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The Effect of LHRH on Rat Conditioned Avoidance Behavior: Interaction With Brain Catecholamines

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NASELLO, A. G., C. R. BYDLOWSKI AND L. F. FELICIO. *The effect of LHRH on rat conditioned avoidance behavior: Interaction with brain catecholamines.* PHARMACOL BIOCHEM BEHAV **37**(4) 639–642, 1990.—LHRH (100 μg/kg, SC) impairs the acquisition of two-way avoidance conditioning. This is partially potentiated by pretreatment with α-methyltyrosine (α-MT; 250 mg/kg IP) or fusaric acid (10 mg/kg IP). L-DOPA (100 mg/kg IP) administered 5 h after α-MT partially reversed its effects. The possible roles of brain catecholamines on the behavioral effects of LHRH are analysed. Other tentative mechanisms of action are also discussed.

LHRH Conditioned avoidance Brain catecholamines Dopamine Norepinephrine

THE development of specific methods for identifying and quantifying luteinizing hormone release hormone (LHRH) has permitted the localization of this peptide in various brain areas in addition to the hypothalamus (8,14). These findings, plus previous studies demonstrating various effects of LHRH, have led authors to propose that this peptide may act centrally, in addition to its classical neurosecretory function. LHRH, injected either subcutaneously or intracerebroventricularly, modifies sexual behavior in castrated and hypophysectomized rats (23, 24, 28). LHRH also potentiates the behavioral effects of L-DOPA and 5-hydroxytryptophan (5-HTP) in pargyline-treated mice, both in intact or hypophysectomized animals (29). This peptide also reduces barbiturate-induced sleeping time when administered centrally (2). In addition, it is able to inhibit the extinction of a pole jumping avoidance response (3). Subcutaneous administration of LHRH impairs the acquisition of a conditioned avoidance response (CAR) in intact as well as in castrated male rats (16), and antagonizes the dose-related impairment of the acquisition of CARs induced by testosterone in castrated animals (18). This effect of LHRH on CAR is dose-dependent (17) and is antagonized by L-DOPA (20). LHRH is also able to antagonize the effects of amphetamine (17) and potentiate the effects of apomorphine (21) on acquisition and retention of CARs and on motor activity. LHRH has been shown to increase and impair the retention of passive and active avoidance conditioning depending on dose and foot shock intensity (19).

Several reports indicate that norepinephrine (NE) and dopamine (DA) participate in the endogenous regulation of LHRH release (11–13, 26). The importance of an undisturbed catecholamine system for the performance of a conditioned avoidance response is well known and studies have shown that central nervous system (CNS) catecholamine mechanisms are involved (1, 4–6, 15). The purpose of the present study was to investigate the interaction of LHRH with α-methyltyrosine, an inhibitor of the synthesis of both DA and NE, and fusaric acid, an inhibitor of the synthesis of NE (9), in terms of their effects on the acquisition of an active avoidance task.

METHOD

Animals

Adult albino male rats of Wistar origin (200–250 g) were used. They were housed in groups of six per cage in a temperature-regulated room (23 ± 2°C) on a 12-h light-dark cycle (lights on from 8:00 to 20:00 h). Food and water were available ad lib. All behavioral experiments were performed between 10:00 and 16:00 h in a sound-attenuated and temperature-regulated room.

Drugs

LHRH, kindly donated by Laboratorios Ayerst Brazil (HRF), was dissolved in 2% benzyl alcohol. α-Methyltyrosine methyl-

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TABLE 1

EFFECT OF LHRH ON ACQUISITION AND RETENTION OF A CONDITIONED RESPONSE[1]

Treatment			Percentage of Conditioned Responses (Mean ± SEM)		
IP	SC	n	Acquisition Session	Difference Within Groups	Retention Session
Vehicle	Vehicle	27	45.6 ± 3.1	$p<0.01$	77.9 ± 4.9
Vehicle	LHRH	28	32.4 ± 3.9		68.2 ± 4.5
α-MT	Vehicle	8	11.5 ± 2.4*	$p<0.05$	66.0 ± 7.7
α-MT	LHRH	8	5.0 ± 1.3*		49.8 ± 13.1*
α-MT+L-DOPA	Vehicle	10	23.2 ± 2.0*	$p<0.025$	67.8 ± 2.3
α-MT+L-DOPA	LHRH	10	16.2 ± 2.1*		66.2 ± 2.1
Fusaric acid	Vehicle	12	31.3 ± 4.9*	$p<0.005$	54.8 ± 7.2*
Fusaric acid	LHRH	9	10.4 ± 2.0*		36.9 ± 11.0*

[1]Influence of pretreatment with vehicle, α-MT (250 mg/kg), αMT+L-DOPA (100 mg/kg) and fusaric acid (10 mg/kg).

*Differences with their controls receiving vehicle IP are significant (at least $p<0.02$).

n: number of animals.

ester (α-MT), L-dihydroxyphenylalanine (L-DOPA) and fusaric acid were obtained from Sigma and were dissolved in saline. All drugs were administered by intraperitoneal injection in volumes of 0.1 ml/100 g body weight except LHRH which was administered subcutaneously (SC).

Schedule of Drug Administration

All drugs were administered prior to the acquisition trials. A group of animals received LHRH 100 μg/kg, SC, or 2% benzyl alcohol, SC, 2 h before beginning the experiment. Another group received α-MT 250 mg/kg, IP, 6 h before the test. These animals were divided into four subgroups that received the following treatments: subgroup A: LHRH 100 μg/kg, SC, 4 h after α-MT; subgroup B: 2% benzyl alcohol, SC, 4 h after α-MT; subgroup C: 2% benzyl alcohol, SC, 4 h after α-MT, then L-DOPA 100 mg/kg, IP, 1 h after 2% benzyl alcohol; subgroup D: LHRH 100 μg/kg 4 h after α-MT, then L-DOPA 100 mg/kg, IP, 1 h after LHRH. In addition, the effects of fusaric acid and its interaction with LHRH were tested. Another group of animals were injected with fusaric acid (FA), 10 mg/kg, IP, 4 h before the test. Two hours after the injection of FA some of them were treated with LHRH 100 μg SC and the others were injected with 2% benzyl alcohol, SC. The solution of FA was made daily.

Active Avoidance Conditioning

The two-way shuttle box used in the experiment has been previously described (25). It consists of a Plexiglas box (50 × 25 × 25 cm) with a grid floor made up of stainless steel bars spaced 12 mm apart. A midline barrier divided the box into two identical compartments. The rats were subjected to two sessions of shuttle avoidance conditioning (acquisition and retention sessions) with an interval of one week between them. Five minutes after the animals were placed in the box they received the first trial. Each trial consisted of the presentation of a buzzer which after 5 s was overlapped with a 0.5 mA foot-shock delivered through the grid floor until the animal escaped to the opposite compartment. A conditioned avoidance response (CAR) was defined as a crossing through the midline barrier within five seconds after the presentation of the buzzer. The interbuzzer interval was 30 s. The animal received 50 trials in each session.

Statistics

The results were analysed by Kruskal-Wallis analyses of variance followed by Mann-Whitney U-test (31). A probability of $p<0.05$ was taken to reflect significant differences for all comparisons made.

RESULTS

Table 1 shows the results obtained. The percentage of conditioned avoidance responses in the acquisition session was lower in the animals treated with LHRH in all groups. The treatment with α-MT also impaired the acquisition of the CAR, which was potentiated by LHRH. L-DOPA partially reversed the decrease induced by both α-MT and by α-MT combined with LHRH.

Fusaric acid also reduced the percentage of CARs and when combined with LHRH this percentage decreased even more. The combination of α-MT with LHRH, fusaric acid with LHRH, and fusaric acid alone also decreased the percentage of CARs in the retention session when compared to their controls receiving vehicle IP.

DISCUSSION

The present study provides further evidence that subcutaneous administration of LHRH in pharmacological doses impairs the acquisition of CARs in male rats. Pretreatment with α-MT also impairs acquisition of the CAR (1,15) and in our experiment this effect was potentiated by LHRH. α-MT inhibits tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis (9), and, as a consequence, the brain levels of DA and NE are decreased (30). In some cases L-DOPA may reverse the behavioral effects of α-MT, either totally or partially (6, 10, 15, 27), however, these effects are not always observed (33). In the experiments described here, the recovery of the CAR by rats treated with L-DOPA was only partial, probably because of the dose used or the duration of action. The potentiation of the disruptive effects of α-MT by LHRH was also observed in the presence of L-DOPA. An additional finding was that α-MT did not modify

the retention of the CARs, although it significantly affected acquisition. This fact disagrees with the results of previous studies which have demonstrated the well-known inhibitory effects of α-MT on memory (6). However, it must be noted that the methyl ester of α-MT was used and that its effects on brain catecholamines have been described (9), but that the behavioral effects are not as well-studied.

Fusaric acid, an inhibitor of dopamine-β-hydroxylase decreases the synthesis of NE (9) and impairs conditioned avoidance behaviors. Previous treatment with fusaric acid also potentiated the impairment effect of LHRH.

It seems that LHRH potentiates the effect of α-MT. Whether DA, NE or both are involved in this effect is unknown. The decrease in the number of conditioned reponses induced by fusaric acid indicates the participation of NE on learning processes. The pretreatment with fusaric acid and LHRH made the impairment of acquisition markedly more evident ($p<0.005$) than that obtained with any other drug association. This suggests that the interaction of LHRH is especially intense with dopaminergic mechanisms. In total or partial absence of NE the relation between DA and LHRH becomes more evident.

Our results are in agreement with previous reports that proposed a dopaminergic mechanism for interaction of LHRH and amphetamine in the acquisition of CARs (17); the potentiation of α-MT by LHRH was expected because the antagonism of LHRH-amphetamine resembles that of α-MT-amphetamine (17).

An interaction between LHRH and DA is supported by anatomical, biochemical and behavioral data. L-DOPA antagonizes the LHRH-induced impairment of conditioning and counteracts the antagonism between LHRH and amphetamine (20). LHRH also antagonizes the effects of apomorphine on conditioning (21) and on other dopaminergic-related behaviors such as motor activity, stereotyped sniffing (21) and yawning and genital grooming (22).

On the other hand, an inhibitory effect of LHRH on DA synthesis has been described in rat in vitro striatal synaptosomes (32). LHRH, subcutaneously injected, is able to decrease the synthesis and release of DA from rat corpus striatum (21). More recently, the effects of localized LHRH administration into the periaqueductal gray on motor activity and grooming behavior has been described (7).

The present study further supports the hypothesis that LHRH has modulatory actions on DA systems, accounting for the effects of this peptide on conditioning and learning. We cannot rule out the possibility that LHRH could modify the activity of brain systems other than catecholaminergic.

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