*Pharmacology Biochemistry & Behavior* Vol 38, pp 273–279 © Pergamon Press plc 1991 Printed in the U S A 0091-3057/91 $3 00 + 00

# Effects of the Oxytocin Fragment Prolyl-Leucyl-Glycinamide on Sexual Behavior in the Rat

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GORZALKA, B B , K A LUCK AND S A TANCO *Effects of the oxytocin fragment prolyl-leucyl-glycinamide on sexual behavior in the rat* PHARMACOL BIOCHEM BEHAV **38**(2) 273–279, 1991 —Prolyl-leucyl-glycinamide (PLG), a natural brain peptide, is identical in structure to the C-terminal of oxytocin Moreover, PLG and oxytocin can act as opiate antagonists Evidence that opiates and oxytocin have significant influences on reproductive behavior suggests that PLG may also be effective Morphine and/or PLG were administered intraperitoneally to male and female rats and sexual behavior was observed PLG (0 1–10 mg/kg) was found to facilitate female sexual behavior in Experiment 1 In Experiment 2, the ability of PLG to facilitate female receptivity was found to be progesterone dependent In Experiment 3, tyrosine-prolyl-leucyl-glycinamide, a putative precursor to PLG, failed to facilitate lordosis In Experiment 4, PLG failed to facilitate male sexual behavior In Experiments 5 and 6, PLG did not affect morphine-induced inhibition of either male or female sexual behavior These data suggest that PLG differentially affects female receptivity and male sexual behavior The current results support the hypothesis that PLG is an active metabolite of oxytocin in the female, but do not provide evidence that PLG functions as an opiate antagonist of sexual behavior

Prolyl-leucyl-glycinamide Oxytocin Sexual behavior Antiopiate Morphine Tyrosine-prolyl-leucyl-glycinamide

PROLYL-LEUCYL-GLYCINAMIDE (PLG), a neuropeptide isolated in hypothalamic tissue of humans, rats and other species (21), is identical in structure to the C-terminal tripeptide of oxytocin. PLG has many diverse behavioral effects. It is known to suppress fluid consumption (29), inhibit aggression (23), antagonize morphine-induced analgesia (11) and attenuate the development of tolerance to ethanol (37). However, the full extent of PLG's biochemical and behavioral effects, as well as its mode of action, has yet to be determined.

Like PLG, the nonapeptide oxytocin also has diverse effects. When released via the neurohypophysis into the peripheral vasculature, oxytocin functions as a hormone (4). In the peripheral system, oxytocin stimulates uterine contractions and breast milk ejection in the female during labour and parturition At central sites, however, oxytocin acts as a neuropeptide (4), influencing reproductive behavior The effects of oxytocin on sexual behavior depend upon the sex of the animal and, at least in the male, are a function of whether the peptide is acting centrally or peripherally. In male rats, peripheral administration of oxytocin decreases the number of intromissions preceding ejaculation, while central administration increases mount and intromission latencies and lengthens the postejaculatory refractory period (36) In female rats, both central (1, 6, 16, 35) and peripheral (1) administration of oxytocin is reported to facilitate sexual receptivity.

Oxytocin may be the precursor of active fragments in the central nervous system (4). Segments generated by proteolysis of oxytocin have been identified in the brain, as have the cleaving-peptidases (4) As well, relatively high oxytocin-related peptidase activity has been identified in the hypothalamus (18).

In addition to their structural similarity, PLG and oxytocin also have a similar behavioral effect both inhibit analgesic tolerance to morphine (2,24) Such structural and behavioral similarities suggest that PLG may be the active metabolite of oxytocin Some authors have claimed evidence that PLG can be generated from oxytocin by aminopeptidase activity (39) Specifically, if incubated with a hypothalamic particulate fraction, oxytocin will undergo stepwise proteolysis from the N-terminal onward, resulting in the formation of PLG (39) Others, however, have been unable to confirm these results and have noted the existence of PLG in nonoxytocinergic neurons (5). Hence, although it is not yet firmly established that PLG is the bioactive metabolite of oxytocin, PLG is clearly an endogenous brain peptide.

The results of radioisotope studies suggest a selective uptake of PLG Following $^{3}$H-PLG administration, the pituitary, pineal and hypothalamus concentrate radioactivity (33). Furthermore, in rat brain tissue, binding to striatum, hypothalamus and cerebral cortex is saturable and reversible, suggesting specificity (9). It is interesting to note that the hypothalamus, a primary area involved in regulation of sexual behavior, is high in oxytocin-related peptidase activity and accumulates PLG.

Considerable research suggests that PLG also has an effect on endogenous opiate receptors (10, 14, 15). Peripherally administered PLG may function either as an opiate agonist or antagonist depending on the dosage and the behavior being observed (10) Mu opiate receptor agonists and antagonists affect sexual behavior in humans, rats and other species (30) Exogenous opiates, such as morphine, consistently inhibit sexual behavior, while exogenous antiopiates, such as naloxone, facilitate sexual behavior

in particular situations (30). Specifically, stimulation of mu opiate receptors inhibits mounts, intromissions and ejaculations in male rodents and lordosis behavior in female rodents (30). Such findings support the possibility that an endogenous opiate system also may inhibit sexual behavior Furthermore, the existence of an endogenous antiopiate system has been hypothesized (15) It is possible that PLG may function as part of such an endogenous antiopiate system, and thus act to facilitate sexual behavior

The following experiments were designed to establish the effects of PLG on sexual behavior in male and female rats. As well, these studies were undertaken to determine whether PLG is the active metabolite of oxytocin and functions as an opiate antagonist.

## GENERAL METHOD

### *Animals and Surgery*

Male and female Sprague-Dawley rats were obtained from the Animal Care Centre at the University of British Columbia from stock originally bred at Charles River Laboratories, Quebec, Canada The experimental females were 90 days old and were sexually naive prior to the first experiment. Two weeks prior to initial testing, the females were ovariectomized bilaterally while under sodium pentobarbital (Somnotol) anaesthetic (65 mg per ml, 0.1 ml per 100 g body weight). Tests of female sexual behavior employed male rats as studs. Studs were selected on the basis of their vigorous copulating performance in previous screening trials. Experimental male rats were selected from a larger group of sexually experienced animals. The selection criterion for males was at least one ejaculation during two 30-minute behavioral tests. Hormone-primed ovariectomized females were used as stimulus females for testing male sexual behavior

Animals were housed in groups of five or six in standard wire mesh cages. Colonies were maintained on a 12/12-h reversed lighting schedule, at 21 ± 1°C. Food and water were available ad lib.

### *Drug Procedures*

Estradiol benzoate (EB) and progesterone (P) (Steraloids) were dissolved in peanut oil in a 0 1 ml volume, administered subcutaneously 48 h and 4 h prior to testing, respectively PLG (Sigma), tyrosine-prolyl-leucyl-glycinamide (TPLG) (Sigma) and morphine sulphate (Ingram and Bell Medical) were dissolved in physiological (0 9%) saline As radioisotope studies have demonstrated that peptides such as PLG cross the blood-brain barrier (33), PLG and TPLG were administered intraperitoneally (IP)

### *Behavioral Testing*

In Experiments 1, 2, 3 and 6, behavioral testing involved presentation of an experimental female to a sexually vigorous male rat in a cylindrical glass testing chamber (29 cm in diameter, 45 cm in height) lined with San-i-cel bedding material If a male failed to mount, the female was placed in a different chamber containing another male. After 10 mounts with pelvic thrusting had occurred, the females were removed The lordosis quotient (LQ) was used as the index of female sexual receptivity; it is calculated as the percentage of mounts eliciting a lordosis response (a concave arching of the back by the female). Immediately prior to these testing sessions, the stud males were given brief access to a fully receptive stimulus female rat (primed with 10 μg EB and 500 μg P)

In Experiments 4 and 5, each experimental male rat was placed in a bilevel Plexiglas chamber (60 cm in length, 16 cm in width and 50 cm in height) (27) and permitted to habituate to the testing arena for five minutes Following the habituation period, a stimulus female (primed with 10 μg EB and 500 μg P) was placed in the testing chamber with each male. The stimulus females were rotated between the males at 10-minute intervals. Testing was terminated after 30 minutes or the first postejaculatory intromission, whichever occurred first

The behavioral parameters recorded and analyzed include mount latency (time from presentation of the first female to the first mount with pelvic thrusting); intromission latency (time from presentation to the first intromission), ejaculation latency (time from the first intromission to the first ejaculation), and postejaculatory interval (time from ejaculation to the next intromission).

All behavioral tests were separated by an interval of at least one week and were conducted during the dark phase of the cycle All behavioral scoring was conducted blind

## EXPERIMENT 1

PLG has a dose-dependent influence on the inhibition of aggressive behavior in mice (23) and the suppression of fluid consumption in rats (29). The present study was designed to establish whether PLG facilitates or inhibits the lordosis response in female rats exhibiting moderate levels of receptivity. Dosages of PLG ranging between 0.1 mg/kg and 10 mg/kg have been established as effective in other behavioral paradigms (20) Accordingly, similar doses were employed in the present study.

### *Method*

In order to establish a moderate baseline of lordosis activity, 24 sexually naive female rats were primed with varying doses of EP and P in a series of preliminary tests These tests established that administration of 10 μg EB and 150 μg P produced a moderate level of lordotic activity which served as a subsequent baseline for examining the effects of PLG

A counterbalanced, repeated measures design was employed Following the pretest, the animals were randomly assigned to one of four groups. Animals were primed with 10 μg EB and 150 μg P. Other investigators have suggested that a peak behavioral effect of PLG occurs one hour following IP injection (11). Therefore, 60 minutes prior to testing, the experimental animals received an IP injection of either 0.1, 1 or 10 mg/ml/kg PLG, while the control group received saline (1 ml/kg).

### *Results and Discussion*

As shown in Fig. 1, the mean LQ scores (± standard error) for the 0.1, 1 and 10 mg/ml/kg PLG-treated animals were higher than those of the control group An analysis of variance revealed that the main effect of PLG was significant, $F(3,69) = 5\ 92$, $p<0.01$. The Tukey method of pairwise comparisons revealed that the LQ scores of animals receiving the 0.1 mg/ml/kg dose ($p<0.001$) and the 10 mg/ml/kg dose ($p<0\ 01$) were significantly greater than those of the control group. Thus it appears that PLG facilitates lordosis in EB- and P-primed female rats

## EXPERIMENT 2

Gorzalka and Lester (16) demonstrated that some quantity of progesterone is necessary for centrally administered oxytocin to facilitate lordosis in estrogen-primed animals. This suggests that oxytocin does not act merely to mimic progesterone. If the facilitatory effect of PLG also were progesterone-dependent, this would be consistent with the hypothesis that PLG and oxytocin have a common mode of action (17). Conversely, if PLG were to facil-

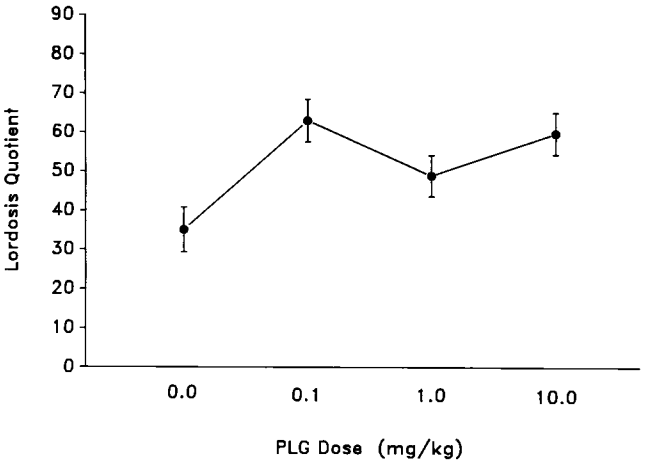


FIG 1 A dose-response curve illustrating the effect of 0, 0 1, 1 and 10 mg/kg prolyl-leucyl-glycinamide (PLG) administered IP to ovariectomized rats primed with 10 μg estradiol benzoate and 150 μg progesterone

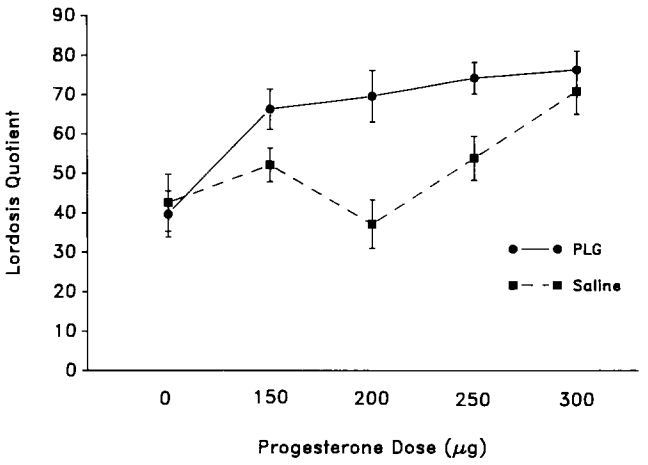


FIG 2 Effect of 10 mg/kg prolyl-leucyl-glycinamide administered IP to ovariectomized rats primed with 10 μg estradiol benzoate and 0, 150, 200, 250 or 300 μg progesterone

itate lordosis in animals receiving EB alone, this would suggest that PLG mimics progesterone. Therefore, in the present study we examined the possibility that PLG's facilitatory effect on sexual behavior is due to a mimicking of progesterone or, alternatively, that it is progesterone-dependent. The PLG dose was kept constant while the progesterone dose was varied systematically

*Method*

Subjects were 24 female rats primed with 10 μg EB and varying doses of P Progesterone was administered to the animals in the following doses and order 250 μg P, 200 μg P, 300 μg P, no P (1 ml/kg peanut oil) and 150 μg P. In addition, one hour prior to testing, animals received an IP injection of either 1 ml/kg saline or 10 mg/ml/kg PLG. The treatment condition of the animals (saline versus PLG) was then reversed so that all animals received PLG and saline at each dose of progesterone.

*Results and Discussion*

The results of Experiment 2 are presented in Fig 2. An analysis of variance revealed significant main effects of both PLG, $F(1,92)=24.47$, $p<0.001$, and progesterone, $F(4,92)=6.63$, $p<0.001$. Furthermore, there was also a significant interaction between progesterone and PLG, $F(4,92)=4\ 70$, $p<0.01$, suggesting that PLG facilitates lordosis behavior in a progesterone-dependent manner. The Newman-Keuls method of pairwise comparisons further revealed that PLG significantly increased the lordosis quotient in animals primed with 150, 200 or 250 μg P ($p<0.05$). However, PLG did not significantly facilitate lordosis in the absence of progesterone. It appears that some quantity of progesterone must be present for PLG to facilitate lordosis behavior in female rats The failure of PLG to significantly facilitate lordosis at 300 μg P is probably a function of ceiling effects obscuring a facilitatory effect

These data are consistent with the progesterone-dependent effects of oxytocin on lordosis behavior (16) The results give further support to the hypothesis that oxytocin and PLG facilitate sexual behavior by some common mode of action.

EXPERIMENT 3

The question of whether oxytocin is the natural precursor to PLG remains a matter of controversy Although it has been reported that oxytocin is converted by brain peptidases to PLG (39), others have failed to replicate this finding. For example, Burbach, de Kloet and de Wied (5) incubated oxytocin with synaptosomal membrane from rat brain and failed to produce PLG Such findings suggest that oxytocin may not be the natural precursor of PLG although both are endogenous to the brain

It is possible that a more recently discovered endogenous neuropeptide, TPLG, rather than oxytocin, may be the natural precursor to PLG. TPLG and PLG have many similar biochemical and behavioral effects; both peptides, administered peripherally, antagonize certain behavioral effects of opiates (15) and both compete for the mu opiate receptor (13,41) Furthermore, TPLG can be degraded to PLG by brain enzyme activity (26). Thus it is possible that TPLG is a natural precursor of PLG.

While PLG and TPLG are structurally related, each may act independently in the CNS Neither oxytocin nor PLG compete with TPLG for binding sites (42). Moreover, TPLG has been shown to serve functions different from those of PLG For example, TPLG increases benzodiazepine binding in vivo, whereas PLG is ineffective (28)

The present study was designed to establish whether TPLG has a facilitatory effect on lordosis behavior If TPLG facilitates sexual responsiveness, it may do so by serving as a precursor to the behaviorally potent peptide, PLG.

*Method*

A counterbalanced, repeated measures design was employed using 24 female rats. Control animals received saline (1 ml/kg IP) and the two experimental groups received TPLG (0.1 mg/ml/kg or 1.0 mg/ml/kg IP). Animals were primed with 10 μg EB and 150 μg P The study was then repeated using a higher P dose (200 μg).

*Results and Discussion*

An analysis of variance failed to reveal a significant effect of TPLG on lordosis at either P dose Lordosis quotients ranged from 61 to 69 The lack of significant differences suggests that peripherally administered TPLG does not serve as a precursor to PLG.

TABLE 1

THE EFFECTS OF PLG ON SEXUAL BEHAVIOR IN MALE RATS

| Behavioral Parameter | Saline | PLG* Dose (mg/kg) 0 1 | 1 0 | 10 |
|---|---|---|---|---|
| ML† | 64 ± 18 | 145 ± 62 | 79 ± 37 | 96 ± 42 |
| IL‡ | 108 ± 26 | 196 ± 75 | 117 ± 38 | 220 ± 61 |
| EL§ | 519 ± 95 | 510 ± 79 | 519 ± 127 | 563 ± 99 |
| PEI¶ | 328 ± 19 | 555 ± 151 | 343 ± 47 | 298 ± 24 |

Note Values are means ± S E M All scores are in seconds
*Prolyl-leucyl-glycinamide
†Mount latency
‡Intromission latency
§Ejaculation latency
¶Postejaculatory interval

TABLE 2a

THE EFFECTS OF PLG AND MORPHINE ON SEXUAL BEHAVIOR IN MALE RATS

| Behavioral Parameter | PLG*/Saline | PLG*/Morphine | Saline/ Saline | Saline/ Morphine |
|---|---|---|---|---|
| ML† | 44 ± 23 | 332 ± 118 | 30 ± 11 | 232 ± 83 |
| IL‡ | 160 ± 59 | 463 ± 143 | 152 ± 65 | 361 ± 109 |
| EL§ | 458 ± 160 | 413 ± 157 | 378 ± 94 | 220 ± 50 |
| PEI¶ | 374 ± 60 | 363 ± 50 | 319 ± 21 | 364 ± 70 |

Note Values are means ± S.E M All scores are in seconds
*Prolyl-leucyl-glycinamide
†Mount latency
‡Intromission latency
§Ejaculation latency
¶Postejaculatory interval

TPLG and PLG have dissimilar effects on lordosis behavior in female rats, yet a seemingly common antagonistic action on the mu opiate receptor (13,41). The possibility exists that PLG and TPLG act on different mu receptor subtypes. It has been suggested that a high-affinity mu receptor is involved in inhibition of lordosis, whereas a low-affinity receptor is involved in the stimulation of lordosis (30). While both PLG and TPLG are selective mu opiate receptor antagonists, it is conceivable that PLG inhibits the putative high-affinity mu receptor (thus facilitating lordosis), whereas TPLG, acting somewhat less selectively, antagonizes both the high- and low-affinity mu receptor subtypes. Consistent with this possibility is evidence that the mu receptor antagonist naloxone reverses both the inhibition and facilitation of female sexual behavior induced by morphiceptin, whereas naloxazone, a more selective mu receptor antagonist, reverses only the inhibition of female sexual behavior induced by morphiceptin (31).

EXPERIMENT 4

The results of Experiment 1 indicate that PLG facilitates sexual behavior in the female rat. The results of Experiment 2 indicate that this facilitatory effect of PLG, like that of oxytocin, is dependent upon progesterone Although peripheral administration of oxytocin facilitates sexual behavior in both males and females, the effect of peripherally administered PLG in males is not known. The present experiment was therefore designed to determine whether PLG facilitates sexual behavior in the male rat in a manner similar to that in the female.

*Method*

A four group counterbalanced repeated measures design was employed. Originally, twenty male rats were randomly assigned to treatment conditions. Due to illness, one rat was sacrificed following the first week of testing reducing the number of males to 19 Testing occurred once weekly for 4 weeks. Males received IP injections of saline, 0.1 mg/ml/kg PLG, 1.0 mg/ml/kg PLG, and 10 mg/ml/kg PLG 60 minutes prior to testing.

*Results and Discussion*

The results of Experiment 4 are presented in Table 1. PLG had no significant effect on sexual behavior Furthermore, no significant differences were found between the different doses of PLG These results contrast with those obtained in female rats.

EXPERIMENT 5

Although PLG did not facilitate sexual behavior in male rats when administered alone, the possibility remains that it may function as an opiate antagonist, perhaps by blocking the effect of an opiate agonist. Naloxone, a potent mu receptor antagonist, does not consistently facilitate sexual behavior when administered in the absence of opiates (30). However, when given in conjunction with morphine, naloxone attenuates morphine-induced inhibition (30). Various behavioral effects of PLG suggest that it also functions as a selective opiate antagonist. PLG blocks the analgesic effects of enkephalins or morphine in the tail-flick test in rats (20) and antagonizes hypothermia and hypomotility induced by beta-endorphin or morphine in rats (40) The evidence cited above suggests that PLG may act in a facilitatory manner by attenuating opiate-induced inhibition of sexual behavior.

The following experiment was designed to investigate the effects of PLG in the presence of morphine, in order to determine whether PLG functions as an opiate antagonist in the control of male sexual behavior.

*Method*

A four group counterbalanced repeated measures design was used Animals were tested weekly for four weeks. Twenty males were randomly assigned to four treatment groups. The animals received IP injections of saline alone, PLG and saline, morphine and saline, and PLG and morphine. PLG (1 mg/ml/kg) or saline (1 ml/kg) was administered 60 minutes in advance. Morphine (3 mg/ml/kg) or saline was administered 30 minutes prior to testing This dose of morphine was selected on the basis of preliminary work in our laboratory which revealed that 3 mg/ml/kg did not inhibit open field behavior nor produce any obvious effects on motor activity. By contrast, 6 mg/ml/kg morphine produced a general motor inhibition in preliminary studies

*Results and Discussion*

The results of Experiment 5 are reported in Tables 2a and 2b. The percentage of animals mounting, intromitting and ejaculating was reduced by morphine but was unaffected by PLG. Mount and intromission latencies appeared to be lengthened by morphine. A repeated measures analysis of variance indicated a significant effect of treatment for mount latency, $F(3,39) = 5\ 33$, $p<0.05$. Only data from animals showing a behavioral response were included in statistical analyses. Pairwise comparison using Tukey's method

TABLE 2b

PERCENTAGE OF MALES EXHIBITING VARIOUS SEXUAL BEHAVIORS

| Behavioral Parameter | PLG*/ Saline | PLG*/ Morphine | Saline/ Saline | Saline/ Morphine |
|---|---|---|---|---|
| Mounts | 95% | 75% | 100% | 75% |
| Intromissions | 95% | 65% | 95% | 65% |
| Ejaculations | 60% | 45% | 60% | 40% |

*Prolyl-leucyl-glycinamide

revealed that the mount latency of the PLG/morphine group was significantly greater than that of the saline or PLG groups ($p<0.05$). No other significant differences were found.

Although these data suggest that PLG does not function as an opiate antagonist with respect to male sexual behavior, such a conclusion may be premature. While there was an inhibitory effect on mount latency, the other three behavioral duration parameters recorded were not significantly inhibited by morphine This finding contrasts with those of other investigators who have found that opiate treatment frequently inhibits not only mount latency, but also other measures of male sexual activity (30) Perhaps the moderate degree of morphine inhibition in the present study prevented observation of a potential reversal by PLG treatment

## EXPERIMENT 6

PLG in the absence of opiates produces differential effects on male and female sexual behavior PLG failed to attenuate morphine-induced inhibition of male sexual behavior in Experiment 5, but facilitated female sexual behavior in the absence of morphine in Experiment 1 However, the effect of PLG, when given in conjunction with opiates, on female sexual behavior has yet to be determined. Contreras and Takemori (10) suggest that at low doses PLG binds at specific PLG binding sites, whereas at higher doses, PLG competes with morphine for mu opioid receptors. If disinhibition by PLG is dose-dependent, then perhaps a dose greater than 1 mg/kg PLG is required to antagonize opiate inhibition of sexual behavior. This experiment was designed to determine whether PLG would attenuate morphine-induced inhibition of female sexual behavior

### *Method*

Prior to the first test, female rats were screened to ensure that they exhibited a high level of receptivity. Animals received 10 μg EB and 500 μg P prior to the screening pretest; those animals with an LQ equal to or less than 40 were eliminated from the study. In total, four animals were eliminated, reducing the subject pool to 20 animals.

Following this pretest, a counterbalanced repeated measures design was employed. Animals were primed with 10 μg EB and 500 μg P. One hour prior to testing, the control group received 1 ml/kg saline IP and the three experimental groups received an IP injection of either 0.1, 1 or 10 mg/ml/kg PLG. Because morphine inhibits lordosis 60 minutes following IP injection (30), in phase 1 of this experiment, all animals received 1.5 mg/ml/kg morphine 1 h in advance. Behavioral testing was conducted once weekly over a four-week period.

Following phase 1, the animals were not tested for two consecutive weeks in an attempt to reduce any possibility of a developing tolerance to morphine In phase 2 of behavioral testing the experimental design was identical to that in phase 1 except that all animals received 3.0 mg/ml/kg morphine rather than 1 5 mg/ml/kg

### *Results and Discussion*

At the 1.5 mg/kg morphine dose the mean LQ's ± S E.M.'s observed for the 0, 0.1, 1.0 and 10 mg/kg PLG groups were 43 ± 7, 67 ± 6, 44 ± 7, and 54 ± 7, respectively Similarly, at 3 0 mg/kg morphine, the mean LQ ± S.E M for each of the above 4 PLG doses was 39 ± 6, 33 ± 5, 50 ± 7, and 44 ± 6 A mean LQ of 86 ± 3 was observed prior to treatment with morphine Thus, at both morphine doses, LQ's were considerably lower than during pretesting. However, the presence or absence of PLG did not appear to be a factor Analyses of variance for both phases of the experiment failed to reveal a significant attenuation by PLG of the morphine-induced inhibition of lordosis.

## GENERAL DISCUSSION

In summary, PLG in the absence of opiates facilitated sexual behavior in the female but not in the male rat. Furthermore, PLG was found to facilitate lordosis behavior in a progesterone-dependent manner Thus our data provide support for the hypothesis that PLG is an active metabolite of oxytocin in females. TPLG failed to facilitate lordosis behavior, suggesting that it does not act as the natural precursor to PLG. PLG failed to antagonize morphine-induced inhibition of sexual behavior in both male and female rats

In the absence of opiates, PLG facilitated sexual behavior in female but not in male rats, indicating the existence of a possible sex difference. This sex difference is reminiscent of that observed with centrally administered oxytocin When administered into the cerebral ventricles, oxytocin facilitated sexual behavior in the female (16), but not in the male (36) It may be that lordosis, a brainstem or spinal reflex, is more sensitive to oxytocin and its fragment PLG than the common measures recorded for male sexual behavior.

Furthermore, the observed sex difference may be due to an interaction with ovarian hormones. Chronic estrogen administration has been shown to increase oxytocin receptor binding in the ventromedial hypothalamic nucleus (VMN) (12) In particular, physiological levels of estradiol maximally increase oxytocin binding in the ventrolateral region of the caudal VMN, an area involved in regulation of sexual behavior, but not in more rostral regions (19). As well, progesterone has been shown to increase oxytocinergic processes in the ventromedial hypothalamus (7) In rats primed with estrogen, progesterone induces expansion of the oxytocin receptor field around the VMN within 4 hours of P treatment (35). Administered into the ventromedial hypothalamus, oxytocin increases the duration of lordosis (34) Moreover, both PLG and oxytocin facilitate sexual receptivity in a progesterone-dependent manner P treatment may also influence the distribution or number of PLG binding sites, thereby permitting the peptide to facilitate lordosis. That low doses of centrally administered oxytocin do not facilitate lordosis in animals primed with EB alone, but do so in animals primed with EB and P, suggests that the P-induced expansion of oxytocin receptors may be a critical step in the facilitation of female sexual behavior It is conceivable that such progesterone dependency may, in part, form a basis for the observed sex difference in PLG's ability to affect reproductive behavior

Studies demonstrating a selective distribution of radioactivity following $^3$H-PLG administration have been restricted to the examination of male animals (9,33). It is possible that PLG binding sites may differ both in location and concentration in the

female and thus contribute in part to the observed differences in the behavioral effects of PLG. The possibility of a sex difference in PLG binding sites is strengthened by the observation that ovarian hormones influence the number and distribution of oxytocin binding sites

PLG may act on both opiate and nonopiate receptors or only on a specific population of opiate receptors (e g., the mu receptor) The effects of PLG on morphine-induced analgesia, tolerance and dependence (10,14) are similar to those of naloxone. Naloxone, however, is behaviorally active in many situations in which PLG is not For example, naloxone affects opiate-induced vas deferens contractions and motor behavior while PLG does not (15). Galina and Kastin (15) suggest that an endogenous opiate antagonist system would most likely involve many highly specific antiopiates, including PLG, rather than a solitary antiopiate peptide. These authors suggest that a system comprised of many specific opiate antagonists would allow for greater diversity of behavior modification Moreover, no single peptide similar in structure and behavioral effects to naloxone has been identified in the mammalian system It, therefore, remains possible that PLG functions as one of several selective endogenous opiate antagonists.

A similarity exists between the effects of naloxone and PLG on sexual behavior in male and female rats. Naloxone consistently facilitates lordosis behavior in females (30). Similarly, in the present series of studies, PLG was found to facilitate lordosis However, the reported effects of naloxone on male sexual behavior are inconsistent. Pfaus and Gorzalka (30) note that opiate antagonists consistently facilitate male sexual behavior only in sexually naive or sluggish rats and in rats copulating to exhaustion. This suggests that naloxone may be capable of facilitating male sexual behavior only in circumstances associated with higher than normal levels of endogenous opiate activity (30) Interestingly, in the current series of studies, PLG did not facilitate sexual behavior in a group of sexually experienced male rats Thus while the results of the present investigations suggest that PLG facilitates female but not male sexual behavior, it remains for future research to establish the basis of this apparent sex difference.

The pharmacological actions of PLG may be the result of conversion to behaviorally active metabolites (3). Following administration of radiolabelled PLG, the major metabolite formed is leucyl-glycinamide (33). Evidence that both leucyl-glycinamide and PLG inhibit the development of tolerance to morphine suggests that metabolites of PLG are bioactive. Moreover, it is conceivable that PLG exerts many of its behavioral effects, including those involving sexual activity, via behaviorally active metabolites.

PLG's ability to affect sexual behavior may, in part, be due to its putative influence on melanocyte stimulating hormone (MSH) release. Several studies provide evidence that PLG does inhibit the release of MSH (8, 22, 39), hence its alternative name, MSH inhibiting factor (MIF). However, Thody and colleagues failed to find a significant effect of PLG on MSH release either in vitro or in vivo (38). In addition, PLG has been shown to antagonize the MSH-induced increase of intraocular pressure and miosis (25). Centrally administered MSH has been shown to inhibit lordosis both acutely and over the long term (32). Therefore, perhaps the facilitation of lordosis by PLG is, in part, a function of MSH inhibition.

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