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Behavioral Effects of Intracerebral Administration of Luteinizing Hormone Releasing Hormone (LHRH) in Rats[1]

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MORA, S., A. AFANI, R. KUSANOVIC, C. TAPIA AND G. DIAZ-VELIZ. *Behavioral effects of intracerebral administration of luteinizing hormone releasing hormone (LHRH) in rats.* PHARMACOL BIOCHEM BEHAV **38**(4) 705–709, 1991.—The effects of LHRH intracerebrally infused on acquisition of conditioned avoidance responses (CARs) and spontaneous motility were studied in adult male rats. The results were the following: 1) LHRH (1 and 2.5 μg/rat) administered through a cannula stereotaxically implanted into the lateral ventricle induced an impairment in the acquisition of CARs along with an increase in global motility, rearing, head shaking and grooming behavior; 2) LHRH 1 μg/rat injected into the hippocampus or nucleus accumbens induced also an impairment in acquisition which is evident 15 min after treatment. In contrast, intrastriatal injection induced an immediate disruption of this behavior; and 3) there is a good dose-response relationship for intrastriatal LHRH between 7.8 and 62.5 ng/rat. The results suggest that the estriatum could be the locus of the LHRH-induced inhibition of CARs. Then the possibility of an involvement of the dopamine nigrostriatal system is discussed.

LHRH Lateral ventricle Striatum Hippocampus N. accumbens Conditioned avoidance responses Spontaneous motor activity Dopamine

SEVERAL reports presented in the last few years have demonstrated that the neuropeptide LHRH is able to induce behavioral effects in the rat which are apparently not related with the release of pituitary hormones. Small doses of LHRH, whether injected subcutaneously (500 ng/rat) or infused via cannulae into the cerebral ventricles, medial preoptic area or the arcuate nucleus (50 ng/rat), potentiate mating behavior in ovariectomized-hypophysectomized estrogen-primed female rats as well as testosterone-primed castrated male rats (18). Nonsexual behavioral effects of LHRH have also been described. Intracerebral administration of LHRH markedly reduces barbiturate-induced sleeping time (3). Large doses of LHRH (1–2 mg/kg, IP) are active in the Everett DOPA/pargyline potentiation test carried out in normal intact and hypophysectomized mice (19). This evidence have led to the hypothesis that LHRH could exert a direct action on the brain.

Reports from our laboratory have demonstrated that LHRH administered subcutaneously is also able to modify the acquisition and retention of conditioned avoidance responses in male rats. Pretraining administration of LHRH induces a dose-dependent and time-dependent impairment in the acquisition of an active avoidance conditioned response (12), and improves the retention of the task when it is injected immediately after training (13). Besides, the neuropeptide has been shown to increase and impair the retention of a passive avoidance conditioned response, according to the intensity of the footshock applied during training (13).

We have observed that pretreatment with LHRH can also modify the conditioned and spontaneous behavioral effects of dopamine (DA) agonists, such as DOPA (14), amphetamine (14) and apomorphine (16). These observations, along with biochemical evidence which demonstrate an inhibitory action of LHRH upon DA synthesis and release (15,22), have led us to postulate that the behavioral effects of LHRH could be the consequence of its interaction with DA systems in the brain.

The present study was designed in order to determine if the intracerebral infusion of LHRH is able to modify the acquisition of the conditioned response or induce other behavioral changes. With this purpose the neuropeptide was administered via a cannula stereotaxically implanted into one of the lateral ventricles of the brain. LHRH was also injected into some other central structures, such as hippocampus, striatum and nucleus accumbens. The results contribute to support the idea that the behavioral effects of LHRH are exerted directly in the brain.

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GENERAL METHOD

Animals

A total of 144 male Sprague-Dawley rats, weighing between 180–200 g, were maintained housed in groups of six per cage under a 12:12 light/dark cycle (lights on from 08:00 to 20:00 h) with free access to food and water.

Surgery

The animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and placed in a David Kopf stereotaxic frame with the skull oriented according to the Fifkova and Marsala atlas (6). A 23-ga stainless steel guide cannula to allow LHRH (Sigma Chemical Co.) or saline intracerebral (IC) injection was affixed to the skull with dental acrylic. The guide cannula was closed with an stainless steel stylet terminating at the guide tip. A recovery period of at least 7 days after surgery was given before testing began. The IC injection was administered through a 30-ga stainless steel cannula which was inserted with its tip extending 1 mm below the guide cannula and connected by polyethylene tube to a Hamilton microsyringe. All the behavioral tests were performed between 10:00 and 16:00 h, and each rat was tested only once.

Behavioral Testing

Spontaneous motor activity. The animals were individually placed in a Plexiglas case (30 × 30 × 30 cm), housed into a sound-attenuated room. The floor of the cage was an activity platform (Lafayette Instrument Co.) connected to an electromechanical counter. After a 15-min period of habituation each rat received IC injection of either saline or LHRH dissolved in saline and then spontaneous motor activity was monitored automatically for 15 min. Simultaneously the following responses were also registered: number of rearings, head shakings and the time (seconds) spent in grooming behavior. Each animal was observed continuously from the moment it was placed on the activity platform until the end of the session, via a Sony video camera (Model AVC-1420) connected to a Panasonic VHS tape recorder (Model PV-4000).

Active avoidance conditioning. The conditioning experiments were carried out with a two-way shuttle box (Lafayette Instruments Co.) composed of two stainless steel modular testing units, which were equipped with an 18-bar insulated shock grid floor, two 28-V DC lights and a tone generator (Mallory Sonalert 2800 Hz). Electric shocks were provided to the grid floor by a Master shock supply (Lafayette Instrument Co.). The animals were individually placed in the shuttle box and, after a 5-min period of habituation, they were trained over 50 trials. Each trial consisted of the presentation of a tone which after 5 s was overlapped with a 0.20 mA foot-shock until the animal escaped to the opposite chamber. The intertone interval was 30 s. A conditioned response (CAR) was defined as a crossing within the first 5 s (tone).

Histology. After behavioral testing, rats were administered an overdose of sodium pentobarbital and decapitated. The brain was removed from the skull and placed in 10% formalin for at least 24 h. The brains were then sectioned and the locations of the cannula tips verified.

Statistics. Analysis of variance (ANOVA) followed by Newman-Keuls or Dunnett multiple comparison procedures, and Student's *t*-test for individual comparisons were applied to evaluate the statistical significance of the results. In all cases differences were considered to be significant when p was equal to or less than 0.05.

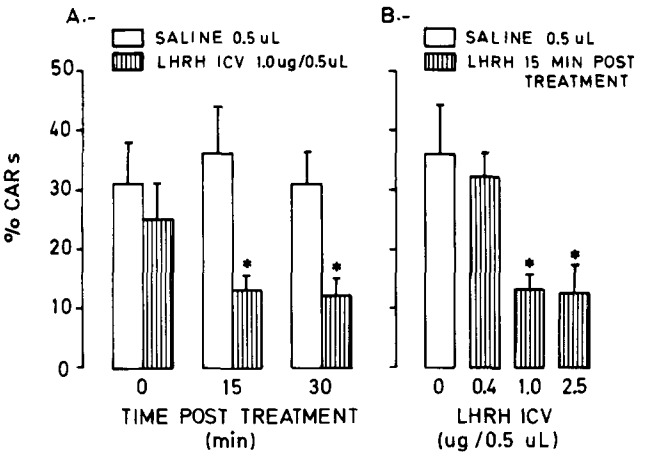

FIG. 1. Effects of intracerebroventricular (ICV) injection of LHRH on the acquisition of conditioned avoidance responses (CARs). The bars represent the mean ± SEM of the percentages of CARs out of 50 trials. Comparisons were made by using Student's *t*-test (A) and one-way ANOVA followed by Dunnett's test (B) (*significantly different from saline group, $p<0.01$). The number of animals in each group was 8–9. For more details see text.

EXPERIMENT 1

PROCEDURE

This experiment was conducted to examine the behavioral effects of intraventricular injection of LHRH. The guide cannula was stereotaxically implanted such that the injection cannula was located in the right lateral ventricle using the following coordinates derived from Fifkova and Marsala: + 1.0 mm with respect to bregma; 1.5 mm lateral to bregma; 4.0 mm below the surface of the skull. Each rat received an intracerebroventricular (ICV) injection of either saline (0.5 μl) or LHRH (0.4, 1.0 or 2.5 μg/0.5 μl) 15 min before the acquisition test. LHRH 1 μg/rat was also administered at 0 and at 30 min before the behavioral test, in order to determine the influence of the time of injection.

RESULTS

Active Avoidance Conditioning

Figure 1 shows that the ICV injection of LHRH impairs acquisition of CARs. This impairment is significant when LHRH is injected 15 or 30 min before the beginning of the test [Fig. 1A, two-way ANOVA for treatment: $F(1,47) = 11.4086$, $p<0.01$]. The 15-min interval between injection and test was used to study the dose-relationship for LHRH ICV (Fig. 1B). One-way ANOVA indicated significant effects of LHRH upon acquisition of CARs, $F(3,31) = 5.3043$, $p<0.01$. Although this study indicated that the effects of LHRH 1 and 2.5 μg/rat were significant, there was not a correlation between the dose and the impairment of the response.

Spontaneous Motor Responses

ICV injections of LHRH induced significant modifications in the four motor behaviors studied. Figure 2A shows that the three doses of LHRH increased general motility [one-way ANOVA, $F(3,46) = 3.4927$, $p<0.05$]. LHRH 0.4 and 2.5 μg/rat significantly increased rearing behavior [Fig. 2B, one-way ANOVA,

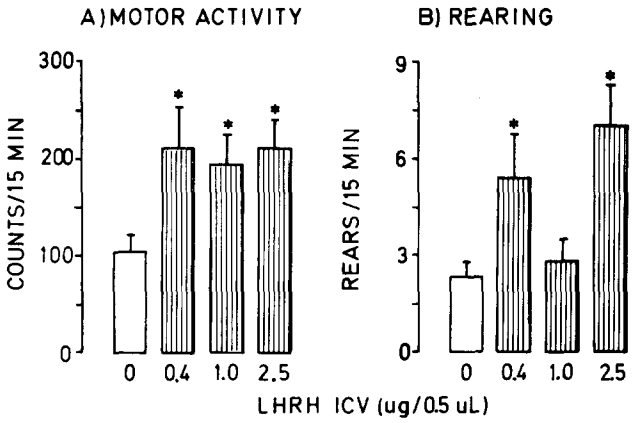

FIG. 2. Effects of ICV injection of LHRH on global activity (A) and rearing behavior (B). Behavioral testings were applied 15 min after LHRH treatment. Each bar represents the mean ± SEM of motility counts or the number of rears, respectively, in 15 min. Comparisons were made by using one-way ANOVA followed by Dunnett's (*significantly different from saline group, $p<0.05$). The number of animals in each group was 8–9. For more details see text.

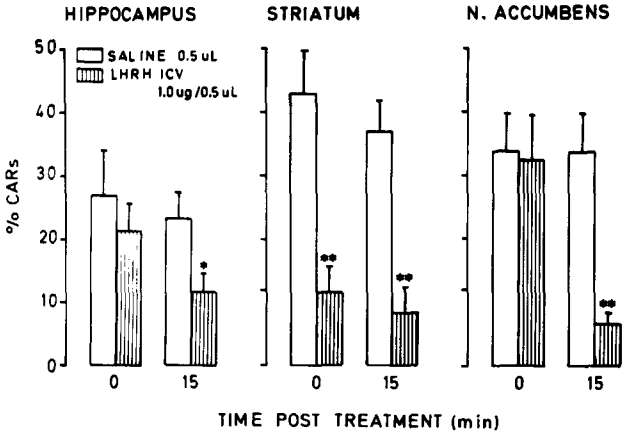

FIG. 4. Effects of LHRH injection into hippocampus, striatum and n. accumbens on the acquisition of CARs. Behavioral testing were performed immediately or 15 min after LHRH treatment. The bars represent the mean ± SEM of the percentages of CARs out of 50 trials. Comparisons were made by using Student's t-test for independent groups (*$p<0.025$). The number of animals in each group was 8–10. For more details see text.

$F(3,46)=6.2528$, $p<0.01$]. Head shaking [Fig. 3A, one-way ANOVA, $F(3,45)=3.8664$, $p<0.05$] and grooming behavior [Fig. 3B, one-way ANOVA, $F(3,46)=4.9382$, $p<0.01$] were also stimulated by the injection of LHRH 1 or 2.5 μg/rat. No correlationship between dose of LHRH and response was observed in any of the behaviors mentioned above.

EXPERIMENT 2

PROCEDURE

This experiment was conducted to determine whether the application of LHRH to specific brain nucleus could reproduce the inhibitory effects of ICV injection of LHRH on acquisition of CARs. Guide cannulae were stereotaxically implanted either into the right striatum (AP −1.5, L +2.5, V −4.5), into the right hippocampus (AP +3, L +2.5, V −4.5) or into the right nucleus accumbens (AP −2.5, L −1.0, V −5.5). Each rat received an intracerebral application of saline (0.5 μl) or LHRH 1.0 μg/0.5 μl). The acquisition test began immediately or 15 min after LHRH.

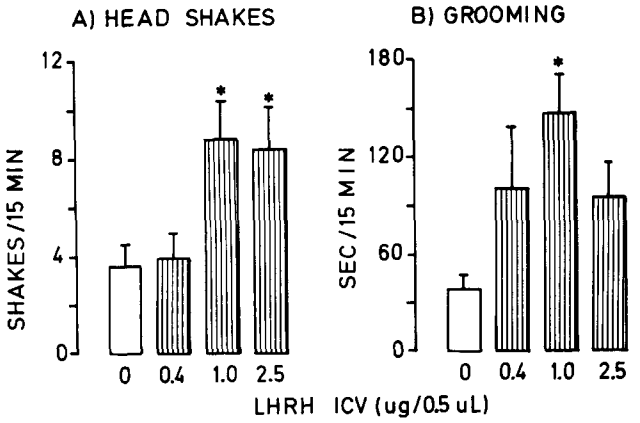

FIG. 3. Effects of ICV injection of LHRH on head shaking (A) and grooming behavior (B). Behavioral testings were applied 15 min after LHRH treatment. Each bar represents the mean ± SEM of the number of shakes and the time in s spent in grooming, respectively, in 15 min. Comparisons were made by using one-way ANOVA followed by Dunnett's (*significantly different from saline group, $p<0.05$). The number of animals in each group was 8–9. For more details see text.

RESULTS

Figure 4 shows that 15 min after the administration of LHRH either into the striatum, hippocampus or accumbens nuclei there is a significant impairment in the acquisition of CARs. Nevertheless, when the test was applied immediately after the injection, the only significant effect was obtained in the striatum.

EXPERIMENT 3

PROCEDURE

Since the results of Experiment 2 indicate that the striatum seems to be more sensitive to LHRH than the other loci studied, this experiment was designed to establish a dose-effect relationship and determine the minimal amount of LHRH which is able to impair the acquisition of CARs. Each animal was intrastriatally infused with one of the following doses of LHRH: 3.91, 7.82, 15.62, 31.25, 62.5, 125, 250, 250, 500 and 1000 ng/rat. The acquisition session began immediately after treatment.

RESULTS

Figure 5 shows the influence of the several doses of intrastriatal LHRH on the acquisition of CARs. One-way ANOVA indicated a significant impairment on this behavior, $F(9,95)=29.4747$, $p<0.01$, which was very well correlated to the dose in the range between 7.8 and 62.5 ng/rat [Pearson's $r=-.99$, $p<0.0005$]. Dunnett's test for comparisons with saline group indicated that LHRH 3.91 and 7.82 enhanced acquisition of CARs.

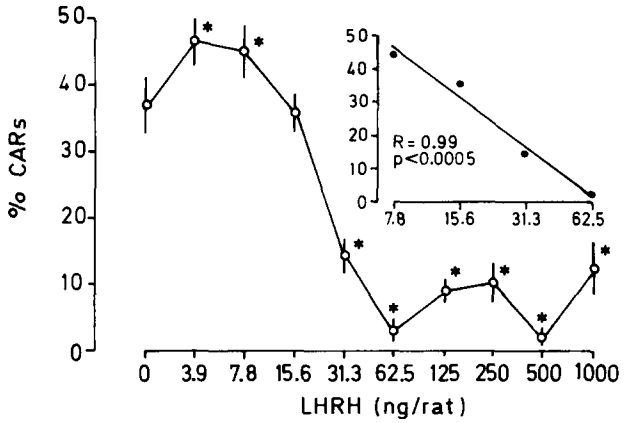

FIG. 5. Effects of intrastriatal injection of several doses of LHRH on the acquisition of CARs. Each point of the principal curve represents the mean ± SEM of the percentages of CARs out of 50 trials. Comparisons were made by using one-way ANOVA followed by Dunnett's test (*significantly different from saline group, $p<0.05$). The upper right curve represents the dose-response analysis in a selected range of doses, assessed by the Pearson correlation test. The number of animals in each group was 9–11. For more details see text.

GENERAL DISCUSSION

The present study demonstrates that the LHRH infusion in the brain affects conditioned and spontaneous behavioral responses in the male rat. Some of these effects are comparable with those observed after subcutaneous injection of the neuropeptide. We have shown previously that LHRH 100 μg SC impairs acquisition of conditioned avoidance responses (CARs) and increases head shakes (13). LHRH brain infusion either through the lateral ventricle or into specific nuclei impaired acquisition of CARs. Nevertheless, the locus where this effect resulted to be more rapid and potent was the striatum. Only the LHRH intrastriatal injection was able to induce immediate effects on the acquisition performance, in contrast with the 15-min delay necessary to observe significant effects after intraventricular, intra-accumbens or intrahippocampus injections of high doses of LHRH (1000 ng). The study of the dose-response relationship indicates an apparently biphasic effect of intrastriatal LHRH on conditioning: whereas small amounts, 3.9 and 7.8 ng, stimulate the acquisition of CARs, increasing dosages induce a dose-dependent inhibition of the response. The maximal disruption was observed after LHRH 62.5 ng.

The present results suggest that the LHRH-induced inhibition of CARs could take place primarily in the estriatum. Although the mechanism of this action is unknown, there is evidence that permits to postulate an involvement of the dopamine nigrostriatal system. This system seems to be of critical importance in the acquisition of a CAR. This suggestion is supported by the studies concerning the effects of DA disruptions on the acquisition of CARs. For instance, animals fail to acquire avoidance responses if trained after DA-depleting intracisternal injections of 6-OH-DA (4), intranigral injections of 6-OH-DA (24) or injection with DA receptors blockers (5) or DA synthesis inhibitors (11). Antipsychotic drugs are evaluated by their ability to inhibit avoidance conditioning (1,17), maybe by a disruption of striatal DA transmission (9,21). In addition, the observation of increased DA release in the rat striatum following brief electric shock to the tail (8) had led to the suggestion that DA mediates negative reinforcement (2). There is evidence that the behavioral effects of LHRH could be correlated with biochemical changes in striatal DA transmission. An in vitro study demonstrated that the incubation of rat corpus striatum synaptosomes in the presence of LHRH has inhibitory effects on DA synthesis (22). Besides, LHRH subcutaneously injected is able to decrease synthesis and release of DA from rat corpus striatum slices (15). The suggestion that LHRH could modify DA activity is indirectly supported by reports about interactions between LHRH SC and DA agonists, such as amphetamine (14), L-dopa (14) and apomorphine (16) which postulate a presynaptic action of LHRH upon DA synthesis and release followed by changes in DA receptors sensitivity.

Significant changes in spontaneous motor responses were also observed immediately after intraventricular infusion of LHRH 1 μg. Global motility, rearing, head shaking and grooming behavior were all stimulated after treatment. This hyperactivity was not evident when the same dose of LHRH was injected into the striatum or hippocampus. Recently (7), it has been reported that an increase in grooming behavior is induced by the infusion of 75 ng LHRH into the mesencephalic periaqueductal gray substance. The frequency of gnawing and head shakes increased as well. Furthermore, intracerebral injections of LHRH or LHRH analogs in various regions produce strong effects on rat sexual behavior (10).

The evidence presented above support the view of a regional selectivity for LHRH-induced effects on behavior and suggest a specific role of LHRH in the CNS, possibly as neurotransmitter or neuromodulator. Immunocytochemical procedures have demonstrated that LHRH is present in neurons and fibers with a wide distribution in the rat CNS (23). In addition, the presence of LHRH receptors in selected brain areas has been reported. The rat hippocampal formation is highly enriched in LHRH receptors and moderate amounts of binding sites could also be seen in the amygdala, the septal area and the perirhinal cortex (20). Although the physiological actions of LHRH in these brain areas are not clearly established, this report contributes to support the idea that the hormones of the hypothalamic-pituitary-gonadal system do not only coordinate sexual or reproductive events, but also are involved in behavioral adaptation.

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