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Mechanism of action of flibanserin in the learned helplessness paradigm in rats

Franco Borsini [a,*], Raffaele Cesana [b]

[a] *CNS Department, Boehringer Ingelheim Pharma KG, Building J63, Birkendorfer Strasse 65, 88397 Biberach an der Riss, Germany*
[b] *Boehringer Ingelheim Italia, Via Lorenzini 8, 20139 Milan, Italy*

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Abstract

The mechanism of action of flibanserin, a 5-HT_{1A} receptor agonist and a 5-HT_{2A} receptor antagonist, was investigated in learned helplessness in rats. The effect of flibanserin (32 mg/kg, i.p. 30 min before testing) on learned helplessness was not antagonized by the (a) 5-HT synthesis inhibitor parachlorophenylalanine (pCPA; 150 mg/kg p.o. × 3 times), which reduced brain 5-HT by 89%; (b) 5-HT_{1A} receptor antagonists (±)-*N*-*tert*-butyl-3-4-(2-ethoxyphenyl)piperazin-1yl-2-phenyl propionamide [WAY100135; 10 mg/kg, i.p. 30 min before flibanserin, or 40 mg/kg, s.c. 15 min before flibanserin] and tertatolol (2.5 and 5 mg/kg, i.p. 30 min before flibanserin); and (c) 5-HT_2 receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI; 3 mg/kg, s.c. simultaneously with flibanserin). The effect of flibanserin on learned helplessness was antagonized by the dopamine D_1 receptor antagonist [*R*]-3-methyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390; 0.1 mg/kg, i.p. 30 min before flibanserin) and by the opioid receptor antagonist naloxone (3 mg/kg, s.c. 15 min before flibanserin). Flibanserin (32 and 64 mg/kg) did not induce conditioned place preference. In conclusion, flibanserin improved rats' performance in the learned helplessness paradigm, by stimulating dopamine D1 and opioid receptors, probably indirectly, since flibanserin has a low affinity for these receptors.

Keywords: Flibanserin; Learned helplessness; WAY100135; Tertatolol; DOI; pCPA (parachlorophenylalanine); SCH 23390; Naloxone; (Rat)

1. Introduction

Flibanserin, formerly known as BIMT 17, is a 5-HT_{1A} receptor agonist/5-HT_{2A} receptor antagonist (Borsini et al., 1998). In contrast to other purported 5-HT_{1A} receptor agonists, it directly and fully activates 5-HT_{1A} mediated effects in the cortex (Borsini et al., 1995a,b). Flibanserin also possesses 5-HT_{2A} receptor antagonistic properties (Borsini et al., 1998; Rueter and Blier, 1999). This dual action on 5-HT_{1A} and 5-HT_{2A} receptors at cortical level has been suggested to be an important mechanism to induce antidepressant effects (Borsini, 1994). Indeed, flibanserin has been shown to induce potential antidepressant-like effects in animal models such as the forced swimming test in mice (Cesana et al., 1995), chronic mild stress in mice and rats (D'Aquila et al., 1997), learned helplessness in rats (Borsini et al., 1997), bulbectomized rats (Borsini et al., 1997), distress call in isolated chicks and muricidal rats (Borsini and Cesana, in press). However, flibanserin does not induce antidepressant-like effects in the differential-reinforcement-of-low rate (DRL 72-s) paradigm (Borsini et al., 1997), the forced swimming test in rats or the tail suspension test in mice (Borsini and Cesana, in press).

In the forced swimming test in mice, flibanserin induces its activity through direct activation of 5-HT_{1A} receptors and, indirectly, via dopamine D_2 receptors (Cesana et al., 1995). With the present work we wanted to evaluate the mechanism of action of flibanserin in another animal paradigm sensitive to antidepressant drugs, the learned helplessness procedure (Willner, 1984). Consequently, the effect of flibanserin was evaluated in the presence of the following treatments: (a) parachlorophenylalanine (pCPA), an inhibitor of 5-HT synthesis (Koe and Weissman, 1966); (b) (±)-*N*-*tert*-butyl-3-4-(2-ethoxyphenyl)piperazin-1yl-2-phenyl propionamide (WAY100135) and tertatolol, selective and non-selective 5-HT_{1A} receptor antagonists, respectively

* Corresponding author. Tel.: +49-7351-547297; fax: +49-7351-5498647.
E-mail address: Franco.Borsini@bc.boehringer-ingelheim.com (F. Borsini).

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(Fletcher et al., 1993a; Jolas et al., 1993); and (c) (±)-2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT_2 receptor agonist (Pranzatelli, 1990). The choice of WAY100135 and tertatolol was based on the fact that these two 5-HT_{1A} receptor antagonists have already been shown to counteract electrophysiological (Borsini et al., 1995a), biochemical (Borsini et al., 1995b) and behavioural (Cesana et al., 1995) effects of flibanserin. Since none of these compounds reduced flibanserin effects in the learned helplessness test, and since it has been suggested that dopamine D_1 and opioid receptors play a role in this test (Besson et al., 1996, 1999; Gambarana et al., 1995a,b; Martin et al., 1990b), the effects of flibanserin were also assessed in the presence of the following treatments: (a) [*R*]-3-methyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH 23390), a dopamine D_1 receptor antagonist (Hyttel, 1983); and (b) naloxone, a μ-opioid receptor antagonist (Satoh and Minami, 1995). We found that both SCH 23390 and naloxone antagonized flibanserin. Such activation of dopamine D_1 and μ-opioid receptors has been reported to play a role in the mechanism of action of several antidepressants (Besson et al., 1999; D'Aquila et al., 1994; Devoize et al., 1984; Gambarana et al., 1995a,b; Gray et al., 1998; Martin et al., 1986; Sampson et al., 1991; Schreiber et al., 1999; Valverde et al., 1994). However, since the stimulation of these receptors has also been involved in reinforcing effects of drugs (Mello and Negus, 1996), we also evaluated flibanserin in this regard, by using the place preference paradigm in rats.

2. Materials and methods

2.1. General

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).

2.2. Animals

Male Wistar rats (Charles River Italia), weighing 200–250 g at the beginning of experiments, were group housed in a regulated environment (21 ± 1 °C, 50–55% relative humidity, 12 h light–dark cycle, light on at 6:00 or 7:00 a.m.), with water and food available ad libitum.

2.3. Learned helplessness in rats

2.3.1. Helplessness induction

Animals were randomly assigned to groups and subjected to one session of inescapable footshock. Electric footshocks were delivered to the animals in eight chambers (22 cm wide × 22 cm long × 27 cm high) with grey Plexiglas walls and covers, equipped with a stainless-steel grid floor (Ugo Basile). A constant-current shocker delivered 60 scrambled, randomized inescapable shocks (15-s duration, 1.2 mA, every 30 ± 24 s) to the grid floor. All the helplessness induction trials were performed in the morning.

2.3.2. Shuttle-box test

It was carried out according to Geoffroy et al. (1990). A single test session took place 4 days after the helplessness induction in the morning. Four two-way shuttle-boxes (Ugo Basile, mod. 7502) were used. After an initial 5-min habituation, a total of 25 unsignalled escape trials were presented to each animal (each trial started with simultaneous onset of a light and a 0.8-mA scrambled electric shock). In the first 10 trials the rats were required to change side once (FR1) and in the following 15 trials the rats had to change side twice (FR2), in order to terminate both shock and light. The shock duration was 15 s max with an intertrial time of 25 s. The number of escape failures (i.e., the failure to respond during the 15-s shock on) and the number of intertrial crossings (i.e., the number of side-to-side crossings during the 25-s shock off) were recorded by means of a computerized data acquisition system (Basilink, Ugo Basile). For the evaluation of the rat's performance, only the FR2 escape responses were analysed. It has already been noted that, in fact, rats exposed to inescapable shock often do learn FR1 shuttle-box response, whereas the FR2 response is always reduced (Maier and Seligman, 1976).

2.3.3. Experimental design

Four groups of 8 or 10 rats each were used as follows: vehicle + vehicle; vehicle + flibanserin; agonist or antagonist + vehicle; agonist or antagonist + flibanserin. Each experiment of interaction (32 or 40 rats) was divided into two experimental blocks of 16 or 20 rats each (four or five rats each group). Flibanserin was injected i.p. 30 min before testing. The dose of flibanserin we used was 32 mg/kg. This dose was selected by a preliminary dose–response study with a few animals. Tertatolol (2.5 and 5 mg/kg, i.p.), SCH 23390 (0.1 mg/kg, i.p.), naloxone (3 mg/kg, s.c.) and DOI (3 mg/kg, s.c.) were administered 30, 30, 15 and 0 min before flibanserin, respectively. WAY100135 was given either intraperitoneally at a dose of 10 mg/kg 30 min before flibanserin or subcutaneously at a dose of 40 kg/kg 15 min before flibanserin. pCPA was given at three oral doses of 150 mg/kg on day 1 at 9:00 a.m. and at 4:00 p.m., and on day 2 at 9:00 a.m. (for a total of 450 mg/kg; Valzelli et al., 1981), and flibanserin was administered 24 h after the last pCPA administration.

2.3.4. Drugs

Flibanserin (Boehringer Ingelheim Italia) was dissolved in the following vehicle: 25% v/v polyethylene glycol-400 in water (1 ml glycol + 3 ml water). (−)Tertatolol hydrochloride (Servier), (±)WAY100135 dihydrochloride (syn-

thesised in the Medicinal Chemistry Department of Boehringer Ingelheim Italia), (*R*)SCH 23390 hydrochloride and (±)DOI (R.B.I.), naloxone (Endo Lab.) and pCPA methyl ester hydrochloride (Aldrich) were dissolved in saline. All the compounds were given at a constant volume of 2 ml/kg, with the exception of pCPA, which was given at a volume of 5 ml/kg. Doses refer to base.

2.3.5. Statistics

The data (FR2 escape failures and intertrial crossings) were expressed as medians and interquartile range. The effect of treatment was analysed by the Kruskal–Wallis test followed by Dunn test. The statistical evaluations were carried out with the program system SAS (SAS Institute, Cary, NC), version 6.07 on a DEC Computer, o.s. VMS.

2.4. 5-HT assay

After completion of the learned helplessness, rats treated with vehicle + vehicle or pCPA + vehicle were killed by cervical dislocation. The brain was removed quickly, the cerebellum was dissected out and the remaining tissues were frozen in dry ice and stored at − 80 °C until assay, which was performed according to the method described by Cesana et al. (1995).

2.5. Place preference in rats

2.5.1. Behavioural procedure

The apparatus consisted of a rectangular plexiglas chamber that is divided into two square-based compartments (30 cm wide × 30 cm long × 38 cm high) by a partition provided by a guillotine door (5 × 7 cm). One compartment has three white walls and some sawdust on the floor, whereas the other compartment has three grey walls and a smooth floor. The back walls of the two compartments and the cover are semi-transparent. Six of these chambers were used. During the experiment, a constant light was provided to each chamber by two 8 W neon lamps placed in front of the semi-transparent walls. The experiment was composed of three phases. During the first phase (pre-conditioning), to determine the baseline preference, rats were allowed free access to both compartments for 15 min on each of 3 consecutive days. On the fourth day, the time spent by the rat in each compartment, during a 15-min session, was recorded and this time was taken as an index of the spontaneous preference of each animal. At the end of this phase, only the rats that exhibited a preference for the grey compartment (about 75%) were used for the following phases of the experiment and assigned to the experimental groups. In fact, in agreement with other authors (Neisewander et al., 1990; Papp, 1988), we have observed that, following conditioning, the vehicle-treated rats that initially preferred the white compartment changed their preference to the grey one, whereas the spontaneous preference for the grey one was maintained. The second phase (conditioning), starting on day 7, lasted 4 days. The guillotine door between the two compartments was closed. Each day, immediately after drug or vehicle injection, the rats were exposed for 45 min to each compartment with an interval of 4 h in balanced order: once in the morning and once in the afternoon. Rats were intraperitoneally given flibanserin (32 and 64 mg/kg) or morphine (2 mg/kg) or vehicle immediately before the exposure to the unpreferred (white) compartment. All the rats received intraperitoneally vehicle immediately before exposure to the preferred compartment. The day following the 4-day conditioning phase, rats were given a preference test (third phase) without administering any compound. The guillotine door, separating the two compartments, was removed allowing free access to the entire apparatus for 15 min. During this period an observer, unaware of the treatment, recorded the time spent in each compartment. Because of considerable individual differences in spontaneous preference, before the conditioning, a matching procedure was used to divide the rats into four experimental groups, each consisting of six animals. The rats were assigned to groups with equivalent means of spontaneous place preference. The sequence of the treatment was chosen according to a complete randomized schedule.

2.5.2. Drugs

Morphine hydrochloride (Salars) and flibanserin were dissolved in the following vehicle: 25% v/v polyethylene glycol-400 in water (1 ml glycol + 3 ml water). All the compounds were given at a constant volume of 2 ml/kg. Doses refer to base.

2.5.3. Statistics

The degree of place preference conditioning was expressed as means ± S.E.M. of the differences between the time spent in the drug-paired compartment during the post-conditioning test and that spent during the pre-conditioning test. Since values were normally distributed and variances homogeneous, the effect of conditioning was evaluated by analysis of variance followed by a two-tailed Dunnett's *t*-test for post-hoc comparisons. The statistical evaluations were carried out

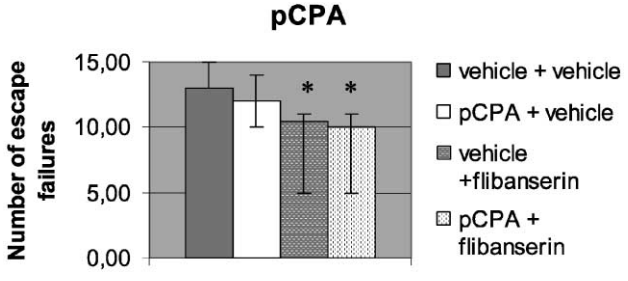

Fig. 1. Effect of pCPA on flibanserin-induced reduction of escape failures in the learned helplessness. Columns represent medians with interquartile of 10 rats. Flibanserin (32 mg/kg) was given intraperitoneally 30 min before testing. pCPA (150 mg/kg × 3 times p.o.) was given 48, 41 and 24 h before flibanserin. * $P < 0.01$ vs. control group.

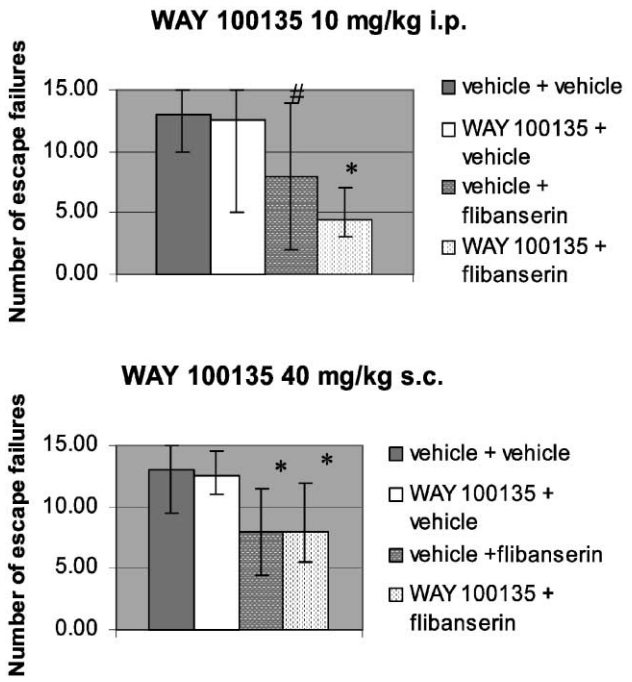

Fig. 2. Effect of (±)WAY 100135 on flibanserin-induced reduction of escape failures in the learned helplessness. Columns represent medians with interquartile of 8 (10 mg/kg) or 10 (40 mg/kg) rats. Flibanserin (32 mg/kg) was given intraperitoneally 30 min before testing. (±)WAY 100135 was given intraperitoneally at 10 mg/kg 30 min before flibanserin or administered subcutaneously at 40 kg/kg 15 min before flibanserin. $^{\#}P<0.07$, $^{*}P<0.01$ vs. control group.

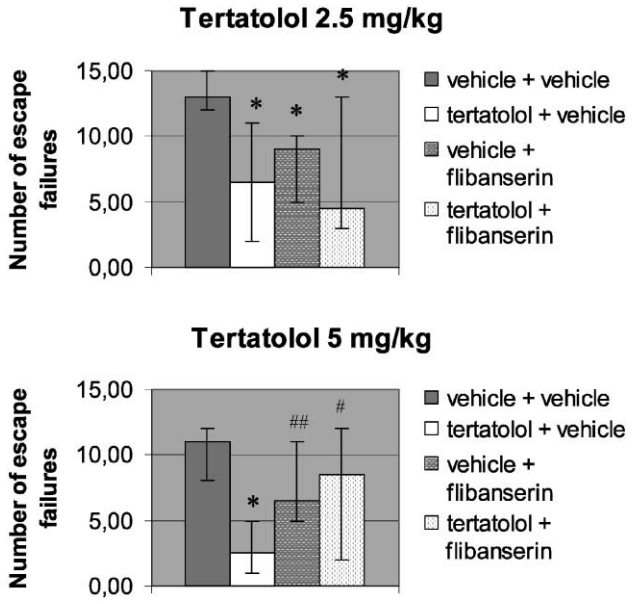

Fig. 3. Effect of (−)tertatolol on flibanserin-induced reduction of escape failures in the learned helplessness. Columns represent medians with interquartile of 10 rats. Flibanserin (32 mg/kg) was given intraperitoneally 30 min before testing. (−)Tertatolol was given intraperitoneally 30 min before flibanserin. $^{*}P<0.01$ vs. control group; $^{\#\#}P<0.05$, $^{\#}P<0.06$ vs. tertatolol-vehicle group.

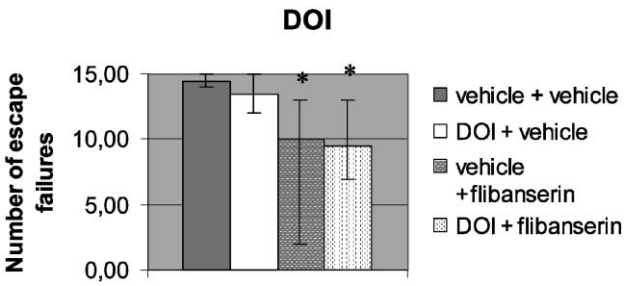

Fig. 4. Effect of (±)DOI on flibanserin-induced reduction of escape failures in the learned helplessness in rats. Columns represent medians with interquartile of 10. Flibanserin (32 mg/kg) was given intraperitoneally 30 min before testing. DOI (3 mg/kg) was given subcutaneously simultaneously with flibanserin. $^{*}P<0.01$ vs. control group.

with the program system SAS (SAS Institute), version 6.07 on a DEC Computer, o.s. VMS.

3. Results

3.1. Learned helplessness

Flibanserin reduced the number of escaping failures in all the experiments with a $P<0.05$, except in the experiment with 10 mg/kg WAY100135 where it reduced the number of escapes with a $P<0.07$ (Fig. 2), and in the experiment with 5 mg/kg tertatolol where the P was >0.07.

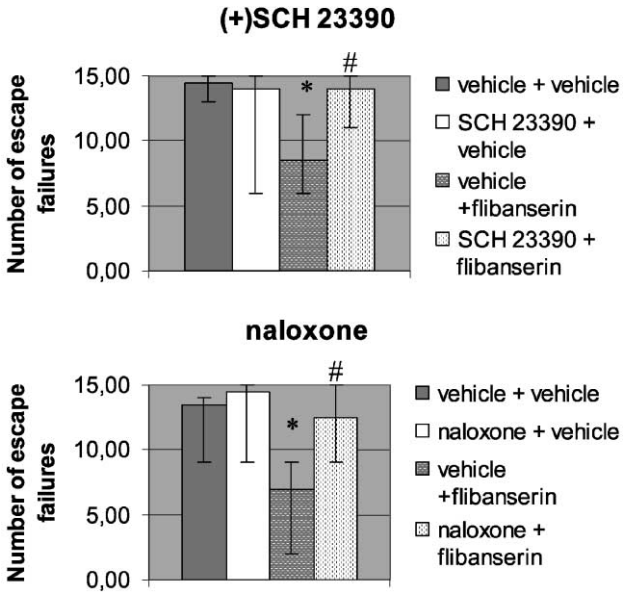

Fig. 5. Effect of (+)SCH 23390 and naloxone on flibanserin-induced reduction of escape failures in the learned helplessness in rats. Columns represent medians with interquartile of 10. Flibanserin (32 mg/kg) was given intraperitoneally 30 min before testing. (+)SCH 23390 (0.1 mg/kg, i.p.) and naloxone (3 mg/kg, s.c.) were given 30 and 15 min before flibanserin respectively. $^{*}P<0.05$ vs. control group; $\#P<0.05$ vs. vehicle + flibanserin group.

pCPA, which per se did not affect the number of escape failures and reduced brain 5-HT by 89% (control group: 569 ± 74 ng/g tissue; pCPA group: 64 ± 22 ng/g tissue), failed to affect flibanserin effect (Fig. 1). WAY 100135 was initially used at 10 mg/kg and given intraperitoneally 30 min before flibanserin. This dose was chosen because it was active to counteract the effect of flibanserin in the forced swimming test in mice (Cesana et al., 1995). WAY 100135, at 10 mg/kg, did not change per se the number of escape failures and did not modify the effect of flibanserin (Fig. 2). In order to completely block 5-HT_{1A} receptors, the dose of WAY100135 was augmented up to 40 mg/kg and given subcutaneously 15 min before flibanserin (Deren-Wesolek et al., 1998). Also at this dosage, WAY100135 did not change per se the number of escape failures and did not modify the effect ($P < 0.07$) of flibanserin (Fig. 2). Given alone, tertatolol (2.5 and 5 mg/kg) significantly reduced the number of escape failures (Fig. 3). The combination of flibanserin and 2.5 mg/kg tertatolol did not appear to change the effects of the individual drugs. However, the combination of flibanserin with 5 mg/kg tertatolol seems to induce an affect that it is smaller than the effect of tertatolol itself (Fig. 3); in this particular experiment, flibanserin effect was not statistically significant ($P > 0.07$). Thus, it seems that flibanserin blocked tertatolol action.

DOI (3 mg/kg) did not change per se the number of escape failures and did not modify the effect of flibanserin (Fig. 4). Both SCH 23390 (0.1 mg/kg) and naloxone (3 mg/kg) did not change per se the number of escape failures and antagonized the effect of flibanserin (Fig. 5). The various groups of animals did not show any difference in the intertrial crossing with respect to the control group (data not shown), with the exception of the animals treated with 5 mg/kg tertatolol and flibanserin (median and interquartile in brackets): saline ± vehicle = 0.5 (0–2.5); saline ± flibanserin = 2.5 (1–6.5); tertatolol ± vehicle = 3 (0.5–7.5); tertatolol + flibanserin = 4.5 mg/kg (1.5–7.5), $P < 0.05$.

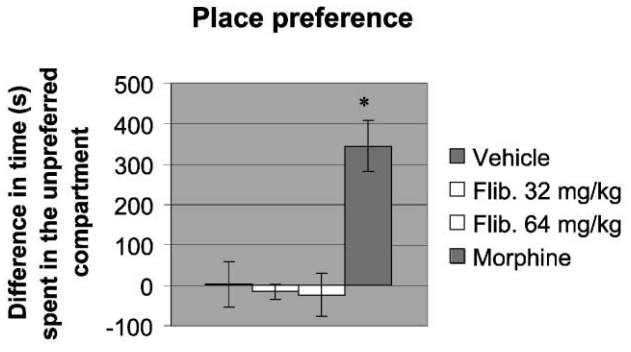

Fig. 6. Effect of flibanserin and morphine in the place preference test in rats. Columns, which represent the means ± S.E.M. of six rats, indicate the difference between the time spent in the unpreferred compartment before and after drug conditioning. Flibanserin (32 and 64 mg/kg) or morphine (2 mg/kg) or vehicle was given intraperitoneally immediately before each 45 min conditioning exposure to the unpreferred compartment for 4 consecutive days. * $P < 0.01$ vs. vehicle.

3.2. Place preference

In contrast to morphine, which induced a significant increase in the time spent in the paired compartment, flibanserin (32 and 64 mg/kg) did not induce conditioned place preference of rats (Fig. 6).

4. Discussion

In agreement with previous results (Borsini et al., 1997), flibanserin reduced the escape failures after a single administration without altering the intertrial crossings. This is a particular important confirmation considering that most antidepressants are only effective after repeated administration (Besson et al., 1999; Borsini et al., 1997; Gambarana et al., 1995a,b; Geoffroy and Christensen, 1993), and most anxiolytics do not reverse established helplessness (Martin and Puech, 1996; Sherman et al., 1982).

The fact that 5-HT depletion, brought about by pCPA, did not alter flibanserin effects suggests that flibanserin action does not require interaction with 5-HT containing neurons.

pCPA itself did not change the number of escape failure, in spite of an 89% reduction in 5-HT brain levels. In a previous study (Edwards et al., 1986), pCPA was found to reduce escape failures and to deplete brain 5-HT by 50–60%. However, other authors have not observed any change in escape failures with pCPA (Anisman et al., 1979) or with similar 5-HT depletion (about 40–70%) by using the selective neurotoxin for 5-HT containing neurons 5,7-dihydroxytryptamine (5,7-DHT; Martin et al., 1990a; Soubrie et al., 1986). Thus, our results support the notion that a large depletion of 5-HT is not necessary for changing performance in the learned helplessness paradigm. pCPA also failed to alter flibanserin effects in the forced swimming test in mice (Cesana et al., 1995). Thus, postsynaptic mechanisms seem to be responsible for the action of flibanserin in these animal paradigms sensitive to antidepressants. Furthermore, it was already suggested that 5-HT_{1A} postsynaptic mechanisms may be important in improving the behaviour in the learned helplessness (Martin et al., 1990a, 1991), and flibanserin behaves as a postsynaptic 5-HT_{1A} receptor agonist (Borsini et al., 1995a,b). Thus, it was surprising that, in contrast to the forced swimming test in mice, where intraperitoneal 10 mg/kg WAY100135 antagonized flibanserin, WAY100135 at the same dose did not counteract flibanserin in the learned helplessness paradigm in rats. Species difference does not seem to account for this discrepancy in the effect of WAY100135, since the same dose of WAY100135 has been found also to antagonize the effects of 5-HT_{1A} receptor agonists in rats (Cervo et al., 1994; Deren-Wesolek et al., 1998). WAY100135 did not counteract flibanserin even at the high dose of 40 mg/kg. In this particular experiment, flibanserin exerted a weak effect ($P < 0.07$), making more difficult to observe an antagonism; however, the median values and interquartile ranges of the

two groups of animals with or without WAY100135, in the presence of flibanserin, were completely overlapping (Fig. 2). Thus, flibanserin exerts its effects in the learned helplessness in rats by activating mechanisms that are different from those that it activates in the forced swimming test in mice. Possibly, the different dosage (16 mg/kg in mice and 32 mg/kg in rats) might account for these different effects. Since WAY100135 has been shown to block 5-HT_{1A} receptor-mediated activity (Fletcher et al., 1993a), one might rule out the activation of 5-HT_{1A} receptors in the mechanism of action of flibanserin in the learned helplessness paradigm. In order to confirm this hypothesis, we used tertatolol as a 5-HT_{1A} receptor antagonist, since it has already been shown that it blocks electrophysiological (Borsini et al., 1995a) and biochemical (Borsini et al., 1995b) effects of flibanserin. However, the results with tertatolol are difficult to interpret, as it improved per se the behavioural performance of rats in the learned helplessness model. The beta-adrenolytic component of tertatolol might play a role in this test, even if it is difficult to understand how. In fact, beta-adrenergic antagonists have been reported either to reduce (Danchev et al., 1989) or not to change the number of escape failures (Martin et al., 1986), and to attenuate the beneficial effect of antidepressants in this test (Martin et al., 1986). Also the antagonism of tertatolol on 5-HT_{1A} receptor cannot explain the activity of tertatolol per se. In fact, WAY100135, which selectively blocks 5-HT_{1A} receptors, did not change the number of escape failures. At present, we do not understand the reason of this strong activity of tertatolol in learned helplessness. The doses we used for tertatolol (2.5 and 5 mg/kg) are in the range of those that have been reported to antagonize 5-HT_{1A} receptor agonist-induced effects in rats (Sanchez et al., 1995; Schreiber et al., 1995). When used at the lower dose, tertatolol failed to antagonize flibanserin. The experiment with 5 mg/kg tertatolol cannot be considered as a valid experiment to reveal an antagonism towards the effect of flibanserin. In fact, in this particular interaction study, flibanserin did not reduce the number of escape failures with significant probability. However, it is worth noting that the effect of 5 mg/kg tertatolol was antagonized by flibanserin, and no additive or synergic effects were seen, as one should have been expected by two compounds exerting the same effect. However, one cannot exclude that the behaviour of rats administered with 5 mg/kg tertatolol and flibanserin may depend on increased motor activity that these rats displayed. Thus, we have not proven in the present study that the effect of flibanserin is mediated by 5-HT_{1A} receptors. However, recently, it has been suggested that 5-HT_{1A} receptor antagonists, by blocking 5-HT_{1A} somatic autoreceptors, may favour rather than reduce the effects of serotonergic drugs (Ahlenius and Larsson, 1999; Bourin et al., 1998; Hashimoto et al., 1997). Actually, a tendency to an increased action of flibanserin was noted after the lower dose of WAY100135. Thus, it might be that the failure of the 5-HT_{1A} receptor antagonists in reducing flibanserin effects might depend on a subtle balance between pre- and postsynaptic 5-HT_{1A} receptor blockade. Nevertheless, this explanation must be considered speculative at the present time.

Flibanserin has been shown to block 5-HT_{2A} receptors (Borsini et al., 1995b, 1998; Rueter and Blier 1999). However, DOI, at a dosage that has been shown to stimulate 5-HT_2 receptors in rats (Arnt and Hyttel, 1989; Millan et al., 1998), did not alter the effect of flibanserin, suggesting that flibanserin blockade of 5-HT_{2A} receptors does not contribute to flibanserin action in the learned helplessness. This was surprising, since 5-HT_{2A} receptor blockade has been reported to have a beneficial effect in the learned helplessness (Rinaldi-Carmona et al., 1992), and flibanserin at 30 mg/kg occupies about 70% of 5-HT_{2A} receptors in the rat brain (Scandroglio et al., 2001). However, it should be noted that the use of only one dose of DOI might be a limitation to investigate the role of 5-HT_2 receptors in the mechanism of action of flibanserin. On the other hand, it was difficult to increase the dose of DOI because of its behavioural effects, which could have interfered with the rat's performance.

The effect of flibanserin was blocked by SCH 23390, a dopamine D_1 receptor antagonist, and by naloxone. A direct action of flibanserin on these receptors may be excluded since flibanserin has a low affinity for these receptors (Borsini et al., 1998). Flibanserin increases dopamine extracellular concentration in the rat brain at 10 mg/kg (Invernizzi R., "Mario Negri Institute", Milan, Italy, personal communication), thus flibanserin may indirectly activate dopamine receptors. A similar hypothesis was also put forward to explain the blocking action of sulpiride, a dopamine D_2 receptor antagonist, of flibanserin effects in the forced swimming test in mice (Cesana et al., 1995). It is worth noting that other 5-HT_{1A} receptor agonists have also been reported to induce some effects that are resistant to blockade of 5-HT_{1A} receptors (Smith and Cutts, 1990; Millan, 1995). Additionally, dopamine receptor antagonists have been reported to reduce some effects of 5-HT_{1A} receptor agonist (Smith and Cutts, 1990; Shippenberg, 1991). To understand how flibanserin interacts with μ-opioid receptors is more difficult, since it has been reported that 5-HT_{1A} receptor stimulation may be reduced by μ-opioid receptor agonists (Powell et al., 1994) rather than by μ-opioid receptor antagonist (Galeotti et al., 1997). However, it must be pointed out that both μ-opioid receptor stimulation (Giacchino and Henriksen, 1998) and flibanserin (Borsini et al., 1998) reduce the firing rate of neurons of prefrontal cortex and hippocampus. Both areas are important for the learned helplessness (Petty and Sherman, 1980; Petty et al., 1982, 1992, 1994). Thus, it may be that the behaviour of animals in our experiments may depend on concurrent neuronal stimulation by endogenous endorphins, released during the footshock stress (Maier, 1984; Kamata et al., 1986; Tejedor-Real et al., 1995), and by flibanserin. Naloxone might reduce the behavioural effects of flibanserin by blocking the effect of endogenous release of

endorphins on neuronal firing. However, this explanation must be considered speculative at the present time. The involvement of dopamine D1 and μ-opioid receptors has already been observed in the mechanism of action of several antidepressants (Besson et al., 1999; D'Aquila et al., 1994; Devoize et al., 1984; Gambarana et al., 1995a,b; Gray et al., 1998; Martin et al., 1986; Sampson et al., 1991; Schreiber et al., 1999; Valverde et al., 1994). Thus, similarly to other antidepressants, flibanserin seems to interfere with dopaminergic and opioid systems without exerting reinforcing properties, as revealed by the lack of effect in the place preference test. The finding that flibanserin did not induce conditioned place preference differentiates flibanserin from other 5-HT_{1A} receptor agonists, such as buspirone, gepirone and 8-OH-DPAT. In fact, those compounds have been reported to induce conditioned place preference (Neisewander et al., 1990; Shippenberg, 1991; Fletcher et al., 1993b). It has been shown that activation of presynaptic 5-HT_{1A} receptors may induce conditioned place preference (Fletcher et al., 1993b). In contrast, stimulation of postsynaptic 5-HT_{1A} receptors may induce conditioned place aversion (Papp and Willner, 1991). Thus, the lack of the effect of flibanserin in inducing conditioned place preference may depend on the fact that flibanserin, differently from other 5-HT_{1A} receptor agonists that preferentially act presynaptically (Borsini et al., 1995a,b, 1998), may equally activate pre- and postsynaptic 5-HT_{1A} receptors (Borsini et al., 1998). Additionally, a role may also be played by the 5-HT_{2A} receptor antagonist properties of flibanserin, as 5-HT_{2A} receptor antagonism has been reported to antagonize drug-induced conditioned place preferences (Nomikos and Spyraki, 1988; see Arolo and McMillen, 1999).

In our experiments, the effects of flibanserin in non-shocked animals were not assessed. We have doubts that non-shocked subjects represent a true control group for drug effects in shocked subjects. The non-shocked group is believed to be useful to evaluate possible drug effects on learning. Flibanserin has not shown any effect in a learning task (Borsini et al., 1999). Additionally, cognition-enhancing drugs do not seem to affect the behaviour of non-shocked rats (Leshner et al., 1978; Cavoy et al., 1988). Thus, we decided not to evaluate the effect of flibanserin in non-shocked subjects.

In conclusion, the mechanism of action of flibanserin in the learned helplessness in rats appears more complex than in the forced swimming test in mice (Cesana et al., 1995). In the learned helplessness test, flibanserin does not activate 5-HT_{1A} receptors and increases dopamine D_1 and opioid receptor activity.

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