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An LH-RH Antagonist Inhibits the Behavioral Effects of the Agonist D-TRP-6-LH-RH in Mice

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KÁDÁR, T., G. TELEGDY AND A. V. SCHALLY. *An LH-RH antagonist inhibits the behavioral effects of the agonist D-TRP-6-LH-RH in mice.* PHARMACOL BIOCHEM BEHAV **41**(4) 665–668, 1992.—The effects of a potent LH-RH receptor antagonist, [Ac-4-Cl-D-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]LH-RH (ORG 30276), on the behavioral actions of the LH-RH agonist, D-Trp-6-LH-RH, were studied in mice. The subcutaneous (SC) administration of 100 μg/kg D-Trp-6-LH-RH inhibited ambulation in an open-field, produced analgesia in the hot-plate and tail-flick tests. These effects of the agonist were totally antagonized by pretreatment with ORG 30276 at a dose of 100 μg/kg SC. In the apomorphine-induced cage-climbing test, both the agonist and the antagonist alone or together suppressed the duration of stereotyped behavior in dose-dependent manner, but, as there was no additive synergism after combined treatments, it seems that the two substances mutually diminish each other's effects. The results indicate that the behavioral effects of the LH-RH agonist can be antagonized by pretreatments with a potent LH-RH antagonist designed to block pituitary LH-RH receptors, with the exception of the suppression of apomorphine-induced cage-climbing, where special type of receptors and/or mechanisms might be involved.

Luteinizing hormone-releasing hormone LHRH agonist LHRH antagonist Analgesia Antistereotypic activity

LUTEINIZING hormone-releasing hormone (LH-RH) is a hypothalamic hormone which also has significant neuromodulator or neurotransmitter functions in extrahypothalamic areas of the brain. The peptide has been detected in the mesencephalon, cerebral cortex, amygdala, septum and olfactory tubercle (8–10, 14–15, 19). A simultaneous localization of LH-RH neuronal systems with catecholaminergic, serotoninergic and noradrenergic projections was also demonstrated (8,18). LH-RH and its precursor molecule were found in the same population of neurons in the olfactory bulb and tubercle, the diagonal band of Broca, the medial part of the preoptic and suprachiasmatic nuclei, the anterior and lateral hypothalamus and several regions of the hippocampus (20). Receptors of LH-RH were also demonstrated in the brain by several techniques, and specific binding sites were revealed in the hypothalamus, amygdala, lateral septum, olfactory nuclei, frontal cortex and hippocampal formation (11,34).

The first behavioral effect of LH-RH to be demonstrated was the facilitation of sexual behavior in experimental animals (27). This LH-RH action was found to be independent of gonadotropins and the pituitary-adrenal axis, since the peptide was active in ovariectomized, hypophysectomized and adrenalectomized rats (19, 28, 32). Detailed research on other types of behavior was carried out by Mora and his associates, who demonstrated that peripheral LH-RH administration impairs learning and facilitates the recall of memory trace in active and passive avoidance paradigms in intact and castrated rats (21–25). In the two-way avoidance shuttle-box test, LH-RH antagonized the effects of testosterone and amphetamine (23–24). Pretreatment with L-DOPA antagonized the inhibitory effects of LH-RH on active avoidance behavior, and also attenuated the LH-RH antagonism of the improved performance induced by amphetamine injection in the same test (26). These studies provided in vivo evidence that LH-RH interacts with other neurotransmitter systems in the brain to exert a wide range of behavioral effects.

The animal experiments were followed by clinical research, but few clinically significant effects on human sexual behavioral functions could be demonstrated (19,29). In a psychophysiological study, McAdoo et al. (17) showed that male volunteers exhibited an increased speed of performance in automatized tasks, and they reported decreased anxiety and fatigue several hours after LH-RH administration.

In our previous studies (12,13), the effects of the SC administration of the potent agonistic analog D-Trp-6-LH-RH were studied in several pharmacological tests in mice, and the peptide was found to exert sedative-anxiolytic, neuroleptic or dopamine antagonist and analgesic activities. In further experiments, the central inhibitory actions of D-Trp-6-LH-RH were demonstrated to be partially reversible by the opiate antagonist naloxone, indicating the possible role of endogenous opioids in the peptide effects. The application of a highly active analog of LH-RH is probably equivalent to a potent as well as sustained excitation of specific LH-RH receptors in the brain, and this receptor binding then triggers the modification of multiple neurotransmitter systems, which might result in an altered behavioral performance. As a first step to check this possibility, the effects of a selective LH-RH antagonist on the behavioral actions of D-Trp-6-LH-RH were investigated in mice.

METHOD

General

Male albino mice of the outbred NMRI strain, weighing 25–35 g, were kept under a 12-h dark–12-h light artificial light-

ing schedule and controlled conditions with free access to commercial laboratory rodent chow and tap water. All animals were used only once. The peptides, i.e., the potent agonistic analog [D-Trp^6]LH-RH (referred to as D-Trp-6-LH-RH in this paper) and the selective LH-RH antagonistic analog [Ac-4-Cl-D-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]LH-RH (ORG 30276), were each dissolved in one drop of 0.1 N acetic acid and were further diluted with saline containing 0.1% BSA. The antagonist (if injected in corn oil) can cause a 100% inhibition of ovulation in the 1–2 μg dose range (4). The dose of D-Trp-6-LH-RH and the antagonist was the same, and in most cases (if not otherwise noted), it was 100 μg/kg. The dose was selected according to previous experiments (12,13), where this dose of D-Trp-LH-RH was found to be effective after peripheral administrations to mice. The antagonist was always injected 10 min prior to D-Trp-6-LH-RH treatment, to allow time for the antagonist to occupy LH-RH receptors. Animals were tested 30 min after the injection with D-Trp-6-LH-RH, as it was found to be the optimal time-point after D-Trp-LH-RH treatments in several experiments (12,13). Animals in the control groups were injected twice with BSA-containing saline.

Open-Field Activity

The locomotor activity was tested in a rectangular open-field box measuring 35 × 35 cm and divided into 5 × 5 cm squares. Ambulation was quantified as number of squares crossed during a 5-min period, 40 min after the first injection.

Analgesic Activity

The analgesic activity was tested in the standard hot-plate and tail-flick tests. The latency until the licking of one hindpaw on a 58°C surface up to 60 s, or the latency to twitching the tail away from the painful heat stimulus produced by a concentrated light source up to 20 s, were measured and recorded in these tests 30 and 60 min after the treatment with D-Trp-6-LH-RH, and the percentage changes in comparison to the predrug-state latencies were calculated. For the calculation of percentage changes of latencies, the following equation (5) was used:

$$\text{Latency increase (\%)} = \frac{T_{treated} - T_{basal}}{T_{maximum} - T_{basal}} \times 100,$$

where $T_{maximum}$, the maximum tested latency was 60 s or 20 s, respectively, and T_{basal} is the predrug state latency.

Apomorphine-Induced Cage-Climbing

In this test (33), 100, 300 and 1000 μg/kg SC doses of the LH-RH analogs were injected alone and in combination. The animals were placed in a 20 × 20 × 20 cm wire-mesh cage and, 20 min following the injection of 1 mg/kg apomorphine IP, the time spent in climbing the wire-mesh walls (grasping the mesh wall with both forepaws and hindlegs) was recorded during a 10-min period. The results are expressed as the time in seconds spent with climbing.

Data Analysis

For statistical analyses, one-way ANOVA, followed by Tukey's test for subsequent pairwise comparisons, was used (1).

RESULTS

From the open-field parameters, the number of squares crossed (ambulation) was observed to be decreased after treatment with D-Trp-6-LH-RH, as shown in Fig. 1. The one-way ANOVA showed significant differences, $F(3,36) = 4.439$, $p<0.01$. The antagonist analog alone did not have a significant effect, while in combination with the agonist it totally antagonized its effect.

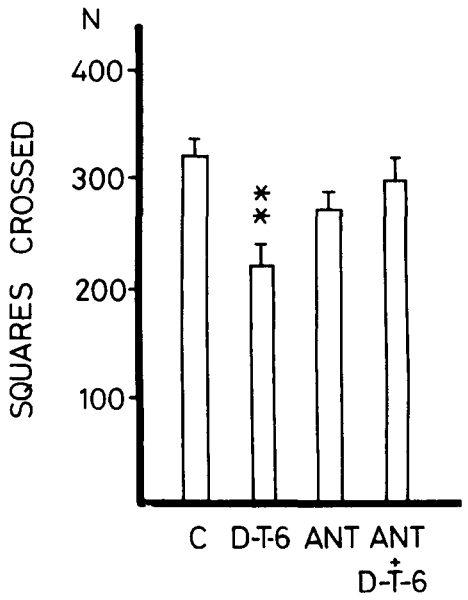

FIG. 1. Locomotor activity after injection with LH-RH analogs. The effects on ambulation (numbers of squares crossed) are shown. (N = 10 in each group.) C: saline-treated control group. D-T-6: D-Trp-6-LH-RH; ANT: ORG 30276 (100 μg/kg SC) **$p < 0.01$ vs. control.

In the hot-plate test, D-Trp-6-LH-RH significantly increased the pain-threshold latency of mice (Fig. 2). The one-way ANOVA revealed significant effect either 30 min or 60 min after the second peptide injection [30 min: $F(3,36) = 4.12$, $p<0.05$; 60 min: $F(3,36) = 6.74$, $p<0.01$]. ORG 30276 did not display a significant analgesic effect, but inhibited that of the agonist. This inhibition reached a level of significance after 60 min, when the latencies in the group with combined treatment dropped below the predrug-state values. In the tail-flick test, the situation was quite similar, $F(3,36) = 10.91$, $p<0.001$, $F(3,36) = 10.11$, $p<0.001$, the only difference being demonstrated in the time-course of the agonist-induced analgesia: the latency increase by D-Trp-6-LH-RH was significant only after 30 min (Fig. 3). The difference between the latency changes of the agonist and the antagonist was significant at both time points.

In the apomorphine-induced cage-climbing test (Fig. 4), the ANOVA also revealed significant differences, $F(9,90) = 30.76$, $p<0.001$. Both D-Trp-6-LH-RH and ORG 30276 inhibited stereotyped behavior in a dose-dependent manner (the antagonist showed a slightly larger inhibition than the agonist in the largest dose). After the combined treatments the inhibition was basically unchanged, moreover, after combined treatments with the largest dose the inhibition was even less than after treatment with either peptide alone.

DISCUSSION

The initial action of LH-RH at the surface of its target cells is the binding to specific membrane receptors. From comparisons of labeled LH-RH agonists or antagonists, several authors

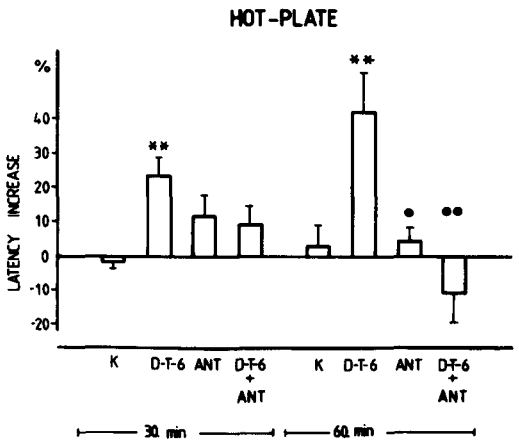

FIG. 2. Hot-plate test. The percentage increases of pain threshold latencies are shown 30 and 60 min after injection with D-Trp-6-LH-RH. (N = 10 in each group.) C: saline-treated control group. D-T-6: D-Trp-6-LH-RH; ANT: ORG 30276 (100 μg/kg SC) *$p<0.05$; **$p<0.01$ vs. control; ●$p<0.05$; ●●$p<0.01$ vs. agonist alone.

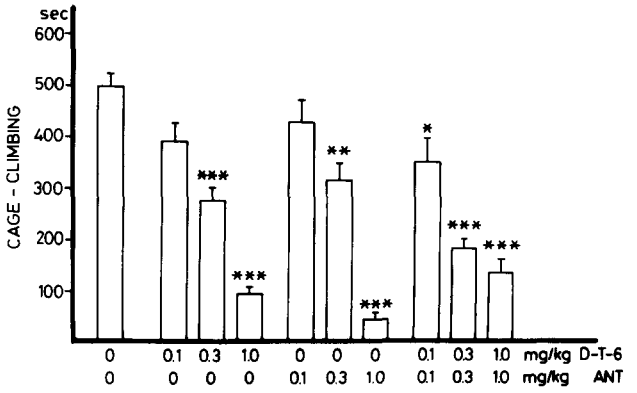

FIG. 4. Apomorphine-induced cage climbing. The duration of stereotyped behavior in seconds during a 10-min session is shown. (N = 10 in each group.) Zero denotes saline treatment. D-T-6: D-Trp-6-LH-RH; ANT: ORG 30276; *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. control.

arrived at the same conclusion that LH-RH agonists and antagonists bind to the same receptor. Perrin et al. (30) found similar kinetic and equilibrium constants for agonists and antagonists and the binding affinities of agonists and antagonists were not significantly different from one another when either an agonist or an antagonist was used as a ragioligand in the receptor assays. This was consistent with the results of another binding study (5). Photoaffinity studies by this latter group (6) led to the same conclusion. Clayton and Catt (2) and Perrin et al. (31) noted that there was a general positive correlation between receptor binding affinity and relative in vitro antagonist activity. The above conclusions were all drawn from research on pituitary LH-RH receptors. As extrapituitary LH-RH receptors are present in high density in specific areas of the brain (10,34), and the binding characteristics of LH-RH receptors in hippocampal membranes were the same as in pituitary membranes (16), it seems that at least some of the brain LH-RH receptors are identical with pituitary LH-RH receptors.

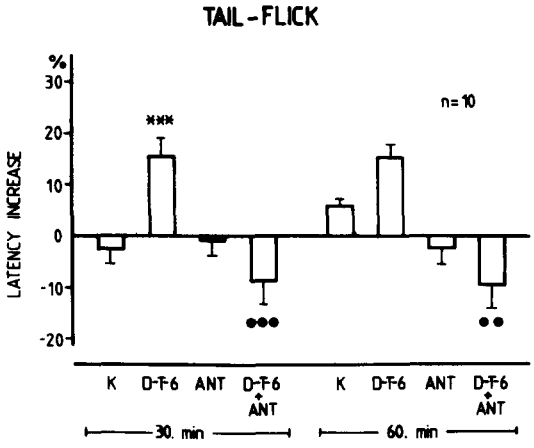

FIG. 3. Tail-flick test. The percentage increases of pain threshold latencies are shown 30 and 60 min after injection with D-Trp-6-LH-RH. (N = 10 in each group.) C: saline-treated control group. D-T-6: D-Trp-6-LH-RH; ANT: ORG 30276 (100 μg/kg SC) ***$p<0.001$ vs. control; ●●$p<0.01$; ●●●$p<0.001$ vs. agonist alone.

The ORG 30276 antagonist is the most potent first-generation LH-RH antagonist with modifications in positions 1, 2, 3, 6, 10, and which has a fairly long biological half-life (4), and, thus, which can maintain an effective competition at the brain receptor level with endogenous LH-RH and the exogenous LH-RH agonist.

If the above supposition is valid, the behavioral actions of D-Trp-6-LH-RH may be mediated through specific LH-RH receptors, and the antagonist ORG 30276 inhibits the effects competitively at the membrane receptor level. This is a possible explanation of the inhibition of the locomotor and analgesic activities of D-Trp-6-LH-RH. The inhibition of apomorphine-induced stereotyped cage-climbing by D-Trp-6-LH-RH was not antagonized by ORG 30276, as the antagonist itself suppressed the behavior.

There are several possible explanations of this finding: 1) the inhibition of apomorphine-induced cage-climbing is not mediated through LH-RH receptors, but a separate indirect mechanism is involved, 2) a special or different type of LH-RH receptor is involved, or 3) the antagonist binds to LH-RH receptors and causes also excitation, thus it seems to be a partial agonist in this effect. The first assumption may be supported by our previous findings that the opiate receptor antagonist, naloxone antagonized the behavioral actions of D-Trp-6-LH-RH. The last assumption may be supported by the in vitro finding that an antagonist can exhibit agonist properties when the antagonist is capable of causing receptor microaggregation (3).

Overall, the present experiments indicate that the behavioral actions of the LH-RH agonist are mediated through specific LH-RH receptors. The site of action of peripherally injected LH-RH analogs is most probably central, since D-Trp-LH-RH injected intracerebroventricularly into rats caused potent behavioral effects with characteristics similar to those demonstrated in mice (Kádár et al., submitted). Possible areas suggested for future investigation by the present findings are the biochemical analysis of LH-RH, neurotransmitter of LH-RH, neuropeptide interactions in the brain, and the changes in parameters of central LH-RH under human pathologic conditions.

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