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Effect of systemic blockade of α_1-noradrenergic receptors on sex behavior and vaginal–cervical stimulation-induced Fos in female rats

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ABSTRACT

It is hypothesized that systemic α_1-noradrenergic antagonists may interfere with the transmission of sensory stimulation, particularly vaginal--cervical stimulation (VCS), which is crucial for reproductive functioning. To determine if α_1-noradrenergic transmission receptor activity is necessary for transmission of sensory information important for VCS-dependent events, we conducted an experiment using prazosin, a α_1-noradrenergic receptor antagonist. First, three doses of prazosin (1.0, 0.5 or 0.1 mg/kg) or the 10% ETOH in sesame oil vehicle were administered i.p. and sexual receptivity was assessed 30 min later in ovariectomized, hormone-treated female rats. The 1 mg/kg dose of prazosin significantly inhibited lordosis quotients and lordosis ratings. This dose of prazosin (1.0 mg/kg) was then administered 30 min prior to VCS or control scapular stimulation (CSS) and Fos-IR was examined in the posterodorsal medial amygdala (MeaPD), the medial preoptic area (mPOA), and the rostral ventromedial hypothalamus (rVMH). VCS significantly increased Fos-IR in all of the brain areas examined. Prazosin treatment inhibited the increase in Fos-IR in the mPOA and MeaPD but not in the rVMH. These results suggest that administration of systemic prazosin may selectively affect sensory inputs to the mPOA and MeaPD and these inputs are relevant for the control of female sexual behaviors by peripheral α_1-noradrenergic activity.

1. Introduction

A growing body of literature indicates an important role for norepinephrine (NE) in mediating sympathetic nervous system activation of sexual arousal in women (Meston, 2000). In particular, modulation of α_2-noradrenergic activity by clonidine, an α_2-noradrenergic agonist has been shown to alter female sexual response in humans (Meston et al., 1997; Meston, 2000; Riley, 1995) and animals (Meston et al., 1996). Despite evidence supporting a role for α_2-noradrenergic activity, the role of peripheral α_1-noradrenergic activity on sympathetic activation of female sexual functioning has not yet been studied.

Systemic α_1-noradrenergic receptor antagonists, such as prazosin, have been widely used for the successful treatment of hypertension (Itskovitz, 1994; Wykretowicz et al., 2008; Zusman, 2000) but sexual dysfunction is a consistently reported side effect in both male and female patients (Grimm et al., 1997; Stevenson and Umstead, 1984; Weiss, 1991). The sexual side effects may be largely explained by changes in muscle contractility and decreases in genital blood pressure due to autonomic inhibition by α_1-noradrenergic receptor antagonists (Hodge et al., 1991; Manolis and Doumas, 2008; Smith and Talbert, 1986).

Studies conducted in rodents suggest an important role for α_1-noradrenergic transmission in the normal display of female sexual behavior. Systemic (Fernandez-Guasti et al., 1985; Thornton et al., 1989; Vincent et al., 1989) and central (Etgen, 1990) administration of α_1-noradrenergic antagonists inhibits hormonally-induced female sex behavior. The ability of noradrenergic antagonists to interfere with sex behavior in female rats has been proposed to be mediated, at least in part, by interfering with estrogen priming in the hypothalamus (Blaustein, 1987; Clark et al., 1985; Montemayor et al., 1990). However, studies in mice have shown that systemically administered prazosin does not readily enter the brain (Stone et al., 2001). Furthermore, behavioral effects of systemically administered prazosin have been observed as quickly as 30 min after administration (Vincent et al., 1989). Collectively, these studies suggest that the behavioral effects of prazosin may not be due to changes in central hormone receptor concentrations.

Other studies have proposed that peripheral NE activity may be crucial for the normal display of female sexual behaviors due to its role in sympathetic control of sexual responsiveness (Meston et al., 1996). Because female sexual behaviors in rodents also depend on the receipt of somatosensory information typically provided by the male during mating (Kow et al., 1979; Pfaff et al., 1977), peripherally-active NE antagonists may affect sexual behavior by interfering with the

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peripheral transmission of sensory information critical for reproductive functioning via changes in the sympathetic activation of sexual response.

During a typical mating interaction with a male rat, a female rat receives tactile stimulation of the flanks, tailbase, perineum and genital area as well as vaginal–cervical stimulation (VCS) from the male's penis during intromissions and ejaculations (Hardy and DeBold, 1972; Kow et al., 1979; Pfaff et al., 1977). VCS has been shown to be crucial for sexual behavior and reproductive success such that the receipt of VCS, alone or in combination with other tactile stimulation, can potentiate the display of lordosis (Bennett et al., 2001), prolong the duration of lordosis (Komisaruk and Diakow, 1973; Rodriguez-Sierra et al., 1975), and abbreviate the duration of sexual receptivity (Bennett et al., 2002; Coopersmith et al., 1996).

The uterus and cervix of the rat are highly innervated by noradrenergic nerve fibers from the sympathetic nervous system (Houdeau et al., 1995, 1998). This sympathetic innervation is crucial for fertilization and pregnancy and may also be important for mediating behavioral responses to VCS (Houdeau et al., 1995, 1998). Genitosensory information received during mating is carried primarily by the pelvic, pudendal and hypogastric nerves and ascends to the brain via the anterolateral columns of the spinal cord, terminating in the A1, A2, A5, and A7 noradrenergic cell groups of the pons and medulla (Berkley et al., 1993a,b; McKenna and Nadelhaft, 1986; Pascual et al., 1992; Zemlan et al., 1978). Although there is evidence for an important role of central α_1-noradrenergic neurons in mediating the effects of VCS on behavior and reproductive success (Cameron et al., 2004a,b; Crowley et al., 1978; Everitt et al., 1975; Hansen et al., 1980, 1981; Northrop et al., 2006), the role of peripheral α_1-noradrenergic transmission in mediating the sensory aspects of mating has not yet been studied. Therefore, it is possible that the negative effects of α_1-noradrenergic antagonists such as prazosin on human and rodent sexual functioning can be explained by pharmacological alterations of the sensory experience of the female during mating.

VCS, provided either artificially or received during mating, has been reliably shown to induce increases in Fos protein, a product of the immediate early gene *c-fos,* in posterodorsal medial amygdala (MeaPD), the medial preoptic area (mPOA), the ventromedial hypothalamus (rVMH) as well as several other areas relevant for reproduction (Erskine, 1993; Greco et al., 2003; Pfaus and Heeb, 1997; Polston and Erskine, 1995; Quysner and Blaustein, 2001; Rowe and Erskine, 1993; Tetel et al., 1993). Fos-IR in these forebrain areas has been shown to be sensitive to changes in sensory information received during mating (Polston and Erskine, 1995) and following transection of the pelvic nerve (Pfaus et al., 2006; Rowe and Erskine, 1993). Therefore, quantification of Fos-IR in these brains areas is a useful tool to measure changes in peripheral sensory input received during VCS.

The aim of the present study is to determine if systemic prazosin, at a dose sufficient to inhibit hormonally induced sex behavior, would also affect the transmission of sensory information associated with VCS. This was determined by examining the effect of a behaviorally effective dose of systemically administered prazosin on induction of Fos-IR in forebrain areas known to be sensitive to mating stimuli. We hypothesize that the behaviorally relevant dose of systemic prazosin will interfere with forebrain Fos-IR induced by VCS.

2. Materials and methods

2.1. Animals

The subjects were adult female Sprague–Dawley rats obtained from Simonsen Laboratories (Gilroy, CA, USA) and housed in the vivarium facilities at Wheaton College. The female rats were approximately 150–175 g and 50 days old at the start of the study. All animals were kept on a 10:14 hour light:dark light cycle (lights ON at 24:00) and were given food and water *ad libitum*. Upon arrival, female rats were housed in groups of 4 in large stainless steel hanging cages. Male rats were used as mating stimuli during behavior tests and were housed individually in stainless steel hanging cages. All animals were allowed to adjust to the vivarium conditions for one week prior to the start of the studies. All procedures used in these studies adhere to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Wheaton College.

2.2. Ovariectomy

At least 1 week after arrival, female rats were ovariectomized through a single midventral incision under ketamine cocktail anesthesia (100 mg ketamine HCL, 20 mg xylazine HCL and 10 mg/ml acetopromazine) in distilled water, delivered i.p. After surgery, animals were allowed to recover for at least one week and were then handled daily to reduce stress related Fos-IR induction on test day.

2.3. Hormone treatment

In each of the three studies described, all female rats were injected subcutaneously with 5 μg/rat of 17β-estradiol benzoate (EB; dissolved in sesame oil in a 0.1 ml volume) 48 h prior to testing or VCS and 0.5 mg/rat of progesterone (P; dissolved in sesame oil in a 0.1 ml volume) 4 hours prior to behavior testing or VCS.

2.4. Dose–response of systemically administered prazosin on hormone-induced sexual receptivity

In order to determine a physiologically relevant dose of systemically administered prazosin, an initial study was conducted to assess the effect of three doses of prazosin on hormone-induced sex behavior in ovariectomized rats. The most effective dose was used in the next stage of the study.

One week after ovariectomy, all female rats ($N=35$) were tested for sexual receptivity with sexually experienced male rats following EB and P administration (as described above) in a behavioral screening test. Each female was placed with a male rat until she received 10 mounts (with or without intromissions and/or ejaculations). A lordosis quotient (LQ; total # of lordosis responses by female during test/total number of mounts from the male X 100) and lordosis rating (LR; total lordosis ratings/total number of mounts from the male) were calculated for each female. To ensure that animals used in the study were maximally responsive to the estrogen and progesterone regimen, only females with LQs greater than 70 in the screening test were included in the study. Four animals were removed from the study due to LQ scores <70.

One week following the behavioral screening test, all female rats ($N=31$) were assigned to 1 of 4 groups in a design based on a previous study (Thornton et al., 1989): 0.1 mg/kg prazosin ($n=8$), 0.5 mg/kg prazosin ($n=7$), 1.0 mg/kg prazosin ($n=8$) or vehicle ($n=8$). All animals were given EB and P (as described above) and, 30 min prior to the behavioral test, all females were given an intraperitoneal injection of prazosin in a 0.1 ml/kg volume (Sigma-Aldrich) or the vehicle (10% ETOH in distilled water in a 0.1 ml/kg volume). All females were then tested for sexual receptivity by experimenters who were blind to treatment conditions. LQs and LRs were calculated for all females.

2.5. Effect of prazosin on VCS-induced Fos-IR in forebrain areas

One week following the dose–response test, all female rats were treated with EB and P (as described above). The hormone treatment was continued in this phase of the study in order to remain consistent

with the behavioral study, allowing for comparison between data from both studies. All females were assigned to 1 of 4 groups that were factorial combinations of 2 stimulation conditions (VCS and control scapular stimulation, CSS) and 2 drug conditions (Prazosin and Vehicle). The 4 experimental groups were: VCS/Vehicle ($n=7$) and VCS/Prazosin ($n=8$), CSS/Vehicle ($n=7$), CSS/Prazosin ($n=7$). Thirty minutes prior to receipt of VCS/CSS, all females were given an intraperitoneal injection of prazosin in a 0.1 ml volume or the vehicle (10% ETOH in distilled water in a 0.1 ml volume). The tissue from 2 animals was not used due to problems with tissue fixation.

2.6. Vaginal–cervical stimulation (VCS)

VCS was administered to all of the female rats using a probe consisting of a 1.0 cc syringe plunger with a flexible rubber tip secured to the end of a force gauge (Wagner Instruments, Greeenwich, CT). This configuration allowed for the experimenter to assess and control the amount of pressure being applied to the vagina and cervix. To administer the VCS stimulation, the female rat was secured briefly by holding the tail and the hindquarters were lifted slightly to allow insertion of the probe into the vagina. The female rats in the VCS group received one 200 g stimulation for 2 s every 30 s for 10 min. This pattern of vaginal–cervical stimulation has been shown to induce Fos protein in forebrain areas of female rats (Tetel et al., 1993). The control scapular stimulation (CSS) group received a similar pattern of stimulation but the probe was placed on the scapular region of the shoulder. After VCS/CSS, the animals were returned to their home-cages for 30 min before perfusion.

2.7. Perfusion

All experimental female animals were given a lethal dose of cholorpent anesthesia (3 ml/animal; 42.5 mg chloral hydrate and 8.6 mg pentobarbital per ml, delivered i.p.) 30 min after VCS/CSS stimulation. The animals were then perfused with 0.09% physiological saline (1 min, 25 ml) followed by 10–15 min of acrolein (2% in 0.1 M sodium phosphate buffer at pH of 7.2) at approximately 100 Hg flow rate of 25 ml/min. The brains were removed and stored at 4 °C in 0.1 M sodium phosphate buffer (pH 7.2) containing 20% sucrose for 48 h. The tissue was cut into series of 8 40 μm sections on a freezing microtome and stored in cryoprotectant (Watson et al., 1986) at −20 °C until they were used for immunocytochemistry.

2.8. Immunocytochemistry

A series of 1 in every 4 sections were rinsed 3 times for 5 min each in 0.05 M Tris-buffered saline (TBS, pH 7.6). To remove residual aldehydes, tissue was pretreated with 1% sodium borohydride for 10 min and rinsed 3 times for 5 min each in TBS. To reduce nonspecific staining and endogenous peroxidase activity, sections were then placed into 1% H_2O_2, 20% goat serum, and 1% bovine serum albumin for 20 min. The sections were incubated for 2 days at 4 °C in a primary antibody solution of TBS containing 0.1% gelatin, 0.02% sodium azide, 0.5% Triton-X-100 (TBS-gel) and 1% normal goat serum and the polyclonal rabbit *c-fos* antiserum (1:30,000, Ab5, Oncogene Science). Following 3 rinses for 5 min each in TBS-gel, the sections were incubated in a biotinylated goat anti-rabbit secondary antibody serum (1 μg/ml) for 90 min at room temperature. Following 2 rinses in TBS-gel for 5 min each and one 5 min rinse in TBS, the sections were incubated in an avidin-biotinylated complex solution (1:100; Vectastain Elite Kit, Vector Laboratories, CA) for 90 min at room temperature. Following 3 rinses in TBS for 5 min each, sections were treated with 0.05% diaminobenzidine and 0.05% H_2O_2 in TBS for 5 min, or until a satisfactory brown reaction product occurred. The sections were rinsed for 5 min in TBS buffer, mounted, and coverslipped with DePex mounting medium for light microscopy.

2.9. Computer imaging and analysis

Cells were considered Fos-IR if they had dark brown nuclear staining with clear boundaries. NIH Image-J (http://rsbweb.nih.gov/ij/) was used to count Fos-IR cells bilaterally from at least two matched sections from each animal for each of the 3 brain areas. Three neuroanatomical areas were chosen for analysis due to previous findings indicating that these areas were responsive to VCS (Auger et al., 1996; Tetel et al., 1993) and were based on the plates provided by Paxinos and Watson (1998): the posterodorsal medial amygdala (MeaPD, plate 33), the medial preoptic area (mPOA, plate 32) and the rostral ventromedial hypothalamus (rVMH, plate 20). Images were captured at 20× magnification and transformed to a grayscale image. Total cell counts were calculated for each image using NIH Image J by an experimenter who was blind to the treatment groups.

2.10. Data analysis

All statistical analyses were conducted using SPSS (Version 17, SPSS Inc., Chicago, IL, USA). Behavioral data were subjected to a one-factor ANOVA and post-hoc comparisons were made using the Mann–Whitney non-parametric post-hoc test. Mean Fos-IR cell counts for each area were subjected to a two-factor ANOVA (VCS × drug). When appropriate, data were subjected to a one-factor ANOVA and post-hoc comparisons were made using the Tukey HSD tests.

3. Results

3.1. Dose–response of systemically administered prazosin on hormone-induced sexual receptivity

There was a significant effect of prazosin administration on LQs [F (3, 30) = 18.55, $p<0.05$, Fig. 1]. Post-hoc analyses reveal that all prazosin-treated females displayed significantly lower mean LQs as compared the vehicle group (Mann–Whitney, $p<0.05$; Fig. 1). In

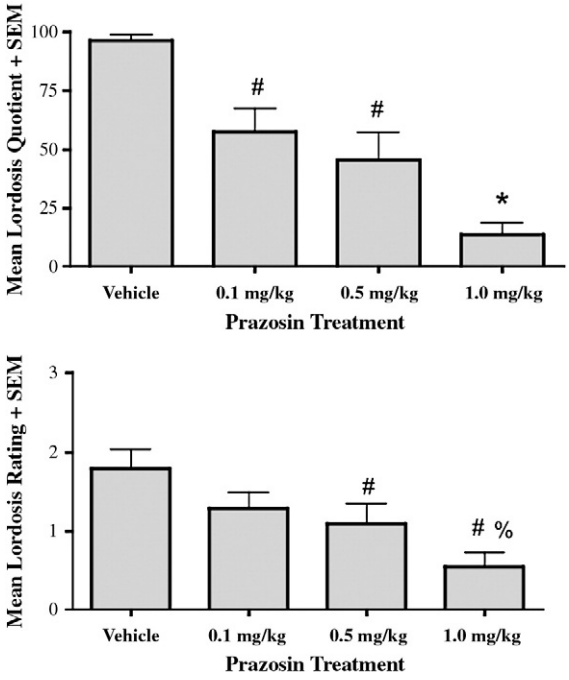

Fig. 1. Effects of prazosin (0.1 mg/kg, 0.5 mg/kg or 1.0 mg/kg) or the 10% ETOH vehicle on mean lordosis quotients (top) and lordosis ratings (bottom) of ovariectomized female rats given estradiol benzoate (5 μg/rat) and progesterone (0.5 mg/rat) 48 and 4 h prior to testing, respectively. Data presented as means + SEM. * = $P<.05$ vs. all other groups. # = $P<.05$ vs. the vehicle group. % = $P<0.05$ vs. the 0.1 mg/kg Prazosin group.

addition, the 1.0 group displayed significantly lower LQs as compared to the 0.1 mg/kg and 0.5 mg/kg groups ($p<0.05$; Fig. 1).

There was a significant effect of prazosin administration on LRs [F (3, 30) = 5.69, $p<0.05$, Fig. 1]. The 1.0 mg/kg group displayed significantly lower LRs as compared to the 0.1 mg/kg and vehicle groups (Mann–Whitney, $p<0.05$, Fig. 1). In addition, the 0.5 mg/kg group displayed significantly lower LRs as compared to the vehicle group ($p<0.05$, Fig. 1).

3.2. Effect of prazosin on VCS-induced Fos-IR in forebrain areas

In the MeaPD, there was an overall effect of VCS administration on Fos-IR [F (1, 105) = 125.793, $p<0.05$, Fig. 2], a significant effect of prazosin treatment [F (1, 105) = 7.437, $p<0.05$, Fig. 2] and a significant VCS × Prazosin interaction [F (1, 105) = 5.315, $p<0.05$, Fig. 2]. A one-way ANOVA followed by post-hoc comparisons revealed that there was a significant increase in the number of Fos-IR cells in the VCS/vehicle group as compared to all other groups [F (3, 105) = 48.313, Tukey, $p<0.05$, Fig. 2]. The number of Fos-IR cells found in the VCS/Prazosin group was significantly greater than both CSS groups but also significantly less than the VCS/Vehicle group (Tukey, $p<0.05$, Fig. 2). Administration of prazosin appears to have partially inhibited the increase in Fos-IR cells observed in the MeaPD after VCS.

In the mPOA, there was an overall effect of VCS administration on Fos-IR [F (1, 102) = 19.422, $p<0.05$, Fig. 3]. There was no significant effect of prazosin treatment [F (1, 102) = 2.294, $p>0.05$, Fig. 3] but there was a significant VCS × Prazosin interaction [F (1, 102) = 3.952, $p<0.05$, Fig. 3]. A one-way ANOVA followed by post-hoc comparisons revealed that there was a significant increase in the number of Fos-IR cells in the VCS/Vehicle group as compared to both CSS groups [F (3, 102) = 8.954, Tukey, $p<0.05$, Fig. 3]. The VCS/Prazosin group was not significantly different from either CSS groups or the VCS/Vehicle, suggesting that administration of prazosin partially blocked the increase in Fos-IR cells observed following VCS.

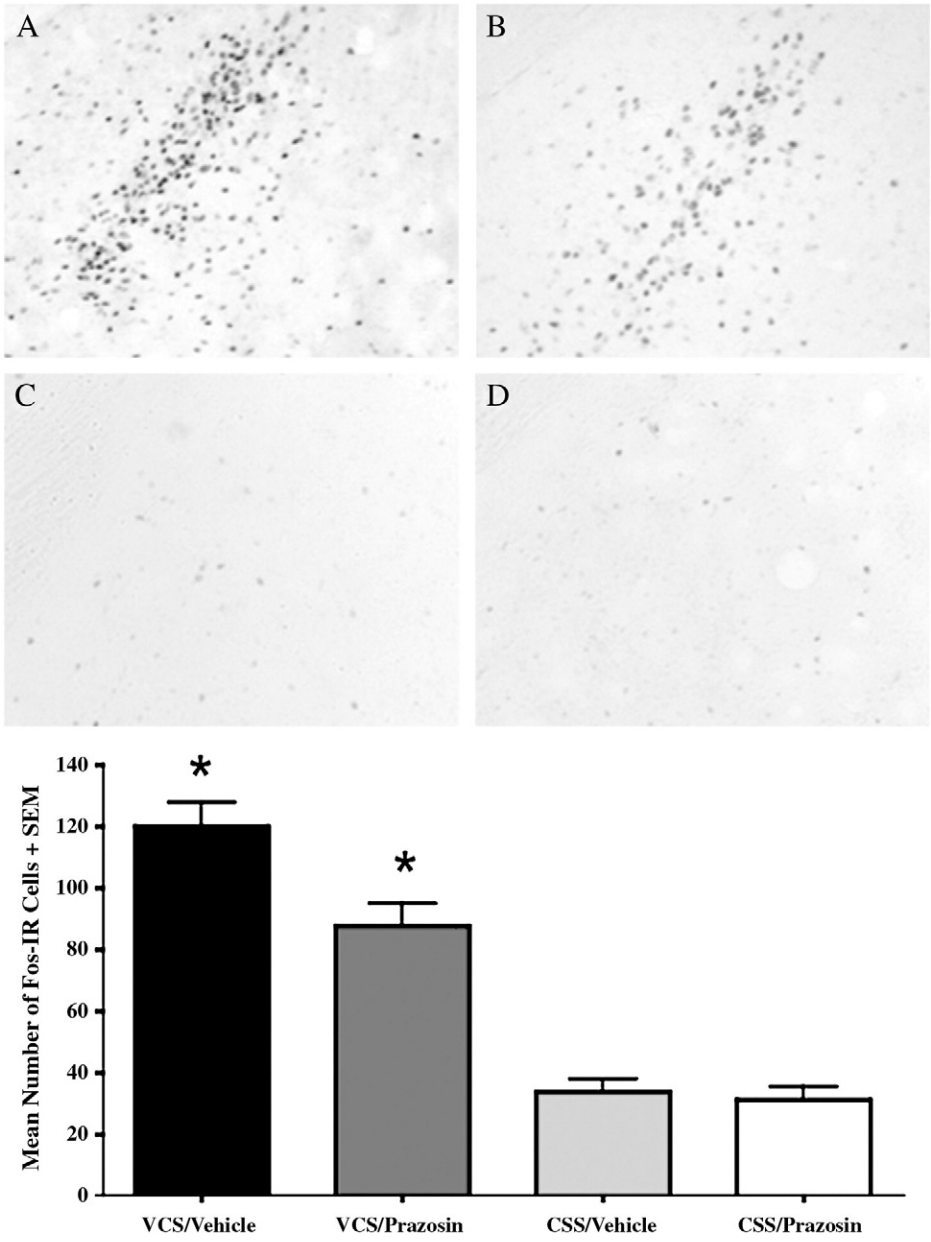

Fig. 2. Effects of prazosin on Fos-immunoreactive (Fos-IR) cells in the MeaPD of hormone-treated ovariectomized female rats. Top: Photomicrographs (20×) depicting Fos induction by vaginal cervical stimulation (VCS) or control scapular stimulation (CSS) following administration of prazosin (1.0 mg kg) or the 10% ETOH vehicle. A = VCS/Vehicle, B = VCS/Prazosin, C = CSS/Vehicle, D = CSS/Prazosin. Bottom: Mean number of Fos-IR cells in each treatment group + SEM. * = $P<.05$ vs. all other groups.

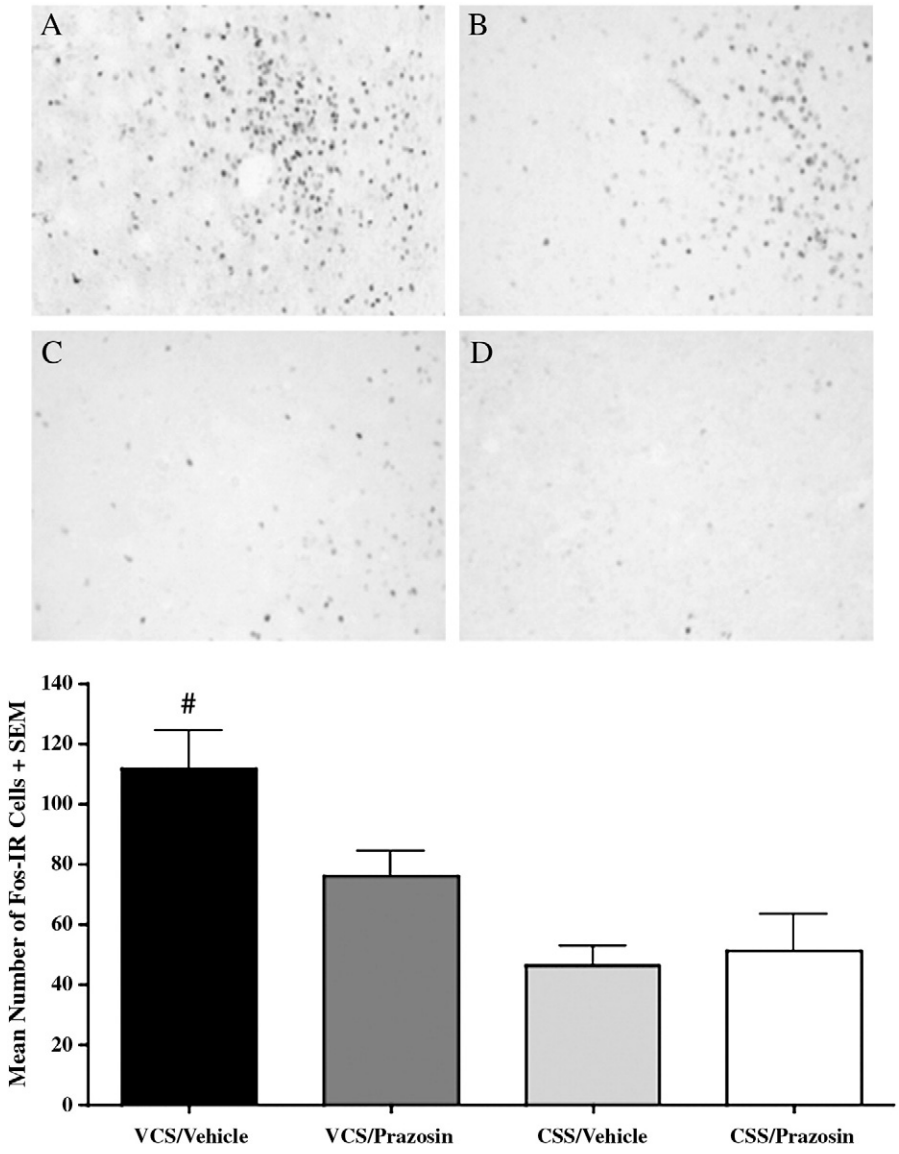

Fig. 3. Effects of prazosin on Fos-immunoreactive (Fos-IR) cells in the mPOA of hormone-treated ovariectomized female rats. Top: Photomicrographs (20×) depicting Fos induction by vaginal cervical stimulation (VCS) or control scapular stimulation (CSS) following administration of prazosin (1.0 mg kg) or the 10% ETOH vehicle. A = VCS/Vehicle, B = VCS/Prazosin, C = CSS/Vehicle, D = CSS/Prazosin. Bottom: Mean number of Fos-IR cells in each treatment group + SEM. # = P<.05 vs. CSS groups.

In the rVMH, there was a significant effect of VCS administration on Fos-IR [F (1, 90) = 29.807, p<0.05, Fig. 4] but there was no effect of prazosin treatment [F (1, 21) = 0.161, p>0.05, Fig. 4] or VCS × Prazosin interaction [F (1, 21) = 0.2.761, p>0.05, Fig. 4]. Therefore, VCS administration caused an expected increase in Fos-IR in the rVMH that was not affected by prazosin treatment.

4. Discussion

The present study examined the effect of a systemic α_1-noradrenergic receptor antagonist on sexual receptivity and a cellular endpoint of VCS in female rats. Our results confirm previous reports (Thornton et al., 1989) that prazosin, given 30 min prior to testing, inhibits sexual receptivity in hormonally-treated ovariectomized female rats. The 1.0 mg/kg dose of prazosin dramatically reduced both lordosis quotients and lordosis ratings as compared to the vehicle. Inhibition of hormone-induced sexual behavior has been demonstrated in previous studies where prazosin was given prior to or concurrently with estrogen or progesterone administration (Blaustein, 1987; Clark et al., 1985; Montemayor et al., 1990). However, given the relatively short latency between drug administration and behavior testing in the present study and the presumed peripheral actions of the drug, it is likely that the behavioral effects of prazosin we observed are not due to potential effects on hormone priming in the brain. Prazosin does enter spinal cord (Tartas et al., 2010) and can therefore alter ascending sensory input carried by noradrenergic pathways. The possibility that prazosin interferes with a "nonhormonal component" of sexual behavior is consistent with a report that systemic prazosin administered 30 min prior to testing inhibited the display of lordosis by castrated male guinea pigs (Thornton et al., 1989). In the castrated male guinea pig, lordosis is dependent on sensory, not hormonal, inputs indicating that α_1-noradrenergic receptors may play an important role in the sensory control of sexual response in rodents (Thornton et al., 1989). If peripheral α_1-noradrenergic receptor activity is important to transmission of sensory information received during mating, the induction of forebrain Fos-IR following VCS would also be expected to change following prazosin administration.

In keeping with previous reports, we observed significant increases in VCS-induced Fos-IR in the MeaPD, mPOA and rVMH of female rats receiving VCS as compared to those receiving the control scapular stimulation (CSS; Erskine, 1993; Greco et al., 2003; Pfaus and Heeb,

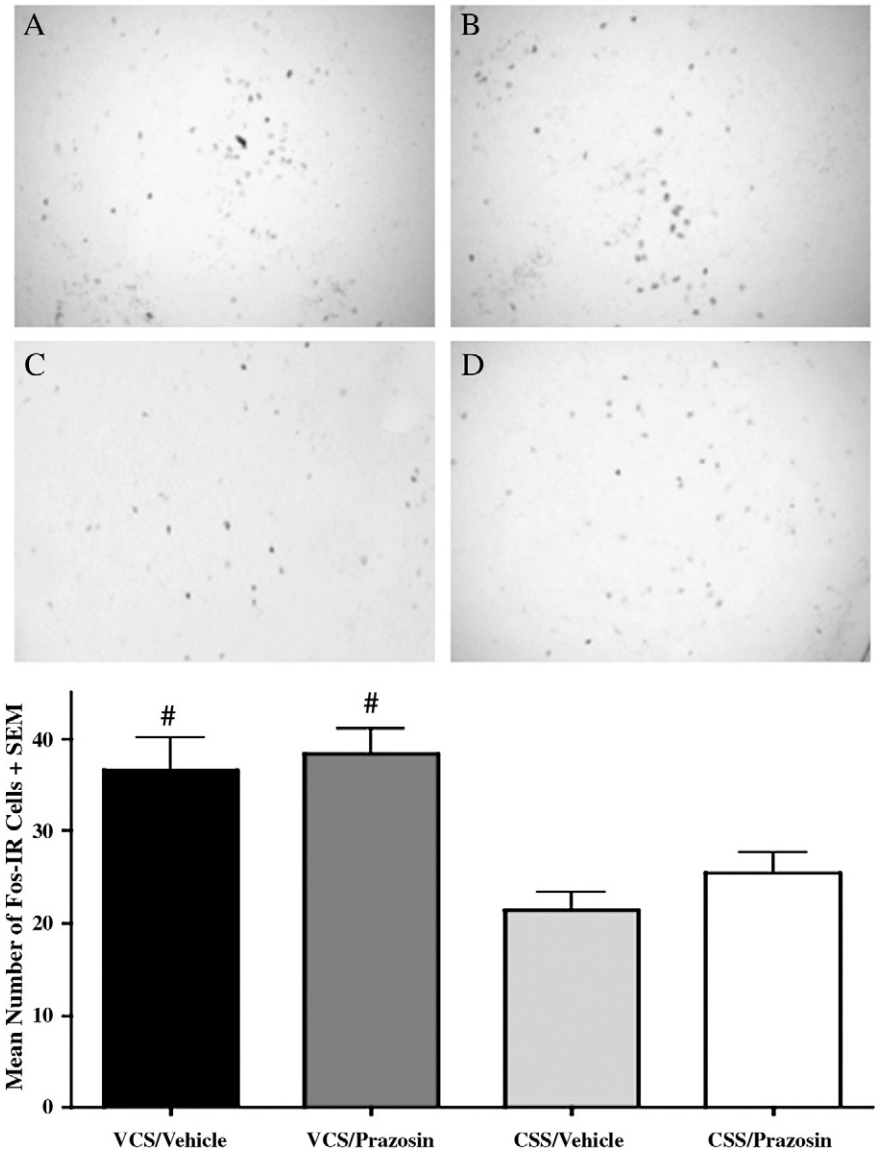

Fig. 4. Effects of prazosin on Fos-immunoreactive (Fos-IR) cells in the rVMH of hormone-treated ovariectomized female rats. Top: Photomicrographs (20×) depicting Fos induction by vaginal cervical stimulation (VCS) or control scapular stimulation (CSS) following administration of prazosin (1.0 mg kg) or the 10%ETOH vehicle. A = VCS/Vehicle, B = VCS/Prazosin, C = CSS/Vehicle, D = CSS/Prazosin. Bottom: Mean number of Fos-IR cells in each treatment group + SEM. # = $P<.05$ vs. CSS groups.

1997; Polston and Erskine, 1995; Quysner and Blaustein, 2001; Rowe and Erskine, 1993; Tetel et al., 1993). We hypothesized that systemically administered prazosin would interfere with the VCS-induced increase in Fos-IR in these forebrain regions. As predicted, prazosin did interfere with VCS-induced Fos-IR but only in the MeaPD and the mPOA.

The mean number of Fos-IR cells reported in the MeaPD for the animals in the VCS/Prazosin group was significantly less than the VCS/vehicle group but greater than either CSS group. The effect was slightly less pronounced in the mPOA such that the VCS/Prazosin group was not significantly different from the VCS/Vehicle group or either CSS group. Therefore, peripheral administration of prazosin at a dose sufficient to inhibit sexual receptivity reduced sensitivity of the MeaPD and mPOA to VCS, suggesting that systemic blockade of α_1-noradrenergic receptors may alter the sensory experience of females receiving VCS.

The present results described for the MeaPD and mPOA are not surprising due to the fact that these brain areas appear to receive similar sensory information from peripheral nerves. Transection of the pelvic nerve significantly reduces Fos-IR induced by both mating stimuli (Rowe and Erskine, 1993) and artificial VCS (Pfaus et al., 2006). Sensory input to both areas appears to be carried primarily by the pelvic nerve since transection of neither the hypogastric nor the pudendal nerves inhibited induction of Fos-IR by VCS (Pfaus et al., 2006). Sensory information relevant to VCS carried by the pelvic nerve appears to be dependent on α_1-noradrenergic activity since prazosin attenuates VCS-induced analgesia in female rats, an effect that is likely mediated by alteration of both ascending and descending noradrenergic pathways (Crowley et al., 1977).

In comparison to previous studies using pelvic nerve transection, prazosin administration resulted in a less dramatic decrease in VCS-induced Fos-IR in the three brain areas examined (Pfaus et al., 2006; Rowe and Erskine, 1993). This suggests that peripheral α_1-noradrenergic activity may be one of many possible mechanisms responsible for the transmission of sensory information to the forebrain from the periphery. In fact, blockade of D_1 dopamine receptors by peripheral administration of SCH 23390 has also been shown to reduce VCS-dependent Fos-IR in the MeaPD and mPOA (Quysner and Blaustein, 2001). Therefore, both norepinephrine and dopamine receptors may play an important role in sensory-mediated reproductive functioning.

Interestingly, the mean number of VCS-induced Fos-IR cells in the rVMH did not change following systemic administration of prazosin, suggesting that the sensory input to this area may not be innervated by α_1-noradrenergic neurons. This result was unexpected due to the fact that pelvic nerve transection reduced VCS-induced Fos-IR in the

VMH as well as the MeaPD and mPOA (Pfaus et al., 2006). However, this discrepancy may be explained by the fact that the present study focused on the rostral region of the VMH whereas other groups have reported findings for the ventrolateral VMH (Pfaus et al., 2006). Future studies could explore these responses of several levels of the VMH to clarify this issue. More important, however, may be the role of central α_1-noradrenergic receptors in the VMH as compared to contributions from peripheral α_1-noradrenergic receptor actions. Central administration of prazosin to the VMH has been shown to disrupt lordosis behavior in hormone primed female rats, prevent VCS-induced facilitation of receptivity and interfere with mating-induced pseudopregnancy (Etgen, 1990; Gonzalez-Flores et al., 2007; Northrop et al., 2006). However, we have found that systemic prazosin did not interfere with induction of pseudopregnancy by VCS (Kirkpatrick and Merrill, 2008), suggesting that alteration of peripheral α_1-noradrenergic receptors may affect specific brain areas differently and, as a result, mediate only specific VCS-dependent events.

Our data suggests an important role for peripheral α_1-noradrenergic receptors in the sympathetic activation of female sexual response. However, the possibility that these effects could also be due to central actions of prazosin has not been ruled out completely. Future studies examining the effects of intracerebroventricular administration or direct administration of prazosin to specific brain regions on VCS-induced Fos-IR might clarify the role of peripheral vs. central α_1-noradrenergic receptors in mediating VCS-dependent events in female rats.

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