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Effects of Neuropeptide Y (NPY) and NPY Agonists on Lordosis in the Female Guinea Pig

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We have previously shown that an NPY antagonist decreases lordosis behavior and that this decrease can be reversed with NPY administration. The present experiments examined whether intracerebroventricular (icv) administration of NPY would facilitate lordosis behavior and whether it would increase feeding behavior in the female guinea pig. Additionally, we examined whether icv administration of a more specific NPY Y1 and/or Y2 receptor agonist would facilitate lordosis behavior. Although NPY (1 μg) increased feeding behavior when it was administered to the lateral ventricle of ovariectomized (ovx) estrogen (i.e., estradiol benzoate; EB) and progesterone- (P) treated guinea pigs, it had no facilitatory effect on lordosis behavior at any of the doses tested (0.5, 1, 5, or 10 μg). In fact, the lower doses had a small, delayed inhibitory effect. NPY also had no effect on lordosis in females treated with EB alone. In contrast, the NPY Y1 agonist (Leu^{31}Pro^{34})NPY significantly facilitated lordosis in ovx EB- and P-treated females. It had no effect in ovx females treated with EB alone. The NPY Y2 agonist NPY(13–36) had a slight, delayed inhibitory effect in ovx EB- and P-treated females. These data are consistent with the hypothesis that NPY can act at a number of receptor subtypes to affect lordosis behavior, and that NPY can facilitate lordosis behavior by acting at Y1 receptors. Furthermore, it appears that this facilitatory effect of Y1 receptors is an effect on some progesterone-mediated component of lordosis, as the Y1 agonist facilitated EB- and P-induced lordosis, but not that induced with EB alone.

Key Words: Neuropeptide Y; lordosis; guinea pig; reproduction; feeding; estrogen; progesterone.

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Introduction

Ovarian hormones act at the hypothalamic/preoptic area of the brain of females of a number of species, including the rat and guinea pig, to induce a series of changes that result in the induction of sexual receptivity in response to mating stimuli (e.g., Young, 1961; Rubin and Barfield, 1983; Pfaff and Schwartz-Giblin, 1988). One of the neural changes that ovarian hormones produce is an increase in NPY in hypothalamic areas of the brain. NPY immunoreactive perikarya and fibers are present in high densities in several hypothalamic nuclei that are important in the control of lordosis behavior (Chronwell et al., 1985; deQuidt and Emson, 1986) and NPY receptors are also found in the hypothalamus (e.g., Dumont et al., 1992). Ovariectomy decreases NPY levels in the hypothalamus and estrogen replacement restores both preproNPY mRNA levels, and NPY peptide levels in the mediobasal hypothalamus (Crowley et al., 1987; Kalra and Crowley, 1992; Sahu et al., 1994). Progesterone injection to estrogen-primed ovx female rats further increases NPY levels (Crowley et al., 1985).

Recently, we have shown that NPY plays a facilitatory role in the control of lordosis behavior in the female guinea pig. That is, icv administration of the NPY receptor antagonist PYX2 decreases lordosis behavior in ovariectomized estrogen- and progesterone-treated female guinea pigs, and this decrease can be reversed with icv administration of neuropeptide Y (Thornton and Carson, 1995). In the present studies, we examined further how NPY might act to facilitate lordosis behavior in the female guinea pig. Because an NPY antagonist decreased lordosis behavior (Thornton and Carson, 1995), we examined whether NPY would facilitate lordosis behavior. First we examined whether NPY would facilitate lordosis in females that were already showing high levels of lordosis responding, i.e., in ovx females treated with estrogen followed by progesterone. As NPY is known to increase feeding behavior in a number of species (e.g., *see* Stanley, 1993), but its effects on feeding behavior have not been examined in guinea pigs, we also documented the effects of NPY on feeding in the female guinea pig. If NPY did affect feeding in the guinea pig, this would con-

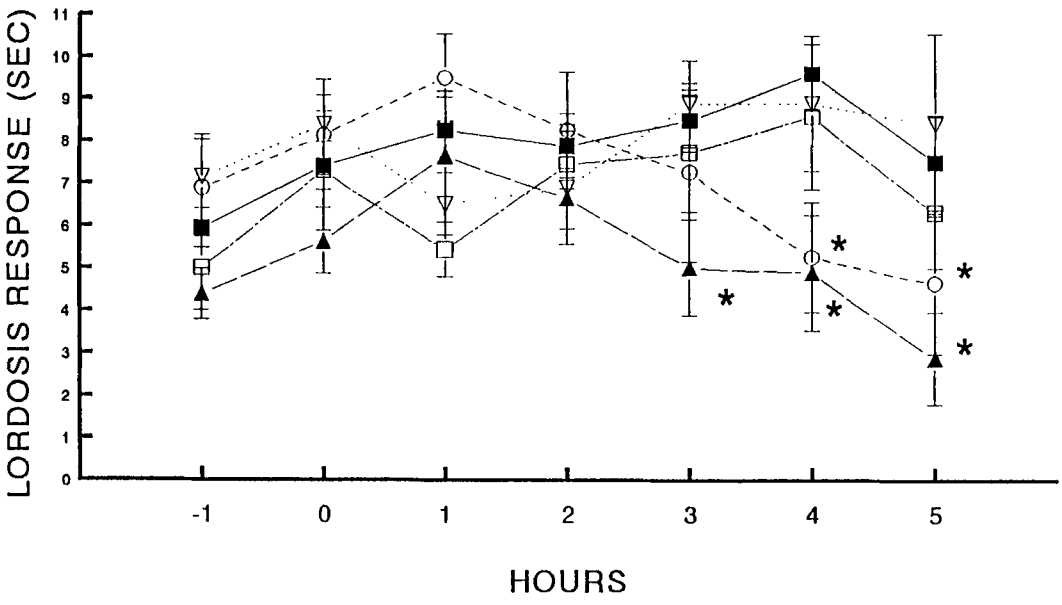

Fig. 1. NPY did not facilitate lordosis in ovx EB- and P-treated female guinea pigs. There was no significant facilitative effect of any of the doses at any of the hours tested. However, the two lower doses of NPY decreased the lordosis response during the later hours of testing (*$p < 0.05$ relative to Control group at same hour). All drugs were infused at hour 0. ■, Control; ○, 0.5 μg; ▲, 1 μg; ▽, 5 μg; □, 10 μg.

firm that we were using biologically active NPY. Secondly, because NPY did not facilitate lordosis behavior in ovx estrogen- and progesterone-treated females, we examined whether NPY would facilitate lordosis behavior in ovx guinea pigs that were showing low levels of lordosis responding (i.e., females that were treated with estrogen alone). Thirdly, we examined which NPY receptor subtype might be important in the control of lordosis behavior. NPY and the NPY antagonist PYX2 can act at a number of receptor subtypes including Y1 and Y2 receptors (Wahlestedt et al., 1986, 1987; Sheikh et al., 1989; Tatemoto et al., 1992). In the present studies, we used the selective Y1 agonist ($Leu^{31}Pro^{34}$)NPY, and the selective Y2 receptor agonist NPY(13–36) (Fuhlendorff et al., 1990; Grundemar et al., 1993) to determine whether an NPY Y1 and/or Y2 agonist would facilitate lordosis in ovx estrogen- and progesterone-treated guinea pigs. Lastly, because the NPY Y1 receptor agonist facilitated lordosis behavior in ovx estrogen- and progesterone-treated females, we examined whether it would also facilitate lordosis in ovx females treated with estrogen alone.

Experiment 1: EB and P + NPY

Because an NPY antagonist significantly decreased lordosis behavior (Thornton and Carson, 1995), we examined whether NPY would facilitate lordosis behavior. In the normal ovarian cycle of the female guinea pig, estradiol levels increase during the late follicular phase, followed by a preovulatory surge of progesterone (Morali and Beyer, 1979). Soon afterwards, females show lordosis behavior and ovulate. Consistent with this, after ovariectomy, female guinea pigs generally show optimal levels of lordosis behavior when treated sequentially with estradiol and progesterone (Dempsey et al., 1936; Collins et al, 1938). In the present experiment, females were ovx and treated with estrogen (i.e., estradiol benzoate), followed by progesterone to induce lordosis behavior.

Experiment 1 Results

When NPY was infused icv into ovx EB- and P-treated female guinea pigs, it had no clear facilitatory effect on lordosis behavior. There was no significant facilitation of the lordosis response (Fig. 1) at any time point, for any of the doses tested. In fact, at the lower doses of NPY (0.5 and 1 μg), the lordosis response was decreased relative to the Control group during the later hours of testing (Fig. 1). There was no effect on the maximum lordosis response (Control group = 10.9 ± 0.92 s, 0.5 μg NPY group = 10.88 ± 1.47 s, 1 μg NPY group = 8.75 ± 1.33 s, 5 μg NPY group = 11.5 ± 1.24 s, 10 μg NPY group = 10.4 ± 1.32 s, NS compared to Control group).

In contrast, NPY did increase the amount of time spent eating. When the proportion of animals that increased the amount of time eating during the hours subsequent to NPY or control treatment was compared, it was seen that seven of nine females who received 1 μg NPY increased the amount of time they spent eating during the hour after NPY infusion (relative to the hour before NPY infusion), whereas only four of 14 control females showed an increase at this same time period (Table 1. Pearson chi square test = 4.701, $p = 0.03$). The effect of NPY on feeding appeared to last for only an hour as there was no significant difference in the proportion of animals that increased their time eating during the second hour after

Table 1
Effect of ICV Infusion of 1 μg NPY on Amount of Time Spent Eating by OVX EB- and P-Treated Female Guinea Pigs

	Seconds spent eating (Mean ± SEM)		Percent (proportion) of animals that increased time eating+	
	NPY	Control	NPY	Control
Hour before++	27.8 ± 12.6	81.4 ± 35.5		
Hour 1 after++	201.2 ± 117.6*	37.4 + 12.2	78% (7/9)**	29% (4/14)
Hour 2 after++	17.2 ± 13.0	21.4 ± 9.0	22% (2/9)	21% (3/4)

+ Percent (and proportion) of animals that increased the number of seconds they spent eating, relative to the hour before NPY or control treatment.

++Time relative to NPY or control treatment.

* $p < 0.05$ relative to hour before for same group.

**$p < 0.05$ relative to Control group at same time.

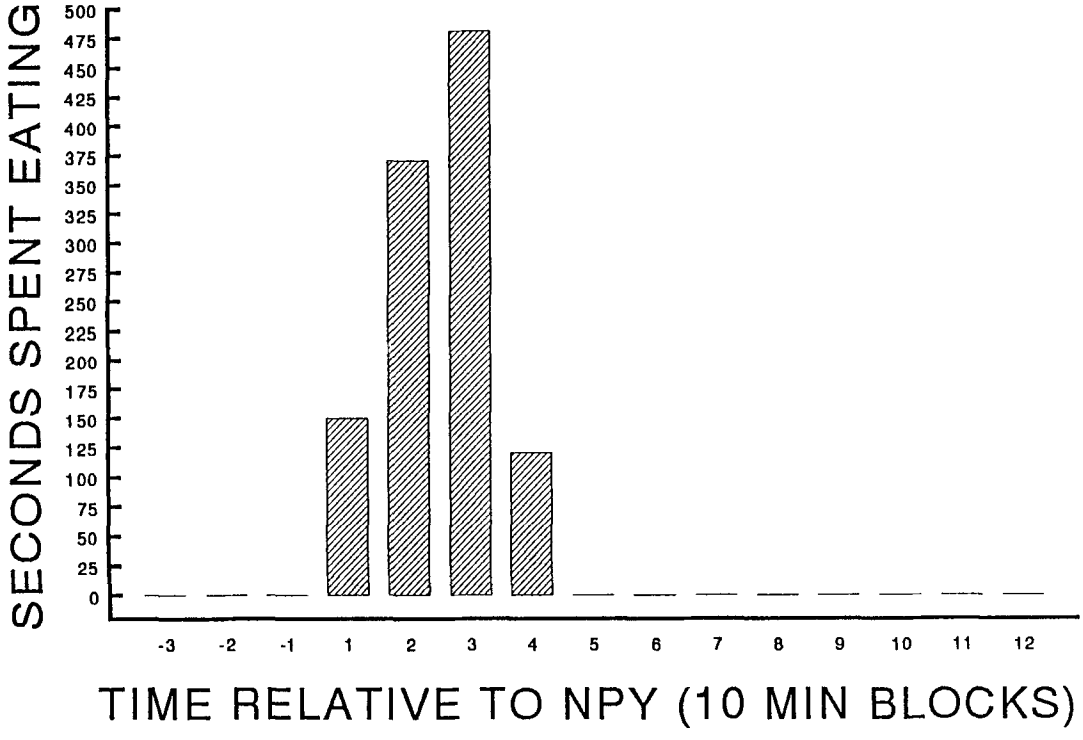

Fig. 2. An example of an animal in which NPY increased the amount of time spent eating.

NPY or control treatment relative to the hour before NPY or control treatment (NPYgroup = two of nine, vs Control group = three of 14, Table 1.). When the mean number of seconds spent eating was examined, it was apparent that the response to NPY was variable, with some experimental animals showing a large increase in time spent feeding, whereas others showed little effect. Overall, the NPY-treated females showed a significant increase in the number of seconds spent eating during the hour after NPY infusion, as opposed to the hour before NPY (hour before = 27.8 ± 12.6 s vs hour 1 after = 210.2 ± 117.6 s, $p < 0.05$, Wilcoxin Test). In contrast, the Control group did not vary significantly across the 2 h (Control group hour before = 81.4 ± 35.5 s vs hour 1 after = 37.4 ± 12.2 s, NS, Wilcoxin Test). Figure 2 shows the feeding behavior across 10-min blocks of an animal that showed a clear response to NPY infusion. Generally, the increase in feeding began in the first or second 10-min block after NPY infusion and lasted for at least 30–40 min.

Experiment 2: EB + NPY

When lordosis is induced in ovx females by estrogen followed by progesterone, as in Experiment 1, relatively high levels of lordosis behavior are produced. To ensure

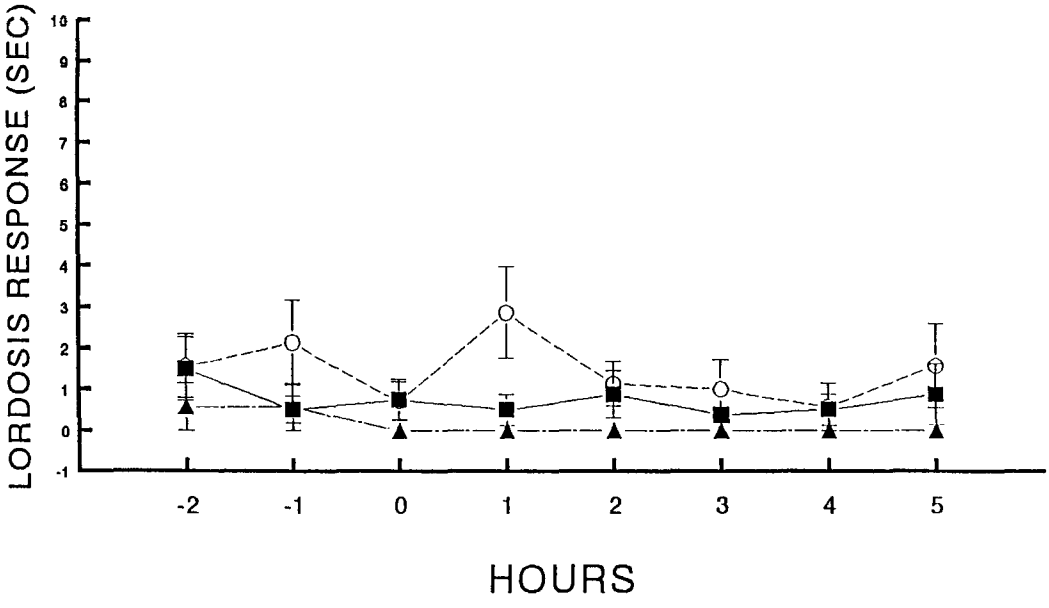

Fig. 3. NPY did not facilitate lordosis in ovx EB-treated female guinea pigs. There was no significant effect of either NPY dose at any of the time points tested (NS compared to Control group at same hour). All drugs were infused at hour 0. ■, Control; ○, 5 μg NPY; ▲, 10 μg NPY.

that the lack of facilitation of lordosis with NPY was not because of a ceiling effect, (i.e., that NPY had no effect because lordosis behavior was already maximal), we used estrogen alone to induce low levels of lordosis responding. We then examined whether NPY would increase these low levels of lordosis responding.

Experiment 2 Results

As shown in Fig. 3, there was no significant effect of either the 5 μg or the 10 μg dose of NPY on the lordosis response at any of the hours tested for ovx females that were treated with EB alone. There also was no significant difference in the maximum lordosis shown after NPY as compared to Controls (Control group = 1.29 ± 0.89 s, 5 μg NPY group = 2.86 ± 1.10 s, 10 μg NPY group = 0.0 ± 0.0 s, NS compared to Control group). There was also no effect on the proportion of animals that showed lordosis (Control group = three of eight, 5 μg NPY group = four of seven, 10 μg NPY group = two of seven).

Experiment 3: EB and P + NPY Y1 or Y2 Agonists

The fact that treatment with NPY does not facilitate lordosis in estrogen or estrogen- and progesterone-primed females suggests that either increasing NPY above endogenous levels has no further facilitatory effect on lordosis behavior over that seen with normal levels of NPY, or that perhaps NPY has opposing actions at two different sites or receptor subtypes. NPY may act at a number of different receptor subtypes, including Y1 and Y2 receptor subtypes (Wahlestedt et al., 1986, 1987; Sheikh et al., 1989). Perhaps NPY acts only at a particular receptor subtype to facilitate lordosis. If so, then perhaps a more specific NPY receptor agonist would increase lordosis without simultaneously activating an inhibitory pathway. To examine this possibility, ovx females were brought into sexual receptivity with estrogen and progesterone priming and were then infused with either an NPY Y1 receptor agonist or an NPY Y2 receptor agonist.

Experiment 3 Results

The NPY Y1 receptor agonist ($Leu^{31}Pro^{34}$)NPY significantly increased the lordosis behavior shown by ovx estrogen- and progesterone-treated female guinea pigs. Although the 0.5 μg dose of the Y1 agonist had no clear effect, the 5 μg dose significantly increased the lordosis response 2 h after treatment, relative to the Control group at the same time point (Fig. 4). When the maximum lordosis response was examined, there was a small increase with the 0.5 dose and a statistically significant increase with the 5 μg dose of the NPY Y1 agonist (Fig. 5).

The NPY Y2 agonist did not facilitate the lordosis behavior of ovx EB- and P-treated females. Neither the 0.5 nor the 5 μg dose increased the lordosis response at any point across the hours of testing (Fig. 6) or the maximum lordosis response (Control group = 10.0 ± 1.45 s, 0.5 μg Y2 group = 11.36 ± 1.36 s, 5 μg Y2 group = 9.9 ± 0.75 s, NS). However, there was a small, but significant decrease in lordosis behavior at hour 5 of testing (relative to control females at the same hour), with the 0.5 μg dose of the NPY Y2 agonist (Fig. 6; Control group = 6.88 ± 1.06 s vs 0.5 μg Y2 group = 4.23 ± 1.17 s, $p < 0.05$).

Experiment 4: EB + Y1 Agonist

Experiment 3 indicated that NPY can act at Y1 receptors to increase lordosis responding. As females were ovx and given estrogen followed by progesterone to induce sexual

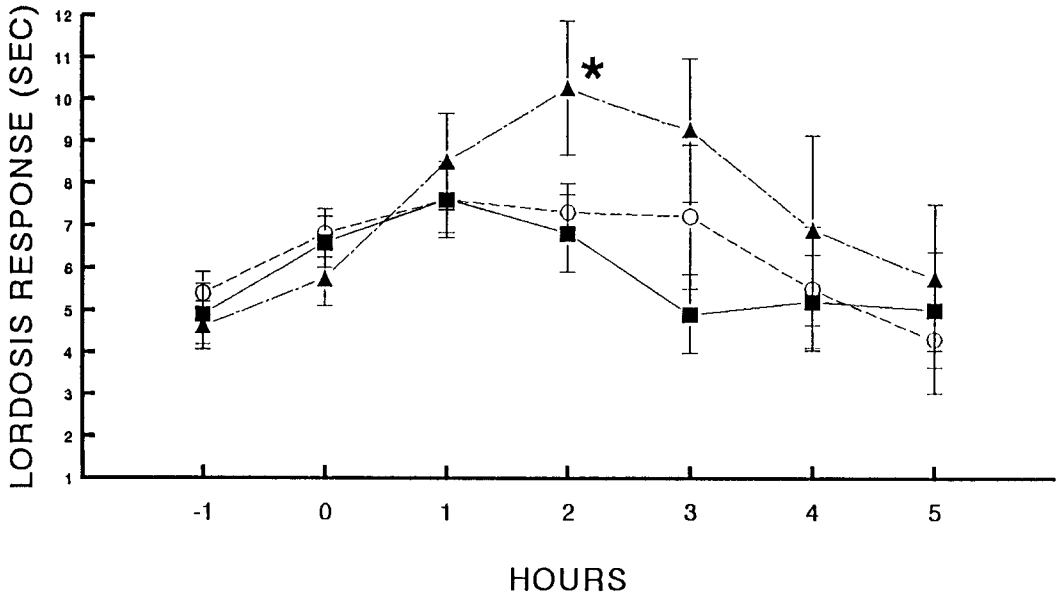

Fig. 4. The NPY Y1 receptor agonist $Leu^{31}Pro^{34}$NPY significantly increased the lordosis response of ovx EB- and P-treated female guinea pigs. Although there was no effect of the 0.5 μg dose, the 5 μg dose of $(Leu^{31}Pro^{34})$NPY increased the lordosis response, which reached statistical significance at hour 2 after infusion (*$p < 0.05$ compared to Control group at same hour). All drugs were infused at hour 0. ■, Control; ○, 0.5 μg Y1; ▲, 5 μg Y1.

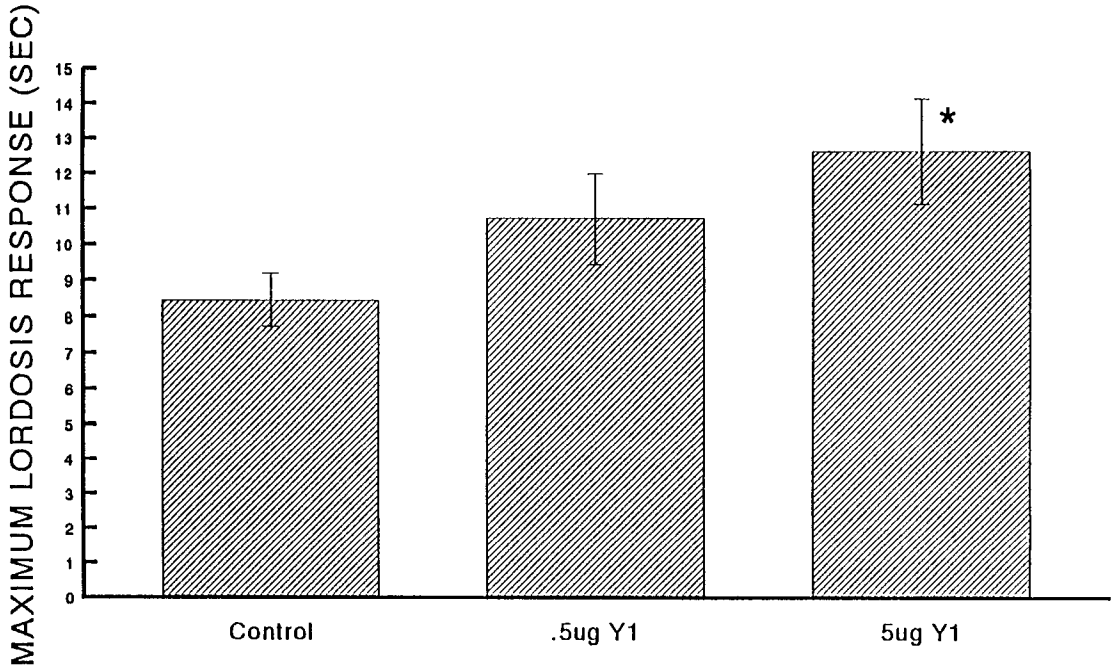

Fig. 5. The NPY Y1 receptor agonist $(Leu^{31}Pro^{34})$NPY significantly increased the maximum lordosis response of ovx EB- and P-treated female guinea pigs (*$p < .05$ compared to Control group).

receptivity, it is unclear whether the Y1 agonist affected some estrogen- and/or some progesterone-mediated component of the lordosis response. In the present experiment, females were brought into sexual receptivity using estrogen alone so that we could examine whether an NPY Y1 agonist would still facilitate lordosis behavior that was not dependent upon progesterone.

Experiment 4 Results

As expected, EB induced a very low level of responding. The Y1 agonist did not facilitate the lordosis behavior shown by females who were given EB alone. The Y1 agonist did not significantly increase the percent of females that showed lordosis. Overall, 37% (10 of 27) of the control females showed EB heats and 45% (5 of 11) of the NPY

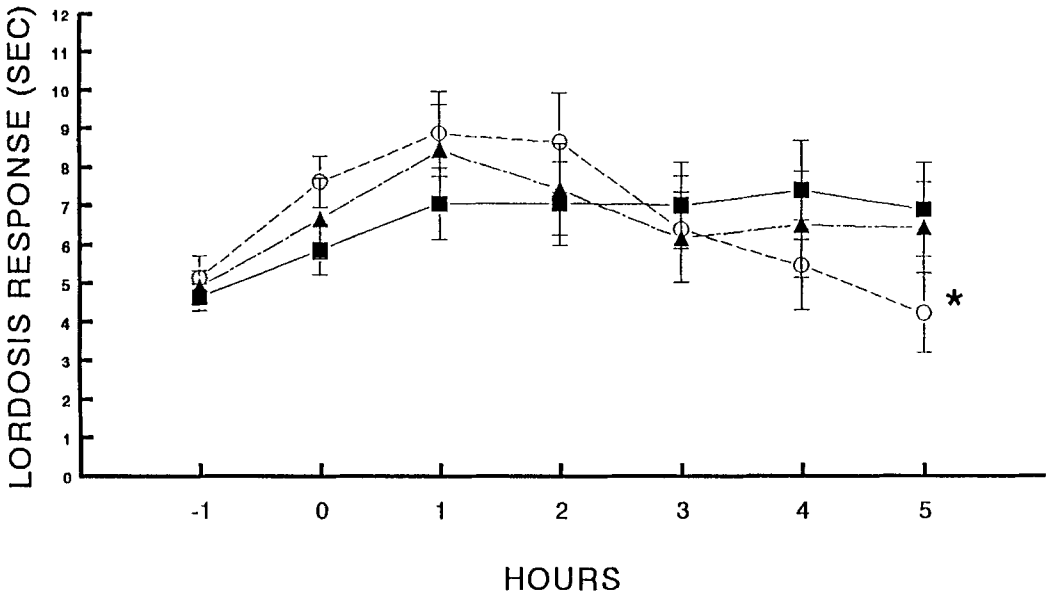

Fig. 6. The NPY Y2 receptor agonist, NPY(13-36), did not increase lordosis behavior in ovx EB- and P-treated female guinea pigs. The lower dose of the Y2 agonist decreased the lordosis response on hour 5 of testing (*$p < 0.05$ compared to Control group at same hour). All drugs were infused at hour 0. ■, Control; ○, 0.5 μg Y2; ▲, 5 μgY2.

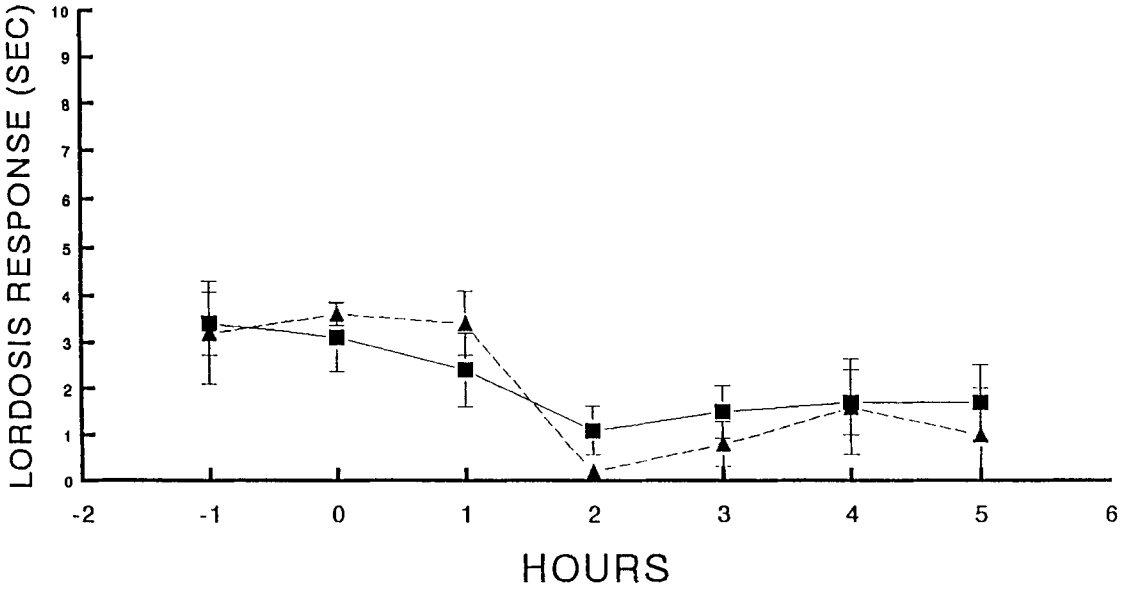

Fig. 7. The NPY Y1 agonist ($Leu^{31}Pro^{34}$)NPY did not significantly increase the lordosis response of females that showed lordosis to EB alone. Drugs were infused at hour 0. ■, Control; ▲, 5 μgY1.

Y1 agonist-treated females showed lordosis to EB alone ($p > 0.05$, NS). Even when only those females that showed lordosis were examined (i.e., responders only), there was still no effect of the NPY Y1 agonist at any of the hours of testing (Fig. 7). There was also no effect of the agonist on the maximum lordosis response shown (Control group = 4.6 ± .52 s, $N = 10$ vs 5 μg Y1 agonist group = 4.4 ± .40 s, $N = 5$, NS).

Discussion

In the present studies, NPY did not significantly facilitate lordosis behavior in ovx female guinea pigs treated with either estrogen and progesterone, or with estrogen alone. However, a more selective NPY Y1 receptor agonist did significantly increase lordosis behavior, specifically in ovx females treated with estrogen and progesterone rather than with estrogen alone. In contrast, a Y2 receptor agonist had no facilitatory effect, and even had a slight inhibitory effect on lordosis behavior in ovx estrogen- and progesterone-treated females.

The facilitatory effect of the NPY Y1 agonist (Leu^{31}-Pro^{34})NPY is consistent with previous research showing that the NPY antagonist PYX2 (Tatemoto et al., 1992) can

decrease lordosis, and that this effect can be reversed with NPY (Thornton and Carson, 1995). Coupled with the finding that the Y2 agonist did not facilitate lordosis, this suggests that the inhibitory effect of PYX2 on lordosis might be via an inhibition of Y1 receptors. A third receptor subtype, currently referred to as Y3 receptors has been proposed (Grundemar et al., 1991; Rimland et al., 1991; Wahlestedt et al., 1991). It is unknown if this receptor subtype plays a role in the control of lordosis, however, it is considered unlikely as the human homolog of this proposed receptor has been cloned, and it does not bind NPY specifically (Herzog et al., 1993).

For reproduction to occur, a female needs to both show sexual behavior, and ovulate. This facilitatory effect of an NPY agonist on lordosis is consistent with what is known about the facilitatory effects of NPY on the release of luteinizing hormone releasing hormone (LHRH), the release of luteinizing hormone (LH), and the control of ovulation. For example, NPY levels increase during the preovulatory period and NPY administration increases LHRH/LH release in ovx EB- and P-treated female rats, and during the preovulatory period (Kalra and Crowley, 1984; McDonald et al., 1985; Sabatino et al., 1990; Bauer-Dantoin et al., 1992). Experiments that immunoneutralized NPY indicate that NPY is necessary for the normal production of an LH surge (Wehrenberg et al., 1989). Moreover, it appears that the facilitatory actions of NPY on LHRH are mediated by NPY Y1 receptors (Kalra et al., 1992; Besecke et al., 1994).

The lack of facilitatory effect with icv NPY was surprising. It is clear that the NPY used was biologically active as it did cause a significant facilitation of feeding behavior. It is also clear that the lack of facilitory effect on NPY was not because lordosis levels were maximal and could not be increased; even when females were induced to show only a low level of lordosis responding, NPY still had no clear facilitory action on lordosis. Recent data (Bauer and Thornton, 1995) indicate that when NPY is infused into the ventromedial hypothalamus (VMH), it will facilitate lordosis behavior. It is unclear why NPY infused into the ventricle did not facilitate lordosis. It is always possible that although the NPY got to the area involved in the control of feeding (the paraventricular nuclei, PVN, perhaps; Stanley and Leibowitz, 1985; Bonavera et al., 1994), it was degraded and/or diluted before it diffused to the area involved in the control of lordosis (the lateral VMH perhaps; Rubin and Barfield, 1983; Pfaff and Schwartz-Giblin, 1988). Alternatively, perhaps NPY acts at two different places in the brain with opposing actions and/or it has opposing actions at two different receptor subtypes. We are currently exploring the possibility that Y1 and/or Y2 receptor stimulation in the POA may inhibit lordosis behavior.

In contrast to the facilitatory effect seen with the Y1 agonist, the NPY Y2 agonist did not facilitate lordosis, and the lowest dose even produced a slight, delayed inhibitory effect. A similar delayed inhibitory effect was also seen with the lowest doses of NPY administered. This inhibitory effect of NPY is partially consistent with the small amount of data available on the effects of NPY on lordosis behavior in the female rat. That is, Clark et al. (1985), found that NPY decreased lordosis in the ovx female rat treated with estrogen and progesterone. However, in that study, NPY appeared to have an immediate inhibitory effect, whereas in the present experiments, any inhibitory effect of NPY was only seen during the later stages of heat. Further research needs to be done to clarify the actions of endogenous NPY on lordosis in the rat, and whether they differ from those in the guinea pig. Although the work by Clark et al. (1985) is suggestive, it cannot alone convincingly demonstrate that endogenous NPY normally plays an inhibitory role in the control of lordosis in the rat as the animals already had endogenous levels of NPY so the administered NPY was in excess of physiological levels. Further experiments, for example, using an NPY antagonist to decrease endogenous levels, need to be done in the rat.

NPY significantly increased the feeding behavior of ovx estrogen- and progesterone-treated female guinea pigs. This is consistent with the effects of NPY in other species. NPY increases feeding behavior in a variety of species including sheep, chickens, rats, hamsters, mice, and snakes (e.g., *see* Stanley, 1993). It has been known for a long time that ovariectomy decreases, and estrogen treatment restores appetite. Recently, it has been suggested that estrogen may affect appetite by decreasing NPY levels and release selectively from the PVN (Bonavera et al., 1994).

Interestingly, in the present studies, the NPY Y1 agonist facilitated lordosis that was induced with estrogen and progesterone, but did not facilitate lordosis which was induced with estrogen alone. As estrogen induced lordosis is independent of progestin receptor stimulation in the female guinea pig (Brown and Blaustein, 1984), this suggests that the Y1 agonist may act on some mechanism that is dependent upon progesterone action. Work with the effects of NPY on LHRH suggest a parallel. That is, if progestin receptors are blocked with a receptor antagonist (i.e., RU 486), NPY will no longer potentiate LHRH-stimulated LH release (Bauer-Dantoin et al., 1993).

Materials and Methods

General

Adult female Hartley guinea pigs (Hilltop Lab Animals, Inc., Scottdale, PA) were allowed to adapt for at least 1 wk before surgery, and were housed with food and water freely available. For surgery, animals were anesthetized with sodium pentobarbital and were then ovariectomized and implanted stereotaxically with a cannula directed at the lateral ventricle. The 20 g guide cannula was implanted using coordinates from the atlas of Luparello (1967) and preliminary studies; anterior/posterior coordinate = +9.6 from

intraural zero, medial/lateral coordinate = 2.5 mm from midline, dorsal/ventral coordinate = 3 mm from dura, incisor bar = −10 mm. At the time of surgery, correct placement of the cannula into the lateral ventricle was determined by infusing a small amount of saline using gravity. Cannulae were attached with dental acrylic, and wires cut 1 mm longer than the cannulae were inserted into them to keep them patent. Animals were allowed at least 1 wk to recover.

All hormones were dissolved in sesame oil and injected subcutaneously (sc) in volumes of 0.1 mL. Neuropeptide Y (Sigma Chemical Co., St. Louis, MO), the NPY Y1 receptor agonist ($leu^{31}pro^{34}$)NPY (Sigma Chemical Co.) and the NPY Y2 receptor agonist NPY(1336) (Sigma Chemical Co.) were dissolved in sterile physiological (0.9%) saline, which also served as the vehicle. All NPY drugs and vehicle were infused icv in 5 μL volumes. They were infused over the course of 60 s and the infuser was then left in the cannula for an additional 60 s to allow the drug to diffuse away from the cannula tip.

Lordosis was tested using the manual stimulation technique (Young et al., 1937; Goy and Young, 1957; Thornton et al., 1987, 1989). This technique consists of stimulating the animals by moving one's hand along the animal's hindquarters and moving rostrally partway along the animal's back. Females that are behaviorally receptive will readily adopt the lordosis posture, whereas unresponsive females will squat or run and vigorously resist stimulation attempts. The time period over which lordosis can be elicited in this manner corresponds to the period of time that the female will be receptive to a male (Young et al., 1935; Goldfoot and Goy, 1970). At each lordosis check, the number of seconds the lordosis posture was held was measured. From data collected in this manner, the following behavioral measures were derived. The lordosis response is the time in seconds that animals exhibited a lordosis response across the hours of testing. All animals were used for each time point. The maximum lordosis response is the longest response shown by an animal at any time during the course of testing subsequent to drug or comparable control treatment. The proportion and percent of animals that responded refers to the proportion/percent of animals that showed a lordosis response at any time during the course of testing. The lordosis response using responders only excluded those animals that did not show a lordosis response over the course of testing. Values are given as mean ± SEM.

Experiment 1: EBP+NPY

Adult ovx, cannulated females were injected with 20 μg of EB followed 40 h later by 0.5 mg P. For the lordosis testing, females were checked for lordosis just before P and hourly thereafter. If they showed a lordosis response for 2 consecutive h, experimental animals were infused icv with either 0.5 ($N = 8$), 1.0 ($N = 9$), 5.0 ($N = 8$), or 10.0 ($N = 7$) μg NPY in 5 μL. Control animals were infused with either 5 μL of the sterile saline vehicle, or they were not infused. As these two control groups did not differ, they were combined into a single control group ($N = 15$). All females were then checked hourly for lordosis for 5 h. For the feeding tests, Control females ($N = 14$) and females that received 1 μg NPY icv ($N = 9$) were watched continuously, and the number of seconds they spent eating was recorded for the hour prior to NPY or control treatment and for 2 h afterwards.

Experiment 2: EB+NPY

Adult ovx, cannulated females were injected with 20 μg EB. Forty hours later, females were checked for lordosis hourly for 3 h. They were then infused with either 5 μg/5 μL NPY ($N = 7$), 10 μg/5 μL NPY ($N = 7$), or were used as controls. Controls were either infused with 5 μL of the saline vehicle or were not infused ($N = 8$). All females were then tested for lordosis hourly for 5 h.

Experiment 3: EBP+ Y1 or Y2 Agonist

Adult ovx, cannulated females were injected with 20 μg EB followed 40 h later by 0.5 mg P. Just before P and hourly afterwards, they were tested for lordosis. If they showed a lordosis response for 2 consecutive h after P, they were infused icv with either the NPY Y1 agonist ($leu^{31}pro^{34}$)NPY (0.5 μg Y1, $N = 10$ or 5 μg Y1, $N = 8$), the NPY Y2 agonist NPY(13–36) (0.5 μg Y2, $N = 13$ or 5 μg Y2, $N = 12$), or were used as controls. As the Y1 and Y2 agonists were run at separate times, they each had their own concurrent control group (Y1 agonist Control group $N = 9$, Y2 agonist control group $N = 16$). Control females were either infused with 5 μL of vehicle, or were not infused. All females were then checked hourly for 5 h.

Experiment 4: EB + Y1 Agonist

Adult ovx, cannulated females were injected with 20 μg EB. Forty hours later they were tested for lordosis for 2 h and then injected icv with either the NPY Y1 agonist ($leu^{31}pro^{34}$)NPY in saline (5 μg Y1; $N = 11$), or were used as controls. Control females were either infused with 5 μL of the saline vehicle, or were not infused. All females were then tested for lordosis hourly for 5 h.

Statistical Analysis

The lordosis response data were analyzed with a two-way (treatment by time) analysis of variance (ANOVA) with repeated measures on time. Planned comparisons were then run using a *t*-Test for Differences Among Several Means (Bruning and Kintz, 1968). The planned comparisons consisted of comparisons between the different treatments within each hour. The maximum lordosis response data were analyzed with two-tailed *t*-Tests for independent groups. For the feeding data, the proportion of animals that increased time eating was analyzed with Pearson Chi square. For the mean number of seconds spent eating data, the nonparametric Wilcoxin Signed-Ranks test for related samples was used. Any comparisons that did not reach a p value of at least 0.05 were considered statistically nonsignificant (NS).

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