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Anti-anhedonic actions of the novel serotonergic agent flibanserin, a potential rapidly-acting antidepressant

Paolo D'Aquila [a], Santiago Monleon [b], Franco Borsini [c], Paul Brain [d], Paul Willner [a,*]

[a] *Department of Psychology, University of Wales, Swansea SA2 8PP, UK*
[b] *Area de Psicobiologia, Facultad de Psicologia, Universidad de Valencia, Ap. 22109, 46071 Valencia, Spain*
[c] *Boehringer Ingelheim Italia, via Lorenzini 8, Milan 20130, Italy*
[d] *Department of Biological Sciences, University of Wales, Swansea SA2 8PP, UK*

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Abstract

Chronic exposure to mild unpredictable stress has previously been found to depress the consumption of palatable sweet solutions and to block the formation of conditioned place preferences; these effects are reversed by chronic treatment with tricyclic or atypical antidepressant drugs. The present study was designed to evaluate the antidepressant-like activity in this model of flibaserin (BIMT-17), a novel serotonergic agent with 5-HT_{1A} receptor agonist and 5-HT_2 receptor antagonist properties. Two experiments were conducted, using rats (experiment 1) and mice (experiment 2). In experiment 1, decreases in sucrose intake were seen in rats exposed to chronic mild stress, but the effect was unreliable in this study, and sucrose testing was terminated after 7 weeks of stress. Beginning after 5 weeks of stress, groups of control and stressed animals were treated daily with vehicle, fluoxetine (5 mg/kg) or flibanserin (5, 10 or 20 mg/kg). After 6 weeks of treatment, all animals were tested for acquisition of food-reinforced place preference conditioning. Conditioning was seen in all groups other than the vehicle-treated stressed animals. We also tested the locomotor stimulant effect of a single injection of the dopamine D_2/D_3 receptor agonist quinpirole (0.2 mg/kg). The effect of quinpirole was potentiated by fluoxetine in control animals, and by both fluoxetine and flibanserin (all doses) in stressed animals. In experiment 2, long-lasting decreases in sucrose intake were seen in mice exposed to chronic mild stress. The effects were reversed by chronic (4 weeks) treatment with fluoxetine (5 mg/kg) or flibanserin (2.5 or 5 mg/kg); the full effect of flibanserin was seen after the first injection. All animals received a single injection of raclopride (0.1 mg/kg) immediately prior to a sucrose intake test on day 27 of drug treatment. Raclopride decreased sucrose intake only in the three drug-treated stressed groups. The results support a rapid antidepressant-like action of flibanserin, and suggest that this effect involves sensitization of dopamine D_2/D_3 receptor-mediated transmission. © 1997 Published by Elsevier Science B.V.

Keywords: Stress; Anhedonia; Sucrose drinking; Place preference conditioning; Reward; Locomotor activity; Flibanserin; Fluoxetine; Quinpirole; Raclopride; (Rat); (Mouse)

1. Introduction

Chronic sequential exposure to mild unpredictable stress suppresses rewarded behaviours in rats and mice. Consumption and preference for palatable sweet solutions are reduced (Willner et al., 1987, 1992; Muscat et al., 1990, 1992; Monleon et al., 1994), animals appear unable to associate rewarding (but not aversive) stimuli with a distinctive environment, as assessed by the place conditioning paradigm (Papp et al., 1991, 1992, 1993a,b) and there is an increase in the threshold for intracranial self-stimulation reward via electrodes implanted in the ventral tegmental area (Moreau et al., 1992, 1993). As stress is implicated in the aetiology of depression (Lloyd, 1980; Kanner et al., 1981; Anisman and Zacharko, 1982; Brown and Harris, 1988), the chronic mild stress paradigm may provide a relatively realistic animal model of the decreased response to rewards (anhedonia) that characterizes melancholia (Klein, 1974; Nelson and Charney, 1981; Fawcett et al., 1983; American Psychiatric Association, 1987).

Chronic treatment with antidepressant drugs reverses the hedonic deficits caused by chronic mild stress (Willner et al., 1992; Willner and Papp, 1997). Drugs shown to be effective include tricyclics (Willner et al., 1987; Muscat et al., 1990; Monleon et al., 1994), the specific monoamine

* Corresponding author. Tel.: +44-1792-295844; fax: +44-1792-295679; e-mail: p.willner@swansea.ac.uk.

uptake inhibitors fluoxetine and maprotiline (Muscat et al., 1992), the atypical antidepressant mianserin (Cheeta et al., 1994; Moreau et al., 1994), and the monoamine oxidase inhibitors moclobemide (Moreau et al., 1993) and brofaromine (Papp et al., 1996). Ineffective agents include chlordiazepoxide (Muscat et al., 1992), D-amphetamine and haloperidol (Papp et al., 1996). In the present study we evaluated the therapeutic efficacy in the chronic mild stress model of flibanserin (BIMT-17), a novel serotonergic agent with 5-HT_{1A} receptor agonist and 5-HT_2 receptor antagonist properties (Borsini et al., 1995a,b), which has antidepressant-like activity in the forced swim and learned helplessness tests (Cesana et al., 1995; Borsini et al., 1997). For comparison purposes, the specific 5-HT uptake inhibitor fluoxetine (Bremner, 1986; Asberg et al., 1986) was included as a positive control.

Antidepressant drugs have traditionally been assumed to exert their clinical effects through an interaction with noradrenergic or serotonergic systems. However, antidepressants also potentiate the locomotor stimulant effects of dopamine D_2/D_3 receptor agonists, after chronic administration. These effects are apparent following systemic administration (Willner and Montgomery, 1981; Martin-Iverson et al., 1983; Arnt et al., 1984; Maj, 1988; Maj et al., 1984a,b) or direct injection of dopamine D_2/D_3 receptor agonists into the nucleus accumbens (Maj, 1988; Maj and Wedzony, 1985, 1988; Maj et al., 1987). We have previously demonstrated that acute administration of dopamine receptor antagonists reduces the consumption of a sweet solution in chronically stressed rats successfully treated with tricyclic or atypical antidepressants, at doses that did not reduce consumption in non-stressed animals or in untreated stressed animals. These data argue strongly that an increase in dopamine receptor responsiveness is responsible for the therapeutic action of antidepressant drugs in this model (Muscat et al., 1990, 1992; Sampson et al., 1991; Cheeta et al., 1994). We therefore also investigated whether flibanserin shares with conventional antidepressants the property of increasing the behavioural response to an acute challenge with the selective dopamine D_2/D_3 receptor agonist quinpirole (Fuller and Hemrick-Luecke, 1985; Sokoloff et al., 1990).

2. Method

2.1. Experiment 1

2.1.1. Subjects

Male Lister hooded rats, from the breeding stock maintained at University of Wales, Swansea were used. At the start of the experiment, the animals were 11 weeks old and weighed 250–300 g. All animals were singly housed, beginning one week before the start of the experiment, except where grouped as part of the stress procedure (see below). They were maintained on a 12:12 h light–dark cycle and tested in the light phase (cf. Willner et al., 1987). Body weight was measured weekly throughout the experiment.

2.1.2. Sucrose intake tests

All animals were first trained to consume a palatable weak (1%) sucrose solution. Training consisted of an initial 48 h exposure to sucrose in place of water, followed by four 1 h tests (2/week over 2 weeks) in which sucrose was presented, in the home cage, following 19 h food and water deprivation; intake was measured by weighing preweighed bottles at the end of the test. Subsequently, sucrose consumption was measured, in 1 h tests (12.00–13.00 h), at weekly intervals for the next 9 weeks.

2.1.3. Stress procedure

On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group ($n = 55$) was subjected for a total of 12 weeks to chronic mild stress; a variety of mild stressors were applied, each for a period of between 0.5 and 20 h. Stressors could occur at any time of day, except during the final two weeks of the study, when stressors were administered at night only, in order not to impinge directly on the behavioural testing procedures conducted during the day. The second group ($n = 55$) was deprived of food and water for 19 h preceding each sucrose intake test, and as required by the place conditioning procedure (see below), but otherwise food and water were freely available in the home cage.

The stress regime applied to the rats was almost identical to that used in other recent studies in this laboratory (D'Aquila et al., 1994), and consisted of: one 19 h period of food and water deprivation immediately prior to the sucrose intake test; one additional 23 h period of food deprivation; two additional (20 h and 7 h) periods of water deprivation; two periods of continuous overnight illumination; three 7 h periods of 45 degree cage tilt; two 24 h periods of paired housing; two periods (17 and 24 h) in a soiled cage (100 ml water in sawdust bedding); two periods (17 and 21 h) of restricted movement (confinement in a mouse cage). All of the individual stressors used were classified as being, at worst, mildly stressful, under the terms of the relevant (U.K.) legislation, the Animals (Scientific Procedures) Act of 1986.

2.1.4. Drug treatment

On the basis of their sucrose intake scores following 5 weeks of stress, each group of rats was divided into five matched subgroups ($n = 11$), using a computerised matching procedure which ensured that the subgroups differed in their mean sucrose intakes by less than 0.1 g. Subsequently, separate groups of control and stressed animals were treated chronically with vehicle, fluoxetine (5 mg/kg), or flibanserin (5, 10 and 20 mg/kg). Drugs were injected daily, between 17.00 and 19.00 h, for a total of 7 weeks.

2.1.5. Locomotor activity

During week 11 of stress (week 6 of drug treatment), all animals were tested, on each of two successive days, in an automated locomotor activity monitoring system. The system consisted of 12 clear perspex chambers ($35 \times 35 \times 20$ cm) with metal grid floors (1 cm mesh), separated by opaque dividers. Each chamber contained two photocells, which monitored locomotor activity by detecting breaks in 2 parallel light beams situated 18 cm apart and 5.5 cm above the floor. Testing took place between 10.00 and 15.00 h. 45 min prior to the second test, animals received a single injection of quinpirole (0.2 mg/kg).

2.1.6. Place conditioning

During week 12 of stress (week 7 of drug treatment) all animals were trained in a food-rewarded place preference conditioning procedure. Place conditioning was conducted in six identical wooden chambers, containing white and black arms ($30 \times 15 \times 15$ cm), with different floor textures (plain wood or wire mesh, respectively), and a central gray area ($15 \times 15 \times 15$ cm). Tests were carried out between 09.00 h and 17.00 h. For the first 3 days the animals were allowed freely to explore the whole chamber for 15 min daily. On day 4 the time spent in each arm was measured in a 10-min pre-conditioning test. On days 5–10, the animals received a series of 30-min training trials, preceded by 22 h of food deprivation, in which the animals were confined on alternate days in each of the two arms. Food pellets (standard lab chow) and a water bottle were freely available in the white arm; the amount of food eaten was measured by weighing the food remaining at the end of each session. No food (or water) was available in the black arm; following confinement in the black arm, the animals were fed for 2 h on their return to the home cage. Changes in side-preference were measured on day 11 in a 10 min post-conditioning test; no rewards were available during this test, and the animals were not food deprived.

2.2. Experiment 2

2.2.1. Subjects

CD1 mice, from the breeding stock maintained at University of Wales, Swansea, were used. At the start of the experiment, the animals were 3 weeks old and weighed 18–22 g. All animals were singly housed, beginning one week before the start of the experiment, except where grouped as part of the stress procedure (see below). They were maintained on a 12:12 h reversed light:dark cycle and tested in the dark phase under dim red light (cf. Monleon et al., 1994). Body weight was measured weekly throughout the experiment.

2.2.2. Sucrose intake tests

All animals were first trained to consume a palatable weak (2%) sucrose solution. Training consisted of an initial 48 h exposure to sucrose in place of water, followed by four 1 h tests (2/week over 2 weeks) in which sucrose was presented, in the home cage, following 3 h of food and water deprivation; intake was measured by weighing pre-weighed bottles at the end of the test. Subsequently sucrose consumption was measured, in 1 h tests (12.00–13.00 h), 3 times weekly for a total of 6 weeks.

2.2.3. Stress procedure

On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group ($n = 51$) was subjected for a total of 6 weeks to a chronic mild stress procedure in which stressors could occur at any time. The second group ($n = 51$) was deprived of food and water for 3 h preceding each sucrose intake test, but otherwise food and water were freely available in the home cage.

The stress regime was identical to that used in our earlier mouse study (Monleon et al., 1994), and consisted of: three 3 h periods of food and water deprivation, one of which was immediately prior to the sucrose test; one additional 16 h period of water deprivation; two periods of continuous overnight illumination; two periods (7 and 17 h) of 45 degree cage tilt; two periods (3 and 5 h) of intermittent exposure to a 2 min, 30 dB, 10 kHz tone, played at iregular intervals; three periods (7, 9 and 17 h) of low intensity stroboscopic illumination (300 flashes/min). All of the individual stressors used were classified as being, at worst, mildly stressful, under the terms of the relevant (UK) legislation, the Animals (Scientific Procedures) Act of 1986.

2.2.4. Drug treatment

On the basis of their sucrose intake scores following 2 weeks of stress, each group of mice was divided into four matched subgroups ($n = 12–13$). Although the difference in sucrose intake between control (2.85 ± 0.17 g) and stressed animals (2.04 ± 0.16 g) was significant overall [$F(1,100) = 11.9$, $P < 0.001$], the larger standard errors of the subgroups led to there being a relatively small separation between control and stressed animals within each pair of subgroups. As the evaluation of drug effects on sucrose intake requires the presence, throughout the experiment, of a significant difference in intake between vehicle-treated control and stressed animals, the separation between control and stressed animals was artificially inflated by eliminating at this stage the animal with the lowest sucrose intake from each control subgroup and the animal with the highest sucrose intake from each stressed subgroup.

Separate groups of control and stressed animals were treated chronically with vehicle ($n = 12$), fluoxetine (5 mg/kg: $n = 12$), flibanserin (2.5 mg/kg: $n = 12$) or flibanserin (5 mg/kg: $n = 11$). Drugs were injected daily, between 17.00 and 19.00 h, for a total of 29 days. 15 min prior to the sucrose intake test on day 28, all animals received a single injection of raclopride (0.1 mg/kg). One further sucrose intake test was carried out on day 30.

2.3. Drugs

The following agents were used in this study: fluoxetine hydrochloride (Lilly, Indianapolis); flibanserin hydrochloride (BIMT-17: Boehringer Ingelheim Italia, Milan); quinpirole hydrochloride (Lilly, Indianapolis); raclopride tartrate (Astra, Södertälje). All drugs were dissolved in distilled water, which was used for control injections, and injected i.p. in a volume of 1 ml/kg (rats) or 10 ml/kg (mice) body weight. Doses of drugs refer to the salt.

2.4. Statistical analysis

Results were analyzed by analysis of variance, supplemented by tests of simple main effects and *F*-tests for contrasts, using the appropriate analysis of variance error term (Winer, 1971). The analyses of data from experiment 1 involved two between-subjects factors (stress/control and drug treatment) and where appropriate, one within-subjects factor (successive tests). In order to optimize the power of the analysis, data from experiment 2 were converted to a random blocks design (Festing, 1994). Animals were ranked, within each of the eight experimental subgroups, according to their sucrose intake on the final test prior to the start of drug treatment (see above); they were then matched highest to highest through lowest to lowest, forming 12 blocks, each containing the animal of equivalent rank in every group. (For the purposes of this analysis, which requires equal group sizes, a dummy subject was created in each of the flibanserin (5 mg/kg) groups, using the group mean values.) Two separate analyses were performed, the first including data collected during the first 24 days of drug treatment, and the second, carried out to evaluate the effects of raclopride, covering the final 3 tests (days 25–30). A further analysis of the first phase of the experiment was carried out in which each group was divided into high and low drinkers, on the basis of a median split of their scores in the final test prior to the start of drug treatment.

3. Results

3.1. Experiment 1

3.1.1. Body weight

In experiment 1 (rats) body weight fell significantly, relative to control animals, during the first 5 weeks of stress (control: 333 ± 3 g; stress: 288 ± 3 g; $n = 55$), and this difference persisted, in vehicle-treated animals, to the end of the experiment [control: 355 ± 3 g; stress: 322 ± 6 g; $n = 11$; $t(20) = 4.9$, $P < 0.001$].

Weight gain over the 7 weeks of drug treatment is shown in Table 1. Analysis of variance revealed a significant stress × drug interaction [$F(4,100) = 2.5$, $P < 0.05$]. Further analysis showed that there were no significant differences among the five nonstressed groups [$F = 0.9$], but the five stressed groups did differ significantly [$F(4,100) = 4.5$, $P < 0.01$], with the fluoxetine and flibanserin (20 mg/kg) groups gaining weight less rapidly than vehicle-treated animals [$F(1,100) = 11.0$, 5.8, respectively, $P < 0.025$].

Table 1
Weight gain (g) in rats over the 7 weeks of drug treatment[a]

	Control	Stress
Vehicle	22.3 (3.6)	36.5 (3.9)
Fluoxetine	14.5 (3.9)	12.3 (6.0)***
Flibanserin (5 mg/kg)	14.6 (9.0)	37.3 (4.4)
Flibanserin (10 mg/kg)	23.0 (3.6)	28.0 (3.3)
Flibanserin (20 mg/kg)	24.3 (4.6)	18.8 (5.7)**

[a]Values are means (with S.E.). At the start of drug treatment, controls weighed 333 ± 3 g and stressed animals 288 ± 3 g; at the end of the experiment, vehicle-treated control animals weighed 355 ± 3 g and vehicle-treated stressed animals 322 ± 6 g.
** $P < 0.02$; *** $P < 0.002$, relative to vehicle-treated stressed animals.

3.1.2. Sucrose intake

In the final baseline test prior to the onset of stress, sucrose intake in rats was identical (mean ± standard error: 11.3 ± 0.3 g) in control and to-be-stressed groups. During the period prior to drug administration, sucrose intake decreased significantly in stressed animals [Fig. 1: $F(1,100) = 23.0$, $P < 0.001$]. However, these changes were somewhat erratic, with significant decreases ($P < 0.001$) seen after 1, 3 and 5 weeks of stress, but no decrease at 2 and 4 weeks. During the subsequent period of chronic drug treatment, sucrose intakes in vehicle-treated animals did not differ significantly between control and stressed animals (Fig. 1). This means that there was no baseline against which to evaluate the effects of chronic drug treatment.

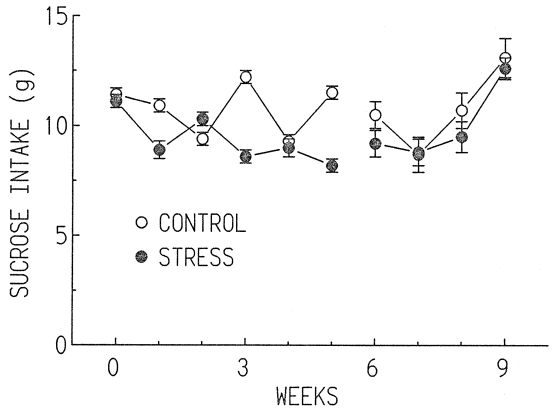


Fig. 1. Sucrose intake (rats) in 1 h weekly tests (experiment 1: rats). Up to week 5, data represent the means (±standard error) of all control and stressed animals ($n = 55$). Drug treatment commenced after 5 weeks of stress, and subsequent data (weeks 6–9) represent the vehicle-treated subgroups ($n = 11$).

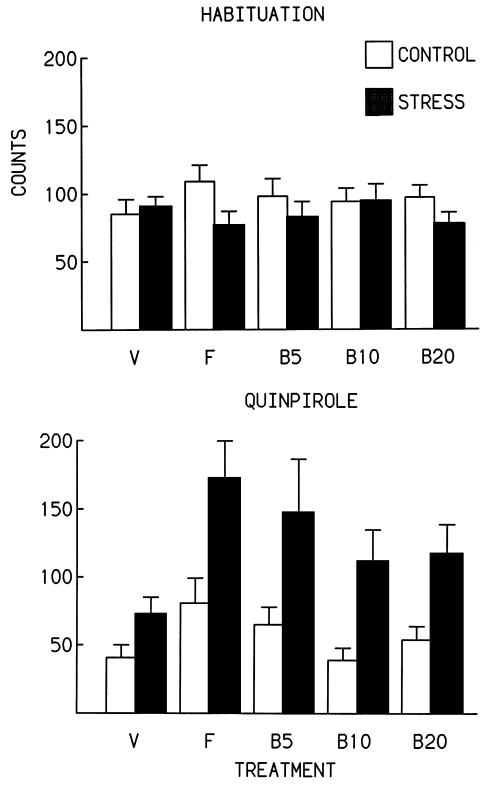


Fig. 2. Locomotor activity (counts) in the habituation session (left) and following administration of quinpirole to rats (right). Values are means (+standard error). Treatments: V, vehicle; F, fluoxetine; B5, flibanserin (BIMT-17) 5 mg/kg; B10, flibanserin (BIMT-17) 10 mg/kg; B20, flibanserin (BIMT-17) 20 mg/kg.

3.1.3. Locomotor activity

On the initial exposure to the locomotor activity monitoring apparatus, all groups of rats showed habituation over the course of the 45 min session (mean ± standard error locomotor counts across all groups: 0–15 min, 46.5 ± 1.5; 15–30 min, 27.2 ± 1.3; 30–45 min, 17.8 ± 1.2), but there were no significant differences between groups (Fig. 2, left; stress: $F = 1.0$; drugs: $F = 0.4$; interaction: $F = 1.1$). However, following quinpirole administration (Fig. 2, right), there was a significant effect of stress [$F(1,100) = 9.1$, $P < 0.01$], which reflects the greater responses to quinpirole shown by drug-treated stressed animals.

In order to examine these effects in greater detail, the effects of quinpirole were expressed as the difference in counts recorded with and without quinpirole (day 2–day 1) in each 15-min period (Fig. 3). Analysis of these data showed significant effects of stress [$F(1,100) = 47.2$, $P < 0.001$], drug treatment [$F(4,100) = 6.5$, $P < 0.001$] and time period [$F(2,200) = 181.7$, $P < 0.001$]. There were also significant interactions of both stress and drug treatment with time period [$F(2,200) = 23.1$, $P < 0.001$; $F(8,200) = 3.4$, $P < 0.001$; respectively], and a significant 3-way interaction [$F(8,200) = 2.4$, $P < 0.02$].

In the first 15 min (Fig. 3, top), all groups were less active on day 2 than on day 1, with the exception of the fluoxetine-treated stressed group. However, by the final 15 min (Fig. 3, bottom), pronounced differences between groups had emerged [stress, $F(1,100) = 70.5$, $P < 0.001$; drugs, $F(4,100) = 7.9$, $P < 0.001$]. Vehicle-treated stressed animals did not differ significantly from vehicle-treated controls [$F(1,100) = 3.9$, NS], but all of the drug-treated stressed groups were more active than their respective drug-treated control groups [minimum $F(1,100) = 8.8$, $P < 0.01$]; the effect was greatest in the group treated with the lowest dose of flibanserin (5 mg/kg). Nonstressed animals treated with fluoxetine were significantly more active than vehicle-treated animals [$F(1,100) = 4.5$, $P <$

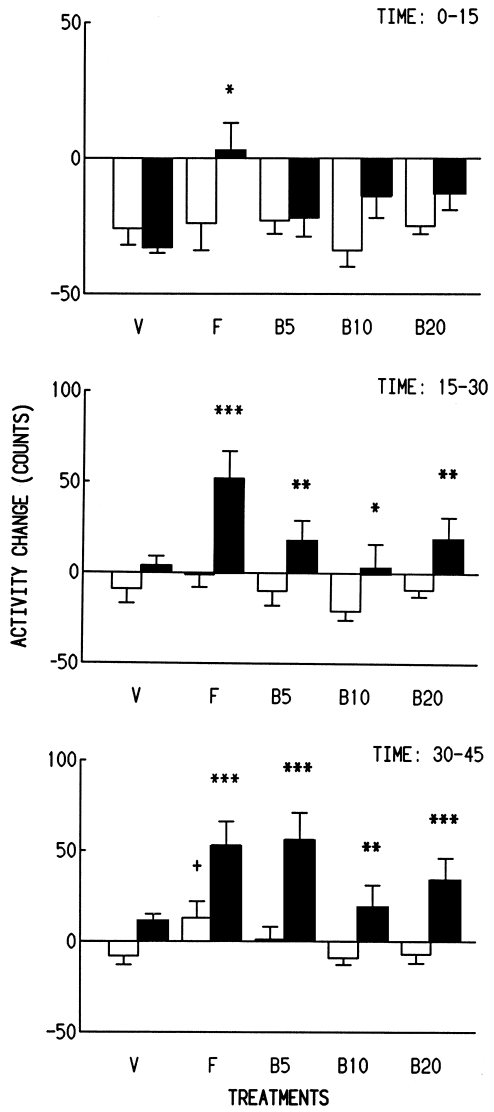


Fig. 3. Change in locomotor activity following quinpirole treatment in rats. Data are the mean (±S.E.) difference in locomotor activity (quinpirole session-habituation session) in each 15 min period. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, relative to corresponding nonstressed group. +, $P < 0.05$ relative to vehicle-treated nonstressed animals. Treatments: V, vehicle; F, fluoxetine; B5, flibanserin (BIMT-17) 5 mg/kg; B10, flibanserin (BIMT-17) 10 mg/kg; B20, flibanserin (BIMT-17) 20 mg/kg.

0.05]. However, this effect was not seen in any of the three flibanserin-treated groups [maximum $F = 0.9$, NS].

3.1.4. Place preference conditioning

Time spent in the white (food-associated) side of the place conditioning apparatus before and after conditioning is shown in Fig. 4. Prior to place conditioning rats spent more time on the black side of the apparatus (mean = 617 s) than on the white side (mean = 96 s). Although the groups receiving different drug treatments varied somewhat in time spent on the white side prior to conditioning, these differences were not significant [$F(4,200) = 1.7$, NS]; however, stressed animals spent more time on the white side than nonstressed controls [$F(1,200) = 10.5$, $P < 0.01$]. After conditioning, time on the white side increased in all five nonstressed groups [$F(1,100) = 34.5$, $P < 0.001$], and in all four of the drug-treated stressed groups [$F(1,100) =$ 17.1, 13.2, 14.8, 11.0 respectively, all $P < 0.002$]. However, the vehicle-treated stressed group showed no sign of conditioning [$F = 0.1$, NS]. Data for time spent in the black side of the apparatus (not shown) were essentially a mirror image of the data shown in Fig. 4.

Food intake on the two place conditioning trials is shown in Table 2. Analysis of variance showed a significant main effect of stress [$F(1,100) = 14.4$, $P < 0.001$], reflecting a lower food intake in all of the stressed groups relative to their respective nonstressed controls. Analysis of simple main effects showed that the difference was significant only in the case of the two flibanserin (5 mg/kg) groups [$F = 4.3$, $P < 0.05$]; in particular, the two vehicle-treated groups did not differ significantly [$F = 3.0$, NS]. There was also a significant main effect of treatment [$F(4,100) = 3.1$, $P < 0.05$], reflecting a tendency for the vehicle-treated animals to eat less than drug-treated animals. The only significant effect revealed by analysis of simple main effects was between control animals treated with vehicle and flibanserin (5 mg/kg); the vehicle-treated stressed animals did not differ significantly from any other stressed group [max $F(1,100) = 3.5$, NS]. Because the vehicle-treated stressed group had the lowest food intakes, albeit not differing significantly on any of the relevant comparisons, we examined the relationship between food intake (mean of two conditioning trials) and change in preference (from pre- to post-test) in this group. In fact, there was a significant negative correlation between these two variables (Spearman's rho = -0.69, $P < 0.02$). Examination of the individual scores revealed that the only three animals in this group to increase their preference for the food-associated side had the lowest food intakes.

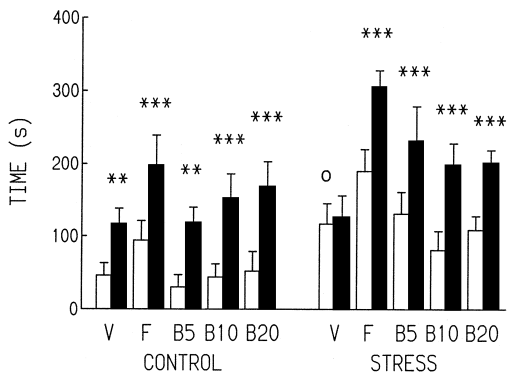


Fig. 4. Effects of chronic mild stress on food-rewarded place preference conditioning in controls and stressed rats. The data are time spent in the food-associated (white) side of the chamber before (open bars) and after (closed bars) conditioning. Comparisons are between pre- and post-conditioning scores: $^{**}P < 0.01$; $^{***}P < 0.001$; ○, not significant. Treatments: V, vehicle; F, fluoxetine; B5, flibanserin (BIMT-17) 5 mg/kg; B10, flibanserin (BIMT-17) 10 mg/kg; B20, flibanserin (BIMT-17) 20 mg/kg.

Table 2
Food intake (g) on place conditioning trials[a]

	Trial 1		Trial 2	
	Control	Stress	Control	Stress
Vehicle	5.6 (0.6)	4.4 (0.6)	5.7 (0.3)	5.3 (0.4)
Fluoxetine	6.8 (0.4)	5.5 (0.3)	6.3 (0.3)	6.0 (0.3)
Flibanserin (5 mg/kg)	6.5 (0.4)	5.8 (0.4)	6.7 (0.2)	5.6 (0.3)
Flibanserin (10 mg/kg)	5.5 (0.4)	5.2 (0.4)	6.0 (0.2)	5.2 (0.4)
Flibanserin (20 mg/kg)	6.2 (0.3)	5.1 (0.6)	6.1 (0.4)	5.7 (0.3)

[a]Values are means (with S.E.).

3.2. Experiment 2

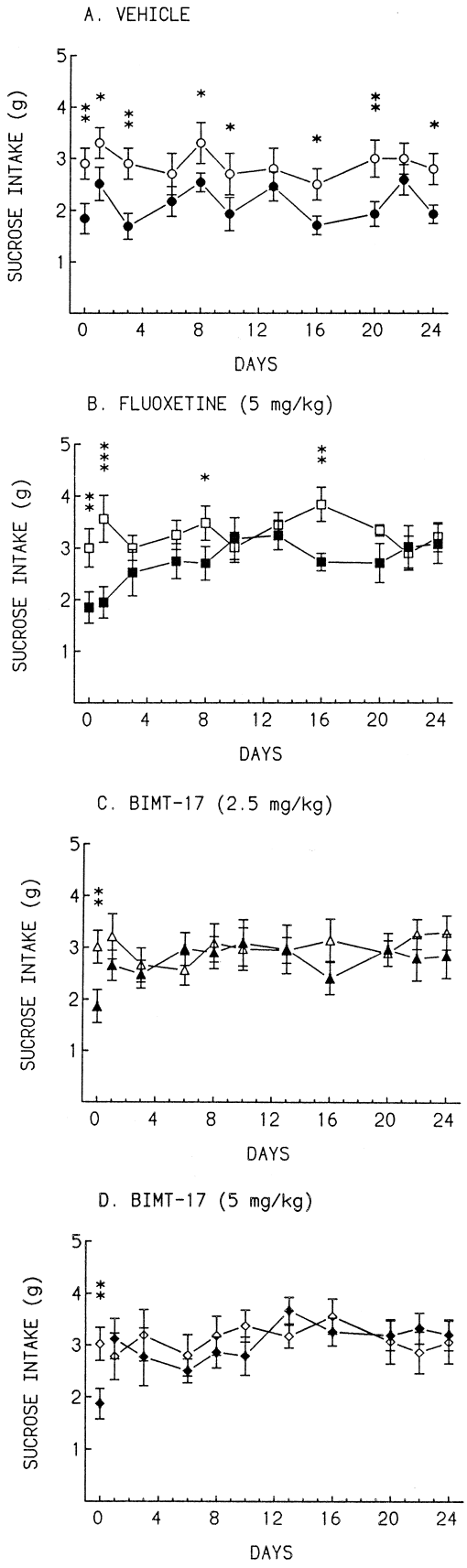
3.2.1. Body weight

In experiment 2 (mice) body weight was not significantly affected by either stress or drug treatment [all F values for main effects and interaction < 1].

3.2.2. Sucrose intake

Analysis of sucrose intake data in mice (Fig. 5) revealed significant main effects of stress [$F(1,11) = 16.5$, $P < 0.002$], drug treatment [$F(3,33) = 3.6$, $P < 0.025$] and time [$F(10,110] = 2.3$, $P < 0.02$], and a significant stress × time interaction [$F(10,110) = 3.3$, $P < 0.001$]. In order to ensure that the data were not distorted by the exclusion of one animal from each group at the start of drug treatment (see Section 2), the data were reanalyzed with an additional factor, high vs. low drinkers. The interactions of this factor with stress and drug treatment were non-significant, as were all three of the higher order interactions with time [max $F = 1.3$], indicating that the effects of stress and drugs were similar in high and low drinkers.

Sucrose intake in vehicle-treated control and stressed mice remained relatively constant across the period of vehicle treatment [control, $F(10,440) = 0.7$; stressed, $F(10,440) = 1.4$; NS]. As a result, the difference in sucrose intake between the two vehicle-treated groups was maintained throughout the experiment [simple main effect of stress: $F(1,44) = 8.9$, $P < 0.01$; stress × time interaction,



$F(10,440) = 0.6$, NS], although the fluctuation in scores from test to test led to the difference not being significant at every time-point (Fig. 5A).

Chronic treatment with fluoxetine did not affect sucrose intake in control mice, but caused a gradual recovery of sucrose drinking in stressed animals, leading to a smaller overall effect of stress [$F(1,44) = 4.3$, $P < 0.05$] and a significant stress × time interaction [$F(10,440) = 2.18$, $P < 0.025$]. The difference between stressed and control animals became nonsignificant after 3 days of fluoxetine treatment, and stressed animals showed a significant improvement from their own pre-drug baseline from day 6 of treatment [$F(1,440) = 4.6$, $P < 0.05$] onwards. However, significant differences between control and stressed animals reemerged on some later trials (days 8 and 16), and the data suggest that three weeks of treatment were needed for complete recovery (Fig. 5B).

In contrast to the gradual onset of action of fluoxetine, flibanserin appeared to exert rapid antidepressant-like effects, particularly at the higher dose. The differences between flibanserin-treated control and stressed animals disappeared after only a single injection, and did not reappear thereafter (Fig. 5C and 5D). As a result, there was no significant effect of stress in either of the flibanserin-treated groups, and the stress × time interactions were also nonsignificant [stress: $F(1,44) = 1.1$, 0.2, respectively; interaction: $F(10,440) = 1.4$, 1.7, respectively; NS]. At the lower dose of flibanserin (2.5 mg/kg), stressed animals showed significant improvements, relative to their own pre-drug baseline, from day 6 of treatment [$F(1,440) = 7.2$, $P < 0.01$] onwards, similar to fluoxetine-treated animals. However, animals receiving the higher dose of flibanserin (5 mg/kg) were improved relative to their own baseline as early as day 1 [$F(1,440) = 9.2$, $P < 0.01$].

A more stringent criterion for antidepressant-like action is to compare drug-treated with vehicle-treated stressed animals. All three drug-treated stressed groups had higher sucrose intakes overall than the vehicle-treated stressed group [$F(1,66) = 4.8$, $P < 0.05$ (fluoxetine); 5.2, $P < 0.05$ (flibanserin, 2.5 mg/kg); 10.0, $P < 0.01$ (flibanserin, 5 mg/kg)]. This effect was significant at 6 of the 11 time-points, and as early as day 3 in the group treated with the higher dose of flibanserin, but at only 3 time-points and not until day 10 in each of the other two drug-treated groups.

In a further attempt to estimate the rate of onset of antidepressant-like effects, the data from drug-treated stressed animals (Fig. 5) were examined for goodness of fit to first-order kinetics (using the Fig. P curve-fitting pack-

Fig. 5. Sucrose intake in mice treated with (A) vehicle, (B) fluoxetine (5 mg/kg), (C) flibanserin (BIMT-17) (2.5 mg/kg), (D) flibanserin (BIMT-17) (5 mg/kg). White symbols, controls; black symbols, stressed animals. Values are means ± standard error. Values shown at day 0 are for the final sucrose intake test prior to the start of chronic drug treatment. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3
Kinetic analysis of improvement in sucrose intake over time[a,b]

	Fluoxetine	BIMT (2.5)	BIMT (5.0)
Asymptotic level of sucrose intake (V_{max})[c]	3.25 (±0.16)	2.97 (±0.06)	3.16 (±0.13)
Days to achieve 50% improvement (K_m)[c]	3.53 (±1.68)	0.57 (±0.33)*	0.32 (±0.47)*
Goodness of fit (r^2)	85.4%	85.5%	57.0%

[a]The Michaelis-Menten equation was fitted to mean sucrose intake values at each time-point.
[b]BIMT = flibanserin.
[c]Values shown are the fitted parameters with estimated standard errors.
* $P < 0.05$ (1-tailed) relative to fluoxetine.

age). The Michaelis-Menten equation provided a good fit to the fluoxetine and flibanserin (2.5 mg/kg) data ($r^2 >$ 85%), but fitted less well to the flibanserin (5 mg/kg) data ($r^2 = 57\%$). The estimated asymptotic levels of sucrose intake were comparable in the three groups, but they differed in the rate of approach to the asymptote: a 50% improvement in sucrose intake required 3.5 days of fluoxetine treatment but was reached in less than a day by both flibanserin-treated groups (Table 3).

3.2.3. Effects of raclopride on sucrose intake

The effects of raclopride were evaluated by comparing sucrose intakes after acute raclopride treatment to intakes on the immediately preceding and following raclopride-free tests (Fig. 6). In control animals sucrose intakes did not differ significantly across days of testing [$F(2,44) = 1.4$, NS]. However, in stressed animals raclopride significantly decreased sucrose intake [$F(1,44) = 15.1$, $P < 0.001$]. Further analysis showed that the effect of raclopride was significant in all three of the drug-treated groups [fluoxetine: $F(1,88) = 3.2$, $0.5 < P < 0.1$; flibanserin (2.5 mg/kg): $F(1,88) = 5.4$, $P < 0.02$; flibanserin (5 mg/kg): $F(1,88) = 7.1$, $P < 0.01$], but not in the vehicle-treated group [$F(1,88) = 0.03$, NS].

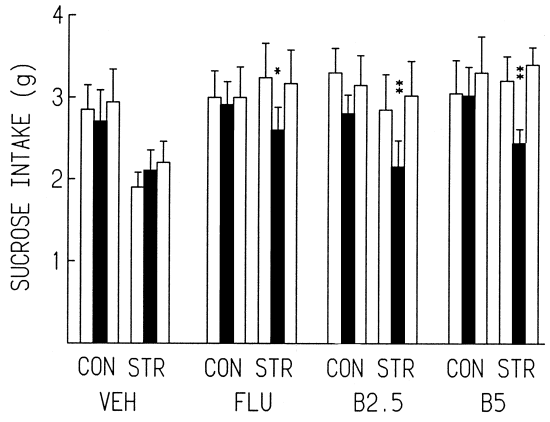


Fig. 6. Effects of raclopride on sucrose intake in mice. Each set of three bars represents intake of a group of animals on three successive tests. The first and third test in each group (white bars) were carried out without acute pretreatment; the second test (black bars) shows the effects of raclopride. The first test of each group corresponds to the final time-points in Fig. 3Fig. 4. Values are means ± standard error. * $P < 0.05$; * * $P < 0.01$ (1-tailed). Treatments: VEH, vehicle; FLU, fluoxetine; B2.5, flibanserin (BIMT-17) 2.5 mg/kg; B5, flibanserin (BIMT-17) 5 mg/kg.

4. Discussion

As in previous experiments using this procedure, chronic sequential exposure of rats to a variety of mild stressors caused a reduction in the consumption of a palatable weak sucrose solution, consistent with a decrease in its rewarding properties (Willner et al., 1987; Muscat et al., 1990; Sampson et al., 1991; Papp et al., 1991, 1992). However, unlike previous studies, the changes in sucrose intake, in rats, were unreliable, and sucrose testing was terminated two weeks after the start of drug treatment. We have found the chronic mild stress procedure to behave in a similarly unreliable manned with respect to sucrose intake in a number of our recent studies (unpublished observations). The reasons for the failure to maintain a prolonged decrease in sucrose intake in this and other experiments remain obscure, but are under active investigation (see Willner, 1997). However, it has always been recognized that the sucrose intake measure could be influenced by a variety of factors unrelated to hedonic tone, and this has on occasion been apparent in earlier studies (e.g. Willner et al., 1994).

In studies using the CMS procedure, sucrose intake has been employed routinely to monitor hedonic status on account of its convenience, not because it is believed to be more valid than other techniques. Despite the absence of usable sucrose intake data in experiment 1, clear evidence of the continued effectiveness of the stress procedure was seen in the place conditioning test. Unlike control animals, vehicle-treated stressed rats failed to show an increase in the time spent in the food-associated environment following conditioning. Vehicle-treated stressed animals tended (nonsignificantly) to consume less food than other groups on the conditioning trials. However, the presence of an inverse relationship between food intake and strength of conditioning rules out the possibility that decreased food intake might be responsible for the lack of conditioning. Against this background, both fluoxetine (5 mg/kg daily) and flibanserin (5–20 mg/kg daily) reinstated normal place conditioning in stressed animals. The effect of fluoxetine replicates our previous observations (Muscat et al., 1992); the effects of flibanserin are clear evidence of an anti-anhedonic action of this drug, comparable to that of fluoxetine.

The second experiment confirmed our previous observation that chronic mild stress is effective in decreasing sucrose drinking in mice (Monleon et al., 1994), and showed that this effect of chronic mild stress was completely reversed by chronic administration of fluoxetine (5 mg/kg daily) or flibanserin (2.5 and 5 mg/kg daily). The

effect of fluoxetine is consistent with earlier studies demonstrating a normalization of sucrose drinking in rats exposed to chronic mild stress, following chronic treatment with fluoxetine (Muscat et al., 1992) or other antidepressants (see Section 1).

Whatever the reason for the recovery of sucrose drinking in stressed rats, the loss of these data had the unfortunate effect of precluding evaluation of the time course of onset of the effects of flibanserin. However, it was possible to evaluate the time course of recovery in experiment 2 (mice). Significantly, on a variety of measures, flibanserin appeared to act more rapidly than fluoxetine, particularly at the higher dose (5 mg/kg). Sucrose intakes are much lower in mice than in rats, and the variability is higher, leading to a relatively small separation of sucrose intake values between stressed and control mice. As a result, sucrose intake in fluoxetine-treated animals relative to controls followed a somewhat erratic course (Fig. 5B), and it is possible that recovery was not complete even after three weeks of treatment. In contrast, flibanserin appeared to exert its full activity after only a single treatment, administered approximately 18 h before the sucrose intake test. This is a potentially important observation: acute effects have not been observed in any previous study of antidepressant action in the CMS model. Acute effects of flibanserin have also been observed in the learned helplessness test in rats (Borsini et al., 1997).

On the basis of the two sets of data reported in this study, flibanserin is predicted to be clinically effective as an antidepressant, and in particular to reverse anhedonia, which in DSM-III (though not in DSM-IIIR or DSM-IV) is definitive of the melancholic subtype of depression (American Psychiatric Association, 1980, 1987). The sucrose intake data further suggest that flibanserin should exert a rapid onset of antidepressant action. Clinical experience with 5-HT_{1A} receptor agonists in the treatment of depression has not been particularly encouraging: the partial agonist gepirone shows limited efficacy, with a typically slow onset of action (Amsterdam, 1992). However, these results do not preclude either antidepressant efficacy or rapid onset for flibanserin, which has a different mechanism of action (see below).

Stressed rats lost weight relative to controls, and this effect was not reversed in any of the drug-treated groups. Indeed, the weight loss was potentiated by fluoxetine and by the highest dose of flibanserin (20 mg/kg). Weight loss is unsurprising as a side effect of treatment with 5-HT receptor agonists (Blundell, 1986; Morley and Flood, 1987; McGuirk et al., 1992). The fact that fluoxetine and flibanserin potentiated weight loss in stressed animals, but not in controls, represents a potential risk in relation to the use of these compounds in depression, which includes weight loss as a prominent symptom (American Psychiatric Association, 1987). However, normal weight gain was seen in stressed rats receiving the lowest dose of flibanserin (5 mg/kg), which was equipotent with the higher doses in reversing the impairment of place conditioning. This suggests that flibanserin should be clinically effective at doses which do not adversely affect body weight.

A recent study has advanced the view that the effects of CMS on sucrose intake may be attributable to loss of body weight (Matthews et al., 1995). In the present study, however, body weight decreases in rats were observed in the absence of a reliable decrease in sucrose intake. By contrast, in mice, where chronic mild stress reliably decreased sucrose intake, there was no loss of body weight; these data are consistent with the majority of other studies (Willner et al., 1996). It has also been suggested that the food deprivation components of the chronic mild stress procedure might be particularly important in decreasing sucrose intake (Forbes et al., 1996; Hatcher et al., 1996). The present study did not directly address this issue, which is discussed elsewhere (Willner, 1997). However, since stressed mice were food deprived for only 6 h/week more than control mice, it seems unlikely that this factor alone could explain their decreased sucrose intake. It is also unclear how food deprivation might account for the impairment of place conditioning in stressed rats, since all animals (stressed and control) were tested under similar conditions of 22 h of food deprivation.

Flibanserin has affinity for cortical 5-HT_{1A} ($K_i = 19$ nM) and 5-HT_2 ($K_i = 133$ nM) receptors, but with the exception of α_1-adrenoceptors ($K_i = 523$ nm), where it acts as an antagonist, flibanserin has negligible affinity for other 5-HT receptors (5-HT_{1B}, 5-HT_{1D}, 5-HT_{2C}, 5-HT_3, 5-HT_4), adrenoceptors (α_2, β_1, β_2), and dopamine (D_1, D_2), muscarinic, or histamine (H_1, H_2) receptor subtypes. Flibanserin elicits a weak serotonergic syndrome in rats at a dose of 64 mg/kg i.p., and antagonizes DOI-induced head twitches in mice at a dose (ED_{50}) of 4.1 mg/kg (Borsini et al., unpublished data). In cortical preparations, flibanserin reduced forskolin-stimulated cAMP accumulation, an effect blocked by the 5-HT_{1A} receptor antagonist tertatolol, and antagonized 5-HT-induced phosphatidylinositol turnover, suggesting that it acts in the cortex as a 5-HT_{1A} receptor agonist and a 5-HT_2 receptor antagonist (Borsini et al., 1995a). This conclusion is supported by electrophysiological studies showing that flibanserin dose-dependently inhibited the firing rate of cells in the medial prefrontal cortex, after intravenous or microiontophoretic application. These effects were still present after destruction of cortical 5-HT terminals, and were antagonized by the 5-HT_{1A} antagonists tertatolol and WAY-100135. A similar decrease in firing rate of the same cells was also seen after microiontophoretic application of 5-HT, suggesting that, on acute administration, flibanserin mimics the action of 5-HT in the prefrontal cortex (Borsini et al., 1995b). This is not true of other 5-HT_{1A} receptor agonists (Borsini et al., 1995a,b).

It is now well established that chronic administration of antidepressant drugs potentiates the responsiveness to ago-

nists of dopamine D_2/D_3 receptors in the nucleus accumbens (Willner and Montgomery, 1981; Martin-Iverson et al., 1983; Arnt et al., 1984; Maj, 1988; Maj and Wedzony, 1985, 1988; Maj et al., 1984a,b, 1987). Both biochemical (receptor binding) and behavioural (place conditioning) studies have shown that stress-induced anhedonia is associated with a decrease in dopamine D_2/D_3 receptor function in the nucleus accumbens, and that recovery from anhedonia is associated with an increase in dopamine D_2/D_3 receptor function (Papp et al., 1993a,b, 1994). It appears, therefore, that the effectiveness of antidepressants in the chronic mild stress model may be mediated by sensitization of dopamine D_2/D_3 receptors in the nucleus accumbens.

In the present study, the locomotor stimulant effect of quinpirole, which, at the dose used, acts as an agonist at postsynaptic dopamine D_2/D_3 receptors, was enhanced by fluoxetine, consistent with the earlier literature; this effect was substantially larger in stressed animals. Flibanserin did not potentiate quinpirole-induced locomotion in control animals, suggesting that the actions of fluoxetine and flibanserin are not identical. Nevertheless, flibanserin did potentiate quinpirole in stressed animals, with the greatest effect at the lowest dose (5 mg/kg). The effectiveness of raclopride in reversing the antidepressant effects of fluoxetine and flibanserin provides further support for an involvement of dopamine synapses in the mechanism of antidepressant action in the chronic mild stress model. These data, in mice, confirm earlier observations that raclopride or other dopamine D_2/D_3 receptor antagonists reversed antidepressant effects on sucrose drinking in rats exposed to chronic mild stress, following chronic treatment with fluoxetine (Muscat et al., 1992) or a variety of other tricyclic and nontricyclic antidepressants (Muscat et al., 1990, 1992; Sampson et al., 1991; Cheeta et al., 1994).

The mechanism by which chronic antidepressant treatment increases behavioural responses to stimulant effects of quinpirole and other dopamine D_2/D_3 receptor agonists is unclear. Chronic antidepressant treatment in control animals does not increase the number of dopamine D_2/D_3 receptors. However, chronic mild stress decreases the number of dopamine D_2/D_3 receptors in the nucleus accumbens, and this effect was completely reversed by chronic treatment with imipramine (Papp et al., 1994). Thus, at the level of the dopamine D_2/D_3 receptor, the effects of chronic antidepressant treatment appear to be greater in animals subjected to chronic mild stress than in control animals. This may provide a basis for understanding the present observation that the potentiation of quinpirole by fluoxetine or flibanserin was greater in stressed animals than in controls. The ability of raclopride to reverse the effects of antidepressant treatment was also greater in stressed animals than in controls: at a higher dose, raclopride decreases sucrose intake in control animals (Phillips et al., 1991), but at the dose used in this study, 0.1 mg/kg, the effect of raclopride is specific to antidepressant-treated stressed animals (e.g. Cheeta et al., 1994; present data). It seems that chronic antidepressant treatment of stressed animals results in supersensitive responses to both agonists and antagonists at dopamine D_2/D_3 receptors. In the present study, both of these effects were particularly clear for flibanserin: stressed animals treated with flibanserin showed a large increase in quinpirole-stimulated locomotion and a substantial suppression of sucrose intake by raclopride; neither of these effects was seen in flibanserin-treated control animals.

These observations suggest that, in common with other antidepressants, the antidepressant-like anti-anhedonic action of flibanserin may involve sensitization of dopamine D_2/D_3 receptors. It remains to be established whether these receptors are located within the mesoaccumbens dopamine system, and if so, whether the antidepressant-like effects of flibanserin are mediated by direct actions within the nucleus accumbens or, as suggested by the electrophysiological evidence (Borsini et al., 1995b), by indirect actions at the level of the prefrontal cortex.

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