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Preclinical Abuse Potential Assessment of Flibanserin: Effects on Intracranial Self-Stimulation in Female and Male Rats

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Abstract

INTRODUCTION—Flibanserin is a serotonin receptor subtype 1A (5HT1A) agonist and 2A (5HT2A) antagonist that has been approved by the Food and Drug Administration for treating female sexual interest/arousal disorder. Little is known about the abuse potential of flibanserin.

AIM—This study examined abuse-related effects of flibanserin in rats using an intracranial self-stimulation (ICSS) procedure that has been used previously to evaluate abuse potential of other drugs.

METHODS—Adult female and male Sprague-Dawley rats with electrodes implanted in the medial forebrain bundle were trained to lever press for electrical brain stimulation under a “frequency-rate” ICSS procedure. In this procedure, increasing frequencies of brain stimulation maintain increasing rates of responding. Drugs of abuse typically increase (or “facilitate”) ICSS rates and produce leftward/upward shifts in ICSS frequency-rate curves, whereas drugs that lack abuse potential typically do not alter or only decrease ICSS rates. Initial studies determined the potency and time course of effects on ICSS produced by acute flibanserin (1.0, 3.2 and 10.0 mg/kg). Subsequent studies determined effects of flibanserin (3.2–18 mg/kg) before and after a regimen of repeated flibanserin administration (5.6 mg/kg/day x 5 days). Effects of the abused stimulant amphetamine (1.0 mg/kg) were examined as a positive control.

MAIN OUTCOME MEASURE—Flibanserin effects on ICSS frequency-rate curves in female and male rats were examined and compared to effects of amphetamine.

RESULTS—Baseline ICSS frequency-rate curves were similar in female and male rats. Both acute and repeated administration of flibanserin produced only decreases in ICSS rates, and rate-decreasing effects of the highest flibanserin dose (10 mg/kg) were greater in females than males. In contrast to flibanserin, amphetamine produced an abuse-related increase in ICSS rates that did not differ between females and males.

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CONCLUSIONS—These results suggest that flibanserin has low abuse potential. Additionally, this study suggests that females may be more sensitive than males to rate-decreasing effects of high flibanserin doses.

Keywords

DRUG ABUSE; SEROTONIN RECEPTOR; SEX DIFFERENCES; INTRACRANIAL SELF-STIMULATION; RAT; FEMALE

INTRODUCTION

Flibanserin is a serotonin receptor subtype 1A (5HT1A) agonist and 2A (5HT2A) antagonist [1, 2] that has shown efficacy in treating female sexual interest/arousal disorder, formerly known as hypoactive sexual desire disorder [3, 4]. Although precise mechanisms of flibanserin effects on sexual behavior are still under study, flibanserin has been shown in rats to alter extracellular levels of dopamine and serotonin in the prefrontal cortex and nucleus accumbens in both male [5] and female rats [6, 7]. The effectiveness of flibanserin to modulate central nervous system monoaminergic signaling suggests that abuse potential is a clinical concern, and in its pre-approval reviews of flibanserin, the Food and Drug Administration requested additional studies on abuse potential [8]. Several different types of procedures are used in preclinical assessment of abuse potential, and these procedures include drug self-administration, place conditioning, and intracranial self-stimulation (ICSS) [9–12]. Presently, little information is available from preclinical studies regarding abuse potential of flibanserin.

In the only preclinical study published to date, flibanserin did not produce a conditioned place preference in male rats [13], but place conditioning studies have not been conducted in females (the sex for which the drug is approved for treatment of sexual interest/arousal disorder), and flibanserin has not been studied in either sex in drug self-administration or ICSS procedures. However, drugs with related pharmacological mechanisms of action as either 5HT1A agonists or 5HT2A antagonists have been tested in both place conditioning and ICSS procedures, and these studies suggest that 5HT1A agonist effects in particular may contribute to abuse potential. Specifically, the 5HT1A agonist 8-OH-DPAT, which has high efficacy at 5HT1A receptors equivalent to that of flibanserin [14], produced both conditioned place preference [15] and facilitation of ICSS [16, 17] at low doses in male rats, although higher doses produced conditioned place aversion and depression of ICSS. Conversely, 5HT2A antagonists have not produced either conditioned place preference [18] or facilitation of ICSS [19, 20]. Taken together, these findings suggest that further preclinical abuse-potential assessment of flibanserin is warranted given the known effectiveness of flibanserin as a high-efficacy 5HT1A agonist.

Accordingly, the goal of the present study was to evaluate abuse-related effects of flibanserin in female and male rats responding in a “frequency-rate” ICSS procedure that has been used extensively to evaluate abuse potential of other drugs [9, 21, 22]. In ICSS procedures, rats equipped with microelectrodes that target a brain reward area are trained to press a lever for pulses of electrical stimulation delivered through the electrode. Increasing

frequencies of electrical brain stimulation maintain increasing rates of ICSS responding, and this relationship can be graphed as a sigmoidal “frequency-rate” curve. Drugs with high abuse potential (e.g. amphetamine) typically increase (or “facilitate”) ICSS rates and produce leftward/upward shifts in ICSS frequency-rate curves. Conversely, drugs that lack abuse potential typically either have no effect on ICSS frequency-rate curves or only depress ICSS. Results using ICSS procedures are usually consistent with results of other procedures for preclinical abuse potential assessment, but ICSS procedures also offer several advantages (e.g. utility for evaluation of drug time course; see [9]). In the present study, initial experiments evaluated the potency and time course of acute flibanserin doses to alter ICSS, starting at a flibanserin dose (1 mg/kg) that is clinically relevant to proposed human dosing (100 mg for the average 75 kg female). For two reasons, subsequent studies evaluated effects of repeated flibanserin doses. First, flibanserin dosing guidelines in humans call for repeated dosing, and full effects of flibanserin on sexual behavior in preclinical studies are also seen primarily following repeated dosing [23]. Second, we have shown previously that repeated drug treatment can unmask expression of abuse-related ICSS rate-increasing effects of some other drugs such as mu opioid receptor agonists [24–27]. Effects of flibanserin were compared to effects of the abused stimulant amphetamine as a positive control.

METHODS

Subjects

For initial studies, a total of five adult female and six adult male Sprague-Dawley rats (Harlan, Frederick, MD) were used, and estrous cycle was not monitored in female rats during the course of the study. A subsequent study was conducted in six additional female Sprague-Dawley rats in which estrous cycle phase was monitored. All rats had ad libitum access to food and water and were housed individually on a 12 h light-dark cycle (6am – 6pm, lights on) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Male rats weighed between 300 and 350g at the time of surgery, whereas females weighed between 200 and 300g. All experiments were performed with the approval of the Institutional Animal Care and Use Committee at Virginia Commonwealth University in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals 8th edition [28].

Assay of Intracranial Self-Stimulation (ICSS)

Surgery—Rats were anesthetized with 2.5% isoflurane (3% in oxygen; Webster Veterinary, Phoenix, AZ, USA) until unresponsive to toe-pinch prior for implantation of stainless steel electrodes (Plastics One, Roanoke, VA, USA). The cathode, which was 0.25 mm in diameter and covered with polyamide insulation except at the flattened tip, was stereotactically implanted into the left medial forebrain bundle at the level of the lateral hypothalamus using previously published coordinates for females [29] and males [30, 31]. Specifically, coordinates in females were 3.8 mm posterior to bregma, 1.6 mm lateral to the midsagittal suture, and 8.7 mm ventral to the skull, and coordinates in males were 2.8 mm posterior to bregma, 1.7 mm lateral to the midsagittal suture, and 8.8 mm ventral to the skull. Three screws were placed in the skull, and the anode (0.125 mm diameter, un-

insulated) was wrapped around one of the screws to act as a ground. Dental acrylic was used to secure the electrode to the screws and skull. Ketoprofen (5 mg/kg) was used as a postoperative analgesic immediately and 24 hrs following surgery. Animals were allowed to recover for at least one week before ICSS training.

Apparatus—Operant conditioning chambers consisted of sound-attenuating boxes containing modular acrylic and metal test chambers (29.2 cm X 30.5 cm X 24.1 cm) (Med Associates, St. Albans, VT). Each chamber had a response lever (4.5 cm wide, 2.0 cm deep, 3.0 cm above the floor), three stimulus lights (red, yellow and green) centered 7.6 cm above the lever, a 2 W house light, and an ICSS stimulator. Bipolar cables routed through a swivel-commutator (Model SL2C, Plastics One) connected the stimulator to the electrode. MED-PC IV computer software controlled all programming parameters and data collection (Med Associates). Throughout training and testing, daily ICSS sessions in female and male rats were conducted concurrently in one room equipped with a total of 12 chambers, and each rat was assigned to one of the chambers for the entire study.

Training—The behavioral procedure was similar to that described previously [9, 30]. A house light was illuminated during behavioral sessions, and lever-press responding under a fixed-ratio 1 (FR1) schedule produced delivery of a 0.5 s train of square-wave cathodal pulses (0.1 ms per pulse) via the intracranial electrode. During brain stimulation, the stimulus lights over the lever were illuminated, and responding had no scheduled consequences. During initial 60 min training sessions, stimulation intensity was set at 150 μ A, and stimulation frequency was set at 158 Hz. Stimulation intensity was then individually manipulated in each rat (100 μ A- 295 μ A; see Results) to identify an intensity that maintained reinforcement rates >30 stimulations/min. Once an appropriate intensity was identified, changes in frequency were introduced during sessions consisting of three consecutive 10 min components, each of which contained 10 consecutive 60 s trials. The stimulation frequency was 158 Hz for the first trial of each component, and frequency decreased in 0.05 log unit steps during the subsequent nine trials to a final frequency of 56 Hz. Each trial began with a 10 s time-out period, during which responding had no scheduled consequences, and five non-contingent stimulations at the designated frequency were delivered at 1 s intervals during the last 5 s of the time out. During the remaining 50 s of each trial, responding produced both intracranial stimulation at the designated frequency and illumination of the lever lights under an FR1 schedule as described above. ICSS performance was considered to be stable when frequency-rate curves were not statistically different over three consecutive days of training as indicated by lack of a significant effect of 'day' in a two-way analysis of variance (ANOVA) with day and frequency as the main effect variables (see Data Analysis below). All training was completed within six weeks of surgery.

Testing—For initial studies, five female and six male rats had a history of acute testing with 4 doses of ($\pm$) 3,4-methylenedioxymethamphetamine (MDMA, results to be published separately), and the present studies were initiated at least 2 weeks after conclusion of studies with MDMA. Prior to initiation of studies with flibanserin, stable baseline ICSS frequency-rate curves were re-confirmed using the criteria described above, and testing was conducted

in four phases to determine (1) flibanserin potency, (2) flibanserin time course, (3) effects of repeated flibanserin administration, and (4) effects of amphetamine as a positive control. Baselines were re-established and brain-stimulation amplitudes were adjusted as necessary between phases. The first phase was conducted over 2 weeks that included 4 separate test periods. The second phase was completed the following week over one test day. The third phase was then completed two weeks following the first two phases to ensure stable baselines. The final phase was completed one week following the third phase.

For studies of flibanserin potency, test sessions consisted of three consecutive 'baseline' components followed first by a treatment interval of 10 min and then by two consecutive 'test' components. Each baseline component is comprised of 10 trials that start at a frequency of 158 Hz that decrease in 0.05 log unit steps during the subsequent nine trials to a final frequency of 56 Hz, as described in Training. Rats are then exposed to three of these components for a total of 30 mins. The two consecutive 'test' components proceed exactly as the 'baseline' components for a total of 20 mins to allow for direct comparisons before and after drug administration. Either vehicle or a dose of flibanserin (1, 3.2 and 10 mg/kg) was administered IP at the start of the treatment interval, and the dose order was varied across rats using a Latin-Square design. Flibanserin time course was subsequently assessed during a single test session, which consisted of three consecutive baseline components followed first by administration of 10 mg/kg flibanserin IP and then by pairs of test components beginning 10, 30, 100 and 180 minutes after flibanserin administration. Potency and time-course test sessions were conducted on Tuesdays and Fridays, and three-component training sessions were conducted on other weekdays ensure that rats are still responding at similar rates in between testing days.

Effects of repeated flibanserin were assessed using a nine-day protocol. On days -2 to 0, test sessions consisted of three consecutive baseline components and data across these three days were averaged to yield pre-flibanserin baseline data as described below. Immediately after conclusion of the last baseline component on Day 0, a cumulative flibanserin dose-effect curve was determined during three consecutive test periods. Each 30-min test period consisted of a 10-min treatment interval followed by a pair of test components. A dose of flibanserin was administered IP at the start of each treatment interval, and each dose increased the total cumulative dose by 0.25 log units. Specifically, the first dose was 3.2 mg/kg, the second dose of 2.4 mg/kg increased the cumulative dose to a total of 5.6 mg/kg, and the final dose of 4.4 mg/kg increased the cumulative dose to a total of 10 mg/kg. On days 1-5, test sessions consisted of three consecutive baseline components followed first by a 10-min treatment interval and then by a pair of test components, and 5.6 mg/kg flibanserin was administered at the start of the treatment interval on each day. The dose of 5.6 mg/kg was chosen based on previous studies in our laboratory that have demonstrated that an intermediate dose of morphine unmasks abuse-related effects for morphine [25, 26]. Finally, on day 6, the cumulative flibanserin dose-effect curve was redetermined as described above during test sessions consisting of three baseline components followed by consecutive 30-min test periods during which cumulative flibanserin doses were administered. An additional test period was added on day 6 to permit testing of an additional flibanserin dose that increased the total cumulative dose to 18 mg/kg.

Effects of 1.0 mg/kg amphetamine were evaluated during a single test session consisting of three consecutive baseline components followed first by a 10-min treatment interval and then by a pair of test components. Amphetamine was administered IP at the start of the treatment interval.

Following these initial studies, a separate group of six drug naïve females was trained and tested with vehicle and doses of 3.2 and 10 mg/kg flibanserin using procedures identical to those described above for dose-effect studies. In addition, estrous cycle phase in these rats was monitored by vaginal lavage following behavioral sessions and evaluation of cell cytology under a microscope at 20X magnification [32].

Data Analysis—Data analysis was performed as previously described [9]. The first baseline component for each day was considered to be a “warm-up” component, and data were discarded. The primary dependent variable was reinforcement rate in stimulations per minute during each frequency trial for all remaining baseline and test components. To normalize these data, raw reinforcement rates from each trial in each rat were converted to percent maximum control rate (%MCR) for that rat. For studies of flibanserin potency, flibanserin time course, and amphetamine effects, MCR was defined as the mean of the maximal rates observed during the second and third baseline components of that test session. For studies of repeated flibanserin, MCR was defined as the mean of the maximal rates observed during the second and third baseline components on day –2 to day 0 (six total baseline components) before initiation of repeated flibanserin treatment. Subsequently, % MCR values for each trial were calculated as [(reinforcement rate during a frequency trial)/(MCR)]×100. For each rat, data from baseline and test components were averaged to yield baseline and test frequency-rate curves. Baseline and test data were then averaged across rats to yield mean baseline and test frequency-rate curves for each manipulation. Results were compared by repeated measures two-way ANOVA with ICSS frequency as one factor and either dose or time as the second factor. A significant main effect of dose or an interaction was followed by the Holm-Sidak post-hoc test, and the criterion for significance was $p<0.05$. Only within sex comparisons were made for these data following flibanserin administration.

To provide an additional summary measure of ICSS performance for use in analysis of sex differences in drug effects, the total number of stimulations per component was determined across all 10 frequency trials of each component. Test data were expressed as a percentage of either (a) the average number of total stimulations per component earned during the baseline components for that day (for studies of flibanserin potency, flibanserin time course, and amphetamine effects) or (b) the average number of total stimulations per component earned during pre-flibanserin baseline components on days –2 to 0 (for studies of repeated flibanserin). Thus, % Baseline Stimulations was calculated as (mean total stimulations during test components/mean total stimulations during baseline components) × 100. These data were then averaged across rats for each experimental manipulation. Results were compared by mixed factor two-way ANOVA (or a Student’s t-test for amphetamine) to compare the repeated-measures factor of either dose or time and the between-subjects factor of sex. A significant ANOVA was followed by the Holm-Sidak post-hoc test, and the criterion for significance was $p<0.05$. Both within and between sex comparisons were made

with these data. Partial eta squared was also determined as a measure of effect size utilizing Microsoft Excel (Redmond, WA).

Verification of electrode placements—Electrode placements were verified by gross dissection. Following termination of experiments, rats were sacrificed by decapitation, brains removed, and suspended in formalin obtained from Fischer Scientific (Waltham, MA; Catalog #305–510) for 48 hours at 4°C. After this fixation period, brains were sliced using a carbon steel razor blade (Ted Pella, Redding, CA) at the level of electrode entry and the most ventral aspect of the electrode track was recorded following examination under a dissecting microscope (4X magnification). This method of verification produced results consistent with previously published methods [33].

Drugs

Flibanserin (1- [2- [-4-(3-trifluoromethyl phenyl)piperazin-1-yl] ethyl] benzimidazol- [1H]-2-one) HCl was synthesized by Dr. Bruce Blough and was dissolved in a solution of 10% ethanol, 20% polyethylene glycol 400 and 70% sterile saline and delivered IP in a volume of 1 ml/kg at all doses except for 10 mg/kg, which was delivered at 2 ml/kg due to limits in solubility. (+)-Amphetamine sulfate was provided by the National Institute on Drug Abuse Supply Program (Bethesda, MD) and was dissolved in sterile saline and delivered at a volume of 1 ml/kg.

MAIN OUTCOME MEASURES

The main outcome measures were (1) ICSS rate maintained by each brain stimulation frequency (for analysis of drug effects on ICSS frequency-rate curves), and (2) summary ICSS rate maintained across all 10 frequency trials (for analysis of sex differences in ICSS effects at different drug doses or pretreatment times).

RESULTS

Initial studies were conducted in two groups consisting of five female rats and six male rats. There were no significant sex differences either in stimulation amplitudes required to maintain ICSS or in baseline measures of ICSS maintained by those stimulation amplitudes. Initial stimulation amplitudes (mean $\pm$ SEM) were $126\mu\text{A} \pm 11$ and $167\mu\text{A} \pm 25$ for female and male rats, respectively. Maximum control rates (MCRs) per trial (mean $\pm$ SEM) were 63.5 ± 1.5 for female rats and 64.5 ± 4.3 for male rats, and total stimulations per component (mean $\pm$ SEM) were 330 ± 17 for female rats and 311 ± 18 for male rats. During testing, amplitudes were adjusted in some rats between study phases to maintain equivalent rates of responding, and final amplitudes (mean $\pm$ SEM) were $137\mu\text{A} \pm 20$ and $175\mu\text{A} \pm 27$, MCRs per trial (mean $\pm$ SEM) were 65.6 ± 2.1 and 61.8 ± 5.0 , and baseline stimulations per component (mean $\pm$ SEM) were 299 ± 21 and 312 ± 18 for females and males, respectively. Student's t-tests indicated that none of these differences were statistically significant (all $p > 0.05$). Figure 1A shows electrode placements in female and male rats. Figure 1B shows mean baseline frequency-rate curves averaged across test sessions during initial studies of flibanserin potency, and these curves were not significantly different.

Figure 2 shows the potency and time course of flibanserin effects on ICSS in female and male rats. There was a significant frequency X dose interaction in both female rats [$F(27, 108) = 5.88$, $p < 0.0001$, $\eta^2p = 0.60$] and male rats [$F(27, 135) = 4.08$, $p < 0.0001$, $\eta^2p = 0.45$]. Flibanserin at doses of 1.0 and 3.2 mg/kg produced no effect on ICSS in either sex, whereas 10 mg/kg decreased ICSS in females at the highest six frequencies (89–158 Hz) (Figure 2A) and in males at the highest four frequencies (112–158 Hz; Figure 2B). Analysis of flibanserin effects on the total number of stimulations per component identified a significant dose X sex interaction [$F(3,27) = 5.04$, $p < 0.01$, $\eta^2p = 0.36$] with greater depression in females at a dose of 10 mg/kg (Figure 2C). In time-course studies, there was a significant frequency X time interaction in both females [$F(36, 144) = 3.64$, $p < 0.0001$, $\eta^2p = 0.48$] and males [$F(36, 180) = 1.67$, $p < 0.05$, $\eta^2p = 0.25$]. In females, 10 mg/kg flibanserin decreased ICSS at five frequencies at 10 minutes, four frequencies at 30 minutes, four frequencies at 100 minutes, and rates of responding returned to baseline after 180 minutes (Figure 2D). In males, 10 mg/kg flibanserin decreased responding for ICSS at two frequencies at 10 minutes, four frequencies at 30 minutes, and one frequency at both 100 and 180 minutes (Figure 2E). Analysis of flibanserin effects on the total number of stimulations per component identified a significant time X sex interaction [$F(4,36) = 5.95$, $p < 0.001$, $\eta^2p = 0.40$, Figure 2F]. In female rats, 10 mg/kg flibanserin decreased this measure of ICSS at 10, 30 and 100 minutes, whereas this measure of ICSS was not significantly decreased at any time in males. ICSS was decreased significantly more in females than in males after 10 min.

Figure 3 shows flibanserin effects on ICSS determined before and after a regimen of repeated flibanserin administration. On Day 0, cumulative doses of flibanserin produced a dose-dependent decrease in ICSS in both female rats {significant frequency X dose interaction [$F(27, 108) = 1.76$, $p < 0.05$, $\eta^2p = 0.31$] and male rats {significant frequency X dose interaction [$F(27, 135) = 1.89$, $p < 0.01$, $\eta^2p = 0.27$]. Specifically, in females (Figure 3A) and males (Figure 3B), doses of 5.6 mg/kg and 10 mg/kg significantly decreased ICSS maintained by at least one frequency of brain stimulation. Although total stimulations per component were decreased to a lower mean level in females than in males by 10 mg/kg flibanserin, this difference was not statistically significant in this cumulative dosing study (Figure 3C). Daily administration of 5.6 mg/kg flibanserin on Days 1–5 produced either no effect or a significant decrease in ICSS maintained by at least one frequency of brain stimulation each day in both female and male rats (data not shown). Comparisons of total stimulations per component showed a significant main effect of sex [$F(1, 45) = 8.89$, $p < 0.01$, $\eta^2p = 0.16$] (data not shown), with greater ICSS decreases in females than males during repeated dosing. On Day 6, effects of cumulative flibanserin were redetermined. There was a significant frequency X dose interaction in females [$F(36,144) = 2.24$, $p < 0.001$, $\eta^2p = 0.36$] and males [$F(36, 180) = 2.52$, $p < 0.0001$, $\eta^2p = 0.34$]. In both females (Figure 3D) and males (Figure 3E), a decrease in ICSS was observed for at least one frequency at all doses tested (3.2–18 mg/kg). For females, total stimulations per component were significantly decreased compared to baseline at the 3.2, 10 and 18 mg/kg doses, whereas total stimulations were only decreased at the 10 and 18 mg/kg doses in male rats (Figure 3F). There were no significant differences between female and male rats when comparing total stimulations per component.

Figure 4 shows that a dose of 1.0 mg/kg amphetamine facilitated ICSS at the lowest six frequencies of brain stimulation (56–100 Hz) in both female and male rats (Figure 4A). There was a significant frequency X dose interaction in females [$F(9,36) = 13.35$, $p < 0.0001$, $\eta^2p = 0.77$] and males [$F(9,45) = 12.88$, $p < 0.0001$, $\eta^2p = 0.72$]. Further, total stimulations per component were increased equally in both female and male rats (Figure 4B), and there was no difference following a Student's t-test ($p > 0.05$).

Based on the initial finding of a sex difference, studies were conducted in a separate group of six drug-naïve females using vehicle and doses of 3.2 and 10 mg/kg flibanserin. During training and testing, vaginal cytology did not provide evidence for cycling and indicated instead that rats were in prolonged period of metestrus/diestrus as defined by the presence of equal numbers of nucleated epithelial cells, cornified epithelial cells and leukocytes (metestrus) or primarily leukocytes (diestrus). Figure 5A shows that 3.2 mg/kg flibanserin had no effect on ICSS in these drug naïve-females, whereas 10 mg/kg significantly depressed ICSS, as there was only a significant main effect of dose [$F(2,27) = 39.88$, $p < 0.0001$, $\eta^2p = 0.75$]. There was no difference in flibanserin effects on ICSS in the two cohorts of female rats. Figure 5B combines flibanserin data for all 11 female rats and compares flibanserin effects in all 11 females with effects in males. There was a significant main effect of dose [$F(2,45) = 30.62$, $p < 0.0001$, $\eta^2p = 0.58$] and a significant main effect of sex [$F(1,45) = 4.50$, $p < 0.05$, $\eta^2p = 0.09$].

DISCUSSION

The abuse potential of flibanserin was evaluated in both female and male rats utilizing a frequency-rate ICSS procedure. There were two main findings. First, flibanserin failed to produce an abuse-related facilitation of ICSS at any brain stimulation frequency after either acute or repeated administration in either female or male rats. In contrast to flibanserin, amphetamine did facilitate ICSS in both females and males, and ICSS facilitation is also produced by many other classes of abused drugs in both sexes (although such effects have been studied primarily in males; [9]). Consequently, these results do not provide evidence for abuse potential by flibanserin. A second finding was that flibanserin produced dose-dependent depression of ICSS, and this effect tended to be greater in females than males; however, the effect size was much smaller for sex differences in flibanserin effects than for dose-dependence of those effects. This evidence for flibanserin-induced depression of positively reinforced operant behavior in rats may be related to clinical evidence for fatigue, somnolence and sedation as undesirable effects of flibanserin in humans [8, 34].

Baseline ICSS in female and male rats

There was a trend in this study for lower brain stimulation intensities to maintain ICSS in females than in males and for ICSS frequency-rate curves to be shifted to the left in females relative to males; however, none of these differences were statistically significant. This agrees with results from previous studies that reported nonsignificant trends for greater sensitivity of female Sprague-Dawley rats to brain stimulation in ICSS procedures [29, 35]. Although this difference has not met criteria for significance in any individual study, the recurrence of this trend raises the possibility that a small sex difference may exist. Notably,

previous studies have suggested that ICSS in females does not vary across the estrous cycle [29, 35], suggesting the magnitude of any sex difference in sensitivity to brain stimulation and expression of ICSS is not obscured by changes across the estrous cycle in females. While we did not initially monitor cycling in females, cycling was monitored in a separate study done in drug naïve females. Females in this study exhibited abnormal cycling that can occur following surgery, stress or vaginal lavage that stimulates the cervix leading to longer diestrus periods or pseudopregnancy that lasts up to 21 days [32, 36–38]. A limitation of the present study is that the effects of flibanserin were not tested during proestrus/estrus or the cycle was not known. This is an important consideration since estrogen and/or progesterone administration can reduce 5HT1A receptors [39] and increase 5HT2A receptors [40]. Despite a role for ovarian hormones in altering serotonergic systems, the dosing guidelines of flibanserin are for once daily dosing irrespective of menstrual cycle stage, and clinical studies suggest that both the efficacy and side effects of flibanserin are similar in both pre- and post-menopausal women [41].

Failure of flibanserin to facilitate ICSS

Flibanserin failed to facilitate ICSS in either female or male rats after either acute or repeated administration and the primary effect of flibanserin was depression of responding. This lack of abuse-related effect in an ICSS procedure agrees with a previous report that flibanserin failed to produce a conditioned place preference in male rats [13]. Conversely, the present results with flibanserin contrast with effects in rats reported for relatively low doses of the 5HT1A agonist 8-OH-DPAT, which usually facilitates ICSS [16, 17] (but see [42]) and produces conditioned place preferences [15]. The present results are not likely to reflect evaluation of an inadequate flibanserin dose range, because flibanserin was tested across a 10-fold dose range from low doses that produced no effect to high doses that only depressed ICSS, and flibanserin doses tested here also produce a range of other behavioral effects in rats (e.g. [43, 44]). Moreover, previous pharmacokinetic studies suggest that the flibanserin doses tested here would be associated with plasma levels spanning levels produced in humans by the recommended therapeutic dose of 100 mg PO [34, 43]. The present results also are unlikely to reflect inadequate efficacy of flibanserin at 5HT1A receptors. Although drugs such as buspirone with low efficacy at 5HT1A receptors generally do not produce abuse-related effects in assays of ICSS, place conditioning, or drug self-administration [16, 45, 46], flibanserin has high efficacy at 5HT1A receptors equivalent to that of 8-OH-DPAT [14]. A more likely possibility is that failure of flibanserin to facilitate ICSS reflects flibanserin effects at targets other than 5HT1A receptors. For example, flibanserin also acts as a 5HT2A antagonist, and the 10 mg/kg dose that depressed ICSS in the present study was found in a previous study to occupy approximately 50% of both 5HT1A and 5HT2A receptors in the prefrontal cortex, hippocampus and midbrain of male rats [47]. Selective 5HT2A antagonists have not been shown to alter ICSS or to block ICSS facilitation by amphetamine or cocaine [19, 20, 48]; however, 5HT2A antagonists can attenuate some neurochemical and behavioral effects of stimulants [49, 50] and may oppose and limit expression of any weak abuse-related effects of flibanserin mediated by 5HT1A receptors. It is likely that the 5HT2A antagonist properties do not contribute to the depression of ICSS by flibanserin as previous studies have found no significant depression

of ICSS with selective 5HT2A antagonists [19, 20]; however, our studies do not rule out that 5HT2A antagonism does not contribute to the depression of ICSS produced by flibanserin.

Flibanserin also failed to facilitate ICSS in female or male rats after repeated administration. This experiment was conducted because previous studies found that the mu opioid receptor agonist morphine produced primarily depression of ICSS after acute administration to morphine-naïve rats; however, repeated treatment with a threshold morphine dose for producing ICSS depression (3.2 mg/kg) resulted in tolerance to ICSS rate-decreasing effects and increased expression of abuse-related ICSS facilitation [25, 26]. These results with morphine suggested that expression of ICSS facilitation by some drugs may be enhanced by repeated drug exposure (also see [24, 27]). However, flibanserin did not display this profile of effects. A dose of 5.6 mg/kg flibanserin was a threshold dose to produce ICSS depression in both female and male rats, but repeated administration of this dose produced little evidence of tolerance to ICSS rate-decreasing effects and no evidence for emergence of ICSS facilitation. This finding agrees with a previous report that repeated flibanserin treatment produced a repeatable decrease in serotonin levels similar to the decrease produced by acute flibanserin at the highest dose tested [7]. An implication of this finding is that abuse-related effects of flibanserin may be unlikely to increase with repeated flibanserin exposure.

Although drug effects in this ICSS procedure correlate highly both with drug effects in other preclinical assays of abuse potential such as drug self-administration and with metrics of clinical abuse, this correlation is not perfect, and some drugs with abuse liability in humans do not reliably facilitate ICSS in rats [9]. Examples include cannabinoid receptor agonists such as Δ^9 -tetrahydrocannabinol [51, 52], the NMDA receptor antagonist ketamine [53, 54], and 5HT2A receptor agonist hallucinogens such as TCB-2 [20]. Thus, while results of the present study suggest little or no abuse liability of flibanserin, it remains possible that flibanserin could produce a constellation of effects sufficient to maintain some patterns of abuse in humans despite a lack of ICSS facilitation in rats. A failure of rats to self-administer flibanserin would certainly provide further evidence that humans are unlikely to abuse flibanserin. Nonetheless, results of this preclinical study are consistent with an apparent lack of evidence for abuse potential of flibanserin in clinical trials as reported in a recent report by the Food and Drug Administration [55].

ICSS depression by flibanserin

Flibanserin doses of 1–10 mg/kg IP produced a dose- and time-dependent depression of ICSS in this study, and these findings agree with previous reports that flibanserin displays similar potency to decrease rates of various other behaviors in rodents [6, 44]. For example, flibanserin doses of 8–16 mg/kg (IP) or 45 mg/kg (PO), which produced similar plasma flibanserin levels, decreased measures of spontaneous locomotion in female and male rats [6, 44]. Moreover, this preclinical evidence for flibanserin effectiveness to decrease rates of operant responding and locomotor activity may be related to evidence for flibanserin-induced fatigue, somnolence and sedation in humans [8, 34].

The present study also found evidence for a sex difference in the magnitude of flibanserin-induced ICSS depression, with depression often being significantly greater in females than

in males. The generality and clinical relevance of this finding remains to be determined. Although flibanserin is indicated for administration exclusively to women, off-label consumption by men should likely be anticipated. Fatigue, somnolence and sedation have emerged as primary side effects of concern with flibanserin [56, 57], and the present results suggest that flibanserin may be less potent or effective to produce behavioral depression in males. However, two caveats warrant mention. First, effect sizes for sex differences were smaller than for dose-dependence in analysis of flibanserin-induced ICSS depression, and sex differences in flibanserin effects were not always statistically significant in the present study (e.g. see Figure 3). These findings suggest that claims of a sex difference in flibanserin effects on ICSS should be viewed with some caution. Second, the relationship of any sex differences in flibanserin-induced depression of ICSS to potential sex differences in flibanserin-induced side effects such as fatigue in humans remains speculative. Nonetheless, the present evidence for greater sensitivity of females to ICSS depression by flibanserin is not likely to reflect a generally heightened sensitivity of females to treatments that depress ICSS. For example, the kappa opioid receptor agonist U50488 was more potent to depress ICSS in males [35] and morphine also decreased rates of responding to a greater degree in males [58]. Consequently, the reliability and underlying mechanisms of sex differences in flibanserin effects on ICSS or other behaviors may warrant further study.

Conclusion

This study found that female and male rats displayed similar sensitivities to brain stimulation reward in an ICSS procedure and similar sensitivities to ICSS facilitation by 1.0 mg/kg amphetamine. In contrast to amphetamine, flibanserin produced dose-dependent depression of ICSS in both female and male rats, suggesting that flibanserin has low abuse potential. Furthermore, repeated administration of flibanserin did not unmask ICSS facilitation, but still exhibited depression of ICSS. Finally, this study provided evidence for statistically significant though small sex differences in the rate-decreasing effects of flibanserin, with greater ICSS depression in females than males.

Abbreviations

8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
ANOVA	analysis of variance
FR	Fixed ratio
Flibanserin	(1- [2- [-4-(3-trifluoromethyl phenyl)piperazin-1-yl] ethyl] benzimidazol-[1H]-2-one)
ICSS	Intracranial self-stimulation
IP	Intraperitoneal
MCR	Maximum control rate
Min	Minute
PO	Per os

S	Second
5HT1A	Serotonin receptor subtype 1A
5HT2A	Serotonin receptor subtype 2A
TCB-2	(4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine
Veh	Vehicle

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Figure 1.

Baseline responses did not differ between female and male rats. Left panel (A) shows electrode placements in female (triangles) and male (circles) rats. Right panel (B) shows baseline ICSS frequency-rate curves in female and male rats. Abscissa: Frequency of electrical brain stimulation in Hz. Ordinate: Percent maximum control reinforcement rate (% MCR). Data presented as mean $\pm$ SEM for five female (triangles) and six male (circles) rats averaged across baselines for the initial dose-effect studies.

Figure 2.

Potency and time course of flibanserin effects on ICSS in female and male rats. Left and middle panels (A, B, D, E) show dose and temporal effects of flibanserin on ICSS frequency-rate curves in both female and male rats. Abscissae: Frequency of electrical brain stimulation in Hz. Ordinates: Percent maximum control reinforcement rate (% MCR). Filled symbols show significant ($p < 0.05$) differences from vehicle (up triangle) (A,B) or baseline (solid line) (D,E). Doses in mg/kg of 1.0 (down triangles) 3.2 (squares) and 10 (hexagons) and times in minutes (min) of 10 (up triangles), 30 (down triangles), 100 (squares) and 180 (circles) were tested. Right panels (C, F) show summary data of total responses per component across all frequencies of brain stimulation expressed as percent of baseline stimulations for dose-effect and time course studies for female (triangles) and male (circles) rats. Abscissa: Dose of drug. Ordinate: Percent baseline stimulation. For dose-effect studies, filled symbols represent a significant difference from vehicle ($p < 0.05$) and asterisks (***) represent a significant sex difference. For time course studies, filled symbols represent a significant difference from baseline ($p < 0.05$), and asterisks (**) represent a significant sex difference. Data presented as mean $\pm$ SEM for five female and six male rats.

Figure 3.

Effects of repeated flibanserin treatment. Left and middle panels (A, B, D, E) show effects of flibanserin on ICSS frequency-rate curves before (A,B) and after (D,E) repeated, daily flibanserin in female and male rats. Abscissae: Frequency of electrical brain stimulation in Hz. Ordinates: Percent maximum control reinforcement rate (% MCR). Filled symbols show significant differences ($p < 0.05$) from baseline (solid line) for 3.2 mg/kg (squares), 5.6 mg/kg (diamonds), 10.0 mg/kg (hexagons) and 18 mg/kg (circles). Right panels (C,F) show summary data of total responses per component across all frequencies of brain stimulation expressed as percent of baseline stimulations for Day 0 and Day 6 for female (triangles) and male (circles) rats. Abscissa: Dose of drug. Ordinate: Percent baseline stimulation. For Day 0, filled symbols represent a significant difference from baseline ($p < 0.05$). For Day 6, filled symbols represent a significant difference from baseline ($p < 0.05$). Data presented as mean $\pm$ SEM for five female and six male rats.

Figure 4.

Effects of 1.0 mg/kg amphetamine in female and male rats. Left panel (A) shows effect of 1.0 mg/kg amphetamine on ICSS frequency-rate curves in both female and male rats. Abscissa: Frequency of electrical brain stimulation in Hz. Ordinate: Percent maximum control reinforcement rate (% MCR). Filled symbols show significant differences from baseline as, $p < 0.05$. Right panel (B) shows summary data of total responses per component across all frequencies of brain stimulation expressed as percent of baseline stimulations for 1.0 mg/kg amphetamine. Abscissa: Dose of drug. Ordinate: Percent baseline stimulation. Data presented as mean $\pm$ SEM for five female (solid grey line and triangles) and six male (solid black line and circles) rats.

Figure 5.

Effects of flibanserin in drug naïve females that were in a prolonged period of metestrus/diestrus. Abscissa: Dose of drug. Ordinate: Percent baseline stimulation. Filled symbols represent significantly different from vehicle and an asterisk represents significantly different between sexes, $p < 0.05$. Left panel (A) shows the effects of vehicle, 3.2 and 10 mg/kg flibanserin in both the drug-naïve females (squares) and those females previously tested (triangles; same data as in Figure 2C). Data presented as mean $\pm$ SEM for 5–6 female rats. Right panel (B) shows the effects of vehicle, 3.2 and 10 mg/kg flibanserin in the combined data from all 11 females (triangles) compared to those of males (circles, same data for males as in Figure 2C). Data presented as mean $\pm$ SEM for 11 female and six male rats.