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Luteinizing Hormone Releasing Hormone (LHRH) in the Periaqueductal Gray Substance Increases Some Subcategories of Grooming Behavior in Male Rats

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GARGIULO, P. A. AND A. O. DONOSO. *Luteinizing hormone releasing hormone (LHRH) in the periaqueductal gray substance increases some subcategories of grooming behavior in male rats.* PHARMACOL BIOCHEM BEHAV **32**(4) 853–856, 1989.— Locomotion, holeboard exploration and grooming behavior were measured in male rats after injection of LHRH or cerebrospinal fluid (CSF) into the mesencephalic periaqueductal gray substance (PGS). LHRH injection at doses of 75 ng did not alter locomotion and rearing but decreased significantly the scores of head dipping and defecation. Several subcategories of grooming responses were evaluated in a home cage-like environment. Head, body and genital grooming increased significantly after injection of 75 ng LHRH. The frequency of gnawing and head shakes increased as well. Lower doses (50 ng) also raised the scores of head and body grooming and produced a tendency for genital grooming increase. Higher doses (100 ng) did not affect genital grooming responses and produced drowsiness in most of animals. These results demonstrate that some motor activities are selectively modified by the localized administration of LHRH in the PGS.

Luteinizing hormone releasing hormone LHRH Locomotion Exploration Grooming Genital grooming
Periaqueductal gray substance

LUTEINIZING hormone releasing hormone (LHRH), a hypothalamic decapeptide, has been demonstrated to potentiate lordotic behavior (16) and facilitate mounting behavior in rats (2,3). Such effects appear to be independent of its gonadotropic actions (18). The decapeptide was found to enhance proceptivity display for copulation in female anthropoid primates (10) but not in female rats (6). Other behavioral actions reported for LHRH are a decrease of acquisition and a facilitation of extinction of an active aversive behavior (15), an increased retention of a passive avoidance response (13), and a modulation of amphetamine effects on avoidance behavior (14). These observations raised the possibility that LHRH may influence other more general parameters of behavior. With the aim of performing a systematic study, we started to measure motor activity in rats. Experiments were designed to administer the decapeptide at different doses into the midbrain central gray (periaqueductal gray substance), a region linked to mating behavior (22), sensory processes and learning (9,21) that is sensitive to LHRH (20,22) and is innervated by LHRH-containing nerve endings (23). In these experiments, locomotor, exploratory and grooming responses were evaluated in adult male rats.

METHOD

Male rats of a Holtzman-derived colony aged 90 days and weighing 240–270 g were used. They were maintained under conditions of controlled temperature (22–24°C) and lighting (lights on 0500–1900 hr). Standard rat chow and water were freely available.

Cannulae Implantation

Animals were anesthetized with ether and stereotaxically implanted with stainless-steel cannulae in the right mesencephalic periaqueductal gray substance (PGS) (17). The implanted cannulae were double barreled and the set was composed of an outer guiding cannula stainless-steel tubing (23-gauge, 15 mm in length) provided with a removable stylet (30-gauge, 15 mm in length) to avoid its obstruction. After surgery, the rats were housed individually and maintained undisturbed for a week recovery period.

LHRH Administration

The groups were defined as follows: Controls: Animals injected with 1 μl of artificial cerebrospinal fluid (CSF) (12); Experimentals: Animals injected with 50, 75 or 100 ng LHRH. Lyophilized LHRH (Elea Laboratories, Argentina) was dissolved in the CSF shortly before use. The freely-moving rats received PGS infusions during a 4-minute time period with a syringe pump provided with a Hamilton microliter syringe. It was connected by a polyethylene tubing to a 30-gauge stainless-steel inner cannula introduced into the guide cannula after removal of its stylet. When the inner cannula was inserted, the tips of both cannulae were at the same level. Rats remained freely moving in their home cages during the infusion.

Locomotor Activity Testing

Scores of locomotion, nonambulatory movements and vertical movements (rearing) were recorded in an animal activity monitor (OVM; Opto Varimex III, Columbus Inst.), 10 min after start of LHRH injection and during 10 min. Fecal boli were scored visually.

Holeboard Exploration

The holeboard was a black painted wooden box, 60 × 60 × 35 cm. The floor was marked off in 20 × 20 cm squares and had 16 holes of 2 cm diameter. The field was illuminated with a 40-W fluorescent lamp. The animals were measured 10 min after the start of LHRH infusion and during 5 min. The frequency of rearing and head dipping, the frequency and time spent grooming and the number of fecal boli were scored.

Grooming Behavior Test

Spontaneous grooming was scored 10 min after LHRH infusion and during 30 min in a cage similar to the home cage. Prior to the test, rats were maintained undisturbed for a 6-min period. The following grooming subcategories were separately scored: face wash, flank scratching, head, body and genital grooming (licking of the genital area). Rearing, digging, gnawing and head shakes were also scored.

Localization of Implant Cannula

Once the experiments were completed all animals were killed by an ether excess, the brains were removed and fixed for at least 72 hr in 10% formaldehyde for histological examination. Frontal sections were then obtained in a cryotome and the site of the implants was checked microscopically.

Data Analysis

Two-way analysis of variance and Scheffé's test of multiple comparisons were used to calculate the significance of differences in motor activity, and one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test for the statistical analysis of holeboard and grooming behavior scores. In all cases, a $p<0.05$ was considered significant. Data are presented as means ± SEM.

RESULTS

Between-groups differences in locomotion, rearing, and nonambulatory movements scored in the OVM test were not significant (results not shown). In contrast, a decrease in the number of fecal boli was found in the 50 ng LHRH group (LHRH: 0.5 ± 0.25, CSF: 1.7 ± 0.3; $p<0.05$). The injection of 75 ng LHRH blocked defecation in most rats (5/6). In doses of 100 ng (n = 8) LHRH did not affect defecation.

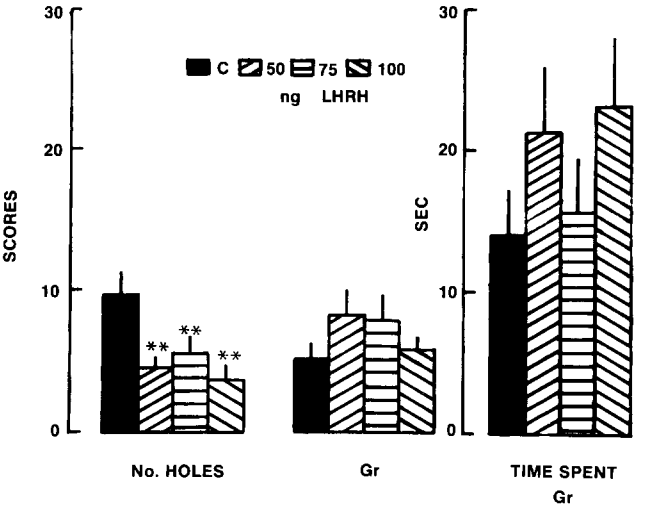

FIG. 1. Histogram representation of holeboard activities of rats injected with different doses of LHRH (hatched bars) in the midbrain central gray: 50 (n = 12), 75 (n = 6) and 100 ng (n = 11). Controls received cerebrospinal fluid (closed bars, C, n = 10). Number of holes explored, grooming scores (Gr) and time spent grooming during a 5-min trial. Mean ± SEM. **$p<0.01$ vs. control.

Between-groups differences in head dipping responses in the hole-board were observed, $F(3,32) = 5.55$, $p<0.01$. This was due to the lower scores of the LHRH-treated groups ($p<0.01$ vs. controls). Differences between LHRH-treated groups in head dipping scores were not found (Fig. 1). Grooming scores in this test showed a tendency to increase in the 50 (n = 12) and 75 ng (n = 6) LHRH-treated animals but differences did not reach significance. The time spent grooming (Fig. 1) and the frequency of rearing and scratching (results not shown) were not affected by the treatments. Holeboard defecation was normal (1–3 boli) in the 50 and 100 ng LHRH-treated groups. In contrast, fecal boli were absent in 5 out of 6 rats treated with 75 ng LHRH.

In the last experiments, performed in novelty cages, between-groups differences in total grooming were not significant. However, LHRH increased significantly several subcategories of grooming. Figure 2 shows that the frequency of head and body grooming increased in the 50 ng LHRH-treated group (n = 9) ($p<0.01$ vs. controls). The injection of 75 ng LHRH (n = 13) caused a further stimulation of the head and body grooming responses ($p<0.01$ and $p<0.05$, respectively, vs. 50 ng group), but scores decreased along the time of observation. The 100 ng LHRH group showed lower scores of head and body grooming than the 75 ng LHRH group ($p<0.05$) but higher than controls ($p<0.01$ and $p<0.05$, respectively). This might be due to the fact that 100 ng LHRH caused drowsiness in half of animals (6 out of 12 rats).

Between-groups differences in the frequency of genital grooming responses were also found, $F(3,24) = 4.68$, $p<0.025$. The PGS administration of LHRH stimulated genital grooming (Fig. 2). In the 75 ng LHRH group the increase reached high statistical significance ($p<0.01$ vs. controls). The response was constant during the 30-min period of observation. A tendency to genital grooming increase was found in the 50 and 100 ng LHRH groups. Genital grooming was infrequent in control rats. The inset of Fig. 2 shows that the means of the summed scores of the subcategories

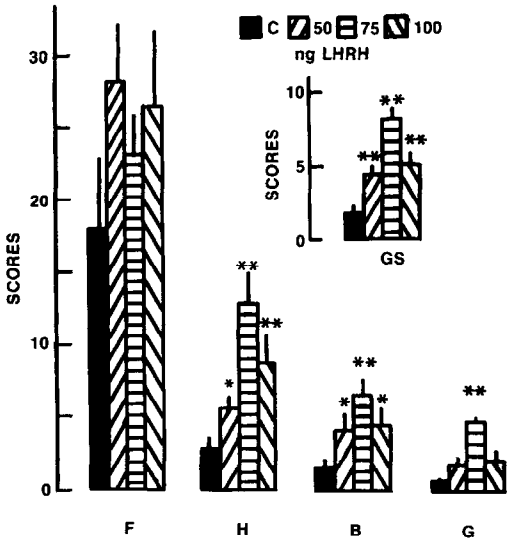

FIG. 2. Histogram representation of grooming behavior of rats injected with LHRH (hatched bars) in the midbrain central gray: 50 (n = 9), 75 (n = 13), and 100 ng (n = 12) or cerebrospinal fluid (closed bars, C, n = 7). Frequency analysis for face (F), head (H), body (B), and genital grooming (G), were determined during 30 min. Upper inset shows the means ± SEM for controls (closed bar) and LHRH-injected (hatched bars) of the additioned H, B and G grooming scores (GS). $^{**}p<0.01$ vs. control.

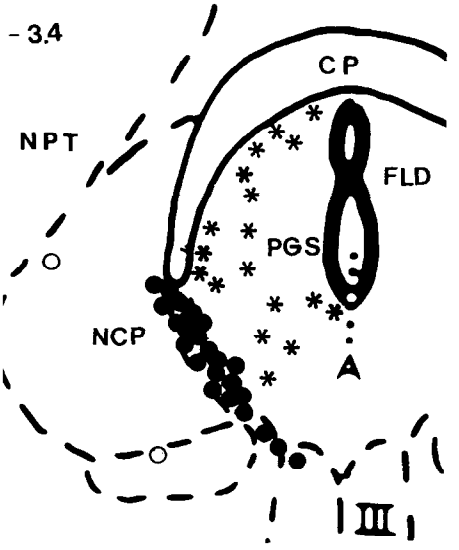

FIG. 3. Coronal section of the rat mesencephalon showing cannulae placements in the periventricular gray substance. Asterisks and dark circles indicate cannulae located in regions where LHRH produced behavioral modifications. Open circles indicate misplaced cannulae. A: Aqueduct; CP: posterior commissure; FLD: dorsal longitudinal bundle; PGS: periventricular gray substance; NPT: posterior nucleus of the thalamus; NCP: bed nucleus of the posterior commissure; III: oculomotor nerve.

modified by the decapeptide were significantly higher than controls. Between-groups differences were found, $F(3,98) = 6.48$, $p<0.01$. This was due to 50 ($p<0.01$), 75 ($p<0.01$) and 100 ng ($p<0.01$) LHRH-treated groups. The increased grooming in the 75 ng LHRH group was accompanied by both increased gnawing ($p<0.01$ vs. control) and head shaking ($p<0.01$ vs. control) (Table 1). As to flank scratching and face washing, between-groups differences were not found. This was the case also for rearing and digging (results not shown). The histological examination revealed that behavioral changes were present in animals showing cannula placed in the dorsal and lateral parts and the border of the central gray substance (Fig. 3).

Grooming scores in animals with displaced cannulae (in the bed nucleus of the posterior commissure) did not differ from controls.

DISCUSSION

These results show that LHRH infusion in the PGS did not affect motor activity. This suggests that such treatment does not cause sedation or a stress response (19) in rats. Since defecation was impaired by LHRH it may be speculated that this compound has an anxiolytic profile. Holeboard experiments show that head dipping decreased after infusion of small doses of LHRH into the PGS. However, holeboard rearing and grooming remained unchanged. It might be, therefore, that the treatment impaired motivation for head dipping.

When grooming was closely examined in a novel environment similar to the familiar cage, it was found that LHRH at all doses used stimulated head and body grooming activities. A dose-response relationship was found only for 50 and 75 ng LHRH, since scores decreased with the highest doses. This inverted U-shaped dose-response curve can be due to the drowsiness produced by the 100 ng dose of LHRH. In a similar manner, the increase of genital grooming was blunted by injection of the high doses.

The pattern of grooming stimulation elicited by LHRH is different from those produced by certain peptides such as CRF (corticotropin-releasing factor). CRF elicits increases in all components of grooming, mainly face washing and excepting flank scratching (7) and is considered as an anxiogenic peptide (8). Vasopressin causes an increase in flank scratching (1). We have found recently that TRH (thyrotropin releasing hormone) injected in the PGS causes an increase in face grooming and other grooming components (unpublished results). On the other hand, a grooming pattern similar to the one reported herein occurs in rats bearing pituitary homografts and high systemic prolactin levels. Prolactin was reported to increase genital grooming without changing face wash and scratching (4). In addition, oxytocin injected ICV produced a dose-dependent linear increase of genital grooming in both male and female rats (5). It is noteworthy that the genital grooming scores found following oxytocin injection and in hyperprolactinemic rats resemble those obtained by us with 75 ng LHRH.

Total grooming responses have been considered as an anxiety

TABLE 1

GNAWING AND HEAD SHAKES SCORES IN LHRH-INJECTED RATS

Behav. Scores	CSF	LHRH ng 50	75	100
Gnawing	10.3 ± 4.4	12.5 ± 3.0	42.3 ± 10.2*	27.7 ± 5.4
Head shakes	0.1 ± 0.1	0.3 ± 0.2	5.9 ± 2.1*	1.7 ± 0.7

Scores are the mean ± SEM for the 30 min following periventricular gray substance injection in rats of cerebrospinal fluid (CSF) (n = 7) or LHRH (n = 9, 13 and 12, respectively).

*$p<0.01$ vs. CSF.

index and a specific rodent response to mild stressful situations (19). The increased grooming in response to novelty stress is mainly due to stimulation of face washing, body grooming and scratching. However, other subcategories of grooming such as genital grooming are not induced by anxiety of stress (8,19). In fact, rats injected with 75 ng LHRH in the PGS, which showed increased genital grooming, displayed normal locomotion scores and a low defecation index suggesting a good adaptation to novelty. Therefore, LHRH exhibited no anxiogenic properties and its actions are apparently related to more specific grooming patterns such as body and genital grooming.

The LHRH microinjection in the PBS induced, in addition, gnawing and head shakes. Gnawing has been classically linked to hypothermia. Consistently, Lomax *et al.* reported that LHRH administration in the preoptic area decreased core temperature (11). However, even though systematic measurements of temperature were not performed here, we have observed that the LHRH infusion in the PGS did not produce changes in rectal temperature.

The PGS is a midbrain site related to sensory functions, behavioral activation (9) and mating behavior (16). The iontophoretic administration of LHRH into the PGS is known to stimulate the neuronal firing (20) at the time that, in the present study, several grooming categories are selectively enhanced. Apparently, there is also a regional selectivity for LHRH-induced grooming. Lack of changes in grooming after infusion into the bed nucleus of the posterior commissure (present results), and in the nucleus accumbens (unpublished results), supports this view.

The mechanism responsible for LHRH effects on behavior remains to be further analyzed. Probably, the LHRH stimulation of genital grooming is in connection to its ability to enhance mating behavior. It has been shown that male rats deprived of genital sensory information displayed increased mounting scores after ICV injection of LHRH (2). However, we cannot rule out the possibility that the effects of LHRH on grooming may be linked to other behavioral actions elicited by LHRH (13–15).

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