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Pharmacological Evidence of Catecholaminergic Involvement in the Behavioral Effects of Luteinizing Hormone Releasing Hormone in Rats[1]

SERGIO MORA AND GABRIELA DIAZ-VELIZ

Departamento Preclinicas, Facultad de Medicina, Division Ciencias Medicas Oriente
Universidad de Chile, P O Box 16038, Santiago 9, Chile

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MORA, S AND G DIAZ-VELIZ *Pharmacological evidence of catecholaminergic involvement in the behavioral effects of luteinizing hormone releasing hormone in rats* PHARMACOL BIOCHEM BEHAV 24(3) 433–438, 1986 —The influence of L-DOPA on the behavioral effects of LHRH was studied in male rats Subcutaneous administration of LHRH (100 μg/kg) caused a significant disruption in the acquisition of a conditioned avoidance response (CAR) and a significant increase in head shaking behavior (HSB) Pretreatment with this hormone antagonized the stimulatory action of amphetamine (1 mg/kg, IP) in acquisition of CARs, spontaneous motor activity (SMA) and rearing behavior (RB) L-DOPA (100 mg/kg, IP), administered after LHRH, stimulated SMA, RB and HSB In addition L-DOPA antagonized the effect of LHRH on acquisition of CARs and counteracted the antagonism between LHRH and amphetamine in acquisition of CARs and SMA These findings indicate that LHRH could exert its behavioral effects through an inhibitory action upon brain catecholamine synthesis The suppression of CARs may be the response to DA antagonism and the interaction with amphetamine could be mediated by an inhibition of both DA and NE activities The possibility of an interaction between LHRH and central serotonin mechanisms is also discussed

LHRH L-DOPA Amphetamine Avoidance behavior Spontaneous motor activity Rearing Head shaking Catecholamines Dopamine

SEVERAL reports presented in the last decade have led to the suggestion that luteinizing hormone releasing hormone (LHRH) could induce pharmacological influences on animal behavior which are independent of its pituitary stimulant properties It has been demonstrated that LHRH, whether administered subcutaneously [16] or infused into the brain [22], facilitates sexual behavior in the rat Small quantities of the neuropeptide injected to ovariectomized-hypophysectomized estrogen-primed female rats as well as testosterone primed castrated male rats potentiate mating behavior [20] In addition, large doses of LHRH potentiate the behavioral effects of DOPA both in normal intact and hypophysectomized mice [21], supporting the hypothesis that LHRH has a direct action on the brain

Recent reports from this laboratory have suggested that LHRH administered subcutaneously can also modify the performance in avoidance conditioning tasks The hormone impairs the acquisition of active avoidance conditioning in normal intact as well as castrated male rats [13] This effect is dose-dependent and time-dependent and it is not followed by subsequent changes in retention of the response, which is assessed a week later [11] Although LHRH disrupts acquisition of conditioned avoidance responses (CARs), when the animals are retested under a no drug condition, performance is comparable to that of animals who received saline before acquisition, suggesting that the associative processes during training remain unimpaired Moreover, there is a significant improvement in the retention performance when the neuropeptide is injected immediately after acquisition of the conditioned task [15] We have also demonstrated that pretreatment with LHRH counteracts some of the stimulatory effects of increasing dosages of amphetamine (0 25, 0 5, 1 and 2 mg/kg IP) In fact, pretreatment with LHRH (100 μg/kg SC) induced a significant displacement of the dose-response curve of amphetamine on several behavioral responses, such as acquisition of CARs, motor activity and rearing [12]

Since the integrity of the dopamine (DA) system is important for the stimulant effects of amphetamine, the ability of LHRH to attenuate the response to amphetamine suggests an interaction between LHRH and DA systems However, LHRH does not appear to block motor activity induced by the direct acting DA agonist apomorphine [14] These data suggest that LHRH could be affecting presynaptic DA mechanisms, e g , synthesis or release of the neurotransmit-

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TABLE 1

INTERACTION BETWEEN LHRH (100 μg/kg SC), L-DOPA (100 mg/kg IP) AND AMPHETAMINE (AMP 1 mg/kg IP) ON AVOIDANCE CONDITIONING

	Conditioned Avoidance Responses CARs		
Treatment	Acquisition session % CARs (mean ± SEM)	Retest session % CARs (mean ± SEM)	Retention* (mean ± SEM)
a Sol + Sal + Sal	38 7 ± 7 7	53 1 ± 9 6	14 4 ± 4 8
b LHRH + Sal + Sal	12 9 ± 3 8 ($p<0$ 05)	38 9 ± 10 0 (NS)	26 0 ± 9 6 (NS)
c Sol + DOPA + Sal	24 0 ± 5 6	39 8 ± 11 9	15 8 ± 9 5
d LHRH + DOPA + Sal	37 3 ± 7 9† (NS)	64 2 ± 8 6† (NS)	26 9 ± 6 2 (NS)
e Sol + Sal + AMP	64 2 ± 5 4†	38 4 ± 8 6	−25 8 ± 6 7‡
f LHRH + Sal + AMP	18 2 ± 4 5 ($p<0$ 0005)	19 3 ± 5 0 ($p<0$ 05)	1 1 ± 2 5† ($p<0$ 005)
g Sol + DOPA + AMP	59 8 ± 5 8†	64 9 ± 8 2	5 1 ± 6 9
h LHRH + DOPA +AMP	58 0 ± 4 9‡ (NS)	46 2 ± 10 0 (NS)	−11 8 ± 9 6† (NS)

*Difference in performance between the two sessions Comparisons between groups were made by using Student's t-test In brackets are indicated differences between LHRH- and Sol-group on each experimental condition Differences with the respective control group (a was compared with c, e and g, and b was compared with d, f and h) are indicated by †$p<0$ 05 and ‡$p<0$ 005

N=9 (number of rats in each group)

Sol=Solvent (Benzyl alcohol 2%) and Sal=Saline

ter In fact, there is evidence that LHRH is able to depress DA synthesis in rat brain slices [24]

To investigate the hypothesis that the behavioral effects of LHRH may be due to an action on the synthesis of DA, we decided to study the influence of L-DOPA on the effects induced by LHRH upon active avoidance conditioning Simultaneously, we also studied the influence of L-DOPA on the interaction of LHRH with amphetamine on active avoidance conditioning and spontaneous motor activity Thus, if LHRH exerts its pharmacological effects through an inhibition of DA synthesis, the addition of L-DOPA must antagonize that inhibition, counteracting both the behavioral effects of LHRH and the interaction of this neuropeptide with amphetamine Briefly, we demonstrate that L-DOPA antagonizes the impairment in acquisition of conditioned avoidance responses (CARs) induced by LHRH In addition, L-DOPA was also able to reverse the antagonism between LHRH and amphetamine in the acquisition of CARs and spontaneous motor activity

METHOD

Animals

A total of 136 male Sprague Dawley rats weighing 200±20 g were used in the experiments They were housed in groups of six per cage in a temperature regulated room (23±2°C) on a 12 hr light-dark cycle (lights were on from 8 00 to 20 00 hr) and they had food and water available ad lib The behavioral experiments were performed between 10 00 and 16 00 hr in a sound attenuated and temperature regulated room

Drugs

Luteinizing hormone releasing hormone (LHRH, Sigma Chemical Co) was dissolved in 2% benzyl alcohol L-DOPA methyl ester hydrochloride and D-amphetamine sulphate were dissolved in saline In all cases the doses to be injected were in a volume of 0 1 ml/100 g of body weight Control animals received the respective solvent

Active Avoidance Conditioning

Apparatus The conditioning experiments were carried out with a two-way shuttle box (Lafayette Instruments Co) composed of two stainless steel modular testing units Each modular chamber was equipped with an 18-bar insulated shock grid floor, two 28 V DC lights and a tone generator (Mallory Sonalert 2800 Hz) Electric shock was provided to the grid floor by a Master Shock Supply (Lafayette Instruments Co)

Procedure Each of the 72 rats used in this experiment was submitted to two sessions of shuttle avoidance conditioning with an interval of 7 days between them In the first, or acquisition session, the animal was trained over 50 trials, in the second, or retest session, it was retrained over the same number of trials Each trial consisted of the presentation of a tone that after 5 sec was overlapped with a 0 25-mA footshock until the animal escaped to the opposite chamber A conditioned avoidance response (CAR) was defined as a crossing within 5 sec Intertone interval was 30 sec "Retention" was considered as the difference in the same animal's performance between the two sessions

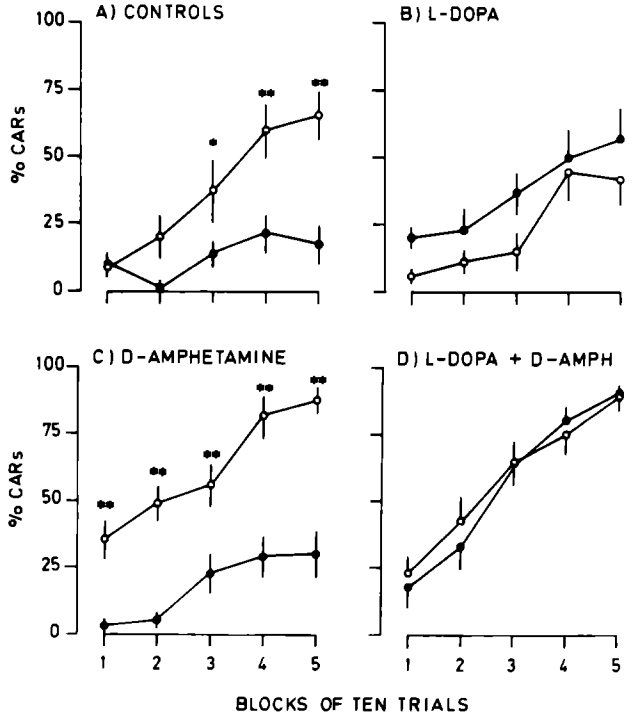

FIG 1 Effects of the interaction between LHRH (100 μg/kg SC), L-DOPA (100 mg/kg IP) and amphetamine (AMP 1 mg/kg IP) on the acquisition of conditioned avoidance responses (CARs) On each group animals were pretreated with solvent (open circles) or LHRH (filled circles) Each point on the curves represents the mean±SEM of the percent of CARs by blocks of 10 successive trials Two-way ANOVA was performed on the data from each experimental condition followed by Newman-Keuls test to assess differences between specific pairs of means (*$p<0$ 05 and **$p<0$ 005) The number of animals on each group was 8

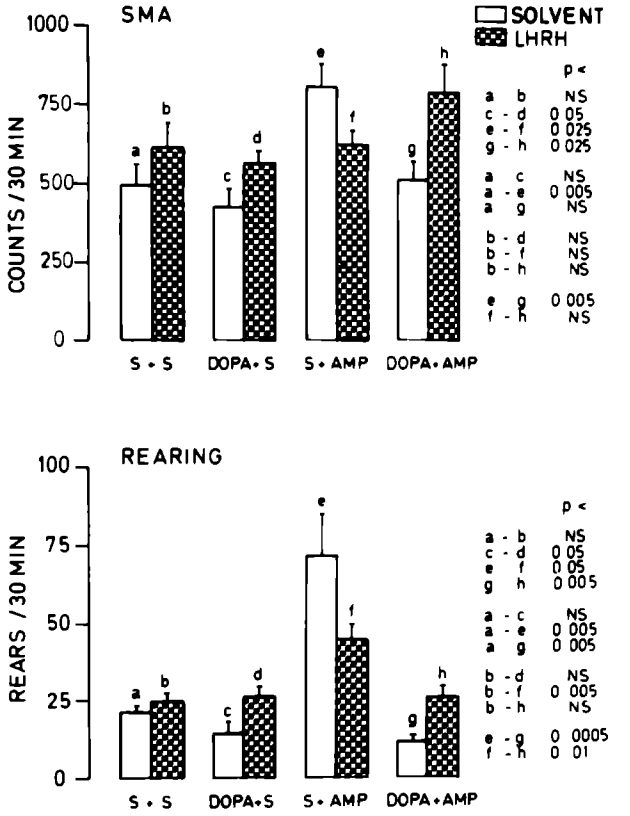

FIG 2 Effects of the interaction between LHRH (100 μg/kg SC), L-DOPA (100 mg/kg IP) and amphetamine (AMP 1 mg/kg IP) on spontaneous motor activity (SMA) and rearing behavior DOPA or saline (S) and amphetamine (AMP) or saline (S) were given 60 and 90 min, respectively, after administration of LHRH or solvent The bars represent the mean+SEM of the total SMA counts and the number of rears in 30 min Comparisons were made by using Student's *t*-test The number of rats on each group was 9

Spontaneous Motor Activity

Apparatus Motor activity was registered by using an activity platform (Lafayette Instrument Co) connected to an electromechanical counter In order to avoid the influence of disturbing noises the platform was placed into a sound-proof chamber and the observations were made through a closed TV-circuit

Procedure Sixty-four animals were individually placed in the platform and the spontaneous motor activity was recorded during a period of 30 min Simultaneously the following responses were also registered number of rearings, number of head shaking and the time spent in grooming behavior

Schedule of Drug Administration

Each animal was injected subcutaneously with LHRH 100 μg/kg or the solvent two hours before the beginning of the acquisition session or motor activity recording The animals also received L-DOPA 100 mg/kg or saline IP and D-amphetamine 1 mg/kg or saline IP, 60 and 90 min after LHRH treatment, respectively No drug was administered prior to the retest session

Data Analysis and Statistics

Student's *t*-test and two-way analysis of variance followed by the Newman-Keuls Multiple Comparison Procedure were used to determine the level of significance of treatment effects Differences were considered to be significant when p was equal to or less than 0 05

RESULTS

Active Avoidance Conditioning

Table 1 summarizes the effects of the interaction between LHRH, L-DOPA and amphetamine on the conditioning performance As previously demonstrated [13], LHRH impaired the acquisition of CARs without modifying the retention of the response Although L-DOPA by itself was not able to induce significant modifications in the performance of CARs, it antagonized the impairing effects of LHRH Amphetamine (AMP) exerted opposite influences on the acquisition and the retention of CARs Whereas the acquisition was significantly enhanced, the retention was severely impaired Pretreatment with LHRH antagonized both the stimulatory effect in acquisition and the impairment in re-

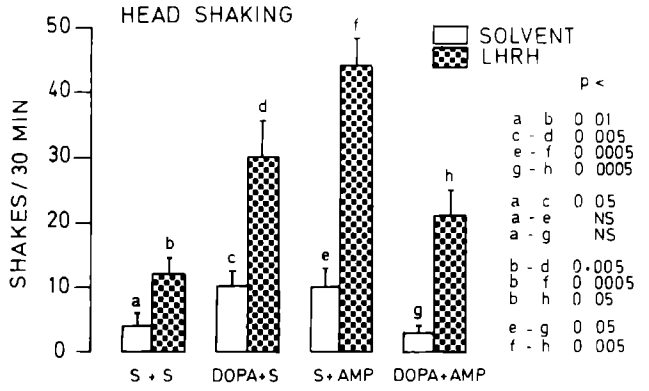

FIG 3 Effects of interaction between LHRH (100 μg/kg SC), L-DOPA (100 mg/kg IP) and amphetamine (AMP 1 mg/kg IP) on head shaking behavior DOPA or saline (S) and amphetamine (AMP) or saline (S) were given 60 and 90 min, respectively, after administration of LHRH or solvent The bars represent the mean+SEM of the number of shakes in 30 min Comparisons were made by using Student's *t*-test The number of rats on each group was 9

tention of CARs induced by AMP L-DOPA did not significantly modify the effects of AMP on the acquisition but it antagonized the amnestic action of this drug ($p<0$ 05) L-DOPA also counteracted the antagonism between LHRH and AMP in both acquisition and retention of the response

The effects of the interaction between LHRH, L-DOPA and AMP on the acquisition rate, expressed as the percent of CARs by blocks of ten successive trials, are presented in Fig 1 Two-way analyses of variance were performed on the data from each curve to assess main effects due to trials and treatment, and the interaction between trials and treatment Statistical differences between specific pairs of means are indicated in the figure It can be observed that LHRH induced a significant inhibition in the acquisition of the response in control animals (Fig 1-A, trials $F(4,89)=8$ 625, $p<0$ 01, treatment $F(1,89)=29$ 557, $p<0$ 01, and interaction $F(4,89)=3$ 226, $p<0$ 05) This effect was not evident when the animals were also treated with L-DOPA (Fig 1-B, trials $F(4,89)=8$ 155, $p<0$ 01, treatment $F(1,89)=6$ 212, $p<0$ 05, and interaction $F(4,89)=0$ 250, $p>0$ 05) Amphetamine increased the acquisition rate, but this effect was completely blocked by LHRH (Fig 1-C, trials $F(4,89)=13$ 756, $p<0$ 01 treatment $F(4,89)=129$ 515, $p<0$ 01, and interaction $F(4,89)=4$ 125, $p<0$ 05) Finally, when L-DOPA was administered in the latter condition, the blocking effects of LHRH were reversed (Fig 1-D, trials $F(4,89)=32$ 6206, $p<0$ 01, treatment $F(1,89)=0$ 156, $p>0$ 05, and interaction $F(4.89)=0$ 279, $p>0$ 05)

Spontaneous Motor Activity and Rearing Behavior

The influence of the interaction between LHRH, L-DOPA and AMP in spontaneous motor activity (SMA) and rearing behavior (RB) is shown in Fig 2 LHRH by itself did not significantly modify SMA nor RB Nevertheless, it significantly reduced the stimulatory effect of AMP in SMA and RB L-DOPA significantly decreased RB without modifying SMA and, when injected after LHRH, it induced a significant increase in both SMA and RB Besides, L-DOPA significantly antagonized the effects of AMP on SMA and counteracted the antagonism between LHRH and AMP on this behavior L-DOPA also antagonized the stimulatory action of AMP on RB in both saline and LHRH treated rats

Head Shaking Behavior

Figure 3 shows the effects of the interaction between LHRH, L-DOPA and AMP on head shaking behavior Shakes are significantly increased by LHRH and L-DOPA but not by AMP On the other hand, whereas both L-DOPA and AMP potentiate shaking behavior induced by LHRH, L-DOPA attenuates shakes caused by AMP in saline as well as LHRH pretreated animals

No significant modification of time spent in grooming behavior was observed after LHRH, L-DOPA or AMP

DISCUSSION

The present results confirm previous reports in showing that the subcutaneous administration of LHRH can affect behavioral processes in the rat The impairment in the acquisition of a conditioned avoidance response (CAR) and the antagonism of the amphetamine-induced effects on conditioning and motor activity were recently reported by us [12] These findings are extended now by showing that the precursor of catecholamines (CA) synthesis, L-DOPA, is able to counteract some of the pharmacological effects of LHRH, suggesting that they could be mediated through an interaction with brain CA, particularly dopamine (DA) This study demonstrates that LHRH interacts with L-DOPA and amphetamine (AMP) In fact, the neuropeptide potentiated the behavioral effects of L-DOPA and, on the other hand, antagonized those induced by AMP, with the exception of head shaking behavior

The basic mechanism of the behavioral actions of LHRH is not understood at present However, if we considered that both L-DOPA and AMP are CA agonists, it might be hypothesized that LHRH alters the activity of CA neurons mediating behavior L-DOPA is a precursor of the synthesis of the CAs and the main psychostimulant effects of AMP are thought to be due to its ability to promote the release of recently synthesized DA [7] The performance of various kinds of conditioned behaviors is probably regulated by central CA of which DA seems of primary importance [4] Thus the acquisition and performance of a CAR is enhanced by AMP [12] and specifically suppressed by neuroleptics [1] CAR performance can also be disrupted by blocking the rate limiting step in the synthesis of DA and norepinephrine (NE) with alpha-methyl-p-tyrosine (AMPT), injected prior to training [2,10] The suppression induced either by neuroleptics or AMPT can, in a large part, be reversed with L-DOPA [3] In addition, the facilitating effects of low doses of AMP injected before active avoidance training are blocked by AMPT [8], and this blocking effect of AMPT is reversed by DL-DOPA [19]

There is a close similarity between the effects of LHRH and those described for neuroleptics and AMPT Indeed, all of them are able to inhibit conditioning and antagonize the stimulatory actions of AMP Interestingly, these are considered characteristic effects of almost all drugs which block central DA receptors [18] The suppression of CARs by AMPT has been reported to be caused by inhibition of the motor, rather than the associative components of CAR performance [9] In the present study, the disruption in the ac-

quisition of CARs induced by LHRH could also be attributed to motor factors However, in our experimental conditions, LHRH suppressed CARs without inducing a significant impairment in motor performance

The hypothesis of an interaction between LHRH and CA neurotransmission is further supported by the motor studies which showed a great similarity to the CAR data LHRH, in a dose producing no significant reduction of total spontaneous motor activity (SMA) and rearing behavior (RB) per se, attenuated the stimulatory action of AMP in both SMA and RB Recently [9], it has been postulated that the stimulatory action of AMP on SMA may be mainly mediated by activation of the DA system and stimulation of RB may occur by activation of the NE system

L-DOPA seems to exert opposite influences on motor activity In fact, whereas it stimulated SMA and RB when injected after LHRH and reversed the antagonism between LHRH and AMP in SMA, it completely blocked the enhancement in SMA and RB induced by AMP The potentiation of the DOPA-induced responses by LHRH and other hypothalamic releasing hormones was demonstrated in a previous report [21], suggesting that these hormones presumably exhibit their activities through dopaminergic systems The antagonism between L-DOPA and AMP was unexpected since they are both CA agonists Any explanation of this finding would be speculative, nevertheless, it has been demonstrated that L-DOPA, according to the dose, has biphasic effects on behavior compatible with a reduction in the release of endogenous dopamine that cause sedation, suppress dyskinetic movements, have an antipsychotic action and potentiate parkinsonism [23]

The evidence presented in this study might be considered rather indirect, but they are in accordance with the hypothesis of an interaction between LHRH and catecholaminergic neurotransmission Both the conditioning and motor activity data support the idea that LHRH, under our experimental conditions, could act by an inhibition of the CA synthesis The suppression of CARs may be the response to DA antagonism, and the interaction with AMP in conditioning and motor activity could be mediated through an inhibition of both DA and NE activities

The possibility of an inhibitory influence of LHRH on DA synthesis has been reported recently An "in vitro" study [24] demonstrated that the incubation of rat corpus striatum slices in presence of LHRH induced a decrease in DA synthesis The authors have suggested that LHRH could exert a negative feedback action on DA neurons, that is, LHRH could inhibit its own release by inhibiting DA synthesis On the other hand, Foreman and Moss [6] have suggested that the stimulatory effects of LHRH upon female lordotic behavior may be mediated through the stimulation of DA neurons Dosages of LHRH could be of primary importance to explain this discrepancy The possibility exists that LHRH effects on DA activity have an inverted-U dose-response relationship

The present study demonstrates that LHRH also induces head shaking behavior and that L-DOPA and AMP potentiate the shakes caused by LHRH The shake response ("wet dog" shakes, head and/or body shakes) is considered a potentially valuable indicator of central activity The neurochochemical and receptor mechanisms involved in the production of shaking behavior remain unclear, although there is evidence that it is a serotonin-dependent behavior [5] Stereotyped head twitches have been induced in the mouse by central serotonin stimulation and benzodiazepines and they were completely blocked by serotonin antagonists such as cyproheptadine and methysergide [17] The possibility that LHRH could interact with brain serotonin has been proposed in a report in which it is demonstrated that LHRH is active in the serotonin potentiation test [21]

We cannot rule out the possibility that LHRH could modify the activity of brain systems other than catecholamines More specific neurochemical studies about the effects of LHRH on monoamines functioning in various brain areas, synthesis and turn-over "in vivo" must be carried out to elucidate the mechanism of the behavioral effects of LHRH

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