schedule. This period was signalled by illumination of the house light alone. Each reinforced response was also signalled by illumination of the magazine light for 0.5 s; b) time out when no food was given. This period was signalled by darkness; c) 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. Shock level was initially set at 0.10 mA for 0.5 s and 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 had been satisfied: a) rates 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.

Following this, drug testing was started. Behavioral testing sessions were held at the same time every day, Monday through Friday. On Tuesdays and Wednesdays, the rats received an injection of vehicle, at the time of drug treatment, before behavioral testing (control session). On Thursdays, they were given an injection of the test compound at the appropriate time before testing (drug session).

Drug Treatment

After training, the animals were assigned randomly to three different experimental groups consisting of eight rats each. In

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE AND DIAZEPAM ON UNPUNISHED AND PUNISHED RATES OF RESPONDING OF RATS

Treatment	Dose (mg/kg)	Number of Lever Presses/min	
		Unpunished	Punished
Baseline		35.9 ± 4.8	0.8 ± 0.1
Chlordiazepoxide	1.0	37.2 ± 4.1	1.5 ± 0.4
Baseline		36.6 ± 3.4	0.9 ± 1.1
Chlordiazepoxide	5.0	30.0 ± 2.8	8.8 ± 1.3*
Baseline		37.6 ± 4.9	1.3 ± 0.2
Chlordiazepoxide	10.0	23.7 ± 3.2*	12.7 ± 1.7*
Baseline		40.2 ± 2.5	1.1 ± 0.1
Diazepam	1.25	37.0 ± 3.2	5.3 ± 1.9*
Baseline		32.7 ± 2.5	1.5 ± 0.2
Diazepam	2.5	21.8 ± 3.4*	9.1 ± 2.1*

Mean responses per minute (± SEM) for at least seven rats.
Chlordiazepoxide and diazepam were given 30 min before testing.
*$p < 0.015$, compared to baseline, Wilcoxon's matched pairs test.

all groups each rat received the treatments in a counterbalanced sequence.

In the first group, the effects on unpunished, punished, and time out responding were recorded after doses of 1.25 and 2.5 mg/kg IP diazepam, 0.06 and 0.125 mg/kg SC 8-OHDPAT, 1-10 mg/kg SC ipsapirone, and 1-10 mg/kg SC (S)-WAY 100135. In the second group, the effects of 1-10 mg/kg IP chlordiazepoxide, 0.001-0.1 mg/kg SC ondansetron, and 0.001-0.1 mg/kg SC tropisetron were assessed. In the third group, the effects of 0.5-2.0 mg/kg SC ketanserin, 0.5-2.0 mg/kg SC ritanserin, 4.0-16.0 mg/kg SC mianserin, and 0.03-0.3 mg/kg SC mesulergine were assessed.

Drugs

Chlordiazepoxide (Roche, Basel, Switzerland), ondansetron (Glaxo, Greenford, Middlesex, UK), ipsapirone (Troponwerke Gmbh and Co., Germany), 8-OH-DPAT (RBI, Wayland, USA), and ketanserin tartrate (R.B.I., Wayland, USA), dissolved in 0.9% NaCl, or vehicle were given 30 min before testing. Tropisetron (R.B.I., Wayland, USA), dissolved in 0.9% NaCl, or vehicle was given 1 h before testing.

Mianserin HCl (Organon, Lanarkshire, Scotland) and mesulergine (Sandoz, Basel, Switzerland), dissolved in distilled water, or vehicle were given, respectively, 30 min and 1 h before testing.

Diazepam (Roche, Basel, Switzerland), dissolved in a mixture of propylene glycol : ethanol : 0.9% sodium chloride (50 : 40 : 10), or vehicle was given 30 min before testing.

Ritanserin (Janssen, Beerse, Belgium), dissolved in distilled water containing two to five drops lactic acid and 20% propylene glycol and brought to pH 5 with 10 N NaOH, or vehicle was given 1 h before testing. (S)-WAY 100135 (Wyeth Research Ltd, Taplow, UK) sonicated in distilled water, was given 1 h before testing. All drugs' doses and appropriate vehicles were administered in a volume of 2 ml/kg.

Statistical Analysis

To assess the effects of drugs, the number of lever presses/min for each individual component (unpunished, punished, and time out periods) on the test day was compared with the mean response rate for the two immediately preceding days by a nonparametric Wilcoxon's matched pairs test. To allow for multiple comparisons, a Bonferroni's correction was used. Each rat was used as its own control.

Animal Care

Procedures involving animals and their care were conducted in conformity with our institutional guidelines, which are in compliance with national 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).

RESULTS

Chlordiazepoxide, 5 and 10 mg/kg, and diazepam, 1.25 and 2.5 mg/kg, significantly increased the rates of punished responding ($p < 0.01$ compared to respective baseline, Wilcoxon's test), while they only reduced unpunished responding at the highest dose ($p < 0.015$ and $p < 0.01$ for chlordiazepoxide 10.0 mg/kg and diazepam 2.5 mg/kg, respectively, compared to respective baseline, Wilcoxon's test) (Table 1).

The effects of drugs with different intrinsic activity at 5-HT_{1A} receptors are shown in Table 2. 8-OH-DPAT, at 0.125 mg/kg, increased the rate of punished responding ($p < 0.01$ compared to respective baseline, Wilcoxon's test) as did ipsapirone at 5 and 10 mg/kg ($p < 0.015$ and $pP < 0.01$ for 5.0 and 10.0 mg/kg, respectively, when compared to respective baseline, Wilcoxon's test). Both drugs also significantly reduced the unpunished rate of responding ($p < 0.01$ for 0.125 mg/kg 8-OH-DPAT and 10 mg/kg ipsapirone, Wilcoxon's test).

TABLE 2

EFFECTS OF 5-HT_{1A} RECEPTOR RELATED DRUGS ON UNPUNISHED AND PUNISHED RATES OF RESPONDING OF RATS

Treatment	Dose (mg/kg)	Number of Lever Presses/min	
		Unpunished	Punished
Baseline		43.4 ± 4.5	0.9 ± 0.3
8-OH-DPAT	0.062	43.2 ± 3.8	2.5 ± 1.3
Baseline		36.1 ± 2.6	2.1 ± 0.7
8-OH-DPAT	0.125	22.5 ± 2.1*	7.2 ± 1.2*
Baseline		39.8 ± 2.2	1.9 ± 0.6
Ipsapirone	1.0	39.1 ± 3.2	3.7 ± 1.3
Baseline		43.1 ± 3.3	1.5 ± 0.4
Ipsapirone	5.0	38.7 ± 3.5	6.9 ± 2.0*
Baseline		37.1 ± 3.4	0.5 ± 0.2
Ipsapirone	10.0	30.3 ± 6.0*	10.3 ± 4.4*
Baseline		41.5 ± 3.1	2.0 ± 0.4
(S)-WAY 100135	1.0	43.7 ± 3.0	2.0 ± 0.4
Baseline		39.2 ± 3.1	1.5 ± 0.4
(S)-WAY 100135	3.0	37.3 ± 3.2	1.0 ± 0.3
Baseline		45.4 ± 3.8	2.0 ± 0.6
(S)-WAY 100135	10.0	43.4 ± 4.5	2.7 ± 1.0

Mean responses per minute (± SEM) for at least seven rats.
Injection times before testing were 30 min for 8-OH-DPAT and ipsapirone and 1 h for (S)-WAY 100135.
*$p < 0.015$, compared to baseline, Wilcoxon's matched pairs test.

(S)-WAY 100135, at doses ranging from 1 to 10 mg/kg, had no effects on unpunished and punished rates of responding. The rate of responding during TO was not modified by any treatment (data not shown).

Neither ondansetron nor tropisetron at doses from 0.001 to 0.1 mg/kg affected unpunished and punished rates of responding (Table 3). Time out was also unaffected.

Table 4 shows the effects of 5-$HT_{2A/2C}$ receptor antagonists on unpunished and punished rates of responding. Ketanserin doses from 0.5 to 2 mg/kg had no effect on punished responding but the highest dose significantly reduced unpunished responding ($p < 0.01$ compared to respective baseline, Wilcoxon's test).

At 2.0 mg/kg, ritanserin slightly but significantly increased the rate of punished responding ($p < 0.015$ compared to respective baseline, Wilcoxon's test). Mianserin, at 8 mg/kg, significantly increased the rate of punished responding ($p < 0.015$ compared to respective baseline, Wilcoxon's test) with no effect on unpunished responding; 16 mg/kg only reduced the rate of unpunished responding ($p < 0.01$ compared to respective baseline, Wilcoxon's test). Mesulergine, at dose of 0.1 mg/kg, significantly increased the rate of punished responding ($p < 0.015$ vs. respective baseline, Wilcoxon's test). Only the highest dose reduced the rate of unpunished responding ($p < 0.01$ compared to respective baseline, Wilcoxon's test). None of these drugs modified the rate of responding during time out period (data not shown).

DISCUSSION

We found that 0.125 mg/kg, but not 0.0625 8-OH-DPAT, significantly increased the rates of punished responding. This dose also significantly reduced unpunished responding. This is in line with previous studies showing that subcutaneous doses of 8-OH-DPAT ranging from 0.1 to 0.5 mg/kg reduce locomotor activity and investigatory behavior in rats (6,15,31). Ipsapirone also increased punished responding and reduced unpunished rates of responding at a dose (5 mg/kg) previously reported to reduce rats' locomotor activity (31). The concomitant presence of reduced unpunished and increased punished responses is not specific for 5-HT_{1A} drugs because similar effects were found with sedative doses of benzodiazepines such as 2.5 mg/kg diazepam and 10 mg/kg chlorodiazepoxide.

Sanger (38) found that 8-OH-DPAT tended to increase punished responding, but the effect did not reach statistical significance probably because of the considerable between-animal variability. We also noticed that 8-OH-DPAT had a weak effect on punished responding in some animals, but nevertheless, the effect of 0.125 mg/kg 8-OH-DPAT on punished responding was highly significant, suggesting that our effects were more consistent than those of Sanger (38). The different route of administration, intraperitoneal in Sanger's study and subcutaneous in the present one, may account for the difference because brain concentrations of 8-OH-DPAT are much higher after SC than after IP 8-OH-DPAT (33).

The fact that 8-OH-DPAT and ipsapirone produced effects on punished responding similar to those of benzodiazepines is compatible with the suggestion that full or partial agonists at 5-HT_{1A} receptors have potential anxiolytic activity (4). Because partial agonists such as ipsapirone are believed to act as agonists at presynaptic 5-HT_{1A} receptors and as antagonists at postsynaptic sites (1,39), 8-OH-DPAT and ipsapirone very probably increase punished responding through stimulation of

TABLE 3

EFFECTS OF TWO 5-HT_3 RECEPTOR ANTAGONISTS, ONDANSETRON AND TROPISETRON, ON UNPUNISHED AND PUNISHED RATES OF RESPONDING OF RATS

Treatment	Dose (mg/kg)	Number of Lever Presses/min	
		Unpunished	Punished
Baseline		43.7 ± 3.5	1.1 ± 0.4
Ondansetron	0.001	42.0 ± 3.5	1.0 ± 0.3
Baseline		38.9 ± 0.8	1.0 ± 0.3
Ondansetron	0.01	41.4 ± 1.8	1.5 ± 0.5
Baseline		40.4 ± 1.9	2.1 ± 0.6
Ondansetron	0.1	41.2 ± 1.9	1.1 ± 0.4
Baseline		39.7 ± 2.7	2.0 ± 0.4
Tropisetron	0.001	41.2 ± 2.7	1.7 ± 0.4
Baseline		43.2 ± 2.0	1.6 ± 0.2
Tropisetron	0.01	43.3 ± 2.0	2.6 ± 0.6
Baseline		42.7 ± 3.6	1.0 ± 0.3
Tropisetron	0.1	41.0 ± 3.6	2.8 ± 1.5

Mean responses per minute (± SEM) for at least seven rats.

Ondansetron and tropisetron were given respectively 30 min and 1 h before testing. Data were analyzed by Wilcoxon's matched pairs test. See text for further details.

TABLE 4

EFFECTS OF 5-HT_2 RECEPTOR ANTAGONISTS ON UNPUNISHED AND PUNISHED RATES OF RESPONDING OF RATS

Treatment	Dose (mg/kg)	Number of Lever Presses/min	
		Unpunished	Punished
Baseline		36.0 ± 5.8	0.8 ± 0.1
Ketanserin	0.5	34.1 ± 5.6	1.0 ± 0.3
Baseline		39.8 ± 5.1	0.7 ± 0.3
Ketanserin	1.0	34.3 ± 5.0	1.5 ± 0.9
Baseline		35.0 ± 5.1	1.4 ± 0.6
Ketanserin	2.0	28.2 ± 4.3*	1.2 ± 0.4
Baseline		40.7 ± 2.6	1.5 ± 0.3
Ritanserin	0.5	38.3 ± 2.8	3.2 ± 1.4
Baseline		36.3 ± 1.8	2.7 ± 0.6
Ritanserin	1.0	35.2 ± 3.2	1.7 ± 0.3
Baseline		38.5 ± 5.4	0.9 ± 0.2
Ritanserin	2.0	35.0 ± 5.9	2.9 ± 0.6*
Baseline		40.2 ± 4.4	0.9 ± 0.2
Mianserin	4.0	36.3 ± 5.3	1.9 ± 0.6
Baseline		31.8 ± 3.3	0.5 ± 0.1
Mianserin	8.0	29.6 ± 3.3	2.1 ± 0.9*
Baseline		40.6 ± 5.0	1.1 ± 0.4
Mianserin	16.0	33.5 ± 2.7*	1.9 ± 0.8
Baseline		31.8 ± 1.9	1.0 ± 0.3
Mesulergine	0.03	31.2 ± 2.4	3.0 ± 1.0
Baseline		34.8 ± 3.6	0.9 ± 0.2
Mesulergine	0.1	34.6 ± 4.1	5.6 ± 2.1*
Baseline		33.3 ± 4.1	0.9 ± 0.1
Mesulergine	0.3	28.0 ± 4.5*	4.0 ± 1.1

Mean responses per minute (± SEM) for at least seven rats.

Injection times before testing were 30 min for ketanserin and mianserin and 1 h for ritanserin and mesulergine.

*$p < 0.015$, compared to baseline, Wilcoxon's matched pairs test.

presynaptic 5-HT_{1A} receptors. This is supported by a recent study in which we found that destruction of 5-HT neurons by intracerebroventricularly injected 5,7-dihydroxytryptamine increased punished responding from day 4 to day 10 after injection and abolished the effect of ipsapirone on punished responding when rates of punished responding had returned to control values 2 week after 5,7-dihydroxytryptamine (8). Moreover, administration of 8-OH-DPAT and ipsapirone into the dorsal raphe significantly increased punished responding in rats (6,20). The results with ipsapirone apparently contrast with those of Przegalinski et al. (35) who found no changes in the anticonflict effect of ipsapirone in animals depleted of brain 5-HT by PCA. 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 PCA (29). Because 5-HT projections of the dorsal raphe nucleus are preferentially affected by PCA (29) and stimulation of 5-HT_{1A} receptors in the raphe medianus also causes anticonflict effects (7), 5-HT neurons spared by PCA may mediate the effect of ipsapirone in the Przegalinski et al. experiments (35).

(S)-WAY 100135 blocks postsynaptic 5-HT_{1A} receptors with no effect on brain 5-HT release and causes anxiolytic-like activity in the mouse light-dark or elevated plus-maze test (37). At doses reported to block postsynaptic 5-HT_{1A} receptors (16), (S)-WAY 100135 did not modify punished or unpunished responding. This agrees with recent findings (10) that (S)-WAY 100135 failed to affect rats' responding in a safety signal withdrawal conflict model. In this study, (S)-WAY 100135 reversed the anxiolytic-like effects of a low dose of buspirone. Taken together, these findings suggest that blockade of 5-HT_{1A} receptors does not cause anxiolytic activity, at least as assessed by conflict procedures in rats.

Because stimulation of presynaptic 5-HT_{1A} receptors reduces 5-HT synthesis and release in various forebrain regions (22,26), reduced availability of 5-HT on some postsynaptic 5-HT receptor might be involved in the anticonflict effect of full and partial agonists at 5-HT_{1A} receptors.

Experiments in various animal models suggest that 5-HT_3 and 5-HT_{2C} are implicated in anxiety (4). However, the effect of antagonists at these receptors on punished responding has never been compared with benzodiazepines and 5-HT_{1A} drugs in the same model. This could well help clarify whether blockade of postsynaptic 5-HT_3 and/or 5-HT_{2C} receptors produces anxiolytic effects similar to those of agents that reduce synaptic 5-HT availability in the rat forebrain.

At doses previously reported to increase social interaction in paired naive rats under high light-unfamiliar conditions (12), neither ondansetron nor tropisetron significantly increased the rates of punished responding. Other authors have not found anxiolytic-like effects of 5-HT_3 receptor antagonists in conflict procedures (13,18,21,27). It remains, therefore, to be seen how the ability of 5-HT_3 receptor antagonists to reduce aversive responding in rat models such as social interaction and the two-compartment exploratory box is predictive of favourable effects on anxiety symptoms in humans.

Kennett et al. (28) recently suggested that blockade of 5-HT_{2C} receptors causes anxiolytic-like effects similar to that of chlordiazepoxide. One aim of the present study was to confirm the results obtained by Kennett et al. with ketanserin and mianserin and extend their findings by investigating the effect of ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist that reportedly alleviates anxiety symptoms in humans (9), and of mesulergine that has high, selective affinity for 5-$HT_{2A/2C}$ receptors with preferential action on 5-HT_{2C} sites (24).

We generally confirmed the results of Kennett et al. (28) in that ketanserin had no effect while the other drugs increased punished responding. The effects, however, were not dose dependent and less pronounced that with benzodiazepines and 5-HT_{1A} receptor full and partial agonists. Mianserin, at dose of 8 mg/kg, but not at 4 and 16, significantly increased the rate of punished responding. Although mianserin has affinity for other binding sites such as histamine H_1 and alpha-adrenoceptors, Kennett et al. (28) proved that antagonists at these receptors had no effect in their test. Mianserin also has moderate affinity for 5-HT_3 binding sites, but this obviously does not explain its effect on punished responding. It is likely, therefore, that its affinity for 5-$HT_{2A/2C}$ receptors is involved.

At 0.1 mg/kg mesulergine increased punished responding while ritanserin had a slightly significant effect only at the highest dose (2 mg/kg). Because mesulergine has a higher affinity for 5-HT_{2C} that ritanserin and its affinity for other receptor types is similar to or lower than that of ritanserin (4,24), the results are compatible with the hypothesis that blockade of 5-HT_{2C} receptors attenuates the punishment-induced suppression of operant responding. As recently discussed by Kennett et al., (28), there are, however, some inconsistencies in the effects of 5-$HT_{2A/2C}$ receptor antagonists in conflict paradigms. More selective 5-HT_{2C} receptor antagonists will, therefore, be required to confirm that blockade of these receptors has anxiolytic-like effects in conflict models.

In conclusion, the present study has shown that anxiolytic-like effects are obtained in a model of punished operant responding with agents that act as agonists at presynaptic 5-HT_{1A} receptors or block postsynaptic 5-HT_{2C} receptors. Further studies are necessary to clarify the exact role of 5-HT_{2C} receptors and how their effect on punished responding is predictive of anxiolytic activity in man.

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