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TROPISETRON INCREASES THE INHIBITORY EFFECT OF MILD RESTRAINT ON LORDOSIS BEHAVIOR OF HORMONALLY PRIMED, OVARECTOMIZED RATS

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Abstract

Ovariectomized rats, hormonally primed with 10 µg estradiol benzoate and 500 µg progesterone are resistant to the lordosis-inhibiting effects of a 5 min restraint experience. However, modulation of the serotonergic (5-HT) system alters this resistance to stress. In the following experiment, ovariectomized Fischer inbred rats were hormonally primed with 10 µg estradiol benzoate and 500 µg progesterone. The effect of 5 min restraint on sexual behavior was examined after bilateral hypothalamic infusion or intraperitoneal (ip) treatment with the 5-HT₃ receptor antagonist, 3-tropanylindole-3-carboxylate hydrochloride (tropisetron). Infusion with 50 or 100 ng tropisetron inhibited lordosis behavior. When rats were infused with 10 or 25 ng tropisetron, rats showed normal lordosis behavior. However, when infusion with 10 or 25 ng tropisetron was combined with 5 min restraint, lordosis behavior was inhibited. These findings are consistent with prior work that has implicated hypothalamic serotonin in control of lordosis behavior and in the effect of mild restraint on the behavior. In contrast to the effects of the intracranial infusion, intraperitoneal injection with 1.0 or 2.0 mg/kg tropisetron did not amplify the effects of restraint.

Keywords

lordosis; female rats; 5-HT₃ receptors; ventromedial hypothalamus; intracranial infusion; intraperitoneal treatment; serotonin

1.0 Introduction

Female rodent sexual behavior includes receptivity (lordosis behavior), proceptivity, and other solicitous behaviors that advertise the female's receptive reproductive state (Erskine, 1989; Pfaff, 2005). Estradiol is required for lordosis behavior while progesterone is not required but facilitates the effect of estradiol (Sodersten, 1981). However, progesterone is

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required for proceptive behavior (Erskine, 1989) and may also enhance sexual behavior by reducing the female's response to mild stress (Truitt et al., 2003; White and Uphouse, 2004). Ovariectomized rats, hormonally primed only with 10 µg estradiol benzoate, show a rapid decline in lordosis behavior after a 5 min restraint experience (White and Uphouse, 2004). The addition of progesterone to the estradiol benzoate priming prevents this restraint-induced decline in the behavior (Truitt et al., 2003; White and Uphouse, 2004). Progesterone's effect on the serotonergic system may contribute to its ability to protect against the effects of mild stress.

Serotonergic modulation of female rat sexual behavior includes the neurotransmitter's activation of several serotonin (5-HT) receptors (Uphouse, 2000). The neurotransmitter has also been implicated in the response to stress (Kirby et al., 1997; Talalaenko et al., 2001) and in the effect of stress on reproductive functioning (Bethea et al., 2005; Bethea et al., 2008). Within the ventromedial nucleus of the hypothalamus (VMN), at least 3 different serotonin receptors contribute to the control of lordosis behavior (Uphouse, 2000). Within the VMN, agonist activation of 5-HT_{1A} receptors inhibits lordosis behavior while 5-HT₂ and 5-HT₃ receptors may play a facilitatory role (Uphouse, 2000). 5-HT_{1A} receptors appear to be important in stopping lordosis behavior even after hormonal priming has initiated a receptive state (Uphouse et al., 1993). 5-HT₂ and 5-HT₃ receptors, in contrast, may be able to override such lordosis inhibition since VMN coinjection of either a 5-HT₂ or a 5-HT₃ receptor agonist with the 5-HT_{1A} receptor agonist, (±)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), attenuates lordosis inhibition characteristic of 8-OH-DPAT (Maswood et al., 1998; Maswood et al., 1996).

We have speculated that this ability of 5-HT₂ and perhaps 5-HT₃ receptors to attenuate 5-HT_{1A} receptor-mediated lordosis inhibition may contribute to the control of sexual behavior during periods of mild stress. In support of this speculation, a 5 min restraint experience amplifies the effect of 8-OH-DPAT on lordosis behavior; and a 5-HT_{1A} receptor antagonist or a 5-HT₂ receptor agonist can prevent restraint-induced lordosis inhibition while a 5-HT₂ receptor antagonist amplifies the effect of restraint (Uphouse et al., 2003; Uphouse et al., 2007).

In the following paper, we have tested the hypothesis that a 5-HT₃ receptor antagonist would amplify effects of restraint on lordosis behavior. It was specifically hypothesized that, after prior treatment with tropisetron, restraint would reduce lordosis behavior of ovariectomized rats, hormonally primed with 10 µg estradiol benzoate and 500 µg progesterone. The results are consistent with this expectation.

2.0 Materials and methods

2.1 Materials

Estradiol benzoate, progesterone, sesame seed oil, and the 5-HT₃ receptor antagonist, 3-tropanylindole-3-carboxylate hydrochloride (tropisetron), were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Isoflurane (AErrane[®]) and suture material (vicryl) were purchased from Henry Schein (Melville, NY). Intracranial (ic) cannulae and dental acrylic were obtained, respectively, from Plastics One (Roanoke, VA) and Reliance Dental (Worth, IL). Decapicone[®] restrainers were from Braintree Scientific, Inc. (Braintree, MA). Other supplies came from Fisher Scientific (Houston, TX).

2.2 General methods

2.2.1 Animals and housing—Adult Fischer (F-344) female rats, purchased from Charles River Laboratories (Wilmington, MA), were housed 2 or 3 per cage in polycarbonate

shoebox cages in a colony room maintained at 25 °C and 55% humidity, with lights on from 12 midnight to 12 noon. Food (Purina LabDiet) and water were available *ad lib*. All procedures were conducted according to PHS policy and were approved by the IACUC at Texas Woman's University.

2.2.2 Surgical procedures, hormone treatments and drug treatments—For intracranial (ic) experiments, when approximately 60 to 90 days of age, females were anesthetized with AErrane[®] and stereotactically implanted bilaterally with 22-gauge stainless steel cannulae directed toward the VMN [atlas coordinates AP 4.38; DV - 7.8; ML $\pm$ 0.4 (König and Klippel, 1963)] as previously described (Uphouse et al., 1992). Approximately two weeks later, rats were ovariectomized under AErrane[®] anesthesia (White and Uphouse, 2004). Two weeks after ovariectomy, rats were injected with 10 μ g of estradiol benzoate followed 48 hr later with 500 μ g progesterone. Hormones, dissolved in sesame seed oil, were injected subcutaneously (sc) in a volume of 0.1 ml/rat. Experimental procedures were initiated 4 to 6 hr after the progesterone. For intraperitoneal (ip) experiments, the rats were ovariectomized and hormonally primed as described for the ic experiments.

Tropisetron was dissolved in deionized water. For ic experiments, tropisetron was administered in a 0.5 μ l volume at concentrations of 10, 25, 50, or 100 ng/bilateral site (20, 50, 100, or 200 ng/rat). For ip injections, 1.0 mg/kg or 2.0 mg/kg doses (1.0 ml/kg) were used.

2.2.3 Handling procedures—Handling began on the day of the estradiol benzoate injection as previously described (Uphouse et al., 2009). Each rat was picked up, held for 15 sec, and turned as in preparation for an ip injection. This procedure was then repeated and constituted a handling session. The procedure was repeated until each rat had received 5 handling sessions. The entire process was repeated the following day.

2.2.4 Restraint procedures—Restraint procedures were as previously described (Uphouse et al., 2007). The female was placed head first into a Decapicone[®] so that her nose was flush with the small opening at the tip of the cone. A longitudinal slit was made along the cone to allow room for the top of the guide cannulae. The slit was then secured with lab tape after the female's nose was flush with the air hole. The base of the cone was gathered around the female's tail and secured tightly with tape. The process of wrapping the female required between 30 and 60 sec. The wrapped female was set aside for 5 min of restraint. Nonrestrained rats remained in their home cages for the 5 min.

2.2.5 Testing for sexual receptivity—On the morning of testing (prior to lights out), rats were moved to the testing room where the males were housed. Testing for sexual behavior, as previously described (Uphouse et al., 1992), was initiated within 1 to 3 hr after colony lights off and 4 to 6 hr after the progesterone injection. Experimenter visibility was aided by red lighting. In a pretest for sexual receptivity, females were placed into the home cages of sexually experienced Sprague-Dawley male rats and behavior was monitored for 10 min or until the male had accomplished 10 mounts; rats with a pretest lordosis to mount (L/M) ratio of 0.7 or higher were included in the remaining experiments. Lordosis quality and proceptivity (hopping and darting), as previously described (White and Uphouse, 2004), were also recorded. Based on the presence or absence of proceptive and resistive behavior, females were categorized as showing or not showing the respective behaviors.

2.2.6 Statistical procedures—L/M ratios, lordosis quality scores and number of mounts by the male were grouped into the pretest interval and five-min intervals after infusion. For the dose response study, data were evaluated by repeated measures ANOVA with dose as

the independent factor. Time relative to infusion was the repeated factor. Effects of dose were compared to their respective pretest with Dunnett's test. For the ic and ip experiments with restraint, data were evaluated with two-factor, repeated measures ANOVA with dose and restraint/nonrestraint as independent factors. Comparisons between means were made with Tukey's test. Proceptive and resistive behavior were compared with the Fisher's Exact Test. Statistical analyses were conducted with SPSS 17 for MacIntosh. Independent pairwise comparisons were performed manually (Zar, 1999). An alpha level of 0.05 was required for rejection of the null hypothesis.

2.3 Specific experiments

2.3.1 IC experiments

2.3.1.1 Dose response: An initial ic dose response analysis was conducted to determine a dose of tropisetron that would not inhibit lordosis behavior. In previous studies, we reported that bilateral hypothalamic infusion with 100 to 500 ng tropisetron dose-dependently reduced lordosis behavior (Maswood et al., 1997). In the current study, rats were infused bilaterally with the water vehicle or with 25, 50 or 100 ng tropisetron to obtain an appropriate dose for later experiments. After infusion, rats were returned to their home cage. Infusions were delivered at a rate of 0.24–0.26 μ l/min. The cannulae remained in place for 2 min following completion of infusion. Fifteen min later, rats were tested for 15 consecutive min for sexual behavior. A total of 15 rats were infused. One rat (25 ng) and 1 rat (vehicle) had cannulae located, respectively, anterior and posterior to the VMN and were excluded from further analysis.

2.3.1.2 IC effects of tropisetron on restraint: After the pretest, rats were bilaterally infused with 10 or 25 ng of tropisetron and returned to their home cage. Intracranial drug concentrations are given as the amount of drug infused per bilateral cannula or one-half the concentration per animal. Fifteen min after infusion, rats were either restrained for 5 min or remained in the home cage. Sexual receptivity was then monitored for 15 consecutive minutes.

A total of 39 rats were used. Of these, 3 rats [25 ng nonrestraint (1 rat) and 10 ng restraint (2 rats)] were found to have cannulae anterior or posterior to the VMN and were excluded from the analysis. In addition, one rat infused with 10 ng tropisetron behaved sporadically after infusion and was excluded from further analysis.

2.3.1.3 Histological procedures: After behavioral testing, rats were anesthetized with AErrane[®] and perfused with 0.9% saline followed by 10% buffered formalin. Brain tissue was placed into 10% buffered formalin for at least 24 h before sectioning. Coronal sections (100 μ m) were stained with cresyl violet and examined for cannulae placement (König and Klippel, 1963).

2.3.2 IP treatments—A total of 49 rats were used in the experiment. After the pretest, rats were injected ip with 1.0 mg/kg or 2.0 mg/kg tropisetron (in deionized water) or the water vehicle and returned to their home cage. Fifteen min later, rats were restrained for 5 min or remained in their home cage for the 5 min. Immediately, thereafter, fifteen min testing began as described above for ic studies.

3.0 Results

3.1 IC dose response

Since tropisetron can reduce lordosis behavior in the absence of restraint (Maswood et al., 1997), we began with a preliminary dose response evaluation to determine a concentration

of tropisetron that would have modest effects on L/M ratios. The results are shown in Figure 1 and cannulae locations are shown in Figure 2. There was a significant effect of dose ($F_{3,9} = 4.20$, $p \leq 0.05$) and time after infusion ($F_{3,27} = 5.93$, $p \leq 0.003$) with doses of 50 and 100 ng tropisetron reducing L/M ratios at every interval after infusion (all $q_{9,4} = 2.37$, $p \leq 0.05$; Dunnett's test relative to the pretest).

There were no significant effects on lordosis quality (data not shown) and only minor, but significant, effects on number of mounts ($F_{3,9} = 5.18$, $p \leq 0.03$). The mean $\pm$ S.E. number of mounts per interval for water, 25, 50 and 100 ng tropisetron, respectively, were 12.16 ± 1.44 , 10.16 ± 0.11 , 11.16 ± 1.31 , and 10.63 ± 1.25 .

From these results, 25 ng tropisetron was selected as the highest dose for evaluation of the effects of restraint.

3.2. Effects of IC tropisetron on restraint

Cannulae locations for rats used in the experiment are shown in Figure 3. Restraint led to a significant decline in lordosis behavior (ANOVA for restraint, $F_{1,31} = 9.50$, $p \leq 0.005$) that was most robust in rats infused with 25 ng tropisetron (ANOVA for dose by restraint, $F_{1,31} = 9.1$, $p \leq 0.005$) (See Figure 4). Rats infused with 25 ng tropisetron and restrained were significantly different from the respective nonrestrained rats at 5 and 15 min after infusion ($q_{93,4} \geq 3.73$, $p \leq 0.05$). After infusion with 10 or 25 ng tropisetron, restrained rats had L/M ratios that were significantly different from their pretest at every interval after infusion ($q_{93,4} \geq 3.73$, $p \leq 0.05$). However, nonrestrained rats also showed a modest decline in the L/M ratio. This was especially evident for 10 ng tropisetron where nonrestrained rats had reduced L/M ratios at 10 and 15 min after infusion. Overall, there was a time-dependent decline in the L/M ratio ($F_{3,93} = 26.68$, $p \leq 0.001$) and a time by restraint interaction ($F_{3,93} = 4.72$, $p \leq 0.005$). A majority (80%) of the rats showed resistive behavior after infusion but this was unrelated to the type of treatment (Fischer's Exact Test, $p > 0.05$).

There was not a significant effect of any factor on lordosis quality (all $p > 0.05$; data not shown). Seventy-four percent of the rats showed proceptivity before the infusion while only 37.1% were proceptive after infusion. Although this may have reflected an effect of the infusion, the difference was not statistically significant and there was no difference across treatment conditions (Fisher's Exact Text, $p > 0.05$).

Mounts were roughly comparable across groups, but there was a significant dose by restraint interaction ($F_{1,31} = 5.76$, $p \leq 0.05$). The mean $\pm$ S.E. number of mounts per interval for 10 and 25 ng tropisetron in nonrestrained and restrained rats, respectively, were 11.09 ± 0.31 , 9.93 ± 0.36 , 9.02 ± 0.20 , and 11.96 ± 0.31 .

3.3 IP experiments

Tropisetron's ability to alter the effect of restraint was less evident when the drug was administered ip (Figure 5). Although there was a significant effect of dose ($F_{2,43} = 4.36$, $p \leq 0.05$), restraint ($F_{1,43} = 4.23$, $p \leq 0.05$), and their interaction ($F_{2,43} = 3.56$, $p \leq 0.05$), the magnitude of these effects were small compared to effects following ic infusion. There was a trend for L/M ratios to decline after the pretest ($F_{3,129} = 3.69$, $p \leq 0.02$); but this was primarily evident for 1 mg/kg tropisetron and there were no interactions between time and any other factor (all $p > 0.05$).

For lordosis quality, there was an overall decline during testing ($F_{3,129} = 5.77$, $p \leq 0.001$) (Figure 6). Restraint significantly reduced lordosis quality ($F_{1,43} = 9.13$, $p \leq 0.005$) and interacted significantly with the time of testing ($F_{3,129} = 5.70$, $p \leq 0.001$). No other effects were significant (all $p > 0.05$).

There was also a time-dependent decline in the number of mounts during the test period ($F_{3,129} = 3.74$, $p \leq 0.02$), but no other effects were significant. Overall, rats received an average $\pm$ S.E. number of mounts of 10.10 ± 0.04 , 9.30 ± 0.71 , 7.36 ± 0.66 , and 7.4 ± 0.55 per test interval for the pretest, 5, 10, and 15 min intervals, respectively.

4.0 Discussion

As hypothesized, after ic infusion of tropisetron, 5 min restraint reduced L/M ratios in rats hormonally primed with 10 μ g estradiol benzoate and 500 μ g progesterone. Ovariectomized rats primed with estradiol benzoate and progesterone are generally resistant to the lordosis-inhibiting effects of this experience (Truitt et al., 2003; White and Uphouse, 2004). The current results thus allow the suggestion that tropisetron increases the lordosis-inhibitory response to 5 min of restraint. Higher doses of tropisetron can inhibit lordosis behavior of nonrestrained gonadally intact or hormonally primed, ovariectomized rats (Maswood et al., 1997). However, bilateral infusion of the lower doses of 10 or 25 ng tropisetron had no effect in the absence of the restraint (current study). Thus, conditions that, when administered alone, were ineffective in reducing lordosis did so when combined together.

The current results are consistent with our working model whereby mild restraint increases the release of 5-HT in the mediobasal hypothalamus that then leads to a reduction in lordosis behavior. Enhanced release of 5-HT will activate multiple receptor subtypes, the relative balance of which determines the behavioral outcome (Uphouse, 2000). Within the VMN, 5-HT's ability to inhibit lordosis resides primarily with the neurotransmitter's activation of 5-HT_{1A} receptors (Uphouse et al., 1992; Uphouse and Wolf, 2004). Coinfusion with either 5-HT₂ or 5-HT₃ receptor agonists protects against this effects of 5-HT_{1A} receptor activation (Maswood et al., 1998; Maswood et al., 1996). Previously, we reported that 5 min restraint increased the lordosis-inhibiting potency of a 5-HT_{1A} receptor agonist and of a 5-HT_{2A/2C} and 5-HT_{2C} receptor antagonist (Uphouse et al., 2003). The current findings extend this model to include the 5-HT₃ receptor. Thus, in the absence of 5-HT₂ or 5-HT₃ receptor activation, restraint leads to lordosis inhibition perhaps through increased activation of 5-HT_{1A} receptors.

How a 5-HT₃ receptor antagonist increases the response to mild restraint is unknown. Unlike the majority of 5-HT receptors, 5-HT₃ receptors are ligand-gated ion channels (Barnes et al., 2009; Hannon and Hoyer, 2008) that exist primarily on presynaptic terminals where they facilitate release of various neurotransmitters, but may also be located on soma and dendrites (Hannon and Hoyer, 2008; Miquel et al., 2002). Many of the neurotransmitters such as norepinephrine (NE) and gamma aminobutyric acid (GABA) that are modulated by 5-HT₃ receptors also influence lordosis behavior (Guptarak et al., 2004; Luine et al., 1991; McCarthy et al., 1991; Vathy and Etgen, 1989). For example, VMN infusion of a GABA_A receptor agonist can prevent 5-HT_{1A} receptor-mediated inhibition of lordosis behavior (Guptarak et al., 2004). Both NE and GABA can also modulate the response to stress (Serra et al., 2007; Shekhar et al., 2005; Van Bockstaele et al.) and may, therefore, contribute to the female's continued mating behavior after the mild restraint. Tropisetron, by blocking 5-HT₃ receptors, might be expected to attenuate this protection and thereby enhance lordosis inhibition after restraint.

It is puzzling that a 5-HT₃ receptor antagonist inhibits a behavior such as lordosis that requires hormonal priming. Estrogen and progesterone facilitate lordosis but antagonize 5-HT₃ receptors (Oz et al., 2002; Wetzell et al., 1998; Wu et al., 2000). Moreover, in some animal models, tropisetron has anxiolytic action [see for review, (Olivier et al., 2000)] which would be expected to reduce, rather than increase, the response to mild restraint. However, the effect of tropisetron on anxiety is not consistent and varies dependent on the animal

model investigated (Olivier et al., 2000). Thus, tropisetron's anxiolytic/anxiogenic effect may vary in different brain areas. This possibility is especially appealing since tropisetron's effect on lordosis after restraint occurred following ic, but not ip, tropisetron. After ip treatment, effects of tropisetron outside the mediobasal hypothalamus may have compensated for hypothalamic effects of the drug. Consistent with this possibility, it is interesting to note that systemic treatment with another 5-HT₃ receptor antagonist, ondansetron, failed to reduce female rat sexual behavior (Tanco et al., 1993).

Finally, although tropisetron was initially thought to be a selective 5-HT₃ receptor antagonist, it is now clear that the drug binds to sites other than the 5-HT₃ receptor (Barnes et al., 2009) and can have functional consequences (Macor et al., 1990; Papke et al., 2005; Rothlin et al., 2003). Therefore, we cannot rule out the possibility that receptors other than the 5-HT₃ receptor contributed to the effects of tropisetron on the response to restraint.

In conclusion, after ic infusion with tropisetron (but not following systemic treatment), a mild 5 min restraint reduced lordosis behavior of rats hormonally primed with estradiol benzoate and progesterone. Since such hormonally primed, ovariectomized rats are resistant to the lordosis-inhibitory effects of this experience, tropisetron appears to have enhanced the effects of restraint. These findings are consistent with a model whereby restraint leads to enhanced activation of serotonin receptors that both inhibit lordosis behavior and protect against this inhibition. The current findings allow the suggestion that 5-HT₃ receptors may contribute to this protection.

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Figure 1. Dose response effect of tropisetron

Ovariectomized rats were hormonally primed with 10 μ g estradiol benzoate and 500 μ g progesterone. After a pretest for sexual receptivity, rats were infused bilaterally through cannulae directed toward the VMN with the vehicle, 25, 50, or 100 ng tropisetron. N's respectively are 4, 3, 3, and 3. Data are the mean $\pm$ S.E. L/M ratios for the pretest (PRE) and 15 consecutive min after the infusion. * Indicates significant difference from the respective pretest L/M ratio (Dunnett's test).

Figure 2. Cannulae locations for rats infused with water, 25, 50, or 100 ng tropisetron
 Shown are the cannulae locations [adapted from (König and Klippel, 1963)] for rats represented in figure 1. Cannulae locations were always bilateral but are represented either on the right or left side of the figure. Cannulae locations for water (open circles) and 100 ng tropisetron (filled circles) are on the right of the figure. Cannulae locations for 25 ng tropisetron (filled circles) and 50 ng tropisetron (open circles) are shown on the left side of the figure.

Figure 3. Cannulae locations for restrained and nonrestrained rats

Shown are the cannulae locations [adapted from (König and Klippel, 1963)] for rats infused bilaterally with either 10 (left side) or 20 ng (right side) tropisetron. Restrained and nonrestrained rats are indicated by filled or open circles, respectively. Cannulae locations were always bilateral but are represented either on the right or left side of the figure.

Figure 4. Effects of ic tropisetron on the response to restraint

Shown in the figure are the mean $\pm$ S.E. L/M ratios for rats infused with 10 or 25 ng tropisetron prior to a restraint or home cage experience. Ovariectomized rats were hormonally primed with 10 μ g estradiol benzoate and 500 μ g progesterone. Four to six hr after progesterone, rats were pretested (PRE) for sexual receptivity and received a bilateral infusion of 10 or 25 ng tropisetron. Fifteen min later, rats were restrained for 5 min or remained in the home cage for a comparable time. Immediately after the 5 min experience, sexual behavior was tested for 15 consecutive min. N's for 10 and 25 ng tropisetron, restrained and nonrestrained rats, respectively, are 7, 8, 8, and 12. * Indicates significant difference from the respective pretest value, within treatment (Tukey's test). ** Indicates significant difference between restrained and nonrestrained rats, within dose and time (Tukey's test).

Figure 5. Effects of ip tropisetron on L/M ratios

Ovariectomized rats, hormonally primed with 10 μ g estradiol benzoate and 500 μ g progesterone, were injected ip with vehicle, 1.0 or 2.0 mg/kg tropisetron. Fifteen min later, rats were restrained or not restrained for 5 min. N's for vehicle, 1.0 and 2.0 mg/kg tropisetron, restrained and nonrestrained rats, respectively are 6, 5, 14, 15, 5, and 4. Sexual behavior was then tested for 15 consecutive minutes. Data are the mean $\pm$ S.E. L/M ratios for the pretest (PRE) and each of 5 test intervals after tropisetron. * Indicates significant difference from the respective pretest value (Tukey's test). ** Indicates significant difference between restrained and nonrestrained rats, within dose and time (Tukey's test).

Figure 6. Effects of ip tropisetron on lordosis quality

Data are the mean $\pm$ S.E. lordosis quality scores for the same rats shown in Figure 4. * Indicates significant difference from the respective pretest value (Tukey's test).