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Zaprinast, a Phosphodiesterase Type-5 Inhibitor, Alters Paced Mating Behavior in Female Rats

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Abstract

Nitric oxide (NO) is the primary mediator of blood flow in female genital tissues and drugs that enhance the activity of nitric oxide, such as phosphodiesterase type-5 (PDE-5) inhibitors, increase vaginal blood flow in anesthetized rats. The goal of the present study was to test the effects of one PDE-5 inhibitor, zaprinast, on the display of sexual behaviors in gonadectomized, estrogen and progesterone-treated female rats. Experiment 1 demonstrates that zaprinast alters paced mating behavior by lengthening the contact-return latency to ejaculation; there is a significant relationship between dose of zaprinast (range 1.5 – 6 mg/kg) and contact-return latency to ejaculation. Experiment 2 illustrates that zaprinast has no effect on preference for an intact male as measured in a No Contact partner preference test. Rats receiving zaprinast tend to exhibit reduced locomotor activity in both experiments. Collectively, these findings demonstrate that modulation of the NO-cGMP pathway using a PDE-5 inhibitor alters the display of paced mating behaviors in rats.

Keywords

Sexual behavior; Nitric oxide; Vaginal blood flow; Sexual dysfunction; Paced mating behavior

1. Introduction

Nitric oxide (NO) is the primary mediator of genital bloodflow in female rats [1]. NO stimulates guanylate cyclase and the production of cyclic guanosine monophosphate (cGMP). cGMP is one of the second messengers modulating intracellular calcium levels in vascular smooth muscle cells and ultimately vaginal muscle tone. Hydrolysis of cGMP into GMP is dependent on phosphodiesterase type 5 (PDE-5) [2–4]. Therefore inhibitors of PDE-5, such as zaprinast or sildenafil citrate (ViagraTM), increase and prolong the effect of cGMP resulting in enhanced vasodilation and increased vaginal blood flow [1,5]. Identification of nitric oxide synthase (NOS), the enzyme necessary for the synthesis of NO, in vaginal tissue from premenopausal women [6], and of PDE-5 expression in human vaginal [7] and clitoral [8] tissue provides additional evidence supporting a role for this pathway in the regulation of genital blood flow in females.

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Direct measurements of PDE-5 inhibitor effects on pelvic-nerve stimulated vaginal bloodflow have been garnered from studies conducted in anesthetized rats [1]. It is not known how the administration of agents that modulate vaginal blood flow such as PDE-5 inhibitors affect the display of sexual behaviors in female rats. One study suggests that PDE-5 inhibitors can modulate the display of sexual behaviors in female hamsters [9]. Specifically, alterations in the pelvic adjustments made by the female hamster that facilitate penile insertion that occurred following the administration of a specific serotonin reuptake inhibitor were ameliorated by zaprinast (3 mg/kg, ip, 20 min before the test), suggesting cross-talk between the serotonin and NO-cGMP pathways in the regulation of female sexual behaviors [9].

Paced mating behavior, the pattern of approach and withdrawal exhibited by a female rat during a sexual encounter with a male rat, is one animal model that has been used to assess female sexual function [10–14]. Paced mating behavior requires the integration of sensory and motivational signals [15], and there is evidence that genitosensory input from the periphery is important for the display of paced mating behavior [16]. Transection of the pelvic and pudendal nerves alters the pattern of paced mating [15]. Furthermore, work from our laboratory has shown that the anti-estrogen, ICI 182,780, a compound that upon systemic administration does not enter the brain, modifies the pattern of paced mating behavior [17]. Collectively, the data showing that PDE-5 inhibitors increase pelvic-nerve stimulated vaginal blood flow in rats [1] and the evidence for a role of peripheral systems in the display of paced mating behavior led us to test in the present experiments the effects of one PDE-5 inhibitor, zaprinast, on the display of sexual behaviors in female rats.

2. Materials and Methods

2.1 Subjects

Female Long-Evans rats weighing approximately 200 g were obtained from Harlan (Indianapolis, IN). Rats were housed individually in hanging metal cages in a light- (12:12, lights off at 1000 h) and temperature-controlled vivarium. Commercial rat pellets and water were freely available. Rats were gonadectomized (GDX) under sodium pentobarbital anesthesia (50 mg/kg, Nembutal; Henry Schein, Indianapolis, IN) approximately 7–10 days before the start of behavioral testing; gonadectomy with hormone replacement allowed us to control the timing and reliability of the expression of sexual behavior. Sexually experienced male Long-Evans rats, aged 3–4 months, were used as stimulus rats. The Institutional Animal Care and Use Committee at Dartmouth College approved the use of rats in these studies and all procedures were conducted in accordance with NIH guidelines.

2.2 Drugs

Experimental female rats received 10 µg estradiol benzoate (EB, Sigma, St. Louis, MO) 48 h and 1 mg progesterone (P, Sigma) 4 h prior to each mating test. Hormones were administered sc in a sesame oil vehicle. Zaprinast (Sigma) was prepared as described previously in a vehicle of 25% dimethylsulfoxide (DMSO) v/v in saline and administered ip, 20 min before testing [9].

2.3 Procedures

2.3.1 Paced mating behavior—Tests for paced mating behavior took place in a clear Plexiglas arena (112.4 cm long × 37.5 cm wide × 31.7 cm high) with wood shavings covering the floor. The arena was divided into three equally sized separate compartments using two clear partitions (36.5 × 31.7 cm) with a 5.0 cm hole in each bottom corner. Opaque Plexiglas partitions (each 36.5 × 31.5 cm) were placed on either side blocking the clear Plexiglas partitions. The experimental and stimulus rats were habituated to their respective compartments of the arena for 5 min before the test began. At the start of the test, one opaque partition was

removed, allowing the experimental rat access to a single male through the clear Plexiglas partition. During the test, the female rat had access to only one male at a time. The test was complete when the experimental rat received 10 mounts with intromissions, including ejaculations when they occurred. When ejaculations were received, the experimental rat was allowed to leave the male rat's compartment and subsequently return, at which point the test timer was stopped, the opaque partition was replaced and the experimental rat was once again confined to the center compartment. The test was resumed immediately upon removal of the other opaque partition, allowing the experimental rat access to the second male rat. This procedure was repeated until the female had received 10 intromissions. Paced mating tests were terminated according to the following criteria: (1) return to the stimulus male rat after receiving a 10th intromission, (2) no intromissions were received within the first 15 min of the test, or (3) if more than 30 min elapsed between any two intromissions. Data for rats that did not receive 10 intromissions or at least one ejaculation during the paced mating tests were not included in the data analyses. Measures recorded during the paced mating test included: (1) sexual receptivity (lordosis), (2) contact-return latency, the time elapsed before the female rat re-enters the male rat's compartment following mating stimulation (i.e., mount, intromission, ejaculation), (3) percentage of exits, the rate of withdrawals by the female rat from the male rat's compartment following mating stimulation, (4) the percentage of total test time the female rat spent with the male, (5) the rate of proceptive behaviors (hops, darts and ear wiggling), (6) the rate of rejection behaviors (kicks and defensive postures), and (7) activity, defined as the rate of entries and exits (arena crossings) from the male rat's compartment.

2.3.2 Partner preference—Behavioral tests were conducted in the tri-compartment arena described above with two pairs of Plexiglas partitions in place at the start of the test to delineate three equal-size compartments. A pair of clear Plexiglas partitions with holes in the bottom corners allowed the experimental female rat to enter into and exit from the stimulus rat compartments. The pair of solid opaque partitions, that blocked both access and view, was removed after a 5-min habituation period that preceded each test to reveal the clear partitions and begin the test. The stimulus rats (an intact male or GDX, EB/P-primed female rat) were housed behind hardware cloth partitions inserted in the middle of each side compartment.

Procedures previously described for partner preference tests in our laboratory were followed [18]. Briefly, an experimental female rat was placed in the center compartment and one stimulus rat was placed in each side compartment. The test began when both opaque partitions were removed allowing the experimental rat to move freely throughout the arena for the 10-min test. Experimental rats could not gain physical access to the stimulus rats that were positioned behind the hardware cloth partitions. Time spent in the compartment in the vicinity of the male and female stimulus rats was recorded as were the number of entries and exits from each compartment. At the conclusion of the partner preference test the rats were tested for sexual receptivity to 10 mounts by a male rat.

For each rat three measures were calculated on each partner preference test: (1) a preference score, defined as the time spent with the intact male divided by the sum of the time with both stimulus rats, (2) a social score, defined as the total combined time the experimental rat spent with the stimulus rats divided by the test duration (600 sec), and (3) activity, defined as the rate of entries and exits from the stimulus animal compartments.

2.3.3 Data analysis—Data from the baseline tests were subtracted from the test data to yield a difference score and the difference scores were then subject to one-way analysis of variance (ANOVA) [18,19]. In Experiment 1, ANOVA with linear contrast analysis was used to examine the dose-response relationship between zaprinast and contact-return latency to ejaculation. The α level was set at $p < 0.05$.

2.4 Experiment 1: Dose-response to zaprinast

The purpose of Experiment 1 was to examine the dose-response characteristics of zaprinast. Approximately one week after gonadectomy, EB- and P-primed rats were tested for paced mating behavior (baseline test). After the baseline tests, the 43 rats were assigned to four groups matched on behavioral measures on the baseline test; Group 1 received the vehicle, Group 2 received 1.5 mg/kg zaprinast, Group 3 received 3 mg/kg, and Group 4 received 6 mg/kg. The dose range of zaprinast selected for testing was based on other published studies of the behavioral effects of systemic zaprinast [9,20]. Tests for paced mating commenced within a week after the baseline test. Three rats did not meet the paced mating behavior criterion and their data were not included in the analysis. Difference scores were computed and examined for a linear relationship between dose of zaprinast and contact-return latency to ejaculation and activity.

2.5 Experiment 2: Zaprinast and partner preference

In Experiment 1 rats receiving zaprinast exhibited a lengthening of the contact-return latency following an ejaculation. In addition to tests of paced mating behavior, the partner preference paradigm has also been used to evaluate sexual behaviors in female rats [11,18]. In the partner preference paradigm, the display of approach behavior and the time spent in the vicinity of an intact male rat are modulated by gonadal hormones [18,21]. In Experiment 2, we tested whether zaprinast altered the display of approach behaviors by a female rat toward a conspecific. Specifically, GDX, EB/P-primed female rats were tested for preference for a sexually active male vs. a GDX, EB/P-primed female rat under No Contact conditions that allowed the exchange of visual, auditory and olfactory cues but prohibited copulation [18,19].

GDX female rats were primed with EB/P and tested for partner preference (baseline). Within one week after the baseline test, the 26 rats were tested a second time; one group received zaprinast ip, 20 min before the test (3 mg/kg, $n = 13$) the other group received the vehicle ($n = 13$). Difference scores on the measures of partner preference and activity were compared between groups using ANOVA.

3. Results

3.1 Experiment 1: Dose-response to zaprinast

Rats in all four groups displayed high levels of sexual receptivity (data not shown). There was a statistically significant relationship between the dose of zaprinast and the contact-return latency to ejaculation [$F(3,36) = 5.3, p < 0.05$; Figure 1], demonstrating that higher doses of zaprinast were associated with lengthened contact-return latencies to ejaculations (Figure 1). The linear relationship between the dose of zaprinast and activity did not attain statistical significance [$F(3,36) = 3.0, p = 0.09$, Figure 2]. There were no significant group differences or linear relationships for contact-return latencies to mounts or intromissions or percentage of exits (Table 1). In addition, there were no group differences in the rate of proceptive and rejection behaviors, the number of mating stimulations (mounts, intromissions or ejaculations) received, or the percentage of the test time spent with the male (data not shown).

3.2 Experiment 2: Zaprinast and partner preference

Rats in Experiment 2 exhibited high levels of sexual receptivity (data not shown). All female rats displayed a preference for the male on both the baseline and test and there were no significant effects of group on preference for male (Figure 2) or social preference (data not shown). There was a significant effect of group on difference scores for activity; rats receiving zaprinast exhibited less activity than controls [$F(1,24) = 5.9, p < 0.05$, Figure 2].

4. Discussion

Zaprinast, a PDE-5 inhibitor, lengthened contact-return latencies following the receipt of an ejaculation. Female rats administered zaprinast continued to display high levels of sexual receptivity and a preference for a sexually active male. Given the known effects of zaprinast to inhibit the activity of phosphodiesterase type-5 [22], the present results support a role for the NO-cGMP pathway in the display of paced mating behaviors.

4.1 PDE-5 inhibitor and sexual function

PDE-5 inhibitors enhance pelvic nerve-stimulated blood flow (e.g., sildenafil, [1]; vardenafil, [23]) although the effects of zaprinast in this model system have yet to be evaluated. Our working model is that the enhanced genital blood flow elicited by zaprinast leads to heightened vaginal sensitivity to mating stimulation manifested behaviorally as a lengthening of contact-return latencies following ejaculation, as exhibited in the present study. The effectiveness of zaprinast to prolong contact-return latencies following the receipt of ejaculation, as opposed to both intromissions and ejaculations, requires further study but could be related to the increased intensity and duration of vaginocervical stimulation accompanying an ejaculation [24].

Modulation of vaginal sensitivity can affect female sexual behavior. Transection of the pelvic nerve renders GDX EB- and P-primed rats less able to discriminate between mounts and intromissions than sham-transected female rats [25]. Pathways between genital tissues (vagina, clitoris) and brain regions known to modulate paced mating behavior have been established [26,27]. The effects of zaprinast on paced mating behaviors observed in the present study, i.e., the lengthening of contact-return latencies to more intense mating stimulation, bear a resemblance to the pattern of paced mating behavior exhibited by rats given a systemic injection of the anti-estrogen ICI 182,780 [17,28]. The results of Experiment 2 demonstrating that preference for a sexually active male conspecific is unaltered in response to zaprinast are consistent with the model that zaprinast modulates sexual sensitivity and response, and not motivation, suggested previously [9]. Recently, the anti-estrogen tamoxifen has been reported to significantly decrease vaginal blood flow compared to controls, underscoring the role of interactions between gonadal steroids, NO and vaginal bloodflow [29]. Collectively, these findings support the contribution of sensory input from the genital area, and genital blood flow, to the expression of paced mating behavior.

4.2 Role of activity

The reduction of activity observed in the partner preference test in rats receiving zaprinast (3 mg/kg) was unexpected. Changes in locomotor activity have been reported following high doses of PDE-5 inhibitors in some studies; a significant reduction in locomotor activity has been observed in response to sildenafil (3×3 mg/kg, po) [30]. In contrast, although high doses of zaprinast (10 mg/kg and higher) suppressed the responding of male rats on a differential rate of low reinforcement schedule, doses equivalent to those used in the present study (0.3–3 mg/kg) did not affect response rate [20]. Similarly, doses of zaprinast up to 2 mg/kg, po, administered to male mice had no effects on locomotor activity [31]. One interpretation of the changes in activity in the present study is that zaprinast had nonspecific effects that resulted in a reduction in activity levels and that the changes in paced mating behavior are an artifact of these nonspecific actions. Several pieces of evidence challenge this view. First, there were no significant group (zaprinast/vehicle) differences in contact-return latency to mounts or intromissions. If zaprinast made the rats more lethargic in general, then contact-return latencies to all types of stimulation would have been predicted to lengthen significantly and this was not the case. Second, the percentage of exits was not affected by zaprinast, and rats treated with zaprinast or with the vehicle were equally likely to move to exit the male compartment. Third,

there were no group differences in the latency to make the initial entry into the male's compartment at the start of the test (data not shown). Collectively, these observations support the view that the effects of zaprinast on paced mating behavior cannot be accounted for by nonspecific effects of the compound on locomotor activity.

The results of the present study provide support for the view that alterations of the NO-cGMP pathway can affect the sexual functioning of female rats, as assessed using the paced mating behavior paradigm. A limitation of the present set of studies is that we did not assess whether the changes in paced mating behavior after zaprinast administration are due to the inhibition of PDE-5 activity in the periphery, perhaps at the level of the vagina, in the central nervous system, or a combination of these sites [32]. Understanding of the role of NO in the display of female sexual behaviors will also be advanced by examining the central and peripheral actions of selective NO inhibitors (e.g., endothelial nitric oxide synthase, [33]) and precursor compounds (e.g., L-arginine, [34]) in future studies.

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A. Ejaculation

B. Activity

Figure 1. Dose-response to zaprinast

Left: Mean $\pm$ SEM (A) contact-return latencies to ejaculations and (B) levels of activity for the baseline (open) and test (solid) for rats receiving the vehicle ($n = 8$), zaprinast at a dose of 1.5 mg/kg ($n = 9$), 3 mg/kg ($n = 9$), or 6 mg/kg ($n = 10$). Right: Difference scores (test - baseline) showed that zaprinast lengthened contact-return latencies following ejaculation in a dose-dependent manner ($*p < 0.05$). There was no significant effect of zaprinast on activity.

A. Preference Score

B. Activity

Figure 2. Zaprinst and partner preference

Left: Mean $\pm$ SEM (A) preference scores and (B) levels of activity are shown for female rats that received zaprinast (3 mg/kg, $n = 13$) or the vehicle ($n = 13$) 20 min before the partner preference test. Right: Difference scores (test -baseline) revealed significantly decreased activity in rats receiving zaprinast compared with controls ($*p < 0.05$ vs. vehicle).

Table 1
Behaviors displayed during paced mating behavior test

Dose of Zaprinas	Contact-Return Latency (sec)			Percentage of Exits	
	Mounts	Intrusions	Mounts	Intrusions	Ejaculations
0 mg/kg	8.7 ± 1.7	20.8 ± 3.3	53.1 ± 12.1	63.0 ± 12.1	100 ± 0
1.5 mg/kg	22.9 ± 13.0	32.2 ± 9.3	36.0 ± 13.5	57.9 ± 9.2	100 ± 0
3.0 mg/kg	14.7 ± 2.6	39.2 ± 8.3	31.5 ± 12.6	51.8 ± 9.9	100 ± 0
6.0 mg/kg	17.2 ± 5.1	37.6 ± 10.7	54.1 ± 12.4	67.6 ± 8.3	100 ± 0

Mean ± SEM are shown.