THE JOURNAL OF
SEXUAL MEDICINE

ORIGINAL RESEARCH

BASIC SCIENCE

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 agonist and 2A antagonist that has been approved by the Food and Drug Administration for treating female sexual interest and arousal disorder. Little is known about the abuse potential of flibanserin.

Aim: To examine abuse-related effects of flibanserin in rats using an intracranial self-stimulation (ICSS) procedure previously used to evaluate the abuse potential of other drugs.

Methods: Adult female and male Sprague-Dawley rats with electrodes implanted in the medial forebrain bundle were trained to press a lever 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 and 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 administration (1.0, 3.2 and 10.0 mg/kg). Subsequent studies determined the effects of flibanserin (3.2—18 mg/kg) before and after a regimen of repeated flibanserin administration (5.6 mg/kg/d for 5 days). Effects of the abused stimulant amphetamine (1.0 mg/kg) were examined as a positive control.

Main Outcome Measures: Flibanserin effects on ICSS frequency—rate curves in female and male rats were examined and compared with the effects of amphetamine.

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

Conclusion: These results suggest that flibanserin has low abuse potential. In addition, this study suggests that female rats might be more sensitive than male rats to the rate-decreasing effects of high flibanserin doses.

J Sex Med 2016;13:338—349.

Key Words: 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 and arousal disorder (formerly known as hypoactive sexual desire disorder).[3,4] Although the precise mechanisms of the effects of flibanserin 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 male[5] and female[6,7] rats. The effectiveness of flibanserin to modulate central nervous system monoaminergic signaling suggests that abuse potential is a clinical concern, and in its preapproval reviews of flibanserin, the Food and Drug Administration requested additional studies on abuse potential.[8] Different procedures are used in the preclinical assessment of abuse potential, and these procedures include drug self-administration, place conditioning, and intracranial self-stimulation (ICSS).[9—12] Currently, little information is available from preclinical studies regarding the 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

Received September 8, 2015. Accepted December 24, 2015.

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http://dx.doi.org/10.1016/j.jsxm.2015.12.031

conditioning studies have not been conducted in female rats (the sex for which the drug is approved for treatment of sexual interest and arousal disorder), and flibanserin has not been studied in either sex in drug self-administration or ICSS procedures. However, drugs with related pharmacologic mechanisms of action as 5HT1A agonists or 5HT2A antagonists have been tested in place conditioning and ICSS procedures, and these studies suggest that 5HT1A agonist effects in particular might contribute to abuse potential. Specifically, the 5HT1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), which has high efficacy at 5HT1A receptors equivalent to that of flibanserin,[14] produced conditioned place preference[15] and facilitation[16,17] of ICSS at low doses in male rats, whereas higher doses produced conditioned place aversion and depression of ICSS. Conversely, 5HT2A antagonists produced neither conditioned place preference[18] nor facilitation[19,20] of ICSS. 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 the 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 relation can be graphed as a sigmoidal frequency—rate curve. Drugs with high abuse potential (eg, amphetamine) typically increase (or "facilitate") ICSS rates and produce leftward and upward shifts in ICSS frequency—rate curves. Conversely, drugs that lack abuse potential typically 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 (eg, utility for evaluation of drug time course; see Negus and Miller[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 woman). For two reasons, subsequent studies evaluated the effects of repeated flibanserin doses. First, flibanserin dosing guidelines in humans call for repeated dosing, and the full effects of flibanserin on sexual behavior in preclinical studies have been seen primarily after repeated dosing.[23] Second, we previously reported that repeated drug treatment can unmask the expression of abuse-related ICSS rate-increasing effects of some other drugs such as μ-opioid receptor agonists.[24—27] The effects of flibanserin were compared with those of the abused stimulant amphetamine as a positive control.

METHODS

Subjects

For the initial studies, five adult female and six adult male Sprague-Dawley rats (Harlan, Frederick, MD, USA) were used, and the 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 the estrous cycle phase was monitored. All rats had ad libitum access to food and water and were housed individually at a 12-hour light-and-dark cycle (lights on from 6 AM to 6 PM) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Male rats weighed 300 to 350 g at the time of surgery, whereas females weighed 200 to 300 g. All experiments were performed with the approval of the institutional animal care and use committee at Virginia Commonwealth University (Richmond, VA, USA) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (eighth edition).[28]

Assay of ICSS

Surgery

Rats were anesthetized with 2.5% isoflurane (3% in oxygen; Webster Veterinary, Phoenix, AZ, USA) until unresponsive to a toe pinch before the 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 stereotaxically implanted into the left medial forebrain bundle at the level of the lateral hypothalamus using previously published coordinates for female[29] and male[30,31] rats. Specifically, coordinates in female rats 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 male rats 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 in diameter, non-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 hours after surgery. Animals were allowed to recover for at least 1 week before ICSS training.

Apparatus

Operant conditioning chambers consisted of sound-attenuating boxes containing modular acrylic and metal test chambers (29.2 × 30.5 × 24.1 cm; Med Associates, St Albans, VT, USA). 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 12 chambers, and each rat was assigned to one of the chambers for the entire study.