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Role of 5-HT_{2A} and 5-HT_{2C} Receptor Subtypes in the Two Types of Fear Generated by the Elevated T-Maze

PAULA O. MORA, CRISTINA FERREIRA NETTO AND FREDERICO G. GRAEFF[1]

Laboratório de Psicobiologia, FFCLRP e Núcleo de Neurociências e Comportamento da Universidade de São Paulo, 14040-901 Ribeirão Preto, São Paulo, Brasil

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MORA, P. O., C. FERREIRA NETTO AND F. G. GRAEFF. *Role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes in the two types of fear generated by the elevated T-maze.* PHARMACOL BIOCHEM BEHAV **58**(4) 1051–1057, 1997.—To study the role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes in anxiety, the behavioral effects of drugs that either block or stimulate these receptors were measured in an animal model of anxiety, the elevated T-maze. One arm of the maze is enclosed by walls and stands perpendicular to the two open arms. Inhibitory (passive) avoidance—representing learned fear—was measured by placing a rat at the end of the enclosed arm and recording the time to leave this arm with the four paws during three consecutive trials. After 30 s, the same animal was placed at the end of one of the open arms and the time to leave this arm with the four paws was recorded. This one-way escape response represents unconditioned fear. The IP injection of the preferential 5-HT_{2C} receptor agonists mCPP and TFMPP (0.1–0.8 mg/kg), 25 min before the experimental session enhanced inhibitory avoidance. In contrast, the same drugs either tended to impair (mCPP) or significantly inhibited (TFMPP) one-way escape. The preferential 5-HT_{2A} agonist DOI (0.03–0.3 mg/kg) did not change either inhibitory avoidance or one-way escape. Inhibitory avoidance was impaired by the selective 5-HT_{2C} antagonists SB 200646A (3.0–30 mg/kg) and SDZ SER 082 (0.1–1.0 mg/kg), by the 5-HT_{2A} antagonist SR 46349B (1.0–10.0 mg/kg), and by the mixed 5-$HT_{2A/2C}$ antagonist ritanserin (0.3–3.0 mg/kg). However, it was not affected by the selective 5-HT_{2A} antagonist RP 62203 (0.25–4.0 mg/kg). All the 5-HT_2 antagonists used were ineffective on one-way escape. Therefore, conditioned fear seems to be tonically facilitated through 5-HT_{2C} receptor stimulation, although the 5-HT_{2A} receptor may also participate in its regulation. Unconditioned fear might be phasically inhibited by 5-HT_{2C} receptor activation. © 1997 Elsevier Science Inc.

Serotonin 5-HT_2 receptors Elevated T-maze Conditioned fear Unconditioned fear Anxiety

IT is widely accepted that 5-HT regulates anxiety. However, there are many inconsistencies in both preclinical and clinical evidence with drugs that modify 5-HT neurotransmission. Among the factors determining such variability of results are the existence of multiple 5-HT pathways and types of 5-HT receptors as well as of different kinds of experimental and clinical anxiety [for reviews, see (5,6,8,17,19,25)]. Therefore, studies with drugs that selectively affect different subtypes of 5-HT receptors and with animal models that clearly specify the type of fear that is being measured may contribute to clarify this issue.

Among 5-HT receptors the 5-HT_2 family seems to be particularly involved in anxiety, because many of the drugs used to treat anxiety disorders affect this type of 5-HT receptor. For instance, chronic administration of several antidepressant drugs that benefit patients with panic or obsessive–compulsive disorder downregulate 5-HT_2 receptors in the rat brain after chronic administration. Although anxiolytic drugs of the buspirone class act primarily on the 5-HT_{1A} receptor, downregulation of 5-HT_2 receptors also follows their repeated administration. Finally, atypical 5-HT_2 receptor antagonists, such as the putative anxiolytic ritanserin, also reduce 5-HT_2 receptor number and/or sensitivity upon prolonged use [for a review of binding studies and individual references, see (44)]. As a consequence, it has been suggested that downregulation of 5-HT_2 receptors may be crucial for the antianxiety action of these different classes of drugs (5,15).

Although the 5-HT_{2B} receptor has recently been identified in limbic areas of the rat brain (9), so far the 5-HT_{2A} and the

[1]To whom requests for reprints should be addressed.

5-HT_{2C} subtypes [former 5-HT_2 and 5-HT_{1C}, respectively, (21)] have been mainly associated with anxiety (34). Nevertheless, the relative contribution of each receptor subtype in anxiety is uncertain. For instance, a line of pharmacological evidence indicates that the anxiogenic effect of the mixed 5-HT receptor agonists mCPP and TFMPP is mediated by the 5-HT_{2C} receptor (26,28), while the majority of binding studies showing 5-HT_2 receptor downregulation have used [^{3}H] ketanserin, a preferential 5-HT_{2A} ligand (44).

New pharmacological tools to assess the specific role of 5-HT_{2A} and 5-HT_{2C} receptors in anxiety have been developed. Among them are: 1) the selective 5-HT_{2A} antagonist SR 46349B, that shows an affinity for the 5-HT_{2A} subtype nearly 20 times higher than for the 5-HT_{2C} receptor (36). Unlike ritanserin and other mixed 5-HT_2 antagonists, chronic administration of SR 46349B has been reported to upregulate 5-HT_{2A} receptors in the rat brain (35). 2) Another selective 5-HT_{2A} receptor antagonist, RP 62203, that has an affinity 5-HT_{2A}/5-HT_{2C} ratio of nearly 160, and does not induce receptor downregulation upon repeated administration (7,41). 3) SB 200646A, a 5-HT antagonist that has a 50-fold selectivity for the 5-HT_{2C} compared to the 5-HT_{2A} receptor (10). 4) A similarly selective 5-HT_{2C} antagonist, SDZ SER 082, having a 30-fold 5-HT_{2C}/5-HT_{2A} affinity ratio (33).

To generate two types of fear in the same rat within one experimental session, we conceived a new animal model of anxiety, named the elevated T-maze (13). This apparatus consists of three arms of equal dimensions elevated from the floor. One arm is enclosed by walls and stands perpendicular to the two open arms. Training of inhibitory (passive) avoidance is made by placing a rat at the end of the enclosed arm for three consecutive trials. In each trial, the time the animal takes to leave the enclosed arm with the four paws is measured. The same rat is then placed at the end of one of the open arms, and the time to leave this arm with the four paws is recorded. The inhibitory avoidance task is assumed to represent conditioned fear, while one-way escape from the open arm is believed to represent unconditioned fear. Supporting these hypotheses, a validating study has shown that the benzodiazepine anxiolytic diazepam and the 5-HT_{1A} ligand ipsapirone markedly impaired inhibitory avoidance, but did not significantly change one-way escape in the elevated T-maze (42). Further task-specific effects of drugs affecting 5-HT neurotransmission, injected either systemically or intracerebrally, have been reported under the same experimental conditions (12,14).

The present study attempts to analyze the role of 5-HT_{2A} and 5-HT_{2C} receptors in the two types of fear seemingly generated by the elevated T-maze. For this, dose–response curves on both inhibitory avoidance and one-way escape tasks were determined for the above selective 5-HT_{2A} and 5-HT_{2C} antagonists SR 46349B, RP 62203, SB 200646A, and SER 082. In addition, some older and less selective compounds have been studied, such as the preferential 5-HT_{2C} agonists mCPP and TFMPP (20), the preferential 5-HT_{2A} agonist DOI (31), and the mixed 5-$HT_{2A/2C}$ antagonist ritanserin (30).

METHOD

Animals

Male Wistar rats, 220–260 g in weight, were housed in groups of four or five with food and water freely available. Lights were on from 0700 to 1900 h. Environmental temperature was kept at 22 ± 1°C.

Apparatus

The elevated T-maze was made of wood and had three arms of equal dimensions (50 × 10 cm). One arm, enclosed by 40 cm high walls, was perpendicular to two opposed open arms. The open arms were surrounded by a Plexiglass rim 1 cm high. The whole apparatus was elevated 50 cm above the floor. The experiments were performed with an observer inside the room. Illumination was provided by a lamp in the ceiling of the room, above the center of the apparatus. The intensity of light at the level of the maze was 45 radiometric lux. Environmental temperature was kept at 22 ± 1°C with an air conditioner that also produced background noise.

Drugs

The 6-(2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one (ritanserin; Jansen, Denmark) was dissolved in a saline-Tween 80 2% solution. The compound 2-{3-[4-(4-fluorophenyl) piperazynil] propyl} naphto [1,8-c,d]isothiazole-1,1-dioxide (RP 62203; Rhone-Poulenc, France) was dissolved in a vehicle containing 10% ethanol and 40% propylene glycol in distilled water. The solution was sonicated for 10 min. The drug *N*-(1-methyl-5-indolyl)-*N'*-(3-pyridyl) urea hydrochloride (SB 200646A; SmithKline Beecham, UK) was dissolved in water acidulated with acetic acid (pH 3). The solution was sonicated for 20 min. The drugs 1-[3-(trifluoromethyl) phenyl] piperazine (TFMPP; RBI, USA), *m*-chlorophenylpiperazine hydrochloride (mCPP; RBI, USA), (±)-1-(2,5-

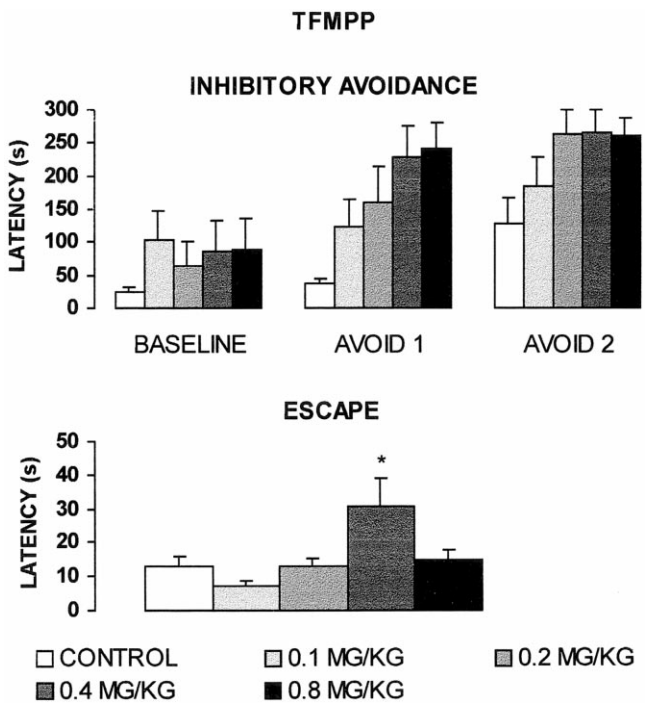

FIG. 1. Facilitatory effect of TFMPP on inhibitory avoidance and attenuating effect on escape in the elevated T-maze. Columns represent the mean and vertical bars the SEM. The latency to leave the enclosed arm (BASELINE, AVOID 1, and AVOID 2) was measured at 30-s intervals beginning 25 min after IP injection of either drug or vehicle. The latency to leave one of the open arms (ESCAPE) was measured 30 s after AVOID 2. The asterisk indicates significant difference from control ($p < 0.05$). $n = 7$–8.

dimethoxy-4-iodophenyl)-2-aminopropane (DOI; RBI, USA), SER 082 (Sandoz, Switzerland) and trans- 4-[(3Z)3-(2-dimethylaminoethyl)oxyimino-3(2-flurophenyl) propen-1-yl]phenol hemifumarate (SR 46349B; Sanofi, France) were dissolved in saline. The latter drug solution was sonicated for 10 min.

Drugs were prepared on the same day of the experiments and injected, IP, in a volume of 1 ml/kg body weight. The doses of the drugs used were selected on the basis of reported studies (4,27,28,36,39,41).

Procedure

On the third and fourth days after their arrival, animals were gently handled for 5–7 min. On the fifth day, they were randomly assigned to different treatment groups, and given SB 200646A (3.0, 10.0, and 30.0 mg/kg), SER 082 (0.1, 0.3, and 1.0 mg/kg), ritanserin (0.3, 1.0, and 3.0 mg/kg), SR 46349B (1.0, 3.0, and 10.0 mg/kg), RP 62203 (0.25, 1.0, and 4.0 mg/kg), mCPP (0.1, 0.2, 0.4, and 0.8 mg/kg), TFMPP (0.1, 0.2, 0.4, and 0.8 mg/kg), DOI (0.03, 0.1, and 0.3 mg/kg), or vehicle injection. After 25 min, each rat was placed at the end of the enclosed arm of the T-maze and the time taken to leave this arm with the four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and avoidance 2) at 30-s intervals. Thirty seconds after the completion of the avoidance task, the rat was placed at the end of the right open arm of the maze, and the time taken to leave the arm with the four paws was recorded (escape). Latency cutoff time was 300 s. After each rat, the maze was cleaned with a 20% alcohol solution to avoid interference of animal odors.

Data Analysis

For the avoidance latency, a two-factor (drug and trial) split-plot ANOVA was used. Whenever a significant drug × trial interaction was found, intergroup comparisons were made at each trial through one-factor ANOVAs, followed by the post hoc Newman–Keuls test. The escape latency was analyzed by one-factor ANOVA followed by the Newman–Keuls test. Variable *n* among doses of the same drug treatment (Table 1 and Figs. 1 and 3) were due to elimination of animals as a result of fall from the open arm (10 rats) or mistaken IP injection (5 rats).

RESULTS

TFMPP

The upper panel of Fig. 1 shows a dose-dependent increase of avoidance latency caused by TFMPP administration. Two-

TABLE 1

EFFECT OF 5-HT$_2$ RECEPTOR AGONISTS AND ANTAGONISTS ON INHIBITORY AVOIDANCE AND ONE-WAY ESCAPE IN THE ELEVATED T-MAZE

	Withdrawal Latency (Mean ± SEM, s)				
	Inhibitory Avoidance			One-Way Escape	
Drug mg/kg	Baseline	Avoidance 1	Avoidance 2	Escape	*n*
mCPP					
0.0	38.89 ± 20.75	120.10 ± 45.26	165.89 ± 42.77	14.22 ± 2.03	9
0.1	74.90 ± 37.66	79.10 ± 32.59	199.30 ± 41.89	16.60 ± 3.56	10
0.2	51.10 ± 28.78	129.70 ± 46.44	236.00 ± 37.42	12.30 ± 1.84	10
0.4	58.00 ± 30.45	219.44 ± 40.34	259.00 ± 22.47	25.67 ± 5.54	9
0.8	169.50 ± 50.27	238.62 ± 41.12	297.50 ± 2.50	23.87 ± 5.21	8
SER 082					
0.0	15.90 ± 2.70	107.90 ± 39.90	164.50 ± 41.90	13.80 ± 2.10	10
0.1	18.60 ± 3.00	104.50 ± 37.30	118.50 ± 40.60	18.80 ± 3.00	10
0.3	14.40 ± 2.30	9.00 ± 1.20	35.30 ± 12.50	17.00 ± 2.40	9
1.0	16.22 ± 5.00	28.50 ± 6.40	144.40 ± 49.40	18.30 ± 3.10	9
Ritanserin					
0.0	14.09 ± 3.16	51.36 ± 25.44	109.82 ± 38.52	33.36 ± 5.53	12
0.3	15.50 ± 3.69	63.75 ± 24.95	173.17 ± 36.65	39.25 ± 6.14	12
1.0	9.25 ± 1.30	12.25 ± 3.68	17.25 ± 4.34*	38.08 ± 7.91	11
3.0	11.50 ± 1.91	42.25 ± 23.71	98.23 ± 35.97	29.67 ± 5.47	11
DOI					
0.0	23.27 ± 4.65	115.91 ± 37.86	190.91 ± 38.89	18.00 ± 2.85	11
0.03	16.90 ± 3.54	52.20 ± 27.98	103.30 ± 34.39	14.20 ± 1.42	10
0.1	15.60 ± 2.36	55.60 ± 27.84	145.50 ± 36.43	14.90 ± 1.69	10
0.3	13.27 ± 3.64	44.00 ± 26.08	86.18 ± 28.19	14.36 ± 1.14	11
RP 62203					
0.0	9.90 ± 1.51	68.90 ± 38.57	182.20 ± 41.91	28.40 ± 5.04	11
0.25	29.36 ± 12.19	83.55 ± 35.57	170.27 ± 44.94	28.90 ± 4.59	11
1.0	13.92 ± 2.53	38.00 ± 24.23	165.83 ± 40.90	29.25 ± 5.06	11
4.0	51.20 ± 26.02	173.30 ± 42.19	214.70 ± 38.78	22.80 ± 4.65	10

*Significant difference from control (Newman–Keuls, $p < 0.05$).

factor ANOVA detected an effect of trial, $F(2, 70) = 29.06$, $p < 0.001$, and of drug, $F(4, 35) = 3.36$, $p = 0.02$. No significant drug × trial interaction was found, $F(8, 70) = 1.48$, $p = 0.180$. The lower panel of the figure shows that 0.4 mg/kg of TFMPP increased escape latency. One-way ANOVA revealed an overall drug effect, $F(4, 35) = 4.19$, $p = 0.007$. Post hoc comparisons with the Newman–Keuls test showed significant differences between the group treated with 0.4 mg/kg and each of the remaining groups ($p < 0.05$). Therefore, TFMPP facilitated inhibitory avoidance and, at one dose, impaired one-way escape.

mCPP

Like TFMPP, mCPP increased avoidance latency in a dose-dependent way. Two-factor ANOVA detected an effect of trial, $F(2, 82) = 31.50$, $p < 0.001$, and of drug, $F(4, 41) = 3.34$, $p = 0.019$. The drug × trial interaction was not significant, $F(8, 82) = 1.08$ $p = 0.385$. One-factor ANOVA revealed a nearly significant effect of drug, $F(4, 41) = 2.43$, $p = 0.062$, on escape latencies. Therefore, mCPP enhanced inhibitory avoidance while tending to impair one-way escape (Table 1).

SB 200646A

The upper panel of Fig. 2 shows that SB 200646A decreased avoidance latency dose dependently. Two-factor ANOVA evidenced an effect of trial, $F(2, 72) = 25.13$, $p < 0.001$, and of drug, $F(3, 36) = 5.58$, $p = 0.003$, as well as a significant drug × trial interaction, $F(6, 72) = 25.13$, $p < 0.001$. Further one-factor ANOVAs evidenced significant between-group differences at baseline, $F(3, 36) = 6.74$, $p = 0.001$, avoidance 1, $F(3, 36) = 2.91$, $p = 0.048$, and avoidance 2, $F(3, 36) = 4.48$, $p < 0.009$ trials. The Newman–Keuls test showed that the three doses of the drug differed from control ($p < 0.05$) at both the baseline and avoidance 2 trials. Nevertheless, no significant difference among treatment groups was detected at avoidance 1. One-way escape was not affected by SB 200646A [one-factor ANOVA, $F(3, 36) = 0.23$, $p > 0.05$]. Therefore, SB 200646A impaired inhibitory avoidance, but did not change one-way escape.

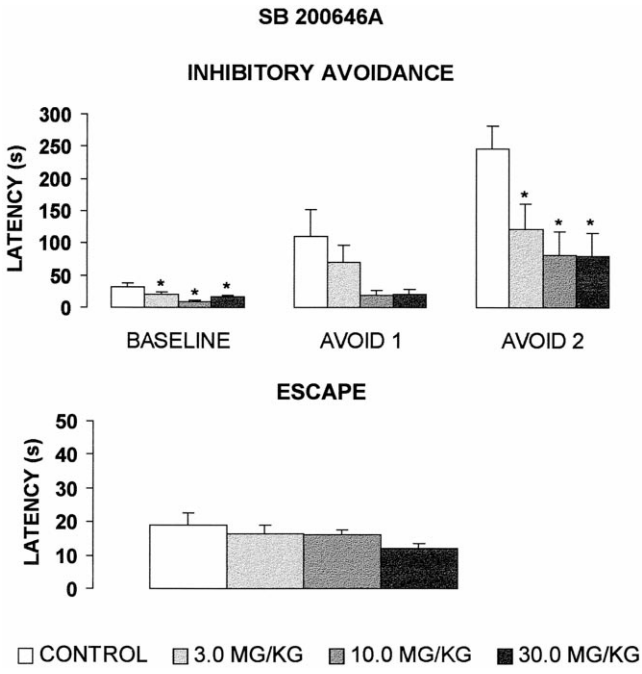

FIG. 2. Anxiolytic effect of SB 200646A on inhibitory avoidance and no effect on escape. $n = 10$. Other specifications in the legend of Fig. 1.

SER 082

The effect of SER 082 is shown in Table 1. Two-factor ANOVA revealed a nearly significant overall drug effect, $F(3, 35) = 2.67$, $p = 0.062$, a significant effect of trial $F(2, 70) = 19.67$, $p < 0.001$, and a significant drug × trial interaction, $F(6, 70) = 2.55$, $p = 0.027$. Further one-factor ANOVAs evidenced significant differences among treatments at avoidance 1, $F(3, 35) = 3.32$, $p = 0.031$, and a nearly significant tendency at avoidance 2, $F(3, 35) = 2.26$, $p = 0.099$. Even at avoidance 1 the Newman–Keuls test did not detect a significant difference between any pair of treatment groups. SER 082 did not change one-way escape [one-factor ANOVA, $F(3, 35) = 0.73$, $p > 0.05$]. Hence, SER 082 had an attenuating effect on inhibitory avoidance that was less clear than that of SB 200646A. This is due to the lack of dose dependence, as the effect reversed at the highest dose of the drug (1.0 mg/kg, Table 1).

Ritanserin

As shown in Table 1, 1.0 mg/kg ritanserin significantly decreased avoidance latency. Two-factor ANOVA showed a significant effect of trial, $F(2, 86) = 20.76$, $p < 0.001$, and of drug, $F(3, 43) = 3.64$, $p = 0.020$. The drug × trial interaction was also significant, $F(6, 86) = 2.71$, $p = 0.019$. Further one-factor ANOVAs evidenced significant between-group differences at avoidance 2 only, $F(3, 43) = 4.66$, $p = 0.006$. The Newman–Keuls test showed that the 1.0 mg/kg group was significantly different from both the control and the 0.3 mg/kg groups ($p < 0.05$). One-way escape was not affected by ritanserin [one-factor ANOVA, $F(3, 43) = 0.49$, $p = 0.692$. Therefore, ritanserin, at one dose, impaired inhibitory avoidance, but had no effect on one-way escape.

DOI

Avoidance latency was not affected by DOI. Two-factor ANOVA detected a significant effect of trial, $F(2, 76) = 27.29$, $p < 0.001$), but neither a significant drug effect, $F(3, 38) = 0.14$, $p > 0.05$, nor a significant drug × trial interaction, $F(6, 76) = 0.35$, $p > 0.05$. The drug also did not significantly change escape latency [one-factor ANOVA, $F(3, 38) = 0.88$, $p > 0.05$]. Thus, DOI was ineffective on both inhibitory avoidance and one-way escape (Table 1).

SR 46349B

The upper panel of Fig. 3 shows that SR 46349B shortened avoidance latency. Two-factor ANOVA showed an effect of trial, $F(2, 62) = 30.99$, $p < 0.001$, and of drug, $F(3, 31) = 2.94$, $p = 0.049$. However, there was no significant drug × trial interaction, $F(2, 62) = 1.20$, $p = 0.316$. The lower panel of the same figure shows an absence of drug effect on escape latency [one-factor ANOVA, $F(3, 31) = 0.03$, $p = 0.994$. Therefore, SR 46349B impaired inhibitory avoidance, but did not change one-way escape.

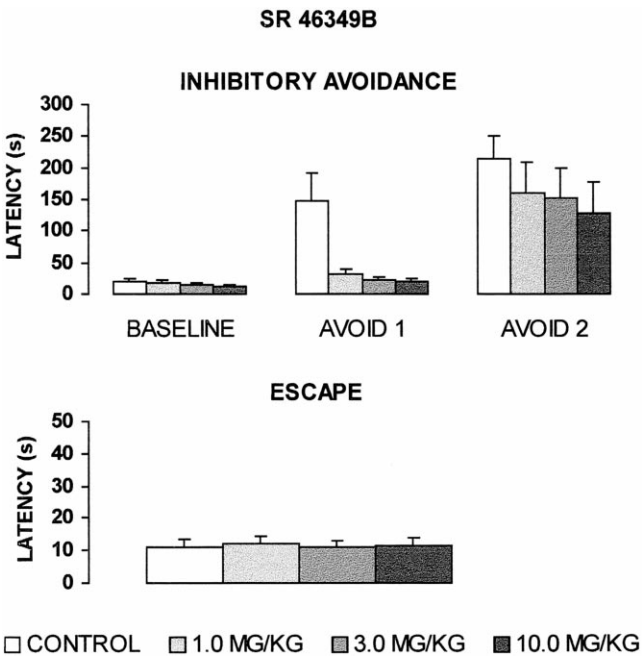

FIG. 3. Anxiolytic effect of SR 46349B on inhibitory avoidance and lack of effect on escape. n = 8–9. Other specifications in the legend of Fig. 1.

RP 62203

RP 62203 did not affect avoidance latency. Two-factor ANOVA showed an effect of trial, $F(2, 78) = 33.72$, $p < 0.001$, but there was neither an effect of drug, $F(3, 39) = 1.97$, $p = 0.135$, nor a significant drug × trial interaction, $F(6, 78) = 0.75$, $p = 0.608$. The drug also did not change escape latency [one-factor ANOVA, $F(3, 39) = 0.72$, $p = 0.548$]. Hence, RP 62203 was ineffective on both inhibitory avoidance and one-way escape tasks (Table 1).

DISCUSSION

The present results show that inhibitory avoidance and one-way escape in the elevated T-maze were differentially affected by the drug treatments used. The two preferential 5-HT$_{2C}$ receptor agonists mCPP and TFMPP facilitated inhibitory avoidance, that is, had an anxiogenic effect in this task. At the same time, escape behavior was impaired, an effect that may be viewed as anxiolytic (see below). On the other hand, the selective 5-HT$_{2C}$ antagonists SB 200646A and SER 082, the selective 5-HT$_{2A}$ antagonist SR 46349B, and the mixed 5-HT$_{2A/2C}$ antagonist ritanserin reduced avoidance without affecting escape behavior. Previous studies have similarly shown that several other drugs, injected either systemically or intracerebrally, had selective effects on the two tasks measured in the elevated T-maze (12,14,42). Such pharmacological specificity of inhibitory avoidance and one-way escape behaviors supports the original assumption that two types of fear are generated by these tasks in the elevated T-maze (13).

Concerning 5-HT$_{2C}$ receptors, the dose-dependent facilitatory effect of the 5-HT$_{2C}$ agonists mCPP and TFMPP on inhibitory avoidance, shown by the present results, indicates that stimulation of these receptors increases conditioned fear. Furthermore, the 5-HT$_{2C}$ antagonists SB 200646A, SER 082, and ritanserin had an anxiolytic effect on inhibitory avoidance, indicating that 5-HT$_{2C}$ receptors are being activated tonically. Some caution, however, is advisable to avoid over interpretation of these results. The 5-HT$_{2C}$ agonists mCPP and TFMPP also have high affinity for the 5-HT$_{2A}$ receptor, where they act as antagonists or partial agonists (18) as well as for the 5-HT$_{1B}$ receptor, acting as agonists (21). Although the subject is still controversial, pharmacological analysis with receptor antagonists having differential affinity for these types of receptors led to the suggestion that the anxiogenic effect of mCPP and TFMPP—demonstrated in several animal models of anxiety (17)—is mediated by the 5-HT$_{2C}$ receptor (26,28). The present results are in agreement with this line of evidence. Second restriction, the antagonist drug ritanserin binds to 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors with comparable affinity (21), and the former may also be involved in the regulation of conditioned fear (see below). Third, the specificity of the anxiolytic effect of the highly selective 5-HT$_{2C}$ antagonist SB 200646A may be questioned, because baseline latency was also shortened by the same doses of SB 200646A that were effective on avoidance 1 and 2 (Fig. 3). Therefore, nonspecific behavioral stimulation or decreased impulse control (40) may be involved in the shortening of avoidance latencies. However, the present result showing that escape latency was not significantly affected by SB 200646A (see below) argues against these alternatives. Moreover, anxiolytic effects of SB 200646A have been reported in the rat social interaction test (29) as well as in the rat Geller-Seifter model and in another conflict test in the marmoset (27). A further alternative is an impairment of learning and memory, because a significant drug × trial interaction was obtained with the three 5-HT$_{2C}$ antagonists used, indicating that acquisition of avoidance was changed by the drugs. In spite of these caveats, the bulk of the evidence favors a participation of the 5-HT$_{2C}$ receptor in the regulation of conditioned fear.

In contrast with the above anxiogenic effect on inhibitory avoidance, the 5-HT$_{2C}$ agonists either tended to (mCPP) or significantly impaired (TFMPP) escape from the open arms of the elevated T-maze, therefore having an anxiolytic effect on this task. Similar results have been reported with escape from electrical stimulation of the dorsal periaqueductal gray—PAG (22–24)—a brain area thought to be critical for the expression of unconditioned defensive behaviors and implicated in panic disorder (5,11,14,15). Therefore, 5-HT$_{2C}$ receptor stimulation seems to decrease unconditioned fear. As a cautionary note, however, it should be pointed out that the presently observed increases in escape latency following mCPP and TFMPP were of low magnitude and not dose dependent. Among alternative interpretations for these results is a drug-induced nonspecific reduction of motor activity. Indeed, hypoactivity is one of the main behavioral effects of mCPP and TFMPP (26) and, accordingly, the present results show that baseline latency of inhibitory avoidance was increased by mCPP and TFMPP (Fig. 1 and Table 1). There is, however, an inherent difficulty with this argument, because behavioral inhibition is an intrinsic manifestation of anxiety (16). Because none of the 5-HT$_{2C}$ receptor antagonists used in the present study affected one-way escape from the open arms, a tonic control of the 5-HT$_{2C}$ receptor on unconditioned fear should be ruled out. Thus, so far, the role of the 5-HT$_{2C}$ receptor in unconditioned fear relies on limited experimental evidence.

Concerning the localization of the 5-HT$_{2C}$ receptors involved in anxiety, there are reported results with local drug injection implicating the hippocampus (43) as well as the dorsal PAG (2). In both these structures the administration of 5-HT$_{2C}$ agonists had anxiogenic effects. Thus, in spite of ex-

tensive data supporting a critical role of the dorsal PAG in unconditioned fear (5,11,14,15), the site of the seemingly anxiolytic effect of systemically administered mCPP and TFMPP on escape behavior [(22,23), and present results] is likely to be localized elsewhere in the brain. Because conditioned fear has been suggested to inhibit unconditioned fear (5), an interesting possibility is that the decrease of unconditioned fear caused by the 5-HT_{2C} agonists is secondary to the enhancing effect of these drugs on conditioned fear.

The role of the 5-HT_{2A} receptor in conditioned fear is far less clear than that of the 5-HT_{2C} receptor. The present results show that the preferential 5-HT_{2A} receptor agonist DOI was ineffective on inhibitory avoidance in the range of doses used. Higher doses of the drug cause increasing frequency of head twitches (37) and, therefore, cannot be explored. Although the mixed 5-$HT_{2A/2C}$ antagonist ritanserin (21) had a modest anxiolytic effect, this may be due to its interaction with 5-HT_{2C} receptors, as discussed above. Also regarding ritanserin, the reported effects of single drug administration on animal models are inconsistent, because anxiogenic, anxiolytic, and null effects have been described (17). While the selective 5-HT_{2A} antagonist SR 46349B presently had a neat anxiolytic effect on inhibitory avoidance, the highly selective agent RP 62203 was ineffective. Subchronic administration of SR 46349B has been reported to attenuate learned helplessness (36). To what extent this antidepressant-like effect is related to the present anxiolytic effect of the drug is not yet clear. The ineffectiveness of RP 62203 shown by the present results contrasts with the reported anxiolytic effect of this drug after single administration in mice exposed to the elevated plus-maze (41).The dose range was the same in both studies, but the route of administration was different—PO in mice vs. IP in rats. Species difference and type of test may be other causes of the discrepancy. In summary, the anxiolytic-like action of SR 46349B on inhibitory avoidance is the only result obtained so far suggesting a (tonic) facilitatory function of the 5-HT_{2A} receptor in conditioned fear. Further evidence is necessary to test this hypothesis.

The present results do not support the participation of the 5-HT_{2A} receptor in unconditioned fear, because neither the agonist DOI (at doses lower than those inducing head twitches) nor the 5-HT_{2A} receptor antagonists SR 46349B and RP 62203 affected escape from the open arm in the elevated T-maze. In spite of this, results reported by Jenck et al. (22) suggest that 5-HT_{2A} receptor stimulation facilitates escape from electrical stimulation of the dorsal PAG. In seeming contrast, however, similar experiments in which drugs were injected directly into the dorsal PAG indicate that 5-HT_{2A} receptor stimulation inhibits aversion generated in the dorsal PAG (1,32,38). The reasons for these discrepancies are not entirely clear. One possibility is that systemically administered drugs act mainly on sites different from the PAG. This could explain the inconsistency among the above results with PAG stimulation. However, it does not apply to the disagreement between the present and Jenck et al. results (22–24). In this case, the difference in procedure—escape from the open arm of the elevated T-maze as opposed to escape from PAG electrical stimulation—seems to be the critical factor.

Three of the drugs used in the present study—mCPP, ritanserin, and SR 46349b—have been assayed in two experimental models of human anxiety, namely the conditioned skin conductance response (CSCR) and the simulated public speaking (SPS) tests. These models are supposed to represent conditioned and unconditioned fear, respectively (6). Regarding conditioned fear, a good correlation between animal and human tests was obtained, because in parallel with the present results with the inhibitory avoidance task in the elevated T-maze, reported results show that mCPP enhanced while ritanserin and SR 46349B decreased anxiety in the CSCR test (3,6). For unconditioned fear, however, the correspondence is incomplete: the 5-HT_{2A} agonist SR 46349B failed to modify both SPS (3) and one-way escape in the elevated T-maze, mCPP tended to impair one-way escape while having no effect on SPS (3), and ritanserin was ineffective on one-way escape but prolonged SPS anxiety (6). Nevertheless, only one dose of each drug was used in healthy volunteers. Moreover, negative correlation, that is, opposite drug effects in the animal model compared to the human tests, has not been observed so far. Therefore, the suggestion that inhibitory avoidance and CSCR may be related to generalized anxiety disorder while one-way escape and simulated public speaking may be associated with panic disorder (5,6) deserves further inquiry.

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