EFFECTS OF PROTEIN KINASE C INHIBITOR ON 5-HT1A AND 5-HT2A/2C RECEPTOR INTERACTION IN THE MEDIOBASAL HYPOTHALAMUS

A DISSERTATION

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To the Dean of the Graduate School:

I am submitting herewith a dissertation written by Amutha Selvamani entitled "*Effects of protein kinase C inhibitor on 5-HT1A and 5-HT2A/2C receptor interaction in the mediobasal hypothalamus*." I have examined this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Molecular Biology.

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To

My parents Revathy Selvamani and Selvamani Subramanian

My grandparents Ammuma and Thatha

My brother Sid

for their love and support without which this journey would not have been possible

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ABSTRACT

AMUTHA SELVAMANI

EFFECTS OF PROTEIN KINASE C INHIBITOR ON 5-HT1A and 5-HT2A/2C RECEPTOR INTERACTION IN THE MEDIOBASAL HYPOTHALAMUS

DECEMBER 2005

Activation of 5-HT_{1A} receptors within the mediobasal hypothalamus (MBH) inhibits female rat lordosis behavior and coinfusion with 5-$HT_{2A/2C}$ receptor agonists attenuates this inhibition. The mechanism by which 5-HT_2 receptors mediate the attenuation of 5-HT_{1A} mediated inhibition of lordosis behavior is unknown. 5-HT_{1A} and 5-HT_2 receptors are coupled to Gi/o/z and Gq/11 proteins, respectively. It has been suggested that 5-HT_2 receptors can induce heterologous desensitization via a protein kinase C (PKC)–induced phosphorylation of 5-HT_{1A} receptors. This investigation tests the hypothesis that PKC inhibitors can attenuate the ability of 5-HT_2 receptor agonists to reduce the lordosis-inhibiting effects of 5-HT_{1A} receptor agonists. In the first experiment, ovariectomized Fischer rats, hormonally primed with 10 μM estradiol benzoate and 500 μM progesterone, received bilateral MBH infusion with the 5-HT_{1A} receptor agonist, 200 ng (±)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), or 200 ng 8-OH-DPAT, plus 2000 ng (+/-)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-$HT_{2A/2C}$ receptor agonist. DOI was able to attenuate the lordosis inhibiting effects of 8-OH-

OH-DPAT. In the second experiment, following hormonal priming, rats were preinfused with either water or 1.0×10^{-1} nmol of the PKC inhibitor, bisindolylmaleimide I HCl (BIM), 30 min or 90 min before infusion with 8-OH-DPAT or with 8-OH-DPAT plus DOI. BIM prevented the DOI mediated attenuation of 8-OH-DPAT mediated inhibition. Varying doses of BIM (1.0×10^{-1} to 1.0×10^{-7} nmol) were used 90 min prior to infusion with 8-OH-DPAT plus DOI in order to see if BIM had a dose-dependent effect on DOI mediated attenuation. The data suggest that BIM dose dependently attenuated DOI's effect. The results of the present study suggest that PKC might play a role in DOI mediated attenuation of lordosis behavior.

TABLE OF CONTENTS

ABBREVIATIONS

8-OH-DPAT.....(±)-8-Hydroxy-2-(di-n-propylamino)tetralin

BIM..... Bisindolylmaleimide I HCl

DOI.....(+/-)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

E.....Estrogen

EB.....Estradiol benzoate

P.....Progesterone

CHAPTER I

INTRODUCTION

The lordosis reflex is a stereotyped posture adopted by a sexually receptive female rat in response to tactile stimulation from the male [37]. After appropriate hormonal priming with the female gonadal hormones estrogen (E) and progesterone (P), female rats exhibit this lordosis posture in response to a mount by the male. Stimulation of cutaneous mechanoreceptors in the flanks, posterior rump, and perineum of the female activate an ascending pathway traveling through the medullary reticular formation and the lateral vestibular nucleus to ultimately terminate in the midbrain [37]. Descending information travels through the lateral vestibulospinal and lateral reticulospinal tracts to terminate on motor neurons of the spinal cord [37]. Modulation of this supraspinal reflex occurs in forebrain areas [61]. Since lesioning of the ventromedial nucleus of the hypothalamus (VMN) significantly reduces lordosis behavior in response to a male's mount, it is believed that the VMN is also a brain area important for the hormonal facilitation of the reflex [37, 44].

Several neurotransmitters including serotonin (5-hydroxytryptamine, 5-HT) are involved in modulation of female rat sexual behavior [20, 30]. Generally, an increase in 5-HT activity is associated with inhibition of lordosis behavior [1], while treatments which decrease 5-HT activity increase lordosis behavior [26]. However, depending on

which 5-HT receptor is activated, 5-HT can either inhibit or facilitate lordosis behavior [30]. Agonists which activate 5-HT_{1A} receptors inhibit [54, 57] while agonist activation of 5-$HT_{2A/2C}$ receptors increases the behavior [55, 60].

The 5-HT_{1A} receptor is primarily coupled to proteins of the $G_{i/o}$ family and its activation is associated with a variety of intracellular responses including inhibition of cAMP production [40]; (Figure 1). Inactivation of Ca^{2+} channels and activation of K^+ channels [34, 35] may also follow agonist activation of 5-HT_{1A} receptors. 5-$HT_{2A/2C}$ receptors are $G_{q/11}$ coupled to phospholipase C and their activation increases diacylglycerol and inositol triphosphate, and leads to activation of protein kinase C (PKC); (Figure 1) and elevation of intracellular Ca^{2+} [25]. Since compounds which elevate levels of cAMP or PKC can increase lordosis behavior [19, 56], it has been suggested that a 5-HT_{1A} receptor mediated decrease in cAMP is responsible for its decrease in lordosis behavior and that an increase in PKC may mediate lordosis facilitatory effects of 5-$HT_{2A/2C}$ receptor agonists [51]. Of particular interest are observations that 5-$HT_{2A/2C}$ receptor agonists can attenuate effects of 5-HT_{1A} receptor agonists on lordosis behavior in rats [28, 52] and that PKC can rapidly desensitize 5-HT_{1A} receptors in a cell culture system [39]. Since endogenous release of 5-HT would be expected to activate both 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors, functional interaction between the receptors is likely to be relevant to the control of lordosis behavior in the female rat. In fact, substantial evidence exists for a functional interaction between 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors [7, 22] and interaction among their transduction mechanisms has

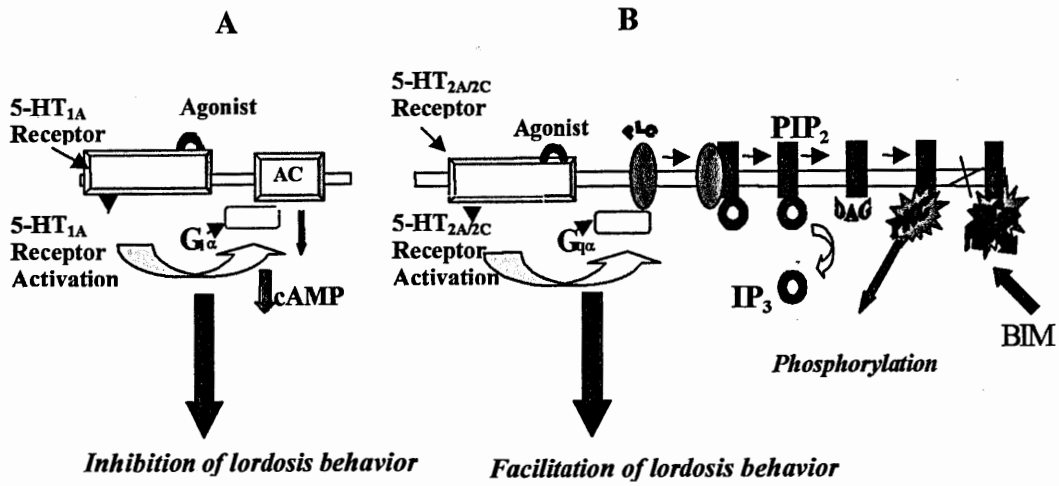


Figure 1. Schematic representation of the possible signal transduction events involved in 5-HT$_{1A}$ receptor (A) and 5-HT$_{2A/2C}$ receptor (B) mediated modulation of lordosis behavior. The X is the site of action of the PKC inhibitor (BIM).

been suggested [9, 17, 48]. Of these hypothesized interactions, an important role for PKC has repeatedly emerged. Evans [9] reported a significant role of PKC in functional interaction between 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors.

How PKC alters the effectiveness of 5-HT_{1A} receptor agonists is not clear, but several possibilities have been suggested. First, at the level of the receptor, three putative phosphorylation site sequences have been identified within the 2nd and 3rd intracellular loop of the 5-HT_{1A} receptor and have been demonstrated to be phosphorylated by PKC; this results in desensitization [39] and loss of high-affinity binding [13].

Secondly, at the level of the G-protein, PKC reduces inhibition of adenylate cyclase by phophorylating $G_{i\alpha 2}$ [45]; it has also been reported that PKC can regulate $\alpha 12$ and αz receptor mediated signaling pathways by preventing their association with $\beta\gamma$ [21]. Thirdly, phosphorylation of $G_{i\alpha 2}$ by PKC results in reduced receptor/G protein coupling [10, 27]. Fourthly, PKC may modulate ion channels and thereby influence their responses to receptor activation. For example the α_1 subunit of the N-type calcium channel has been shown to be phosphorylated by PKC, and this in turn results in reduced ability of the channel to undergo subsequent G-protein dependent downregulation [62]. Although all of these mechanisms have not been investigated for 5-HT_{1A} receptor function, PKC could act via one or more of these mechanisms to alter the effectiveness of 5-HT_{1A} receptor agonists.

Considering the functional interaction between 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors and the role PKC plays in the cross-talk between them, it was therefore hypothesized that

the 5-$HT_{2A/2C}$ receptor attenuation of the 5-HT_{1A} receptor's inhibition of lordosis behavior might require PKC. If so, a PKC inhibitor should prevent 5-$HT_{2A/2C}$ receptor mediated attenuation of the effects of a 5-HT_{1A} agonist. The present experiments were designed to test this hypothesis. Bisindolylmaleimide I HCl (BIM), a member of the family of bisindolylmaleimide compounds, acts as a competitive inhibitor to the ATP binding site of PKC. BIM has a high selectivity for α, β_I, β_{II}, γ, δ, and ε isozymes of PKC [11, 47]. This inhibitor was used in the experiments.

The following were the specific hypotheses.

1. A 5-$HT_{2A/2C}$ receptor agonist, (±)-(2,5-dimethoxy-4-iodophenyl)-amino-propane HCl (DOI), will attenuate the lordosis inhibiting effects of 5-HT_{1A} receptor activation.
2. The PKC inhibitor, BIM, will prevent the 5-$HT_{2A/2C}$ receptor mediated attenuation of 5-HT_{1A} receptor mediated inhibition of lordosis behavior and the effect of BIM will be dose-dependent.

CHAPTER II

MATERIALS AND METHODS

MATERIALS

Estradiol benzoate (EB), progesterone (P), sesame seed oil, the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), and the 5-$HT_{2A/2C}$ receptor agonist, DOI, were purchased from Sigma Chemical Co. (St. Louis, MO). Isoflurane (AErrane®) was obtained from Pitman-Moore (Mundelein, IL). Intracranial (i.c.) cannulae were obtained from Plastics One (Roanoke, VA). Dental acrylic was purchased from Reliance Dental Mfg Co. (Worth, IL). Suture materials were purchased from Henry Schein (Melville, NY). All other supplies were purchased from Fisher Scientific (Houston, TX).

GENERAL METHODS

Animals and housing conditions

Female rats (CDF-344) were purchased from Sasco Laboratories (Wilmington, MA) or were bred in the TWU animal facility from stock obtained from Sasco Laboratories. Rats were housed three or four per cage in polycarbonate shoebox cages in a housing area maintained at 22 °C and 55% humidity with a 12:12-h dark-light cycle (lights on at 12.00 a.m.). Food and water were available ad lib.

Surgical procedures

When at least 60 days of age and 140-170 g body weight, rats were anesthetized with AErrane® and were implanted with 22-gauge stainless steel guide cannulae directed toward the ventromedial nucleus of the hypothalamus (VMN) (atlas coordinates from König and Klippel, anterior +4.38, DV -7.8, ML ± 0.4) as previously described [57]. Bilateral ovariectomy was performed immediately after implant surgery [16] and the rats were allowed to recover for at least 2 weeks. All procedures were carried out according to PHS policy and were approved by the IACUC at Texas Woman's University.

Hormonal priming

Hormonal priming began 2-3 weeks after ovariectomy and all hormone injections were administered between 9 and 10 a.m. Rats were injected with 10 μg EB followed 48 h later with 500 μg P. Hormones were dissolved in sesame seed oil and injections were given subcutaneously (SC) in a volume of 0.1 ml per rat.

Intracranial treatment

8-OH-DPAT and DOI were dissolved in saline and BIM was dissolved in deionized water. Receptive females had their dummy cannulae replaced with 28 gauge stainless steel internal cannulae, attached by tubing (i.d.= 0.58 mm; o.d. = 0.96 mm) to a BAS (CMA/100) microinjector. All infusions were delivered at a rate of 0.24-0.26 μl/min to a final infusion volume of 0.5 μl per bilateral site.

Behavioral testing procedures

Behavioral testing took place during the dark phase of the light-dark cycle. Red lighting was used to facilitate visibility. Four to 6 h after P, females were screened for sexual receptivity by placing the female in the home cage of a sexually experienced male and observing the behavior for 10 mounts. This screening for sexual receptivity is referred to as the pretest (PRE). After this pretest, the female was infused with the appropriate 5-HT receptor agonists. When effects of BIM were examined, rats were preinfused with BIM (1×10^{-1}, 5×10^{-2}, 2.5×10^{-2}, 1×10^{-3}, 1×10^{-5}, or 1×10^{-7} nmol) or deionized water. After 30 min or 90 min, rats were infused with the appropriate 5-HT receptor agonist. Sexual behavior after infusion with 5-HT compounds was monitored for 30 consecutive min as previously described [53]. Sexual receptivity was quantified as the lordosis to mount (L/M) ratio (e.g. number of lordosis responses by the female divided by the number of mounts by the male). When a female's L/M ratio fell below 0.7 for two consecutive intervals after drug infusion, the female's behavior was considered to have been inhibited by the drug. Lordosis quality was recorded on a scale of 1-4 as previously described [53]. The absence of a lordosis response was given a score of 0.0. Minimal arching of the back was given a score of 1.0. An intermediate reflex was scored as 2.0; a normal reflex was scored as 3.0, and an exaggerated reflex was scored as 4.0.

Histological procedures

After behavioral testing, rats were anesthetized with AErrane® and perfused with phosphate-buffered saline followed by 10% buffered formalin. Brain tissue was placed in 10% buffered formalin for at least 24 h before sectioning. Coronal sections (100 μm) were stained with cresyl violet and examined for cannulae placement according to the atlas of König and Klippel [18]. Rats with cannulae placements outside the vicinity of the VMN or its most anterior extension were excluded from the data analysis. All rats with cannulae in the third ventricle were excluded.

Statistical methods

Data were grouped into the pretest interval and five consecutive 5 min intervals after treatment. The data were analyzed by repeated measures ANOVA with time after 5-HT receptor agonist infusion as the repeated measure and type of agonist infusion as the independent factor. When effects of BIM were examined, data were evaluated by 2- way repeated measures ANOVA with type of preinfusion and 5-HT receptor agonist condition as independent factors. Time dependent differences, within treatment, were compared to the pretest interval with Dunnett's test; differences between groups within a time interval were compared by Tukey's test. The statistical reference was Zar [63] and an α level of 0.05 was required to reject the null hypothesis.

SPECIFIC METHODS

1. A 5-$HT_{2A/2C}$ receptor agonist, DOI, will attenuate the lordosis inhibiting effects of 5-HT_{1A} receptor activation.

After the pretest for sexual behavior, ovariectomized hormonally primed, sexually receptive females were infused with 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. Sexual behavior was monitored for 30 consecutive min after infusion. After testing was complete, rats were perfused transcardially and cannulae locations were determined as previously described [32, 35]. Data were grouped into the pretest interval and six consecutive 5 min intervals after treatment. The data were analyzed by repeated measures ANOVA with time after infusion as the repeated measure and type of infusion as the independent factor.

2. The PKC inhibitor BIM will prevent the 5-$HT_{2A/2C}$ receptor mediated attenuation of 5-HT_{1A} receptor mediated inhibition of lordosis behavior.

Hormonally primed ovariectomized rats were tested for sexual receptivity as above. Receptive rats were infused with varying concentrations of BIM (1×10^{-1} to 10^{-7} nmol) or deionized water. Thirty and ninety min later, rats were infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. Sexual behavior was monitored for 30 consecutive min after infusion with the 5-HT receptor active drugs. After completion of testing, rats were perfused transcardially and cannulae locations were

determined as previously described [32, 35]. Data were grouped into the pretest interval and six consecutive 5 min intervals after treatment. The data were analyzed by repeated measures ANOVA with time after 5-HT receptor agonists as the repeated measure and type of agonist and preinfusion conditions as the independent factors.

CHAPTER III

RESULTS

1. Effects of DOI on the lordosis inhibiting effects of 8-OH-DPAT.

Coinfusion with DOI attenuated the effects of 8-OH-DPAT on lordosis behavior in rats (Figure 2A). There was a significant effect of drug ($F_{1,21} = 5.27$ $p \leq 0.05$), time after infusion ($F_{6,126} = 9.95$, $p \leq 0.0001$), and the time by drug interaction ($F_{6,126} = 2.62$, $p \leq 0.05$). Rats infused with 8-OH-DPAT showed L/M ratios significantly lower than in the pretest at 10 min and in every interval thereafter (Dunnett's $q_{126,7} \geq 2.57$, $p \leq 0.05$). For rats coinfused with 8-OH-DPAT and DOI, the L/M ratios were significantly lower than the pretest at the 5, 15, 20, and 25 min time intervals (Dunnett's $q_{126,7} \geq 2.57$, $p \leq 0.05$). L/M ratios of rats infused with 200 ng 8-OH-DPAT were significantly lower than those of rats coinfused with 200 ng 8-OH-DPAT and 2000 ng DOI at all time intervals except the first 5 min interval after infusion (Tukey's, all $q_{126,2} \geq 2.77$, $p \leq 0.05$). Eleven out of 13 rats infused with 8-OH-DPAT showed a decline in lordosis behavior, while only 6/10 of the rats coinfused with 8-OH-DPAT plus DOI showed such a decline. Moreover, for 2 rats infused with 8-OH-DPAT plus DOI, the decline did not occur until 20 min after infusion while, for the majority of 8-OH-DPAT rats, inhibition occurred within 10-15 min of the infusion. There was no significant effect of type of drug infusion

Figure 2

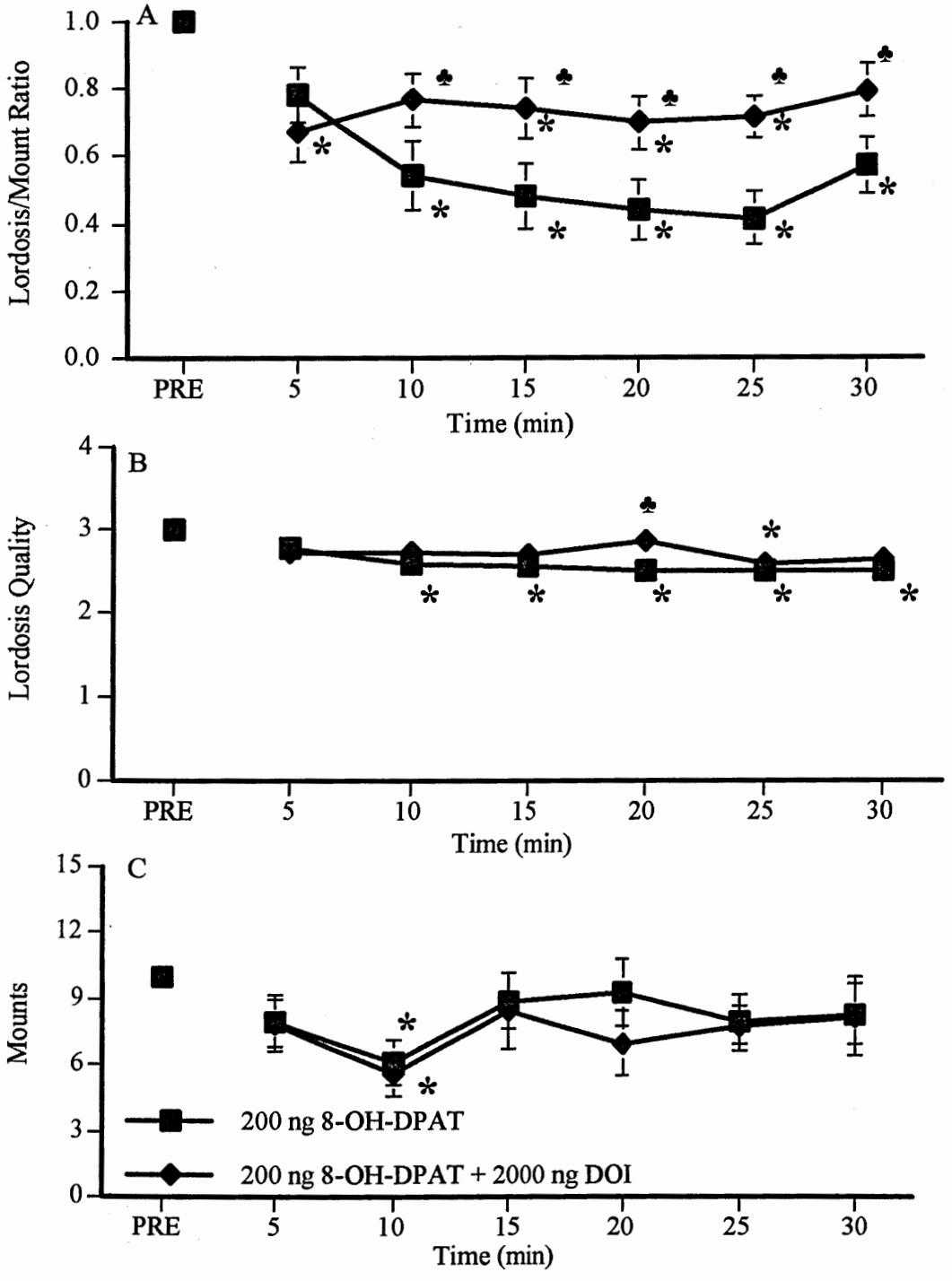

A
Lordosis/Mount Ratio
1.0
0.8
0.6
0.4
0.2
0.0
PRE
5
10
15
20
25
30
Time (min)
B
Lordosis Quality
4
3
2
1
0
PRE
5
10
15
20
25
30
Time (min)
C
Mounts
15
12
9
6
3
0
200 ng 8-OH-DPAT
200 ng 8-OH-DPAT + 2000 ng DOI
PRE
5
10
15
20
25
30
Time (min)

Figure 2: Effect of 8-OH-DPAT infusion and 8-OH-DPAT and DOI coinfusion on lordosis behavior in ovariectomized rats.

Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity (PRE). After the pretest for sexual behavior, females were infused with 200 ng 8-OH-DPAT or were coinfused with 200 ng 8-OH-DPAT and 2000 ng DOI. The number of subjects for 8-OH-DPAT and 8-OH-DPAT plus DOI rats were 13 and 10, respectively. Data in Figure 1A are the mean ± S.E. L/M ratios before infusion (PRE) and for six consecutive 5 min intervals after infusion. Data in Figure 1B and 1C, respectively, are the mean ± S.E. lordosis quality and number of mounts at each test interval. Shamrocks indicate a significant difference between behavior of rats infused with 8-OH-DPAT and rats coinfused with 8-OH-DPAT/DOI within each 5 min time interval. Asterisks indicate a significant difference from the pretest.

on lordosis quality (Figure 2B). However, for 4 of the 8-OH-DPAT treated rats, the lordosis quality fell to zero during testing so their quality scores were excluded from the ANOVA. It is important to note that all 4 of these rats were infused with 8-OH-DPAT. Both groups showed a small decline in lordosis quality during the testing so that there was a significant effect of time after infusion ($F_{6,102} = 4.61$, $p \leq 0.05$) but the time by type of drug infusion was not significant ($F_{6,102} = 0.85$, $p > 0.05$). Rats infused with 8-OH-DPAT showed lordosis quality significantly lower than in the pretest at 10 min and every interval thereafter (Dunnett's $q_{102,7} \geq 2.64$, $p \leq 0.05$). For rats coinfused with 8-OH-DPAT and DOI, the lordosis quality was significantly lower than the pretest at only one time interval (25 min) (Dunnett's $q_{102,7} = 2.57$, $p \leq 0.05$). Mounts received showed a significant effect of time after infusion ($F_{6,126} = 3.73$, $p \leq 0.05$) but there was not a significant interaction between time and type of drug infusion ($F_{6,126} = .418$, $p \leq 0.05$) (Figure 2C). For both rats infused with 8-OH-DPAT and those coinfused with 8-OH-DPAT and DOI, number of mounts were significantly lower than in the pretest only at the 10 min time interval (Dunnett's $q_{126,7} = 2.57$, $p \leq 0.05$).

Histological analysis was used to determine location of cannulae. Cannulae locations from rats infused with 8-OH-DPAT and 8-OH-DPAT plus DOI are shown in Figure 3.

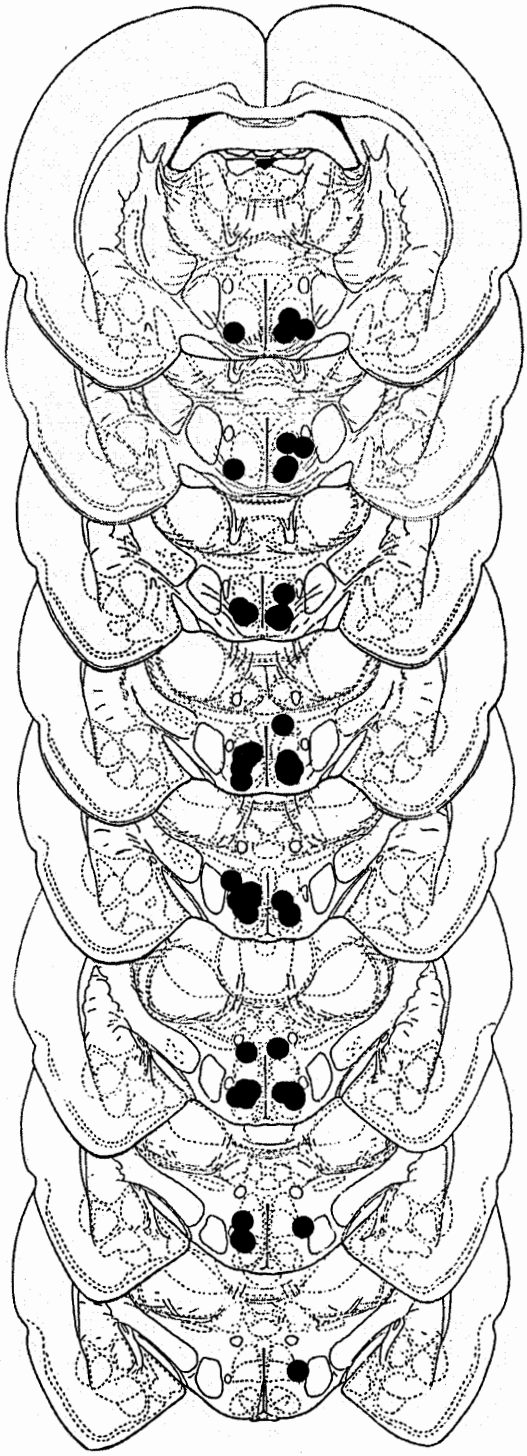

A 5660 µ
A 5340 µ
A 5150 µ
A 4890 µ
A 4620 µ
A 4380 µ
A 4230 µ
A 4110 µ

Figure3: Histological locations of cannulae on coronal sections of the brain from rats infused with 200 ng 8-OH-DPAT and coinfused with 200 ng 8-OH-DPAT plus 2000 ng DOI.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4110 μ to A 5660 μ. ● on the left side of the coronal section indicates the location of cannula sites from rats infused with 200 ng 8-OH-DPAT. ● on the right side of the coronal section indicates the location of cannula sites from rats coinfused with 200 ng 8-OH-DPAT plus 2000 ng DOI.

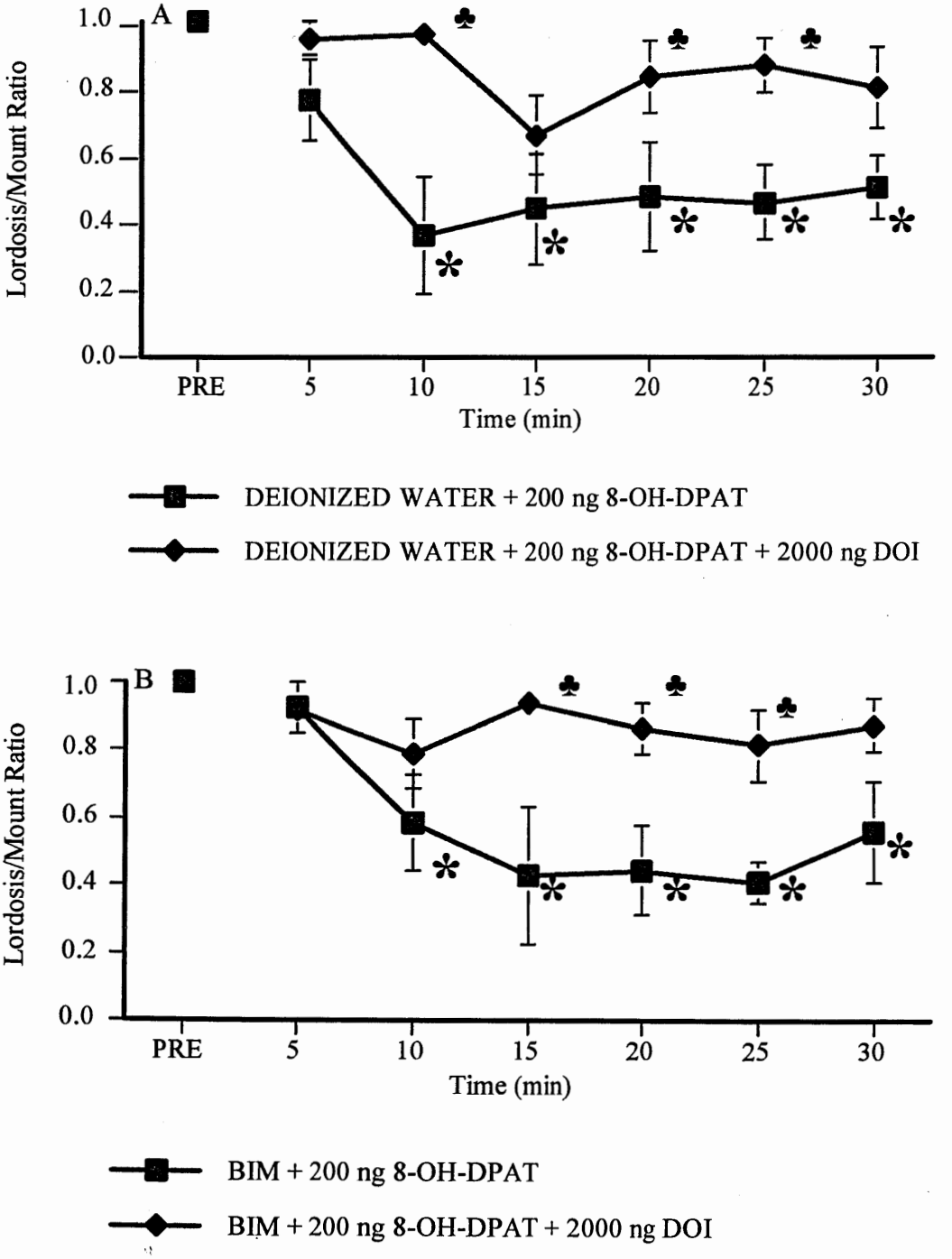
2. Effect of the PKC inhibitor, BIM, on DOI's ability to attenuate effects of 8-OH-DPAT.

a. Administration of 1 x 10^{-1} nmol BIM, 30 min before 5-HT receptor agonists.

In the next experiment, rats were preinfused with 1 X 10^{-1} nmol BIM or deionized water 30 min before infusion with 8-OH-DPAT or 8-OH-DPAT plus DOI. Of 31 rats used in the experiment, 8 had cannulae outside the target area or had poor histology and were excluded from the analysis. For the remaining rats, whether pretreatment was with water or with BIM, every rat infused with 8-OH-DPAT showed a decline in the L/M ratio during the testing period. In contrast, only 2 rats (1 preinfused with water and 1 preinfused with BIM) infused with DPAT and DOI showed such a decline. Independent of the type of pretreatment, 8-OH-DPAT reduced L/M ratios leading to a significant main effect for the second infusion ($F_{1,19} = 16.00$, $p \leq 0.0008$) (Figure 4A and 4B). There was also a significant effect of time after the second infusion ($F_{6,114} = 10.12$, $p \leq 0.0001$) and a significant interaction between time and type of second infusion ($F_{6,114} = 3.22$, $p \leq 0.006$). There was not a significant effect of type of pretreatment and none of the interactions with pretreatment were significant (all $p > 0.05$).

Rats preinfused with deionized water and 8-OH-DPAT plus DOI had significantly higher L/M ratios than their 8-OH-DPAT counterparts at 10, 20, and 25 min time intervals after infusion. DOI was also effective in decreasing effects of 8-OH-DPAT in rats preinfused with BIM at the 15, 20, and 25 min time intervals (Tukey's $q_{114,4} = 3.68$, $p \leq 0.05$). In rats preinfused with deionized water or BIM and then infused with 8-OH-DPAT, there was a significant difference from their pretest at 10 min and every interval

Figure 4


A
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0.4
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Lordosis/Mount Ratio
PRE
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20
25
30
Time (min)
DEIONIZED WATER + 200 ng 8-OH-DPAT
DEIONIZED WATER + 200 ng 8-OH-DPAT + 2000 ng DOI
B
1.0
0.8
0.6
0.4
0.2
0.0
Lordosis/Mount Ratio
PRE
5
10
15
20
25
30
Time (min)
BIM + 200 ng 8-OH-DPAT
BIM + 200 ng 8-OH-DPAT + 2000 ng DOI

Figure 4: Effect of preinfusion with 1 x 10^{-1} nmol of the PKC inhibitor, BIM, or water 30 min before 8-OH-DPAT infusion or 8-OH-DPAT and DOI coinfusion on lordosis behavior.

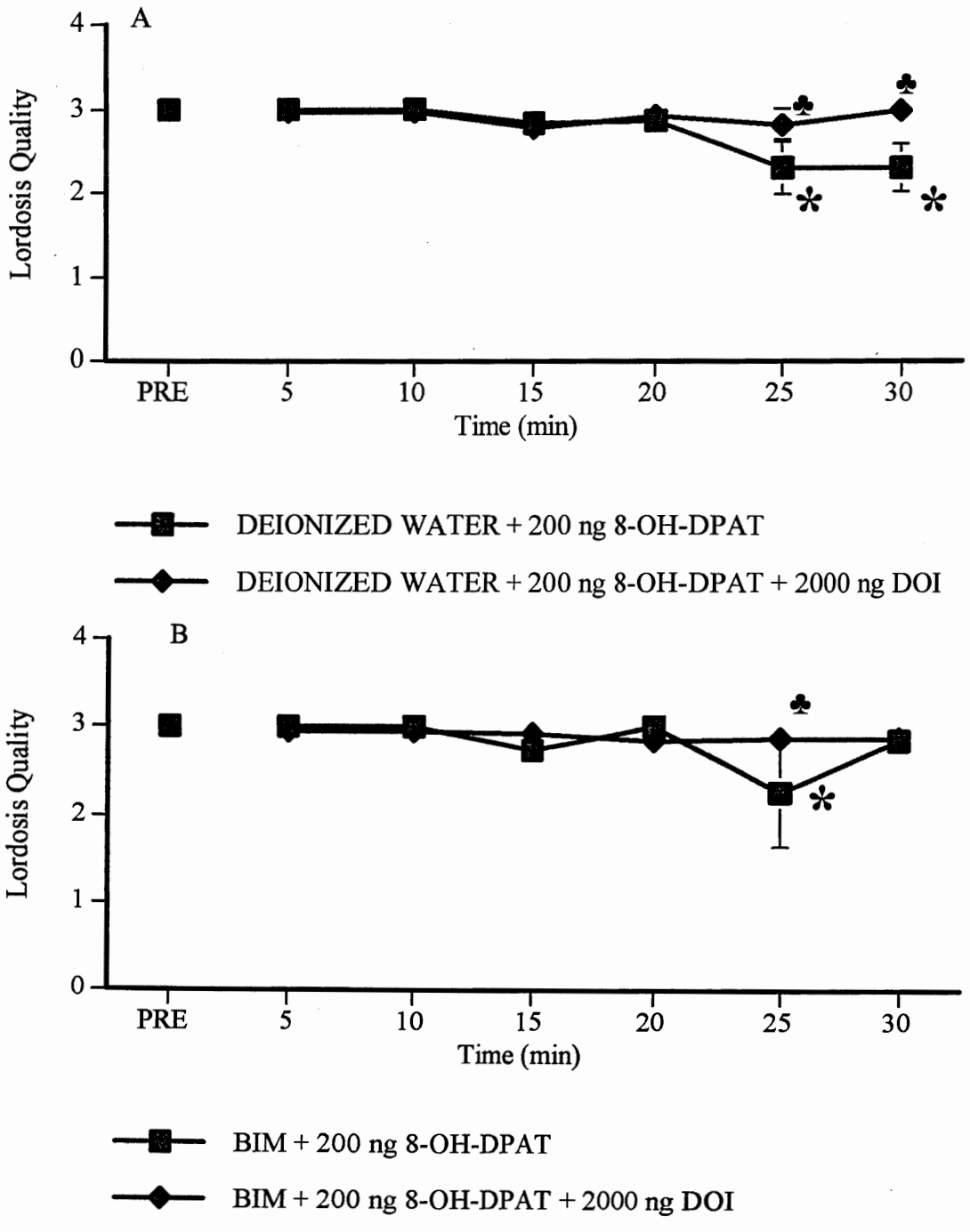
Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity (PRE). After the pretest for sexual behavior, rats were infused with either deionized water or BIM. Thirty min later they were infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. The number of subjects for rats preinfused with deionized water (Figure 4A) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 6 and 4, respectively. The number of subjects for rats preinfused with BIM (Figure 4B) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 6 and 7, respectively. Data in Figures 4A and 4B are the mean ± S.E. L/M ratios before infusion (PRE) and for six consecutive 5 min intervals after infusion. Shamrocks indicate a significant difference between rats infused with 8-OH-DPAT or 8-OH-DPAT plus DOI within preinfusion condition. Asterisks indicate a significant difference from the pretest.

thereafter (Dunnett's $q_{114,7} = 2.64$, $p \leq 0.05$). Regardless of the type of preinfusion, rats infused with 8-OH-DPAT plus DOI were never significantly different from the pretest (all $p > 0.05$).

L/M ratios of 6 rats (3 water 8-OH-DPAT and 3 BIM 8-OH-DPAT) fell to zero during the test and could not be included in the ANOVA. For the remaining rats, there were no significant main effects for lordosis quality although there was a significant interaction between time after the second infusion and the type of second infusion ($F_{6, 78} = 3.45$, $p \leq 0.005$) (Figure 5A and 5B). The significant interaction resulted from the slightly lower quality scores by 25 min from rats infused with 8-OH-DPAT. In rats preinfused with water, the 8-OH-DPAT plus DOI infused rats had significantly higher lordosis quality than the 8-OH-DPAT treated rats at the 25 and 30 min intervals after infusion. In rats preinfused with BIM, the 8-OH-DPAT plus DOI rats had significantly higher lordosis quality than the 8-OH-DPAT rats at the 25 min interval after infusion (Tukey's $q_{78,4} = 3.68$, $p \leq 0.05$). In rats preinfused with deionized water, 8-OH-DPAT rats differed from their pretest interval at 25 and 30 min after infusion. In rats preinfused with BIM, 8-OH-DPAT rats differed significantly in lordosis quality from the pretest only at the 25 min time interval (Dunnett's $q_{78,7} = 2.6$, $p \leq 0.05$).

There were no significant main effects of treatment on the number of mounts received by the female (Figures 6A and 6B). Although there was a significant three way interaction ($F_{6,114} = 2.3$, $p \leq 0.04$), this did not reflect any consistent effect of treatment parameters.

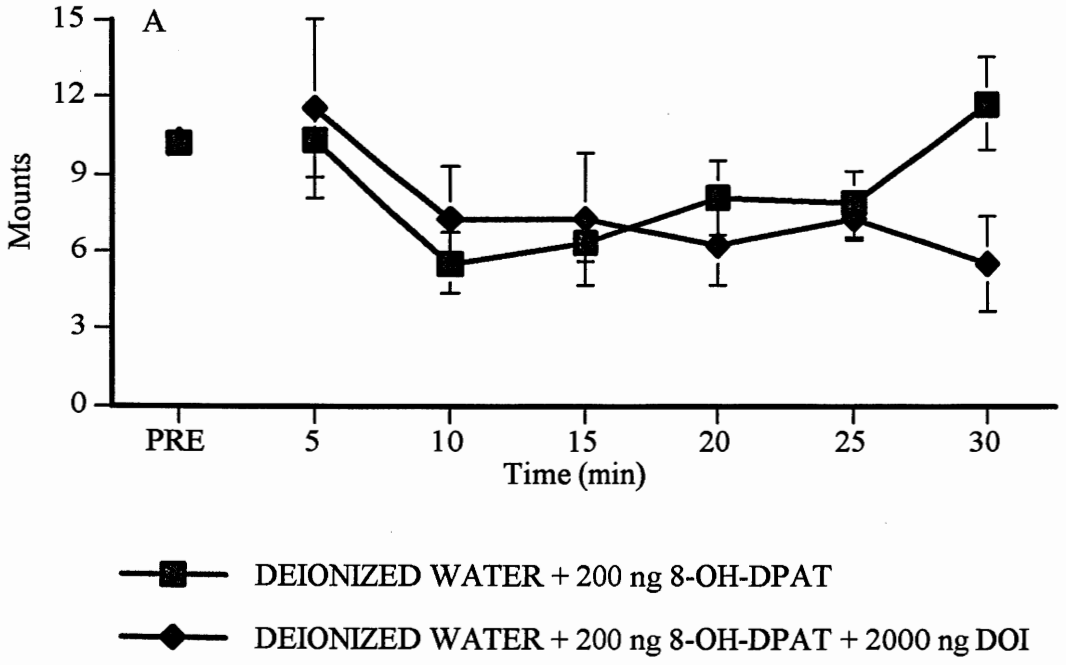
Figure 5


A
Lordosis Quality
4
3
2
1
0
PRE
5
10
15
20
25
30
Time (min)
DEIONIZED WATER + 200 ng 8-OH-DPAT
DEIONIZED WATER + 200 ng 8-OH-DPAT + 2000 ng DOI
B
Lordosis Quality
4
3
2
1
0
PRE
5
10
15
20
25
30
Time (min)
BIM + 200 ng 8-OH-DPAT
BIM + 200 ng 8-OH-DPAT + 2000 ng DOI

Figuure 5: Effect of preinfusion with 1 x 10^{-1} nmol BIM or water 30 min before 8-OH-DPAT infusion or 8-OH-DPAT and DOI coinfusion on lordosis quality.

Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity (PRE). After the pretest for sexual behavior, rats were infused with either deionized water or BIM. Thirty min later they were infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. The number of subjects for rats preinfused with deionized water (Figure 5A) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 6 and 4, respectively. The number of subjects for rats preinfused with BIM (Figure 5B) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 6 and 7, respectively. Data in Figures 5A and 5B are the mean ± S.E. lordosis quality before infusion (PRE) and for six consecutive 5 min intervals after infusion. Shamrocks indicate a significant difference between rats infused with 8-OH-DPAT or 8-OH-DPAT plus DOI within preinfusion condition. Asterisks indicate a significant difference from the pretest.

Figure 6


A
15
12
9
6
3
0
Mounts
PRE
5
10
15
20
25
30
Time (min)
DEIONIZED WATER + 200 ng 8-OH-DPAT
DEIONIZED WATER + 200 ng 8-OH-DPAT + 2000 ng DOI

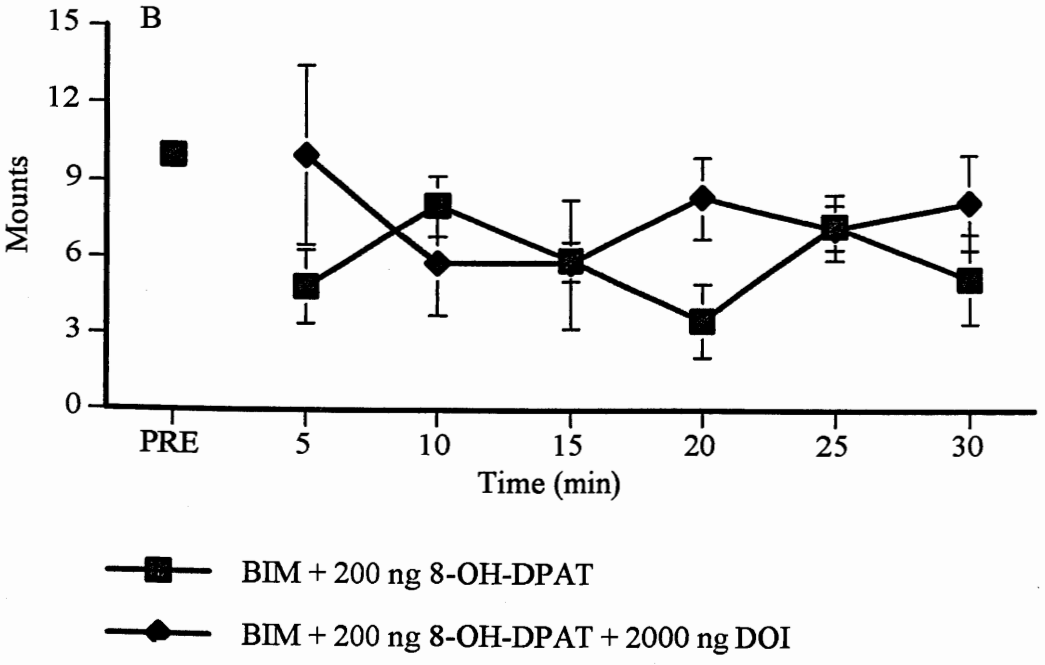

B
15
12
9
6
3
0
Mounts
PRE
5
10
15
20
25
30
Time (min)
BIM + 200 ng 8-OH-DPAT
BIM + 200 ng 8-OH-DPAT + 2000 ng DOI

Figure 6: Effect of preinfusion with 1 x 10^{-1} nmol of the PKC inhibitor, BIM, or water 30 min before 8-OH-DPAT infusion or 8-OH-DPAT and DOI coinfusion on number of mounts.

Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity (PRE). After the pretest for sexual behavior, rats were infused with either deionized water or BIM. Thirty min later they were infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. The number of subjects for rats preinfused with deionized water (Figure 6A) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 6 and 4, respectively. The number of subjects for rats preinfused with BIM (Figure 6B) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 6 and 7, respectively. Data in Figures 6A and 6B are the mean ± S.E. number of mounts before infusion (PRE) and for six consecutive 5 min intervals after infusion.

Histological analysis was used to determine location of cannulae. Cannulae locations from rats preinfused with deionized water before infusion with either 8-OH-DPAT or 8-OH-DPAT plus DOI are shown in Figure 7; those of rats preinfused with 1×10^{-1} nmol BIM before infusion with either 8-OH-DPAT or 8-OH-DPAT plus DOI are shown in Figure 8.

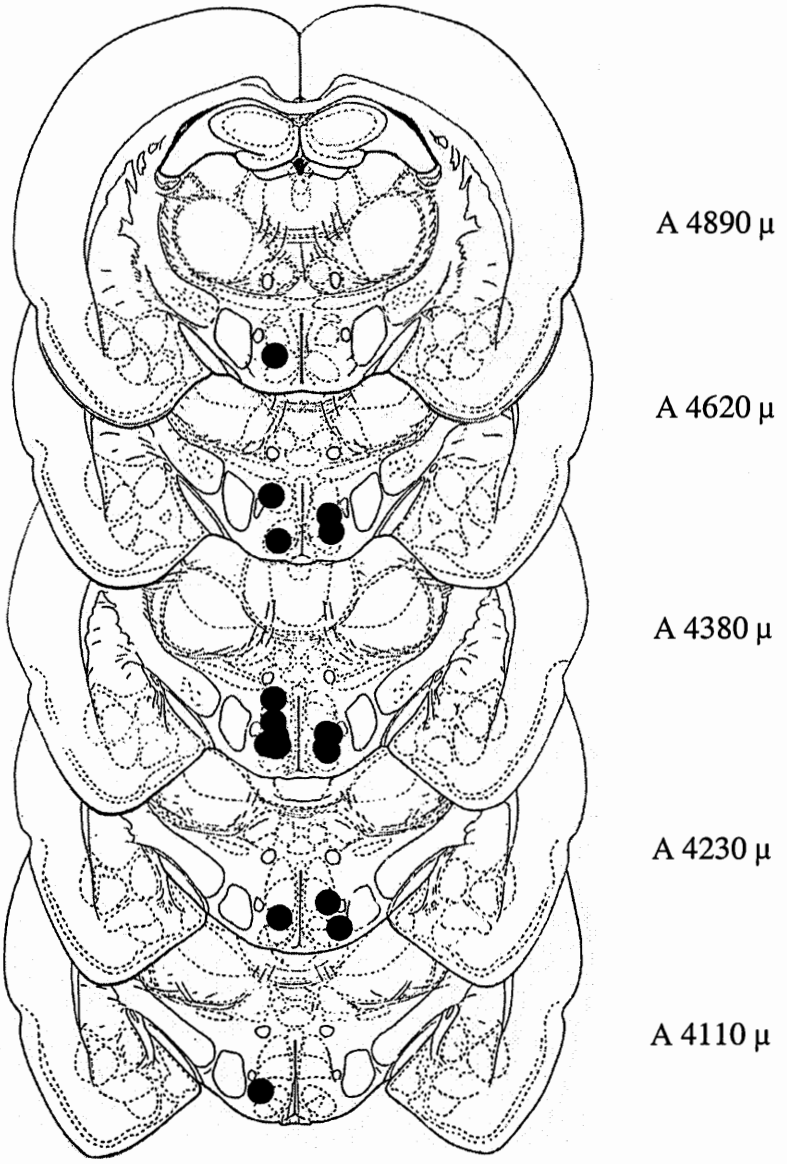


Figure 7: Histological locations of cannulae on coronal sections of the brain from rats preinfused with deionized water 30 min before either 200 ng 8-OH-DPAT infusion or 200 ng 8-OH-DPAT plus 2000 ng DOI coinfusion.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4110 μ to A 4890 μ. ● on the left side of the coronal section indicates the location of cannula sites from rats infused with 200 ng 8-OH-DPAT. ● on the right side of the coronal section indicates the location of cannula sites from rats coinfused with 200 ng 8-OH-DPAT plus 2000 ng DOI.

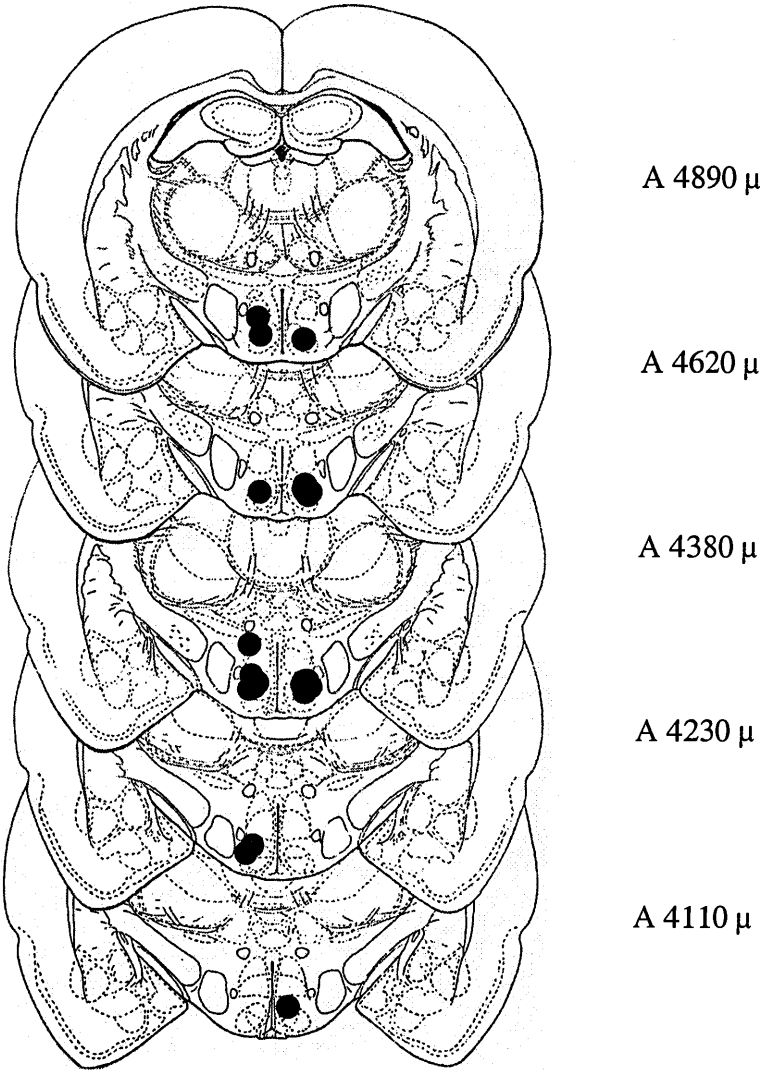


Figure 8: Histological locations of cannulae on coronal sections of the brain from rats preinfused with 1 x 10^{-1} nmol BIM 30 min before either 200 ng 8-OH-DPAT infusion or 200 ng 8-OH-DPAT plus 2000 ng DOI coinfusion.

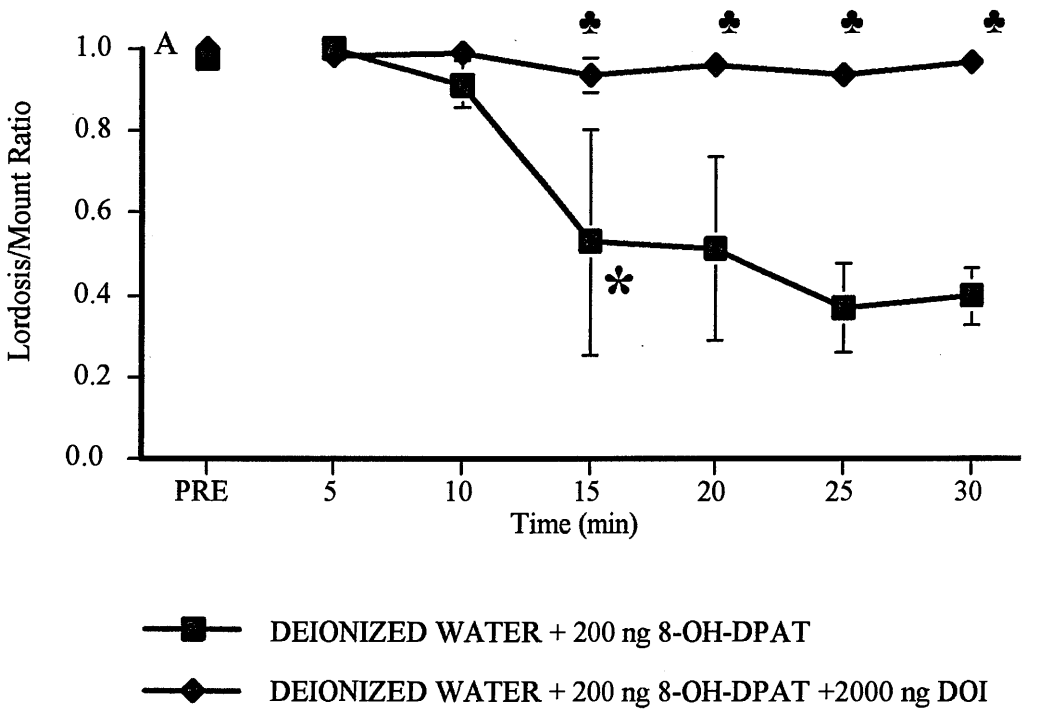
The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4110 µ to A 4890 µ. ● on the left side of the coronal section indicates the location of cannula sites from rats infused with 200 ng 8-OH-DPAT. ● on the right side of the coronal section indicates the location of cannula sites from rats coinfused with 200 ng 8-OH-DPAT plus 2000 ng DOI.

b. Administration of 1 x 10^{-1} nmol BIM, 90 min before 5-HT receptor agonists.

When BIM was infused 90 min before the 5-$HT_{2A/2C}$ receptor agonist, the PKC inhibitor attenuated the effects of DOI (Figure 9). Forty-one rats were used for the experiment. Trevino et al. [49] previously found 8-OH-DPAT to be less effective in reducing lordosis behavior following infusion into the most anterior portion of the VMN; therefore 8 rats were omitted from the data analysis because their cannulae were located in the anterior hypothalamus. Data from two additional rats were omitted because of poor histology. These 10 rats included 5 rats preinfused with BIM (1 8-OH-DPAT/DOI and 4 8-OH-DPAT rats) and 5 preinfused with water (2 8-OH-DPAT/DOI and 3 8-OH-DPAT rats) and showed basically the same pattern of behavioral responses that are described below for infusions into the VMN.

The effects of BIM or water preinfusion on the response to 8-OH-DPAT or 8-OH-DPAT plus DOI are shown in Figures 9A and 9B. 8-OH-DPAT significantly reduced lordosis behavior whether rats were preinfused with BIM or water. DOI attenuated 8-OH-DPAT's inhibition of the lordosis response but only in rats preinfused with water. Preinfusion 90 min earlier with BIM effectively eliminated DOI's ability to attenuate the effects of 8-OH-DPAT (Figure 9B). There was a significant main effect of type of pretreatment (water versus BIM); ($F_{1,27} = 18.65$, $p \leq 0.0002$), type of second infusion (8-OH-DPAT versus 8-OH-DPAT plus DOI) ($F_{1,27} = 6.27$, $p \leq 0.02$), and their interaction ($F_{1,27} = 4.25$, $p \leq 0.05$). Overall, L/M ratios declined with time ($F_{6,162} = 18.81$, $p \leq 0.0001$) and interacted significantly with type of pretreatment ($F_{6,162} = 3.53$, $p \leq 0.003$) as

Figure 9


A
1.0
0.8
0.6
0.4
0.2
0.0
Lordosis/Mount Ratio
PRE
5
10
15
20
25
30
Time (min)
DEIONIZED WATER + 200 ng 8-OH-DPAT
DEIONIZED WATER + 200 ng 8-OH-DPAT +2000 ng DOI

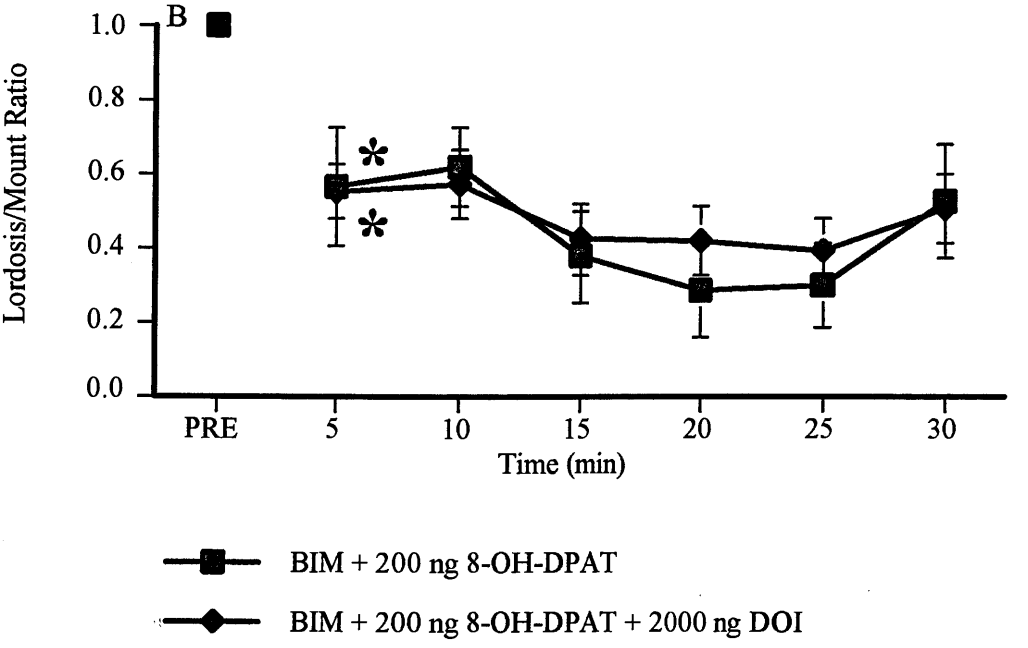

B
1.0
0.8
0.6
0.4
0.2
0.0
Lordosis/Mount Ratio
PRE
5
10
15
20
25
30
Time (min)
BIM + 200 ng 8-OH-DPAT
BIM + 200 ng 8-OH-DPAT + 2000 ng DOI

Figure 9: Effect of preinfusion with 1 x 10^{-1} nmol of the PKC inhibitor, BIM, or water 90 min before 8-OH-DPAT infusion or 8-OH-DPAT and DOI coinfusion on lordosis behavior.

Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity during a pretest (PRE). After the pretest for sexual behavior, rats were infused with either deionized water or BIM. Ninety min later they were then infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. The number of subjects for rats preinfused with deionized water (Figure 9A) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 4 and 9, respectively. The number of subjects for rats preinfused with BIM (Figure 9B) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 5 and 13, respectively. Data in Figures 9A and 9B are the mean ± S.E. L/M ratios before infusion (PRE) and for six consecutive 5 min intervals after infusion. Shamrocks indicate a significant difference between rats infused with 8-OH-DPAT or 8-OH-DPAT plus DOI within preinfusion condition. Asterisks indicate the first time interval within treatment conditions at which there was a significant difference from the pretest.

well as with the second infusion ($F_{6,162} = 3.37$, $p \leq 0.004$). The three-way interaction was not significant. Whether preinfused with water or BIM, every rat infused with 8-OH-DPAT showed a reduction in the L/M ratio for at least 2 consecutive 5 min intervals. Similarly 12 of 13 of the rats preinfused with BIM and then infused with 8-OH-DPAT plus DOI exhibited such a decline in lordosis behavior. In contrast, none of the rats that were preinfused with water and then infused with 8-OH-DPAT and DOI showed a reduction in the lordosis response.

Rats preinfused with deionized water and 8-OH-DPAT plus DOI had significantly higher L/M ratios than their 8-OH-DPAT counterparts at 15 min and this lasted throughout the testing period (Tukey's $q_{162,4} \geq 3.63$, $p \leq 0.05$). In rats preinfused with deionized water, 8-OH-DPAT rats differed from the pretest at 15, 20, 25, and 30 min time interval (Dunnett's $q_{162,7} \geq 2.57$, $p \leq 0.05$). In rats preinfused with BIM the L/M ratios did not significantly differ between the 8-OH-DPAT and 8-OH-DPAT plus DOI treated rats (Tukey's $q_{162,4} \geq 3.63$, $p \leq 0.05$). In rats preinfused with BIM, both 8-OH-DPAT and 8-OH-DPAT plus DOI treated rats had significantly lower L/M ratios than the pretest at every test interval (Dunnett's $q_{162,7} \geq 2.57$, $p \leq 0.05$).

Lordosis quality scores are shown in Figure 10. Since L/M ratios for 7 rats (1 BIM-8-OH-DPAT, 5 BIM 8-OH-DPAT plus DOI, and 1 water 8-OH-DPAT) dropped to zero during the experiment, lordosis quality scores could not be computed for these rats and their data were omitted from the overall ANOVA. For the remaining rats, there was a slight reduction in lordosis quality for rats preinfused with BIM (main effect for

Figure 10

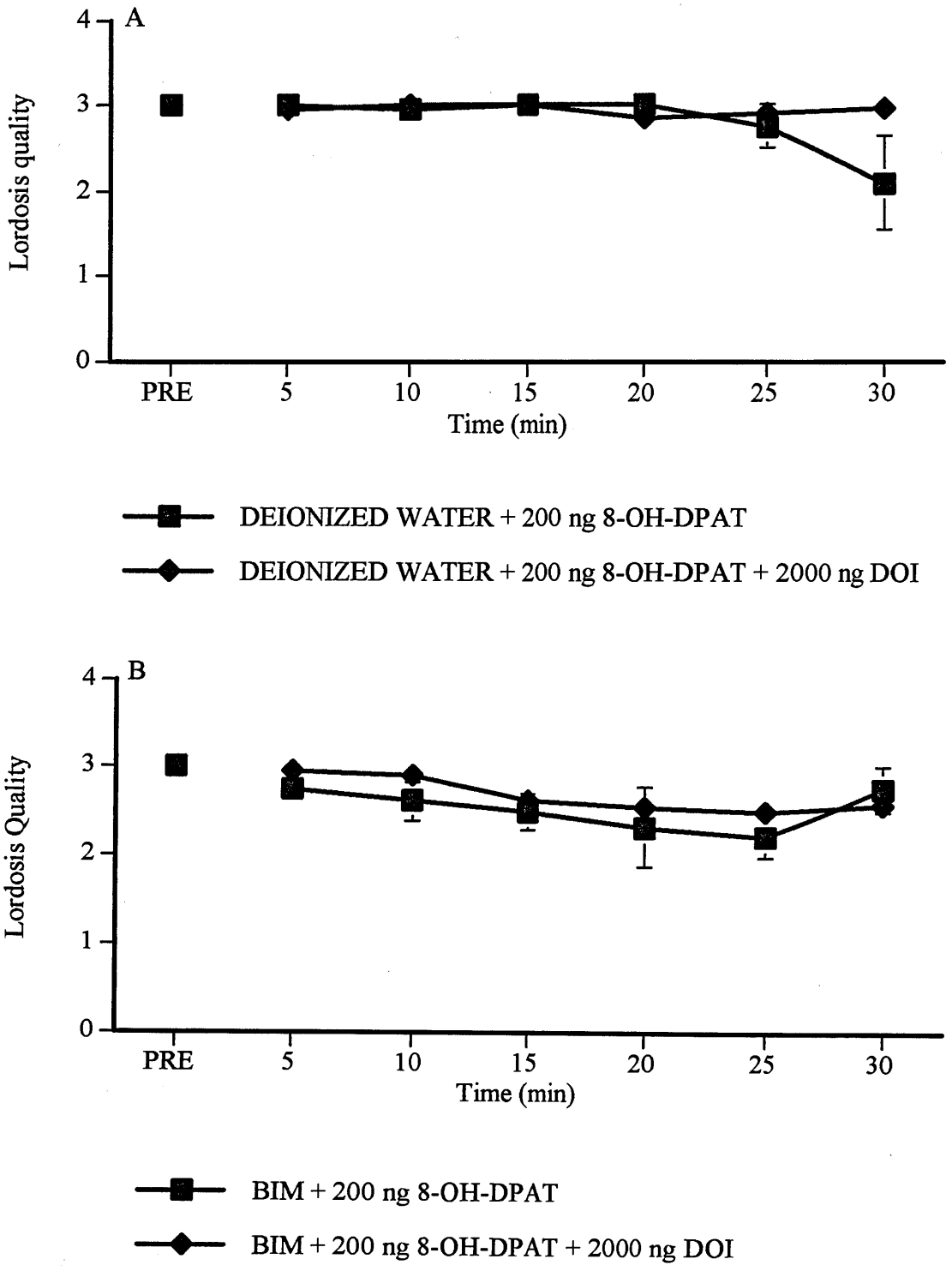

A
Lordosis quality
4
3
2
1
0
PRE
5
10
15
20
25
30
Time (min)
DEIONIZED WATER + 200 ng 8-OH-DPAT
DEIONIZED WATER + 200 ng 8-OH-DPAT + 2000 ng DOI
B
Lordosis Quality
4
3
2
1
0
PRE
5
10
15
20
25
30
Time (min)
BIM + 200 ng 8-OH-DPAT
BIM + 200 ng 8-OH-DPAT + 2000 ng DOI

Figure 10: Effect of preinfusion with 1 x 10^{-1} nmol of the PKC inhibitor, BIM, or water 90 min before 8-OH-DPAT infusion or 8-OH-DPAT and DOI coinfusion on lordosis quality.

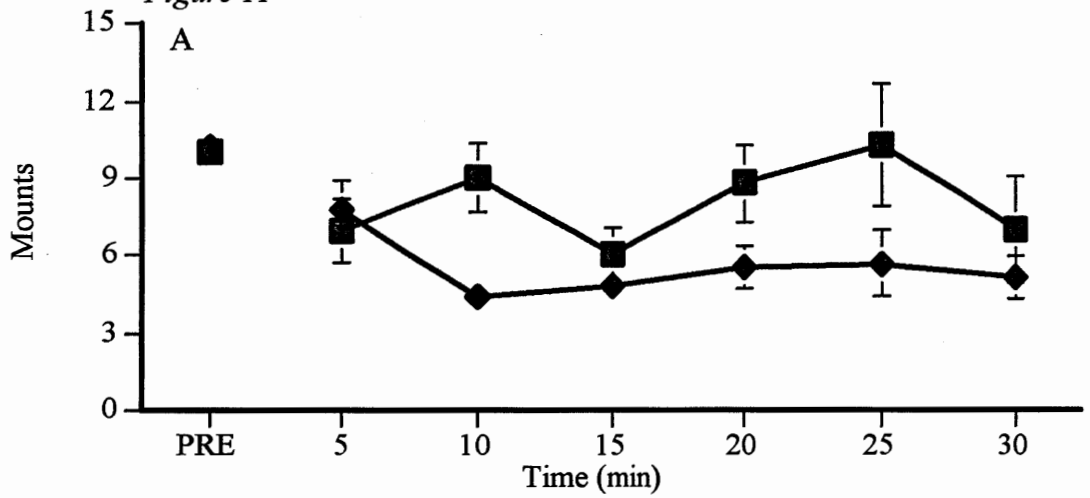
Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity (PRE). After the pretest for sexual behavior, rats were infused with either deionized water or BIM. Ninety min later they were then infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. The number of subjects for rats preinfused with deionized water (Figure 10A) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 4 and 9, respectively. The number of subjects for rats preinfused with BIM (Figure 10B) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 5 and 13, respectively. Data in Figures 10A and 10B are the mean ± S.E. lordosis quality before infusion (PRE) and for six consecutive 5 min intervals after infusion.

pretreatment, $F_{1,20} = 7.66$, $p \leq 0.02$). Across time, lordosis quality declined ($F_{6,120} = 5.93$, $p \leq 0.0001$) and both the the two way and three-way interactions with pretreatment were significant (respectively $F_{6,120} = 3.47$ and 3.44, $p \leq 0.004$). In rats preinfused with deionized water, 8-OH-DPAT decreased the lordosis quality only at the 30 min time interval (Tukey's $q_{120,4} = 3.68$, $p \leq 0.05$). In rats preinfused with BIM and then infused with 8-OH-DPAT, lordosis quality was significantly lower than the pretest at the 15, 20, and 25 min time intervals (Dunnett's $q_{120,7} = 2.60$, $p \leq 0.05$).

Number of mounts per test interval are shown in Figure 11. Mounts per test interval were roughly comparable for all treatment groups. A significant main effect of the second infusion ($F_{1,27} = 4.95$, $p \leq 0.05$) reflected the slightly lesser number of mounts in rats infused with the combination of 8-OH-DPAT plus DOI. Although there was a significant difference in the number of mounts over the test interval ($F_{6,162} = 2.47$, $p \leq 0.05$), there was never a significant interaction with either pretreatment or type of infusion (all $p > 0.05$).

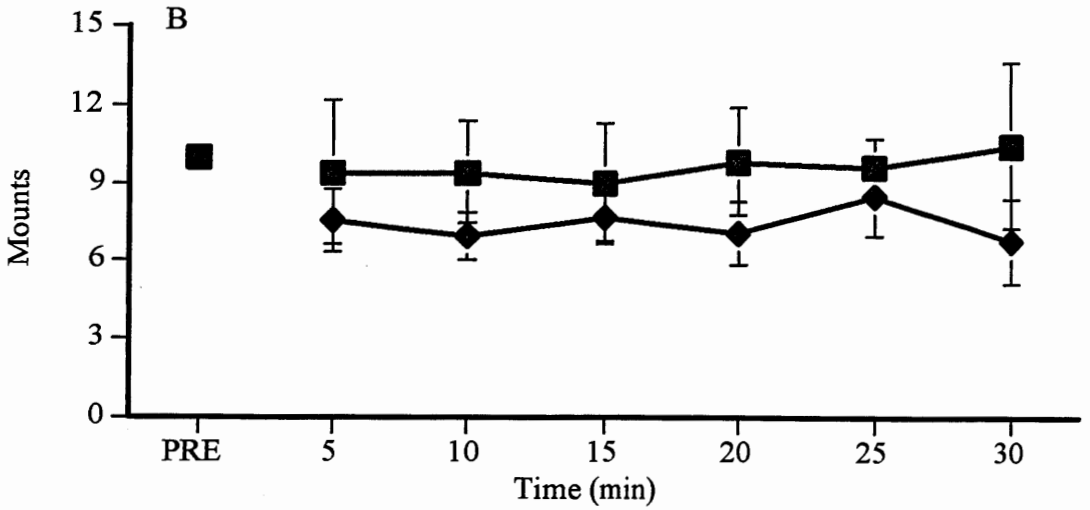
Histological analysis was used to determine location of cannulae. Cannulae locations from rats preinfused with deionized water 90 min prior to infusion with either 8-OH-DPAT or 8-OH-DPAT plus DOI are shown in Figure 12; those of rats preinfused with 1×10^{-1} nmol BIM prior to infusion with either 8-OH-DPAT or 8-OH-DPAT plus DOI are shown in Figure 13.

Figure 11



DEIONIZED WATER + 200 ng 8-OH-DPAT

DEIONIZED WATER + 200 ng 8-OH-DPAT + 2000 ng DOI



BIM + 200 ng 8-OH-DPAT

BIM + 200 ng 8-OH-DPAT + 2000 ng DOI

Figure 11: Effect of preinfusion with 1 x 10^{-1} nmol of the PKC inhibitor, BIM, or water 90 min before 8-OH-DPAT infusion or 8-OH-DPAT and DOI coinfusion on number of mounts.

Ovariectomized rats, with bilateral cannulae, were hormonally primed with 10 μg EB and 500 μg P. Four to six h after the P injection, rats were tested for sexual receptivity (PRE). After the pretest for sexual behavior, rats were infused with either deionized water or BIM. Ninety min later they were then infused with either 200 ng 8-OH-DPAT or 200 ng 8-OH-DPAT plus 2000 ng DOI. The number of subjects for rats preinfused with deionized water (Figure 11A) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 4 and 9, respectively. The number of subjects for rats preinfused with BIM (Figure 11B) and infused with 8-OH-DPAT or 8-OH-DPAT plus DOI were 5 and 13, respectively. Data in Figures 11A and 11B are the mean ± S.E. number of mounts before infusion (PRE) and for six consecutive 5 min intervals after infusion.

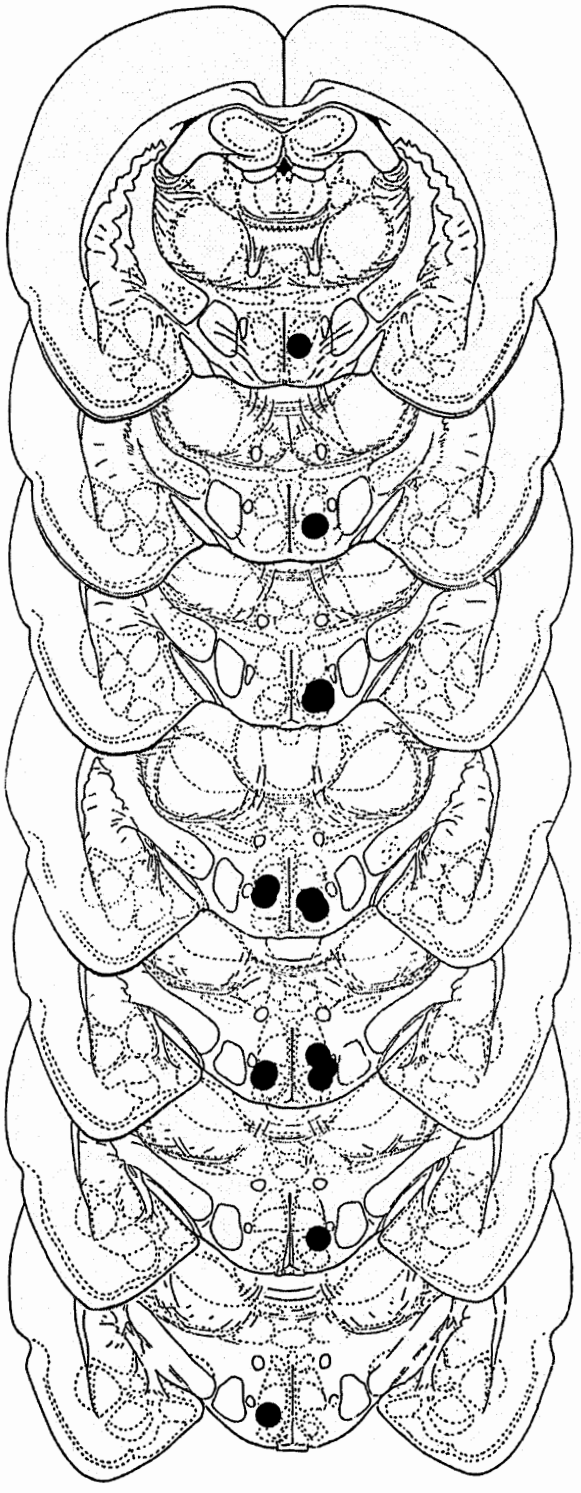

A 5150 µ
A 4890 µ
A 4620 µ
A 4380 µ
A 4230 µ
A 4110 µ
A 3990 µ

Figure 12: Histological locations of cannulae on coronal sections of the brain from rats preinfused with deionized water 90 min before either 200 ng 8-OH-DPAT infusion or 200 ng 8-OH-DPAT plus 2000 ng DOI coinfusion.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 3990 μ to A 5150 μ. ● on the left side of the coronal section indicates the location of cannula sites from rats infused with 200 ng 8-OH-DPAT. ● on the right side of the coronal section indicates the location of cannula sites from rats coinfused with 200 ng 8-OH-DPAT plus 2000 ng DOI.

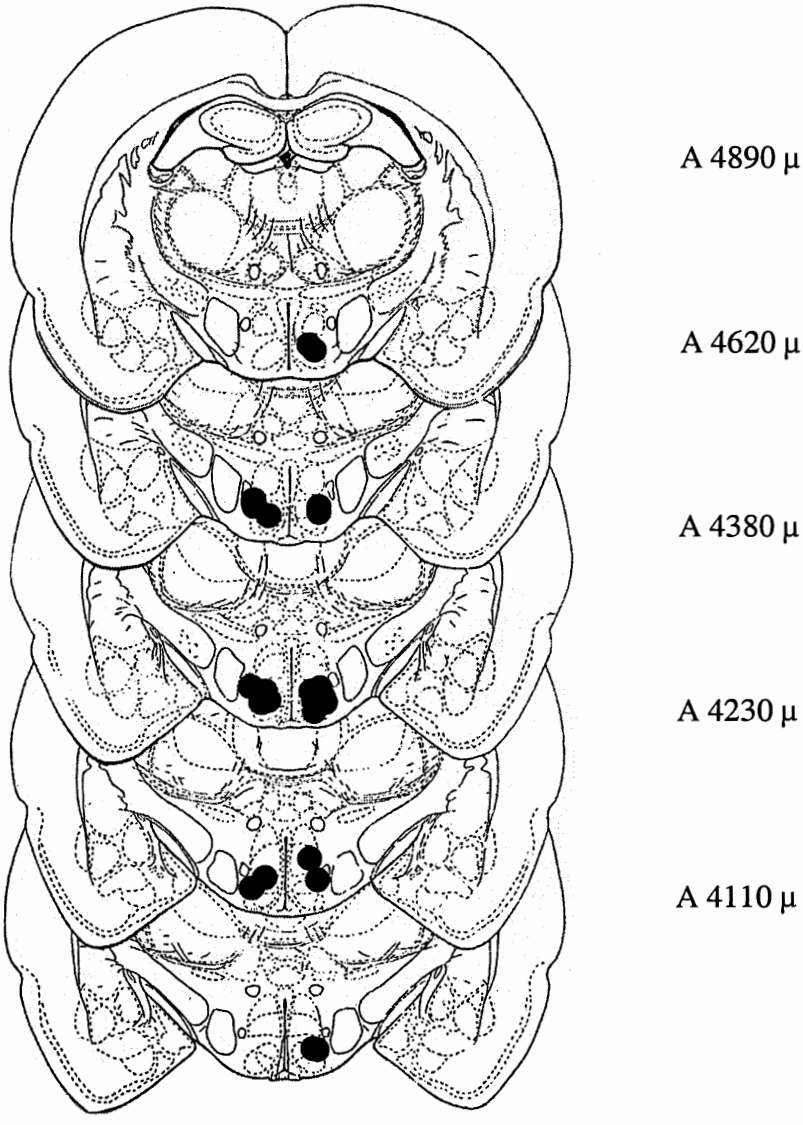


Figure 13: Histological locations of cannulae on coronal sections of the brain from rats preinfused with 1 x 10^{-1} nmol BIM 90 min before either 200 ng 8-OH-DPAT infusion or 200 ng 8-OH-DPAT plus 2000 ng DOI coinfusion.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4110 µ to A 4890 µ. ● on the left side of the coronal section indicates the location of cannula sites from rats infused with 200 ng 8-OH-DPAT. ● on the right side of the coronal section indicates the location of cannula sites from rats coinfused with 200 ng 8-OH-DPAT plus 2000 ng DOI.

3. Effect of varying concentrations of the PKC inhibitor, BIM, on DOI mediated attenuation of 8-OH-DPAT induced inhibition of lordosis behavior.

When varying concentrations of BIM were evaluated for their ability to attenuate the effects of DOI, there was an overall effect of concentration ($F_{6,26} = 8.31$, $p \leq 0.0001$) and a significant concentration by time interval interaction ($F_{36,156} = 1.67$, $p \leq 0.02$).

A concentration of BIM as low as 1×10^{-5} nmol was effective in attenuating the effects of DOI (Figure 13). In fact, every dose of BIM greater than or equal to 1×10^{-5} nmol reduced the ability of DOI to attenuate the effects of 8-OH-DPAT. In contrast, a concentration of 1×10^{-7} nmol BIM was ineffective. Consequently, there was limited evidence for dose-responsivity over the concentration range of 1×10^{-5} to 1×10^{-1} nmol BIM. In rats preinfused with 1×10^{-5} nmol BIM and then infused with 8-OH-DPAT plus DOI, L/M ratios were significantly different from the pretest at 15, 20, and 25 min time after infusion. Rats preinfused with 1×10^{-3} nmol BIM were significantly different from the pretest at 10 min and at every interval thereafter. Rats preinfused with 2.5×10^{-2}, 5×10^{-2}, and 1×10^{-1} nmol BIM were significantly different from the pretest at every interval after infusion (Dunnett's $q_{156,7} \geq 2.57$, $p \leq 0.05$).

Rats preinfused with either water or 1×10^{-7} nmol BIM were significantly different from those treated with all other doses throughout the testing period (Tukey's $q_{156,7} \geq 4.17$, $p \leq 0.05$). In agreement with the prior experiments, there was not a significant effect of treatment on lordosis quality ($F_{4,17} = 1.01$, $p > 0.05$) (Figure 15) or of a time by treatment interaction ($F_{24,102} = 0.98$, $p > 0.05$).

Figure 14

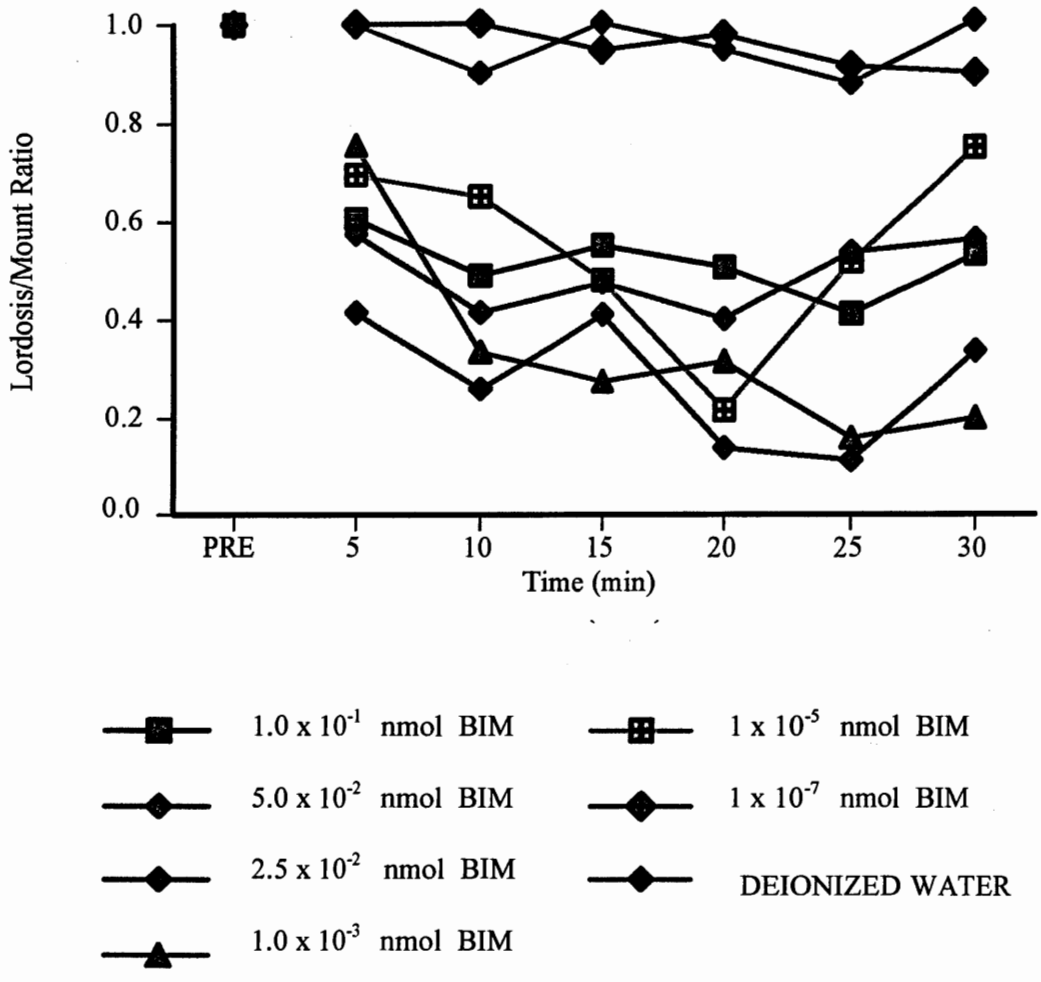

Lordosis/Mount Ratio
1.0
0.8
0.6
0.4
0.2
0.0
PRE
5
10
15
20
25
30
Time (min)
1.0 x 10-1 nmol BIM
5.0 x 10-2 nmol BIM
2.5 x 10-2 nmol BIM
1.0 x 10-3 nmol BIM
1 x 10-5 nmol BIM
1 x 10-7 nmol BIM
DEIONIZED WATER

Figure 14. Effect of varying concentrations of the PKC inhibitor, BIM, on DOI mediated attenuation of 8-OH-DPAT lordosis behavior.

Ovariectomized rats were hormonally primed with 10 μg EB followed 48 h later with P. Four-six h later, rats were pretested for sexual behavior (PRE). They were then infused with one of several concentrations of BIM or the water vehicle. Ninety min later, rats were infused with 200 ng 8-OH-DPAT plus 2000 ng DOI. Data are the mean ± S.E. L/M ratios for the pretest (PRE) and 6 consecutive 5 min intervals after the second infusion. The number of subjects per treatment were as follows: 0 , n = 4; 1 x 10^{-7} nmol BIM, n = 6; 1 x 10^{-5} nmol BIM, n = 3; 1 x 10^{-3} nmol BIM, n = 3; 2.5 x 10^{-2} nmol BIM, n = 3; 5 x 10^{-2} nmol BIM, n = 7; and 1 x 10^{-1} nmol BIM, n = 7. For ease of viewing, S.E. error bars have been omitted. The largest S.E. was 0.33 in the 1 x 10^{-3} nmol BIM group at 10 min and the overall MS was 0.048.

There was no significant effect of treatment on number of mounts ($F_{6,26} = 1.59$, $p > 0.05$) (Figure 16) or of a time by treatment interaction ($F_{36,156} = 0.91$, $p > 0.05$).

Histological analysis was used to determine location of cannulae. Cannulae locations from rats preinfused with 1×10^{-1} or 5×10^{-2} nmol BIM 90 min prior to infusion are shown in Figure 17; those infused with 2.5×10^{-2}, 1×10^{-3}, or 1×10^{-5} nmol are shown in Figure 18; and the rats infused with 1×10^{-7} nmol and deionized water are shown in Figure 19.

Figure 15

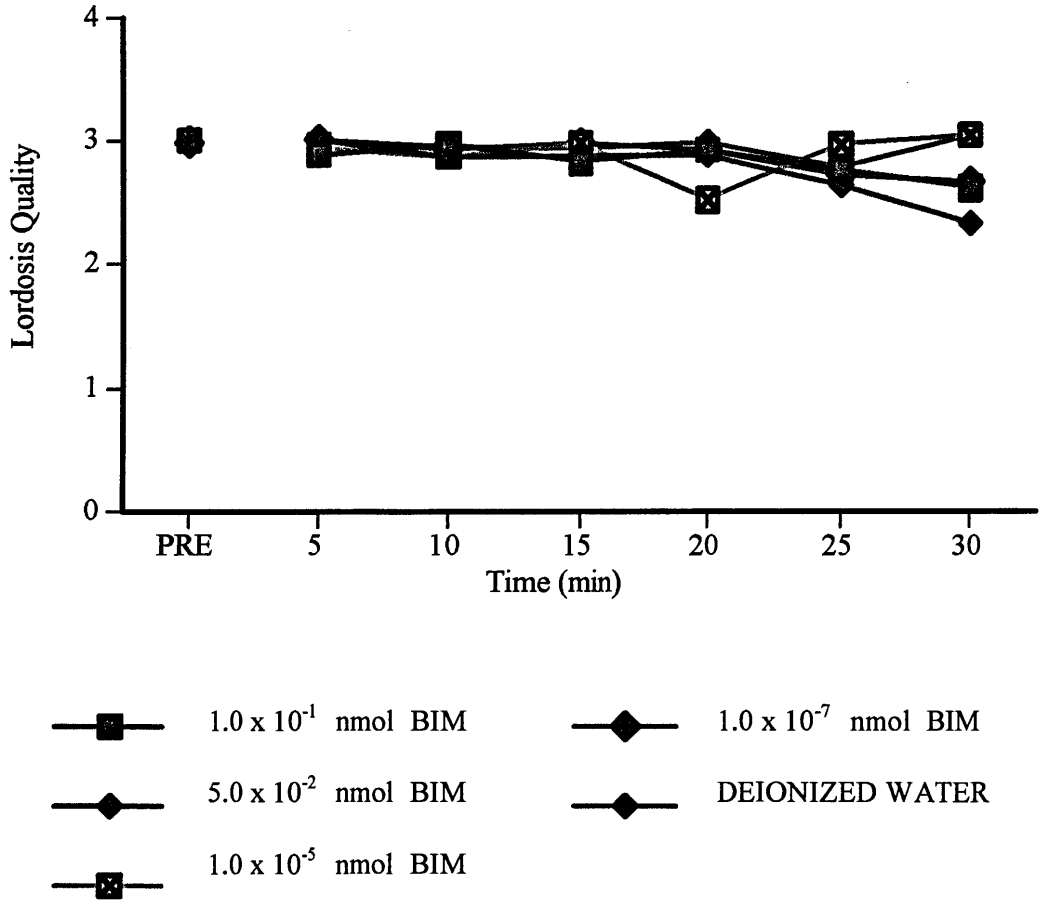

Lordosis Quality
4
3
2
1
0
PRE
5
10
15
20
25
30
Time (min)
1.0 x 10-1 nmol BIM
5.0 x 10-2 nmol BIM
1.0 x 10-5 nmol BIM
1.0 x 10-7 nmol BIM
DEIONIZED WATER

Figure 15. Effect of varying concentrations of the PKC inhibitor, BIM, on lordosis quality.

Ovariectomized rats were hormonally primed with 10 μg EB followed 48 h later with P. Four-six h later, rats were pretested for sexual behavior (PRE). They were then infused with one of several concentrations of BIM or the water vehicle. Ninety min later, rats were infused with 200 ng 8-OH-DPAT plus 2000 ng DOI. Data are the mean ± S.E. L/M ratios for the pretest (PRE) and 6 consecutive 5 min intervals after the second infusion. The number of subjects per treatment were as follows: 0 , n = 4; 1 x 10^{-7} nmol BIM, n = 6; 1 x 10^{-5} nmol BIM, n = 3; 1 x 10^{-3} nmol BIM, n = 3; 2.5 x 10^{-2} nmol BIM, n = 3; 5 x 10^{-2} nmol BIM, n = 7; and 1 x 10^{-1} nmol BIM, n = 7. Data from rats treated with 0.025 and 0.001 nmol BIM were not used in the analysis because all three rats treated with 0.025 nmol BIM and two of the three rats treated with 0.001 nmol BIM had a L/M ratio of 0 and therefore a quality score of 0. For ease of viewing, S.E. errors have been omitted. The largest S.E. was 0.5 in the 1 x 10^{-5} nmol BIM group at 20 min and the overall MS was 0.08.

Figure 16

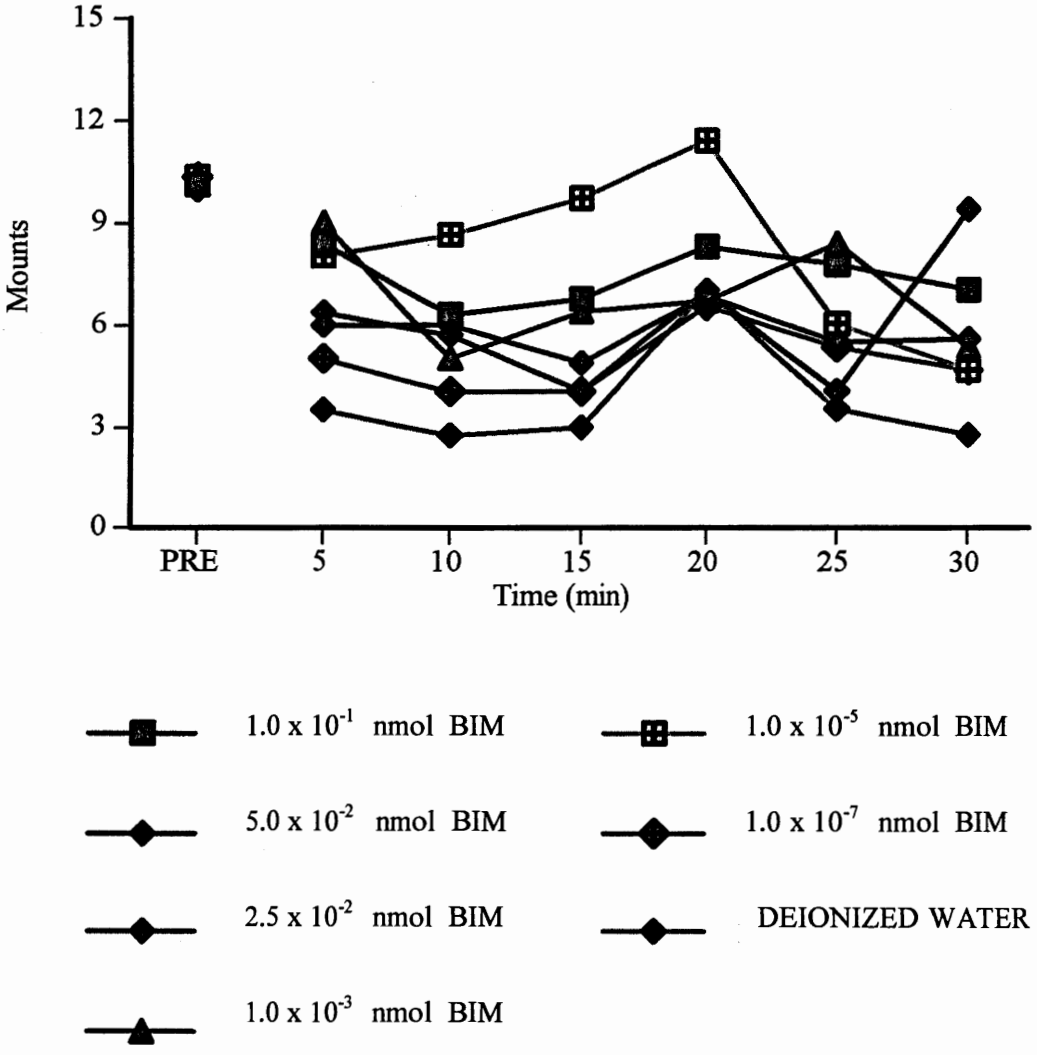

15
12
9
6
3
0
Mounts
PRE
5
10
15
20
25
30
Time (min)
1.0 x 10-1 nmol BIM
5.0 x 10-2 nmol BIM
2.5 x 10-2 nmol BIM
1.0 x 10-3 nmol BIM
1.0 x 10-5 nmol BIM
1.0 x 10-7 nmol BIM
DEIONIZED WATER

Figure 16. Effect of varying concentrations of the PKC inhibitor, BIM, on number of mounts.

Ovariectomized rats were hormonally primed with 10 μg EB followed 48 h later with P. Four-six h later, rats were pretested for sexual behavior (PRE). They were then infused with one of several concentrations of BIM or the water vehicle. Ninety min later, rats were infused with 200 ng 8-OH-DPAT plus 2000 ng DOI. Data are the mean ± S.E. L/M ratios for the pretest (PRE) and 6 consecutive 5 min intervals after the second infusion. The number of subjects per treatment were as follows: 0 , n = 4; 1 x 10^{-7} nmol BIM, n = 6; 1 x 10^{-5} nmol BIM, n = 3; 1 x 10^{-3} nmol BIM, n = 3; 2.5 x 10^{-2} nmol BIM, n = 3; 5 x 10^{-2} nmol BIM, n = 7; and 1 x 10^{-1} nmol BIM, n = 7. For ease of viewing, S.E. errors have been omitted. The largest S.E. was 5.60 in the 2.5 x 10^{-2} nmol BIM group at 20 min and the overall MS was 7.33.

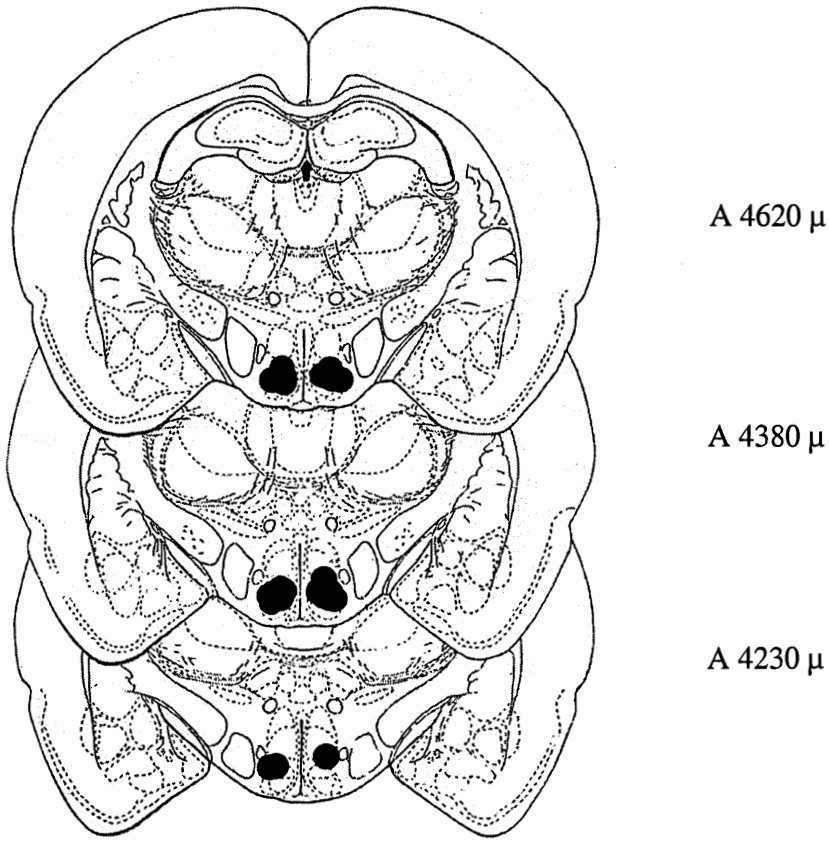


Figure 17: Histological locations of cannulae on coronal sections of the brain from rats preinfused with 1 x 10^{-1} or 5 x 10^{-2} nmol BIM 90 min before infusion with 200 ng 8-OH-DPAT plus 2000 ng DOI.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4230 µ to A 4620 µ. ● on the left side of the coronal section indicates the location of cannula sites from rats preinfused with 1 x 10^{-1} nmole BIM. ● on the right side of the coronal section indicates the location of cannula sites from rats preinfused with 5 x 10^{-2} nmol BIM before 200 ng 8-OH-DPAT plus 2000 ng DOI.

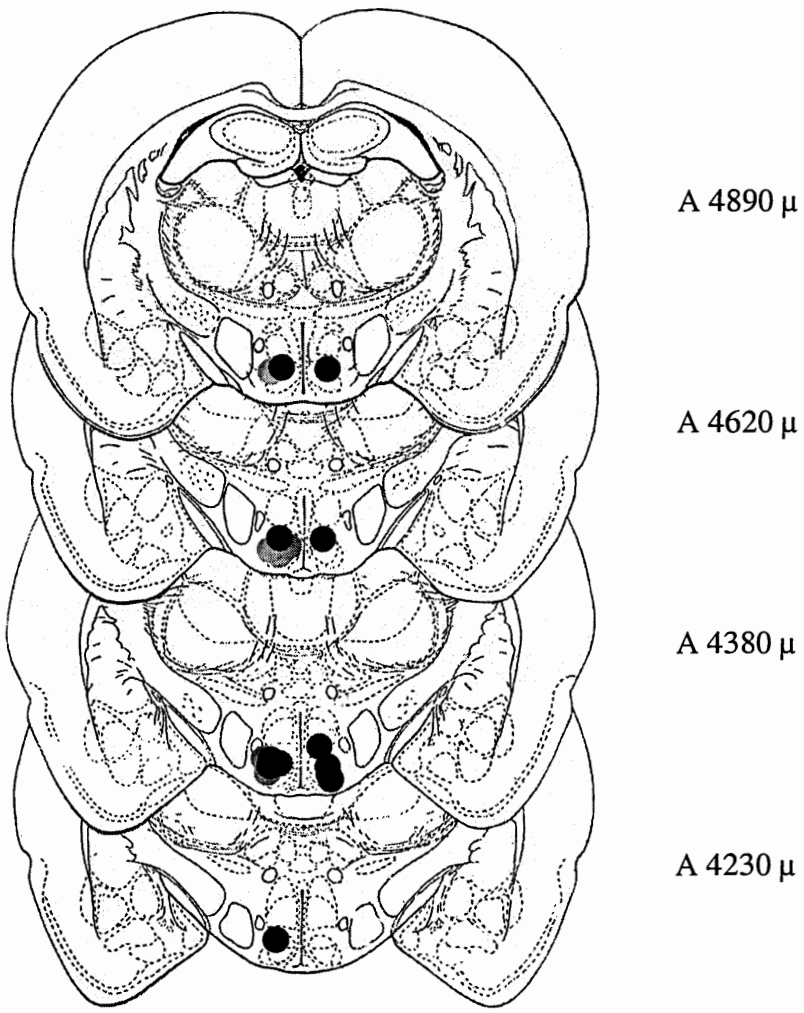


Figure 18: Histological locations of cannulae on coronal sections of the brain from rats preinfused with 2.5 x 10^{-2}, 1 x 10^{-3} or 1 x 10^{-5} nmol BIM 90 min before infusion with 200 ng 8-OH-DPAT plus 2000 ng DOI.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4230 µ to A 4890 µ. ● on the left side of the coronal section indicates the location of cannula sites from rats preinfused with 2.5 x 10^{-2} nmol BIM. ● on the right side of the coronal section indicates the location of cannula sites from rats preinfused with 1 x 10^{-3} nmol BIM. ● on the left side of the coronal section indicates the location of cannula sites from rats preinfused with 1 x 10^{-5} n mol BIM before 200 ng 8-OH-DPAT plus 2000 ng DOI.

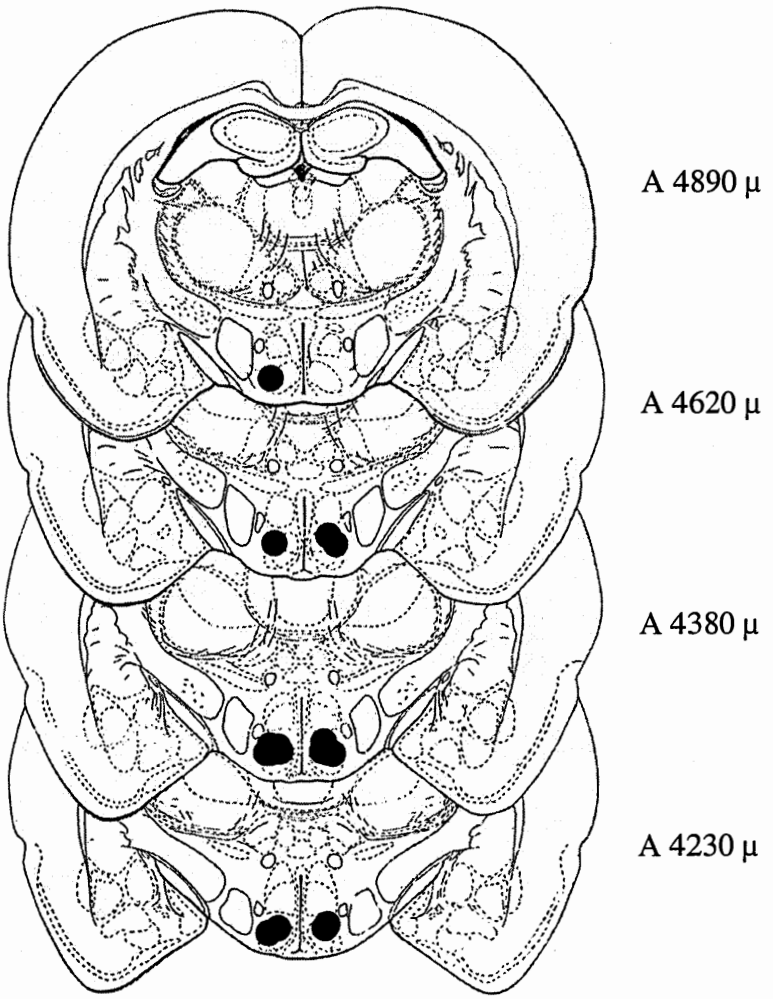


Figure 19: Histological locations of cannulae on coronal sections of the brain from rats preinfused with 1 x 10^{-7} nmol BIM or deionized water 90 min before infusion with 200 ng 8-OH-DPAT plus 2000 ng DOI.

The figure represents coronal sections at the level of the ventromedial nucleus of the hypothalamus (VMN) adapted from König and Klippel [18] from A 4890 µ to A 4230 µ. ● on the left side of the coronal section indicates the location of cannula sites from rats preinfused with 1 x 10^{-7} nmole BIM. ● on the right side of the coronal section indicates the location of cannula sites from rats preinfused with deionized water before 200 ng 8-OH-DPAT plus 2000 ng DOI.

CHAPTER IV

DISCUSSION

The major findings of this study are: 1) Within the mediobasal hypothalamus, activation of $5HT_{2A/2C}$ receptors with DOI attenuates the lordosis inhibiting effects of $5HT_{1A}$ receptor activation; 2) The PKC inhibitor, BIM, prevents this DOI mediated attenuation.

The present study provides evidence that the $5HT_{1A}$ receptor agonist, 8-OH-DPAT, inhibits lordosis behavior in a rat hormonally primed with 10 μg EB and 500 μg P and that this decrease is attenuated by DOI. This is consistent with previous reports that hypothalamic infusion of DOI attenuates the inhibitory action of 8-OH-DPAT on lordosis behavior of sexually receptive, intact, proestrous rats [52] or of ovx rats hormonally primed with 0.5 μg of E and 500 μg P [60].

In the intact naturally cycling female rat, both E and P induce sexual receptivity [6, 58]. In ovx rats, a period of 16-18 h of E priming followed by P treatment is required to elicit the entire repertoire of sexual behavior exhibited by an intact sexually receptive female [43]. However, in ovx rats, P treatment is not required for lordosis if the dose of E is sufficiently large [36]. Progesterone, therefore, reduces the dose of E required for the reflex [5, 33].

Both E and P influence the response to serotonergic compounds. An ovariectomy-induced increase in hypothalamic serotonin-1A receptor signaling as measured by levels of Gz protein has been shown to be prevented by estradiol [38]. Lakoski [23] reported that E decreased the ability of 8-OH-DPAT to suppress 5-HT neuronal firing and E treatment reduces the potency of 8-OH-DPAT in inhibiting lordosis behavior [15, 49, 54]. Progesterone, following E treatment, has also been reported to decrease the ability of a 5-HT_{1A} receptor agonist to inhibit lordosis [50]. At proestrous and following E treatment, increases in $5HT_{2A/2C}$ receptor density have been reported [4, 46]. An E induced increase in 5-$HT_{2A/2C}$ receptor function is consistent with the finding by Sinclair-Worley and Uphouse [42] that E decreased the effectiveness of a 5-$HT_{2A/2C}$ receptor antagonist in decreasing lordosis behavior. In earlier work, Wilson suggested that P, after E priming, increased $5HT_2$ receptor function [59]. So both hormones may contribute to an accentuation of 5-$HT_{2A/2C}$ receptor action. Therefore the balance between 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors is likely to be altered by hormonal treatment. Given evidence that both E and P manipulate the response to 5-HT_{1A} and 5-HT_2 active drugs, it was necessary to confirm that the priming conditions did not alter the ability of DOI to attenuate the lordosis inhibiting effects of 8-OH-DPAT.

Considerable evidence exists for functional interaction between 5-HT_{1A} receptors and 5-$HT_{2A/2C}$ receptors [7, 14, 22]. Opposing effects of $5HT_{1A}$ and 5-HT_2 receptors in different behaviors (sleep, sexual behavior, reflexes, temperature regulation) have been reported [12]. There is also evidence of functional interaction among these receptors

involving an additive effect. Arnt et al. [3] reported that simultaneous stimulation of 5-HT_{1A} and 5-HT_{2A} receptors with 8-OH-DPAT and DOI, respectively, accentuated forepaw treading induced by 8-OH-DPAT. The results from the present study are consistent with suggestions that $5HT_{2A/2C}$ receptors can influence the effects due to 5-HT_{IA} receptor activation.

Potential mechanisms involved in the interaction between the 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors would depend primarily on the location of the receptors (i.e. whether the receptors are located in the same, or different, cells). Activation of 5-$HT_{2A/2C}$ receptors could decrease the effect of 5-HT_{1A} receptors on lordosis behavior either by altering neuronal systems which impact the effect of 5-HT_{1A} receptors or by a direct effect on the 5-HT_{1A} receptor. If the receptors are present on two different cells, the mechanisms involved might include differential modulation by 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors of other neuronal systems. Activation of 5-$HT_{2A/2C}$ might lead to release of neurotransmitters that facilitate lordosis behavior so that attenuation of 5-HT_{1A} receptors mediated effect could occur. There is evidence for norepinephrine neurons terminating within the MBH [31]. Maswood et al. [29] suggested that norepinephrine attenuated 8-OH-DPAT's lordosis inhibiting effects when both compounds were coinfused into the VMN. Etgen et al. [8] reported that activation of $NE_{\alpha 1}$ receptors facilitated lordosis behavior. Therefore it is possible that activation of 5-$HT_{2A/2C}$ might lead to release of norepinephrine and thereby attenuate 5-HT_{1A} receptor mediated inhibition of lordosis behavior.

If both 5-HT_{1A} and 5-HT_2 receptors are present on the same cell, interaction of their transduction mechanisms is a possibility. 5-HT_{1A} receptors are Gi/Go coupled receptors, the activation of which leads to a decrease in Ca^{2+} conductance [35], increase in K^+ conductance via pertussis toxin–sensitive G protein (i.e. Gi/Go) [2] and decrease in cAMP production [40]. 5-$HT_{2A/2C}$ receptors are Gq/11 coupled to phospholipase C and their activation leads to PI hydrolysis resulting in an increase of DAG and IP3, which in turn leads to activation of PKC and elevation of intracellular Ca^{2+} [25]. Interaction among the 5-HT_{1A} and 5-HT_2 receptor's transduction mechanisms has been previously suggested [9, 17, 48]. PKC was shown to reduce the effectiveness of the 5-HT_{1A} receptor by mediating 5-HT_{1A} receptor desensitization [39]. PKC was also shown to reduce 5-HT_{1A} receptor-G-protein coupling [10, 27] and to reduce the ability of N-type Ca^{++} channels to undergo G-protein dependent downregulation [62]. Therefore, it is reasonable to consider that PKC might play a role in the attenuation of 5-HT_{1A} receptor mediated lordosis behavior.

In the current studies, it was hypothesized that a 5-$HT_{2A/2C}$ receptor mediated increase in PKC was important in the 5-$HT_{2A/2C}$ receptor attenuation of the effects of the 5-HT_{1A} receptor agonist on lordosis behavior. If so, the PKC inhibitor, BIM, should block the protective effects of DOI and this expectation was confirmed. BIM would block PKC mediated phosphorylation of 5-HT_{1A} receptors and would increase the potency of the 5-HT_{1A} receptor ligand. By blocking phosphorylation of Gα subunits, BIM would increase the effectiveness of 8-OH-DPAT binding to the 5-HT_{1A} receptor and

thereby increase its effect on lordosis behavior. By blocking phosphorylation of those Gα subunits which modulate Ca^{2+} channel opening, BIM could decrease 8-OH-DPAT's ability to close Ca^{2+} channels. Alternatively, BIM's attenuation of the effects of DOI could have resulted from mechanisms completely independent of these intracellular 5-HT_{1A}-5-$HT_{2A/2C}$ receptor interactions.

There is evidence for PKC–mediated reductions in 5-HT_{IA} receptor system responsiveness [13, 24, 39]. Factors that elevate cAMP or PKC can increase lordosis behavior [20]. Kow et al. [19] reported that activation of PKC in the VMN of the hypothalamus facilitates lordosis behavior. Therefore, it is possible that inhibitory effects of 5-HT_{1A} receptors and facilitatory effects of 5-$HT_{2A/2C}$ receptors simply cancel each other out. However, activation of PKC with the phorbol ester, phorbol 12,13-dibutyrate, reduced the efficacy of the $5HT_{1A}$ receptor agonist to inhibit forskolin stimulated cAMP and this effect was blocked by the PKC inhibitor, staurosporine [9]. Moreover, an increase in cAMP attenuates the lordosis inhibiting effects of 8-OH-DPAT [56]. Thus, it is possible to speculate that DOI reduces transduction mechanisms responsible for 8-OH-DPAT mediated inhibition of lordosis behavior and that BIM prevents these effects of DOI.

The effect of BIM was time dependent. To our knowledge, BIM has been used in two other in vivo studies. In one study, 0.1 mg/kg BIM was administered intravenously 15 min and intraperitoneally 30 min before assessing the involvement of PKC in preventing cell loss due to ischemia in retinal tissue [41]. In the second study

30 μM BIM were infused intrastriatally 90 min prior to evaluating MDMA-induced increase in extracellular concentration of dopamine in the striatum and BIM had a inhibitory effect on MDMA-induced dopamine release [32]. Based on these two studies the 30 min and 90 min time points were chosen for our study. Infusion of BIM 90 min before coinfusion with 8-OH-DPAT and DOI prevented the DOI-mediated attenuation of 8-OH-DPAT mediated inhibition of lordosis behavior. This effect could not be observed when the interval between BIM infusion and coinfusion with 8-OH-DPAT plus DOI was 30 min. Our observations agree with work by Nair et al. [32], who have found BIM to be effective at 90 min in inhibiting PKC activity. In our study, one of the possible reasons for BIM's ineffectiveness at 30 min could be the comparatively low dose of BIM used. At a higher dose, it might have been possible to observe an effect of BIM.

Unexpectedly, the effect of BIM was not dose-dependent over the range of concentration from 1.0×10^{-1} nmol to 1.0×10^{-5} nmol BIM used. All concentrations above 10^{-5} were effective in attenuating DOI's effect but 1.0×10^{-7} nmol of BIM was not effective in attenuating the DOI effect. Investigating concentrations between 1.0×10^{-5} nmol and 1.0×10^{-7} nmol of BIM could have possibly resulted in a dose-response curve.

Uphouse [51] suggested that a putative lordosis threshold exists in receptive female rats. Activation of 5-HT_{1A} receptors moves the female below this threshold; 5-$HT_{2A/2C}$ receptors attenuate these effects and thereby move the female above the threshold. Therefore, it is conceivable that at a concentration of 10^{-5} nmol, by inhibiting PKC, BIM inhibits 5-$HT_{2A/2C}$ receptor effects, thereby pushing the female below its

PKC, BIM inhibits 5-$HT_{2A/2C}$ receptor effects, thereby pushing the female below its lordosis threshold. And any concentrations of BIM higher than 10^{-5} nmol will effectively keep the female below this threshold.

Even though studying the effect of more doses and time-points would have strengthened this study, we conclude the following: 1) BIM is able to prevent 5-$HT_{2A/2C}$ receptor mediated facilitation of lordosis behavior when administered 90 min prior to infusion with 8-OH-DPAT plus DOI but not 30 min prior to infusion; 2) PKC may be involved in the cross-talk between the 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor in regulating lordosis behavior.

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