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Behavioral Effects of Flibanserin (BIMT 17)

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BORSINI, F., A. BRAMBILLA, N. GRIPPA, AND N. PITSIKAS. *Behavioral effects of flibanserin.* PHARMACOL BIOCHEM BEHAV **64**(1) 137–146, 1999.—Flibanserin is a 5-HT_{1A} agonist that, in contrast to other 5-HT_{1A} receptor agonists, is capable of activating 5-HT_{1A} receptors in frontal cortex. Flibanserin also behaves as an antagonist at 5-HT_{2A} receptors. This compound has been described to be a putative fast-acting antidepressant owing to these properties. In the present study, the effect of flibanserin was investigated in several behavioral paradigms different from animal models of depression. Intraperitoneal flibanserin, at doses of 4–8 mg/kg, antagonized *d*-amphetamine– and (+)SKF-10047– induced hypermotility in mice and rats. At doses of 8–16 mg/kg, flibanserin exerted anxiolytic-like effects in the light/dark exploratory test and stress-induced hyperthermia in mice, and antagonized *d*-amphetamine– and apomorphine-induced stereotypy in rats. At the dose of 16 mg/kg, flibanserin reduced spontaneous motor activity in rats. At the dose of 32 mg/kg, flibanserin did not exert any clear effect on spontaneous motor activity in mice, or on the elevated plus-maze and the water maze in rats. © 1999 Elsevier Science Inc.

Flibanserin Light/dark exploratory test Stress-induced hyperthermia Elevated plus-maze Motor activity Hypermotility Stereotypy Water maze Rat Mouse

FLIBANSERIN has been reported to behave as a full agonist on serotonin 5-HT_{1A} receptors located in different brain regions (10). This spectrum of agonist activity differentiates flibanserin from other claimed 5-HT_{1A} agonists. In fact, considering adenylate cyclase as an index of 5-HT stimulation, buspirone behaves as a partial agonist in hippocampus and is devoid of any agonist activity in the cortex (10). Moreover, 8-OH-DPAT behaves as a full agonist in hippocampus and as an antagonist in the cortex (11). In addition, buspirone also has affinity for dopamine D_2 receptors (70), and 8-OH-DPAT also has affinity for 5-HT7 (66) and 5-HT1D β receptors (8). In contrast, flibanserin appears to be quite selective for 5-HT_{1A} and 5-HT_{2A} receptors (10), being an antagonist on the latter receptor. Actually, this antagonist action on 5-HT_2 receptors is desiderable. In fact, *in vitro*, the blockade of 5-HT_2 receptors has been reported to potentiate the stimulation of 5-HT_1 receptors (47), and the stimulation of 5-HT_2 receptors has been reported to antagonize the effect of 5-HT_{1A} receptor activation (3). Thus, it is conceivable that physiologically released 5-HT, through stimulation of 5-HT_2 receptors, may attenuate the effect of drug-induced 5-HT_{1A} receptor activation Thus, it appears that flibanserin may represent a new tool to investigate the function of 5-HT_{1A} receptor activation in the brain, above all in the cortex where 5-HT_{2A} and 5-HT_{1A} receptors may coexist in the same cell (3).

It has already been shown that flibanserin induces antidepressant-like effects in some animal models of depression, i.e., the forced swimming test in mice (21), bulbectomized rats and the learned-helplessness test (12), and chronic mild stress (32). Flibanserin showed a fast onset of action in the last two tests, where antidepressants are effective only after a repeated treatment. However, it was inactive in the differential-reinforcement-of-low rate 72-s (DRL 72-s) test (12). Flibanserin also induced a low degree of serotonergic syndrome, without inducing forepaw treading, and antagonized DOI-induced head twitches (10).

The aim of the present work was to evaluate the effects of flibanserin in a wide spectrum of behavioral paradigms. The light/dark exploratory test and stress-induced hyperthermia in mice, and the elevated plus-maze test in rats were used to assess the anxiolytic-like properties of flibanserin. The Morris water maze test in rats was used to evaluate its effects on learning. Furthermore, the activity of flibanserin was investigated on either spontaneous or drug-stimulated motor activity in rats and mice.

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METHOD

Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies (EEC Council Directive 86/609, = J L 358,1, Dec. 12, 1987; NIH Guide for the Care and Use of Laboratory Animals. NIH publication no. 85-23, 1985).

The statistical evaluations were carried out by N.G. with the SAS program system (SAS Institute Inc., Cary, NC), version 6.07 on a DEC Computer, o.s. VMS.

Animals

Male CD1 mice, weighing 20–24 g, and male Sprague–Dawley rats, weighing 150–175 g or 200–220 g for the water maze (both species from Charles River, Italy) were used. Mice (20 to a cage) and rats (10 to a cage, 55 × 37.5 × 19.5 cm) were housed in a regulated environment (21 ± 1°C, 50–55% relative humidity, 12 L:12 D cycle, lights on at 0700 h—except for the light/dark test where lights were off at 0700—with food (Mucedola s.r.l., Italy) and water available ad lib. Rats were maintained in this environment for 5–7 days before the beginning of the experiments. Mice for the light/dark experiments were kept on a reversed light cycle for at least 2 weeks.

Dark/Light Exploratory Test in Mice

Apparatus. The apparatus consisted of a white open-topped box (45 × 27 × 27 cm) divided into two compartments communicating by a door. One of the chambers was large (3/5) and brightly lit, and the other was smaller (2/5) and maintained under a low level of illumination. The floor of each compartment was marked into 9-cm squares.

Procedure. The experiments were performed between 0900 and 1200 h, during the dark phase of the cycle. Each mouse was tested by placing it in the center of the white area and allowing it to explore the apparatus for 5 min. The time spent by the animal in the light chamber and the number of transitions between the two compartments were recorded. An increase in these two measurements has been suggested to be indicative of a possible anxiolytic effect (49).

This behavior and motor activity (i.e., line crossings with all four paws and rearings) were recorded on videotape and evaluated by an observer unaware of the treatment.

Treatment. Eight mice for each group were used. Vehicle or drug was randomly injected intraperitoneally 30 min before the 5-min testing. Three separate experimental sessions were performed: (a) vehicle and flibanserin at doses of 2 and 4 mg/kg; (b) vehicle and flibanserin at doses of 8 and 16 mg/kg; and, (c) vehicle and the anxiolytic drug lorazepam, used as reference compound, at doses of 0.1 and 1 mg/kg.

Statistics. Results are expressed as the median with an interquartile range, because of the nonnormal distribution of the values. Data of sessions (a) and (b) were analyzed by one-way ANOVA followed by Dunnett's *t*-test. The group of 16 mg/kg flibanserin was excluded from the analysis due to the observed maximum sedative effect of the drug. In session (c), data relevant to transition, rearings, and line crossings were analyzed by one-way ANOVA followed by Dunnett's *t*-test, whereas data relevant to time spent in the lit area were analyzed by Kruskal-Wallis test followed by Dunn test vs. control.

Stress-Induced Hyperthermia in Mice

Procedure. This test was performed according to (13). Mice, maintained in the experimental room and handled for 3 days before the beginning of the experiments, were divided in groups of 16 per cage (24 × 26 × 14 cm). The rectal temperature was measured by inserting a thermistor probe for a length of 2 cm into the rectum of the mouse that was restrained manually. Digital recording of the temperature was determined to the nearest 0.1°C by means of a U. Sachs instrument. The probe, dipped into silicon oil before insertion, was held in the rectum for about 20 s. Rectal temperature was recorded in the first four and last four removed mice from the same cage. The intermediate 8 animals were removed without any probe insertion in the rectum and became the first and last four experimental animals of the following day. After rectal temperature measurements, mice were returned to the same cage. Rectal temperature was measured by an observer unaware of the treatment.

Treatment. Vehicle or drug was randomly injected intraperitoneally 30 min before the rectal measurement. Two separate experimental sessions were performed: (a) vehicle and flibanserin at doses of 8 and 16 mg/kg; and (a) vehicle and buspirone at doses of 5 and 10 mg/kg. Sixteen animals for each group were used in session (a) and eight in session (b). The experiments were performed between 1400 and 1600 h.

Statistics. Results were expressed as the median with an interquartile range, because of the nonnormal distribution of the values. Data were analyzed by Wilcoxon test for independent samples and Kruskal-Wallis test followed by Dunn test vs. control.

Elevated Plus-Maze in Rats

Apparatus. This consisted of a plus-shaped maze with two 50-cm length open and two 50-cm length enclosed arms, with 10-cm walls, placed 50 cm above the floor of the room. The arms were connected by a central 10 × 10 cm square

Procedure. The test involved placing the animal in the center of the maze (10 × 10 cm) facing one closed arm, and allowing it to explore for a 5-min period (57). During this time, the number of open- and closed-arms entries and the time (seconds) spent by the animal in each arm were recorded on videotape and evaluated by an observer unaware of the treatment. An entry was recorded when the animal entered an arm with all four legs.

Treatment. Eight rats per group were used. Vehicle or drug was randomly injected intraperitoneally 30 min before the 5-min testing. Three separate experimental sessions were performed: (a) vehicle and flibanserin at doses of 2 and 4 mg/kg; (b) vehicle and flibanserin at doses of 8 and 16 mg/kg; and, (c) vehicle and the anxiolytic drug lorazepam, used as a reference compound, at doses of 0.0625, 0.125, and 0.25 mg/kg.

Statistics. Results are expressed as the median with an interquartile range, because of the nonnormal distribution of all values. Data of sessions (a) and (b) were analyzed by one-way ANOVA (sometimes preceded by a monotonic transformation) followed by Dunnett's *t*-test. In session (c), data were analyzed by Kruskal–Wallis followed by Dunn's test vs. control.

Motor Activity in Mice and Rats

Apparatus. This consisted of two (20 × 20 × 20 cm, for mice) or one (40 × 40 × 30 cm, for rats) Plexiglas boxes housed within an outer chamber equipped with two rows of 16 photocells. The first set of photocells was sited 4 cm above the grid floor, whereas the height of the second set was sited at 6 cm for mice and 13 cm for rats above the grid floor. The lower photocell row served to measure horizontal activity, while the higher row served to detect vertical movements. The number

of interruptions by each animal of the photoelectric beams was recorded by a computer system (Activity Monitor mod. 540, Giunta Scientific Instruments). Four chambers, for a total of eight cages for mice or four for rats, at the same time were used.

Spontaneous Motor Activity

Procedure. In the experiment with mice, the animals were placed in the apparatus for a 30-min habituation period. Thereafter, they were given vehicle or the drug and immediately returned to the apparatus, and horizontal and vertical motor activity was monitored for 60 min.

In the experiment with rats, animals were removed from their home cage, injected with vehicle or the drug, and immediately placed in the apparatus for a 60-min recording period.

Treatment. Eight animals for each group were used. Vehicle or drug was randomly injected intraperitoneally just before the motor activity measurement. In mice, only one experimental session was carried out: vehicle and flibanserin at doses of 8, 16, and 32 mg/kg. In rats, two separate experimental sessions were performed: (a) vehicle and flibanserin at a dose of 8 mg/kg; and (b) vehicle and flibanserin at a dose of 16 mg/kg. The experiments were performed between 0900 and 1600 h.

Statistics. Data are expressed as the mean ± SEM. Differences between groups were tested by one-way ANOVA followed by Dunnett's *t*-test when necessary. The analysis was preceded by a monotonic transformation of the data to achieve a normal distribution.

d-Amphetamine- and (+)SKF10047-Induced Hypermotility

Procedure. Mice were individually placed into the apparatus for 15 min prior to treatment. Then the animals were randomly injected with intraperitoneal vehicle or flibanserin and placed again in the apparatus for 15 min. Thereafter, vehicle or *d*-amphetamine (2.5 mg/kg IP) or (+)SKF10047 (20 mg/kg SC) was administered, and the mice again put in the apparatus. Thirty minutes later horizontal motor activity was recorded for 10 min. When rats were used, they were randomly administered IP with vehicle or flibanserin and put individually in the apparatus, where 15 min later they received vehicle or *d*-amphetamine (1.25 mg/kg IP) or (+)SKF10047 (20 mg/kg SC). Then, immediately, horizontal motor activity was recorded for 60 min.

Treatment. Six or eight mice, and eight rats for each group were used. The experimental design consisted of four groups: vehicle + vehicle; vehicle + psychostimulant; flibanserin (one dose) + vehicle; flibanserin (one dose) + psychostimulant. This scheme was repeated for each dose of flibanserin. The doses of flibanserin were 2, 4, and 8 mg/kg in mice, and 4 and 8 mg/kg in rats. The experiments were performed between 0900 and 1600 h.

Statistics. Data were expressed as the median with an interquartile range or as the mean ± SEM. Data were analyzed by factorial analysis of variance followed by Student's *t*-test according to Bonferroni criterion, or by the Kruskal–Wallis test followed by the Wilcoxon test when necessary.

Stereotypy in Rats

Procedure. Rats were individually habituated to Perpex cages for 15 min and then randomly treated with flibanserin or vehicle. Fifteen minutes later, animals were injected with *d*-amphetamine or apomorphine. Thereafter, behavioral observations, lasting 15 s, were made every 15 min up to the disappearance of the *d*-amphetamine or apomorphine effects. Stereotypy was scored from 0 to 4, according to Costall and Naylor (25,26): 0 = no stereotyped behavior; 1 = periodic sniffing; 2 = continuous sniffing; 3 = periodic licking or biting of the grid; 4 = continuous licking or biting of the grid. The experiments started at 0900 h. Stereotypy score was evaluated by an observer unaware of the treatment.

Treatment. Five rats for each group were used. Vehicle or flibanserin, at doses of 8 and 16 mg/kg, was administered intraperitoneally. *d*-Amphetamine was given intraperitoneally at a dose of 10 mg/kg. Apomorphine was administered subcutaneously at a dose of 5 mg/kg.

Statistics. Results are expressed as the mean ± SEM of area under the curve, calculated by the trapezoidal rule. Data were analyzed by one-way analysis of variance followed by Dunnett's test.

Water Maze in Rats

Apparatus. The water maze consisted of a PVC circular tank (145 × 45 cm), filled to 40 cm with water made opaque by addition of food dye (coffee color, Bayo, Italy) and maintained at 27 ± 1°C. A transparent squared platform (11 × 11 cm) was placed in a fixed position (not in the center) in the tank, 1 cm below the water surface. Extra-maze cues were provided in the room to help the rat locate the platform.

Procedure. This was performed according to (59). Three days prior to the start of experimental trials, the rats were gently placed in the pool with no platform for 1 min of habituation. For the experimental trials the animal was gently put in the pool, close to and facing the wall in one of the four equally spaced quadrants. Rats were allowed to swim freely until they found the escape platform. If the rat failed to find the platform within 60 s, the observer placed the rat on it. Each rat performed four trials for 4 consecutive days. The intertrial interval was 30 s, during which rats remained on the platform. The time spent to reach the platform (latency), the swimming path length and the swimming speed were recorded. On day 5, rats performed the spatial probe trial. This trial consisted of removing the platform from the pool and allowing the rat to swim for 60 s in search of it. The time spent in each of the four quadrants of the tank was calculated as a percentage over 60 s. A persistent preference to navigate in the quadrant where the platform had been previously placed was taken as an index that the animal had acquired the task and remembered it. The behavior of the animals was recorded on videotape.

Treatment. Two different experimental sessions were performed; (a) vehicle and 16 mg/kg flibanserin; and (b) vehicle, 8 mg/kg flibanserin, 32 mg/kg flibanserin, 2 mg/kg buspirone, and 2 mg/kg diazepam. The animals, 10 and 12 for each group in the first and second sessions, respectively, were injected intraperitoneally with vehicle or drug once a day, 30 min before starting behavioral testing. In both sessions, animals were randomly divided into the experimental groups.

Statistics. The log-rank test based on the Kaplan–Meier method was utilized each day separately to analyze the effects of flibanserin, buspirone, and diazepam on rat latencies (28,42). The comparison between each of the treated groups vs. the control group were made according to the closed testing procedure, in order to maintain the experimentwise error rate equals the required level α of the overall test. Because the majority of experimental values were right-censored data (the trials were stopped after 60 s), the four observations of each day were summarized with the second highest value. This summarizing value was arbitrarily selected because

TABLE 1

EFFECT OF FLIBANSERIN AND LORAZEPAM IN THE DARK/LIGHT EXPLORATORY TEST IN MICE

Treatment	Dose (mg/kg)	Time in Lit Area (seconds)	Transitions (Number)	Line Crossings (Number)	Rearings (Number)
Vehicle	—	129 (102.5–152.5)	24.5(23–27)	142.5(122–158)	59.5(52–65)
Flibanserin	2	127 (109–143.5)	24 (20.5–28.5)	139.5(118.5–154.5)	55.5(46.5–62)
Flibanserin	4	155.5 (123–164)	27 (20–33)	150 (136.5–161.5)	56 (54.5–71)
Vehicle	—	128 (71–141)	16.5(15–20.5)	127.5(113–145)	67.5(53–75)
Flibanserin	8	176.5 (149–185)*	36 (30.5–39.5)†	162 (131.5–188.5)	55.5(41–62.5)
Flibanserin	16‡	>300 (298.5–300)	0 (0–1.5)	7.5(0–27.5)	2 (0–15.5)
Vehicle	—	105 (76.5–117)	20 (17.5–30)	145.5(120.5–182)	45.5(40.5–50.5)
Lorazepam	0.1	108 (80.5–127)	17.5(7–20)	121 (71.5–143)	29 (15.5–35.5)†
Lorazepam	1	195.5 (117.5–300)	2.5(0–4)†	14.5(6.5–37)†	1.5(0.5–5)†

Data are median with interquartile range from eight mice. Drugs were administered IP 30 min before the test. Exploratory behaviors were recorded for 5 min. Statistics: one-way ANOVA followed by Dunnett's *t*-test. In the experiment with lorazepam, data concerning the time spent in the lit area were analyzed by a Kruskal–Wallis test followed by Dunn test vs. respective vehicle.

*$p < 0.05$, †$p < 0.01$ vs. respective vehicle.

‡This dose was excluded from the analysis due to the observed maximum sedative effect of the drug.

medians cannot be calculated. Swimming path length and swimming speed data, which were normally distributed with homogeneous variances, were analyzed by ANOVA with a split-plot design. As far as the spatial probe trial was concerned, only the time spent by each rat in the quadrant, where the platform was, was statistically analyzed; the difference between the experimental groups were analyzed by one-way ANOVA followed by Duncan's test for multiple comparisons.

Drugs

Flibanserin, synthesized as BIMT 17 chloride in the Department of Medicinal Chemistry of Boehringer Ingelheim, Italy, was dissolved in 2% glycerol formale + 2% Mulgofen + Tris HCl 50 mM, pH 7.4. Lorazepam (Prodotti Gianni) was dissolved with a few drops of Tween 80 and diluted with 0.2% tylose. Diazepam as Valium® (Hoffmann–LaRoche) was suspended in the commercial vehicle consisting of propylene glycol, ethanol, and saline (40:10:50). *d*-Amphetamine sulphate (Recordati), (+)SKF-10047 (R.B.I.), and buspirone chloride (Bristol-Myers) were dissolved in 0.9% NaCl. The doses of the drugs refer to the base form. Vehicle or the drug was administered in a volume of 10 or 20 (spontaneous motor activity) ml/kg in mice and in a volume of 5 ml/kg in rats.

RESULTS

Dark–Light Exploratory Test

Vehicle-treated animals spent only a small part of their time in the lit compartment (Table 1). Flibanserin, at 2 and 4 mg/kg, did not modify mice behavior (Table 1). The dose of 8 mg/kg increased the time spent in the lit compartment and reduced the number of transitions, without affecting the number of either line crossings or rearings. In contrast, the dose of 16 mg/kg strongly decreased the number of transitions, line crossings, and rearings, and concurrently increased the time spent in the lit area. Lorazepam, at 1 mg/kg, increased the time spent in the lit compartment and decreased the number of transitions, the dose of 0.1 mg/kg being almost ineffective. The motor activity was significantly reduced with lorazepam at 1 mg/kg (Table 1).

Stress-Induced Hyperthermia

In the vehicle-treated mice, the body temperature of the last removed animals was significantly higher than that of the first removed mice (Table 2). Flibanserin did not induce hypothermia in the first removed animals at any dose. At 16 mg/kg, but not at 8 mg/kg, flibanserin prevented stress-induced hyperthermia. Also, buspirone, at 5 mg/kg, prevented stress-induced hyperthermia. Buspirone, at 10 mg/kg, induced hypothermia in the first removed animals.

Elevated Plus-Maze

Vehicle-treated animals spent only a little part of their time in exploring the open arms of the apparatus. In contrast to 0.125 and 0.250 mg/kg lorazepam, flibanserin (2, 4, 8, and 16 mg/kg) did not change any behavioral parameter observed in the elevated plus (data not shown).

TABLE 2

EFFECT OF FLIBANSERIN AND BUSPIRONE ON STRESS-INDUCED HYPERTHERMIA IN MICE

Treatment	Dose (mg/kg)	Rectal Temperature (°C) "First"	Rectal Temperature (°C) "Last"
Vehicle	—	37.7 (37.4–38.0)	38.4 (38.1–38.6)†
Flibanserin	8	37.2 (36.9–37.9)	38.3 (37.7–38.7)†
Flibanserin	16	37.3 (36.9–37.4)	37.6 (36.0–38.1)§
Vehicle	—	37.1 (36.9–37.3)	38.4 (38.1–38.5)†
Buspirone	5	37.0 (36.7–37.2)	37.5 (36.9–37.7)§
Buspirone	10	36.7 (36.2–36.9)‡	37.2 (36.8–37.6)*‡

Values represent median with interquartile range of 16 (experiment with flibanserin) or 8 (experiment with buspirone) mice. Drugs were administered IP 30 min before rectal temperature recording. "First" and "last" refer to the rectal termperature of the first and last four animals removed from the cage.

*$p < 0.05$, †$p < 0.01$ vs. respective "first" (Wilcoxon test for independent samples).

‡$p < 0.05$, §$p < 0.01$ vs. respective vehicle (Dunn test).

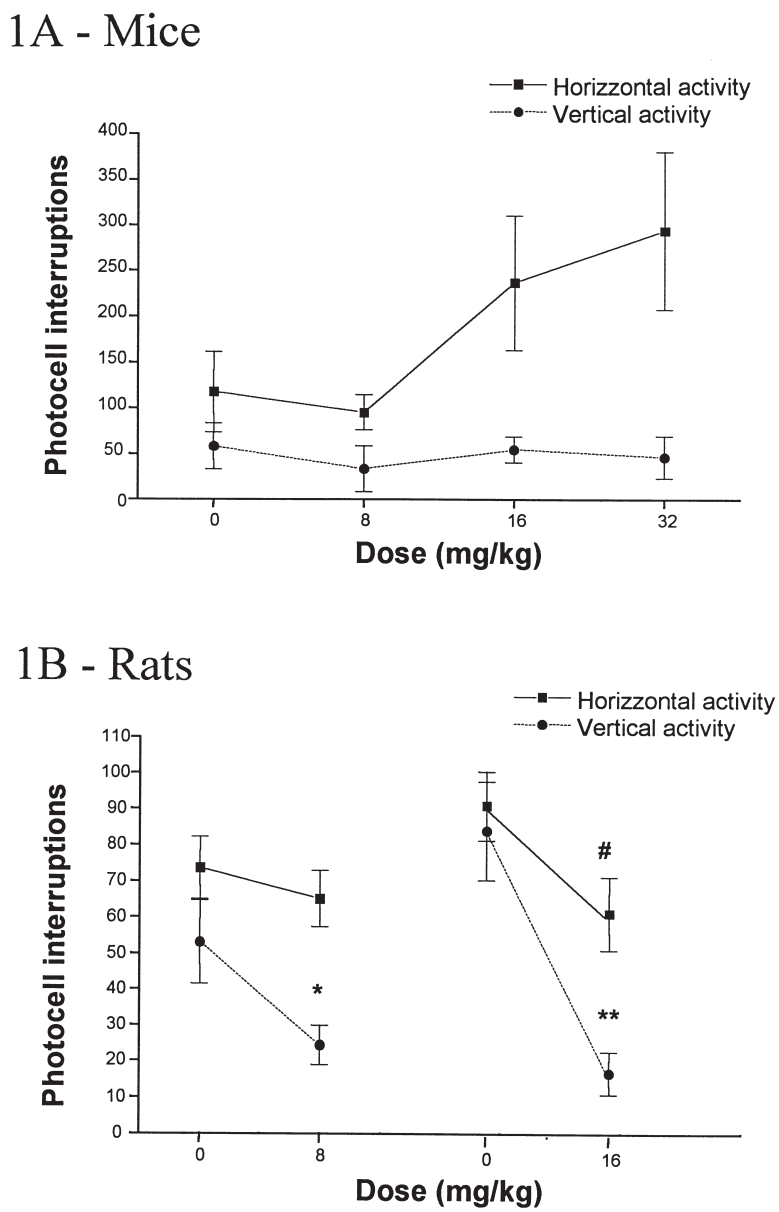

FIG. 1. Effect of flibanserin on spontaneous motor activity. Values represent mean ± SEM from eight animals. Motor activity was counted for a 60-min period, immediately after the IP drug administration. Mice (1A), but not rats (2A), were habituated to the apparatus for 30 min before the recording. #$p = 0.051$, *$p < 0.05$, **$p < 0.001$ vs. respective vehicle (one-way ANOVA).

Spontaneous Motor Activity

In mice, no dose of flibanserin significantly changed horizontal and vertical motor activity, even if 16 and 32 mg/kg flibanserin showed a trend ($p = 0.076$) to increase horizontal motor activity (Fig. 1A). In rats, flibanserin reduced vertical activity at 8 and 16 mg/kg, whereas a decrease in horizontal activity was observed only at the dose of 16 mg/kg (Fig. 1B).

Psychostimulant-Induced Hypermotility

d-Amphetamine (Table 3) and (+)SKF10047 (Table 4) increased motor activity in both mice and rats. The lower doses of flibanserin, 2 mg/kg in mice and 4 mg/kg in rats, were ineffective in altering *d*-amphetamine hypermotility. Flibanserin, at doses of 4, $F(1, 20) = 6.75$, $p < 0.05$, and 8 mg/kg in mice, and at the dose of 8 mg/kg in rats, $F(1, 28) = 7.63$, $p < 0.05$, antagonized the *d*-amphetamine-induced hypermotility (Table 3). Flibanserin, at doses of 4, $F(1, 28) = 24.64$, $p < 0.01$, and 8 mg/kg, $F(1, 20) = 9.22$, $p < 0.01$, in mice, and at the dose of 8 mg/kg, $F(1, 28) = 22.9$, $p < 0.01$, in rats, also antagonized the (+)SKF10047-induced hypermotility (Table 4). Flibanserin, at a dose of 2 mg/kg did not alter (+)SKF10047-induced hypermotility in mice.

TABLE 3

EFFECT OF FLIBANSERIN ON D-AMPHETAMINE-INDUCED HYPERMOTILITY IN MICE AND RATS

Species	Treatment	Dose (mg/kg)	Photocell Interruptions	
			Saline	*d*-Amphetamine
Mice	Vehicle	—	0.5 (0–3)	357 (282–579)*
	Flibanserin	2	18.5 (7–126)	395.5 (137–487)
	Vehicle	—	1 (0.26)	495.5 (405–650)*
	Flibanserin	4	16.5 (1–79)	208.5 (54–373)†
	Vehicle	—	4 (0–14)	412 (345–690)*
	Flibanserin	8	3.5 (0–13)	204 (74–264)†
Rats	Vehicle	—	30 ± 6.7	392 ± 73*
	Flibenserin	4	18 ± 4.3	373 ± 81
	Vehicle	—	23 ± 6.5	416 ± 45*
	Flibanserin	8	30 ± 5.7	182 ± 41†

Values represent median with interquartile range or mean ± SEM of six to eight animals. Flibanserin was administered 15 min before IP *d*-amphetamine (2.5 mg/kg in mice and 1.25 mg/kg in rats). Motor activity was recorded for 10 min starting 30 min after *d*-amphetamine administration in mice, or for 60 min starting immediately after *d*-amphetamine administration in rats. A Kruskal-Wallis test followed by a Wilcoxon test was used for the experiment with 8 mg/kg flibanserin in mice. All the other experiments were analyzed with factorial ANOVA followed by Student's *t*-test according to the Bonferroni criterion.

*$p < 0.01$ vs. respective vehicle + saline group; †$p < 0.05$ vs. respective vehicle + *d*-amphetamine group.

Stereotypy

d-Amphetamine-induced stereotypy lasted 240 min. Flibanserin, at doses of 8 and 16 mg/kg. reduced the intensity of *d*-amphetamine-induced stereotypy (Fig. 2). Apomorphine-induced stereotypy lasted 105 min. Flibanserin, at a dose of 16 mg/kg, but not at a dose of 8 mg/kg, attenuated the intensity of apomorphine-induced stereotypy (Fig. 2).

TABLE 4

EFFECT OF FLIBANSERIN ON (+)SKF-10047-INDUCED HYPERMOTILITY IN MICE AND RATS

Species	Treatment	Dose (mg/kg)	Photocell Interruptions	
			Saline	(+)SKF-10047
Mice	Vehicle	—	23 (0–51.5)	306 (229–326.5)*
	Flibanserin	2	0 (0–39)	203.5 (124–249)
	Vehicle	—	0 (0–33)	330.5 (195.5–385)*
	Flibanserin	4	59.5 (0–83.5)	80.5 (22–152.5)†
	Vehicle	—	7 (1–8)	197 (141–212)*
	Flibanserin	8	14.5 (0–43)	24.5 (7–95)†
Rats	Vehicle	—	24 ± 8.1	352 ± 65*
	Flibanserin	8	28 ± 9.1	57 ± 20

Values represent median with interquartile range or mean ± SEM of six to eight animals. Flibanserin was administered IP 15 min before SC 20 mg/kg (+)SKF10047. Motor activity was recorded for 10 min starting 30 min after (+)SKF-10047 administration in mice, or for 60 min starting immediately after (+)SKF-10047 administration in rats. Factorial ANOVA followed by Student's *t*-test according to the Bonferroni criterion was used for statistical analysis.

*$p < 0.01$ vs. respective vehicle + saline group; †$p < 0.01$ vs. respective vehicle + (+)SKF-10047 group.

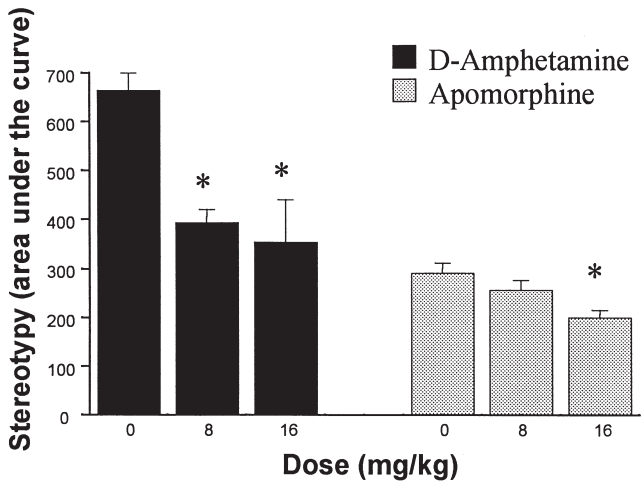

FIG. 2. Effect of flibanserin on stereotypy induced by *d*-amphetamine or apomorphine in rats. Values represent mean ± SEM of area under the curve, calculated for 240 min in case of *d*-amphetamine, and for 105 min in case of apomorphine. Each experimental group consisted of five rats. Animals were observed for 15 s every 15 min starting from IP 10 mg/kg *d*-amphetamine or SC 5 mg/kg apomorphine administration. Flibanserin or vehicle was given IP 15 min before psychostimulants. Stereotypy was scored from 0 to 4 according to Costall and Naylor (25,26). Statistics: one-way ANOVA followed by Dunnett's test. *$p < 0.01$ vs. respective vehicle.

Water Maze

During the days, vehicle-treated rats reduced their latency in finding the platform, the length of swimming path (Table 5), but failed to change their swimming speed. Vehicle- and 8, 16 (data not shown), and 32 mg/kg flibanserin-treated rats did not show any statistical difference in any of these parameters (Table 5). Latencies displayed by buspirone- and diazepam-treated animals were significantly higher than those expressed by vehicle-treated rats. Length of swimming path was only increased by diazepam. Swimming speed was unaltered by both buspirone and diazepam. In the spatial probe trial, buspirone worsened the behavior of animals (Table 5).

DISCUSSION

Flibanserin seems to possess anxiolytic-like effects in the light–dark exploratory test and the stress-induced hyperthermia. The effect of flibanserin in these two tests seems to be specific. In fact, it appears at the dose that does not alter motor activity in the light/dark exploratory test, and does not reduce body temperature in the stress-induced hyperthermia. Because flibanserin does not possess affinity for benzodiazepine receptors (10), it seems that its serotonergic component may be important in exerting anxiolytic-like effects. An anxiolytic-like effect has been reported with other 5-HT_{1A} agonists in both light/dark exploratory test (5,27,38,51) and stress-induced hyperthermia (39,48,56) in mice. In contrast, 5-HT_2 antagonists failed to induce such an effect in both tests (27,36,48). However, 5-HT_2 blockade has been reported to potentiate 5-HT_{1A} agonists in the light/dark exploratory test in both mice (27) and rats (64). Such an interaction between 5-HT_{1A} and 5-HT_2 receptors has not yet been investigated in the stress-induced hyperthermia model. Thus, the dual mechanism of action of flibanserin, as 5-HT_{1A} agonist and 5-HT_{2A}

TABLE 5

EFFECTS OF FLIBANSERIN, BUSPIRONE, AND DIAZEPAM IN THE WATER-MAZE TASK IN RATS

Treatment	Dose (mg/kg)	Days 1	2	3	4	5
Latency (s)						
Vehicle	–	>60 (53.5->60)	32 (20.5–56.5)	24 (20.5–31.5)	28.5 (11–38.5)	
Flibanserin	8	>60 (>60->60)	49.2 (22->60)	23.5 (16–37.5)	11.5 (7.5–27.5)	
Flibanserin	32	>60 (>60->60)	51.5 (24.5->60)	47.5 (21.5->60)	44.5 (15->60)	
Buspirone	2	>60 (>60->60)	>60 (49->60)	>60 (29.5->60)	45 (25->60)	
Diazepam	2	>60 (>60->60)	>60 (>60->60)	>60 (>60->60)	>60 (>60->60)	
Length of the swimming path (cm)						
Vehicle	–	760 ± 50	526 ± 71	491 ± 84	468 ± 85	
Flibanserin	8	814 ± 58	626 ± 69	462 ± 77	295 ± 46	
Flibanserin	32	772 ± 58	638 ± 99	626 ± 107	492 ± 99	
Buspirone	2	868 ± 57	770 ± 51	586 ± 75	660 ± 97	
Diazepam	2	853 ± 78	886 ± 105	1,049 ± 106	1,058 ± 100	
Swimming speed (cm/s)						
Vehicle	–	18 ± 1	14 ± 1	18 ± 2	20 ± 1	
Flibanserin	8	17 ± 1	18 ± 1	19 ± 2	19 ± 2	
Flibanserin	32	15 ± 1	18 ± 2	17 ± 2	16 ± 1	
Buspirone	2	18 ± 1	18 ± 2	16 ± 1	20 ± 2	
Diazepam	2	15 ± 1	17 ± 2	20 ± 2	19 ± 1	
Percentage of time spent in the 1st quadrant during the spatial probe trial						
Vehicle	–					33.9 ± 3.1
Flibanserin	8					40.5 ± 3.6
Flibanserin	32					30.8 ± 4.9
Buspirone	2					21.6 ± 2.9
Diazepam	2					29.1 ± 3.1

Values represent the median with interquartile range or the mean ± SEM of 12 rats. Flibanserin, buspirone, and diazepam were given IP each day, 30 min before behavioural testing. Statistics: test of equity for latency ($p < 0.01$ from the second day); split-plot for length of the swimming path [day × group, $F(12, 162) = 3.5$, $p < 0.01$] and swimming speed [day × group, $F(12, 162) = 1.54$, not significant]; ANOVA for the probe trial, $F(4, 55) = 3.7$, $p < 0.01$.

antagonist, might contribute to the flibanserin effect at least in the light/dark exploratory test.

Differently from its activity in anxiolytic-sensitive animal models in mice, flibanserin is inactive in the elevated plus-maze in rats. However, it should be pointed out that the effect of other 5-HT_{1A} agonists in this test is unclear. In fact, 5-HT_{1A} receptor agonists have been reported to exert either anxiolytic-like (23,30,31) or anxiogenic-like (23,35,53,68) effects, or even to be inactive (22,30,46,54). 5-HT_2 receptor antagonists have also been shown to exert anxiolytic-like (29,55) and anxiogenic-like (58) effects or even to be inactive (62). It has been suggested that the differences in effects may depend on the dose, animal species, and experimental conditions (35,37,40).

Flibanserin effects on motor activity seem test dependent. A clear reduction in motor activity was observed in mice after 30 min from flibanserin administration in the light/dark exploratory test, performed during the dark phase, but was not observed in the automated cages, during the light phase [see also (21)]. Similarly, in rats, flibanserin reduced motor activity when measured with photocells but did not alter the motor pattern of animals in the water maze (present results) and the learned-helplessness test (12). 5-HT_{1A} agonists have been reported to reduce motor activity at shorter times in both rats (1,15,52) and mice (5–7,38), and to increase it at longer times in both rats (34,41) and mice (7,34). However, in both experiments we performed in mice, the motor effects were measured at the same time and for a similar time period [present results; (21)]. Because it has been reported that 5-HT_2 blockade may reduce the increase in ambulation induced by a 5-HT_{1A} agonist (69) and considering the role of 5-HT_{1A} and 5-HT_{2A} receptors in some stressful conditions, further experiments are clearly needed to understand the complex interaction between flibanserin and environment (test) on motor activity.

However, at doses that do not interfere with spontaneous motor activity, flibanserin reduced *d*-amphetamine–induced hypermotility in both mice and rats. In addition, flibanserin also reduced (+)SKF-10047–induced hypermotility in rats. These effects have been shown to be displayed by dopamine antagonists, but flibanserin does not possess affinity for either dopamine D_1 or D_2 receptors (10). In addition to dopaminergic antagonists, this type of drug-induced hypermotility has been reported to be reduced by 5-HT_{1A} receptor agonists (61) and 5-HT_2 receptor antagonists (4,65). Thus, it is possible that the dual mechanism of action of flibanserin may play an important role in reducing drug-induced dopaminergic stimulation. This notion is also confirmed by the fact that flibanserin also blocks apomorphine- and amphetamine-induced stereotypy. The reduction of dopaminergic stimulation in basal ganglia by 5-HT_{1A} agonists and 5-HT_2 antagonists has already been reported (60,67). In contrast, flibanserin increases dopaminergic transmission in the forced swimming test (21). Dopaminergic mesolimbic and striatal systems have been shown to mediate the effect on motor activity and stereotypy (24), but very little is known about the dopaminergic system that is activated in the forced swimming test (14), although the dopaminergic system in the nucleus accumbens has been claimed

to play a role (18–20). Thus, it appears that flibanserin may inhibit or activate different dopaminergic brain pathways. That activation of 5-HT_{1A} receptors may induce discrete effects on the dopaminergic system has also been recently reported (61). In fact, these authors showed that 8-OH-DPAT attenuates locomotor but not discriminative effects of amphetamine and cocaine in rats.

The aforementioned effects of flibanserin may well be explained as a positive interaction of its 5-HT_{1A} agonist and 5-HT_{2A} antagonist activity, where the latter activity may enhance the former one. The only test where 5-HT_2 antagonism may counteract the 5-HT_{1A} agonism is the water maze. In fact, flibanserin does not impair rat performance in this test. In contrast, a deficit in learning has been reported by 5-HT_{1A} stimulation [present results; (16,43,50)], whereas some results suggest that the blockade of central 5-HT_2 receptors may have some beneficial cognitive effects (17). Thus, 5-HT_2 antagonism brought about by flibanserin may potentiate some actions or antagonize some detrimental ones induced by its 5-HT_{1A} activity.

The behavioral tests where the effect of a single administration of flibanserin was evaluated can be divided in two categories: those where flibanserin exerts effects similar to other 5-HT_{1A} agonists, and those where flibanserin induces effects different from other 5-HT_{1A} agonists. The first category is comprised of the light/dark exploratory test, stress-induced hyperthermia, reduction in motor activity, antagonism of psychostimulant-induced hypermotility and stereotypy, and the forced swimming test (21). The effect of flibanserin in these tests might be mediated by 5-HT_{1A} receptors, and may or may not benefit from concurrent 5-HT_{2A} receptor blockade. The second category is comprised of the Morris water maze, differential-reinforcement-of-low-rate 72 s (DRL 72-s), and learned helplessness (12). At present, it is difficult to say if the effects of flibanserin in this latter category of tests solely depends on its full and wide agonist spectrum of activity on 5-HT_{1A} receptors (which apparently differentiates flibanserin from the other 5-HT_{1A} agonists) or on combined activity as a 5-HT_{1A} agonist and 5-HT_2 antagonist.

There is a series of compounds claimed to be 5-HT_2 antagonists and 5-HT_{1A} agonists, as is flibanserin. These were compared in their antipunishment effects in the pigeon (45). There was no homogeneous picture of effects. In fact, two of the compounds, WY 50324 and S 14671, exhibited substantial effects in the pigeon conflict procedure; two others, FG 5974 and CGS 18102A, showed limited effects; and one, LEK 8804, was inactive. Thus, it seems that something else besides 5-HT_2 antagonism may play a role in their mechanism of action in this test. This suggests that the type of 5-HT_{1A} agonism may be important, and is particularly relevant in light of the heterogeneous pharmacology of 5-HT_{1A} receptors (9,33). For example, flibanserin induces 5-HT_{1A}–mediated electrophysiological effects in the cortex that are different from those exerted by S 14671 (11). Moreover, LEK 8804, which is weaker than WY-50324 in the conflict procedure and in producing lower lip reaction (44), is stronger than WY-50324 in inducing forepaw treading in rats (44).

As far as the 5-HT syndrome is concerned, with the exception of LEK 8804, an apparent common property among compounds with a mixed 5-HT_{1A} agonism/5-HT_2 antagonism (i.e., CGS-18102A, FG 5893, FG 5974, flibanserin, HT-90B, LY 165163, and WY-50324) is the low incidence of forepaw treading (2,10,41,44). However, it is difficult to ascertain whether the low incidence of serotonergic syndrome exerted by mixed 5-HT_{1A} agonist/5-HT_2 antagonists depends on this dual mechanism or solely on activity (full vs. partial) on particular 5-HT_{1A} receptors located in certain brain areas.

In conclusion, the results show that the 5HT_{1A} agonist/5-HT_{2A} antagonist flibanserin behaves as other 5-HT_{1A} agonists in some anxiolytic-sensitive and antipsychotic-sensitive tests. Differently from other 5-HT_{1A} agonists, it does not impair performance in the water maze test in rats, is inactive in the DRL 72-s paradigm, but improves, after a single administration, animal behavior in the learned-helplessness test (12). These findings, together with the possible preferential postsynaptic action of flibanserin (11,21,63), make this drug a new tool to better understand the pharmacology of 5-HT_{1A} receptors.

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