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Temporal Characteristics of Appetitive Stimulus Effects of Luteinizing Hormone-Releasing Hormone in Male Rats

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DE BEUN, R., E. JANSEN, N. E. GEERTS, J. L. SLANGEN AND N. E. VAN DE POLL. *Temporal characteristics of appetitive stimulus effects of luteinizing hormone-releasing hormone in male rats.* PHARMACOL BIOCHEM BEHAV **42**(3) 445–450, 1992.—Conditioned place preference, induced by intraperitoneal injections of 5 μg/kg luteinizing hormone-releasing hormone (LHRH), was studied by varying the interval between the injection of LHRH and the conditioning sessions. Place preference was investigated for five presession intervals (0, 15, 45, 75, and 120 min) in separate groups of gonadectomized male rats provided with a subcutaneous testosterone implant. It was shown that the presession interval is an important parameter in the development of LHRH-induced conditioned place preference. Place preference was not observed after conditioning with intervals of 0, 75, and 120 min. With 15 and 45 min, however, a reliable preference was induced by LHRH. This study provides insight into the onset and offset of the appetitive stimulus properties of LHRH in male rats.

Conditioned place preference LHRH Time dependent Male rats

THE peptide luteinizing hormone-releasing hormone (LHRH) has been reported to induce conditioned place-preference (CPP) in rats. Pairing of IP injections of LHRH with a distinctive environment was found to increase approach behavior toward this environment (5,6). This finding may be taken as evidence for an unconditioned appetitive stimulus effect of LHRH (13,20,21,26,31).

Several experiments have now shown that stimulus properties of LHRH depend upon specific parameters. LHRH-induced CPP could thus far only be established in male rats and not in female rats. In males, CPP was produced in gonadectomized (GDX) animals with either an SC testosterone (T) or estradiol (E_2) implant and also in gonadally intact males, but not in GDX animals without an SC steroid implant (5,6). In females, on the other hand, there was a lack of effect of LHRH in GDX animals with either an SC E_2 or T implant. Neither did CPP develop in intact females nor in GDX animals without steroid substitution [(6); unpublished results]. Besides demonstrating a sex-dependent effect, these findings also indicate that in male rats the level of circulating sex steroids is a critical factor for LHRH-induced CPP, as there was a lack of effect in GDX animals without an implant and thus with extremely low levels of circulating sex steroids (5,30). In addition, the LHRH-induced CPP in males was found to be dose dependent. In GDX animals with an SC T implant, 5 and 1 μg/kg resulted in CPP (1 μg being less effective than 5 μg), whereas 200 ng/kg did not (5).

The qualitative nature of the subjective effects that a drug produces is the principal determinant of the potential to act as an unconditioned stimulus (US). However, the magnitude of a drug-induced CPP effect and even its quality (appetitive or aversive) has been found to depend upon various parameters. For instance, variables like route of administration, dose, vehicle used, interval between drug administration and conditioning session, number of conditioning sessions, and stimulus effects on perception and locomotion may all influence the development of CPP (20,22,26,31).

Among these variables, the presession interval (PI) has been reported to be a crucial variable in the acquisition of CPP, affecting both quantitative as well as qualitative aspects of the stimulus effect. It appears that drug-induced CPP requires substantial overlap between exposure to the environmental cues [the conditioned stimuli (CS)] and the affective stimulus effects (2,3,9,11,22,27). Injections at some time prior to the conditioning sessions are effective, but the same injections with substantially longer PIs or just after the sessions are usually ineffective in establishing CPP. The available data thus suggests that appetitive stimulus effects of drug treatment

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have to be present during the conditioning sessions to induce CPP. In contrast to other classical conditioning paradigms (4,26,31), forward (or delay) conditioning in a CPP design appears to be a weak design for detecting appetitive stimulus properties of drugs (2,3,9,11,22,27). Interestingly, some drugs (nicotine, amphetamine) that induce CPP when injected prior to the association sessions have been found to induce conditioned place aversion (CPA) when injected immediately after the sessions (9–11).

The purpose of the present study was to expand the CPP results obtained with LHRH by varying the PI to gain insight into the time course of the appetitive stimulus properties of this hormone. In this article, the onset and offset of the appetitive stimulus properties of systemically injected LHRH were investigated in GDX male rats provided with an SC T implant by injecting a single dose (5 μg/kg, IP) of the hormone at different intervals prior to the conditioning sessions. In addition to the already used PI of 15 min (5,6), PIs of 0, 45, 75, and 120 min were introduced.

METHOD

Subjects

Sixty male Wistar rats were used (HSD/CPB; Zeist, The Netherlands), 12 animals per experimental group. All rats were 5 weeks old when they arrived at our laboratory and were maintained in groups of four per cage under a reversed light/dark cycle (lights off from 0700 1900 h). After arrival, they were handled twice a week during 3 weeks. Food (standard pellets, Hope Farms B.V., Woerden, The Netherlands) and tapwater were supplied ad lib. Room temperature was kept constant at 19.5–21°C. At the age of 6 weeks, subjects were gonadectomized under hypnorm anesthesia. During anesthesia, they also received a silastic SC T implant to preserve the negative feedback of T on LHRH and gonadotropins. All behavioral sessions were conducted during the dark phase of the subject's light/dark cycle (in monochromatic red light). Testing started when subjects were 8 weeks of age (mean body weight was 235 g) and ended at the age of 10 weeks (mean body weight was 287 g).

Apparatus and Experimental Conditions

Both the adaptation session and preference test took place in a two-compartment preference box. The apparatus was described in detail previously (5) and will at present only be described briefly. One compartment had black walls, whereas the other compartment had white walls. The black and white parts of the box were of equal size and were separated by a small area with grey walls. The floor of all three parts of the box was grey. Frequencies of entrance and duration of time spent on the three different locations were registered by infrared beam interruption. Conditioning sessions took place in separate black and white boxes that were similar to the compartments of the test box. To mask sudden noises, a radio was always tuned on a station broadcasting popular music, providing a background noise of 65–75 dB(A) in the experimental room.

Drugs

Synthetic LHRH (LHRH acetate salt, peptide content approximately 87%, Sigma Chemical Company, St Louis, MO) was dissolved in 0.9% NaCl. Solutions of LHRH or an equal volume of vehicle were injected IP in a volume of 1 ml/kg. Small samples of the solution were stored at −80°C and an amount required for 1 day was warmed to room temperature just prior to experimentation. For the silastic T implants (length 1.1 cm, i.d. 1.6 mm, o.d. 2.5 mm, Dow Corning Corp., Midland, MI), 4-androsten-17 β-*ol*-3-one testosterone was used (Steraloids Inc., Wilton, CT).

Procedure

Three weeks after arrival at the laboratory and 2 weeks after the gonadectomy, behavioral sessions started with an adaptation session. During this session, an unconditioned baseline preference was established by allowing animals free access to the black and white compartments of the preference box for 60 min (under nondrug condition). From the next day on, subjects were injected daily with LHRH or vehicle and thereafter placed in one of the two conditioning boxes for 30 min. LHRH and vehicle injections were alternated during 8 days, LHRH thus being associated four times with one of the boxes (black or white) and vehicle four times with the other box. The treatment design was fully balanced: For half the animals of each group ($N = 6$), LHRH injections were paired with placement in the black box; for the other half, it was paired with the white box. Half the animals in each subgroup ($N = 3$) started their association sessions in the white box (and consequently finished in the black box), whereas the other animals started their conditioning sessions in the black box and finished in the white box. In all experimental groups ($N = 12$), 5 μg/kg LHRH was used. The parameter that varied between groups was the PI: Five different presession intervals were studied—0, 15, 45, 75, and 120 min [data of the 15-min condition were previously published in another article (5)]. Twenty-four hours after the last conditioning session, the development of CPP was tested. Similar to the adaptation session, animals were not injected prior to the preference test and were allowed free access to the black and white compartments for 60 min. Time spent on the side of the box associated with LHRH injections before (adaptation session) and after (preference test) conditioning was compared and taken as an index for LHRH-induced CPP. Number of entrances into the two compartments of the box was taken as an index for locomotor activity.

Statistics

Data (time spent on LHRH-associated side) were submitted to analysis of variance (ANOVA) with repeated measures. The design consisted of one between-subjects factor (PI, five levels) and two within-subjects factors (conditioning, two levels: pre- and postconditioning data; and period, four levels: four subsequent periods of 15 min within the 60-min adaptation session and preference test). Additional ANOVAs for separate experimental groups were also conducted and posthoc analysis took place with two-tailed paired *t*-tests. Results were considered significant when $p < 0.05$.

RESULTS

Time spent on the side of the preference box associated with LHRH injections both during the adaptation session (unconditioned preference) and preference test (conditioned preference) is presented in Fig. 1.

There was a significant effect of conditioning, $F(1, 55) = 5.36, p < 0.05$, which was to a certain extent dependent upon the PI used: the PI × conditioning interaction showed a sta-

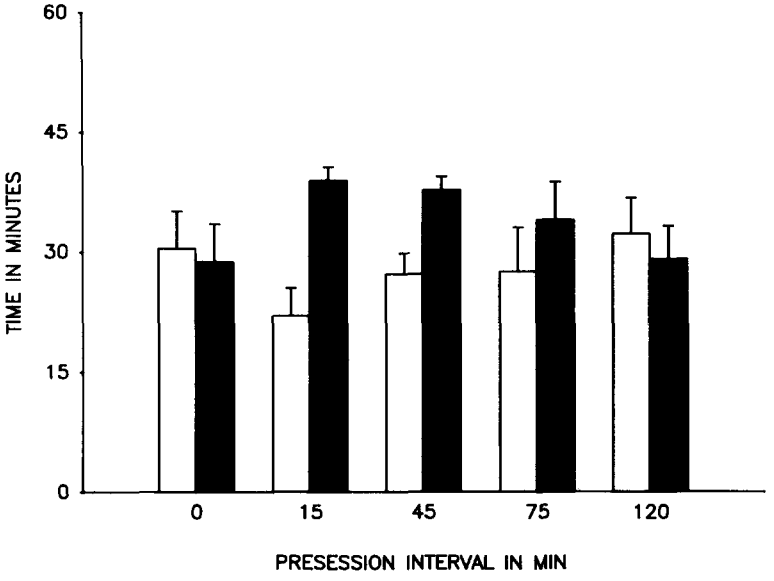

FIG. 1. Time spent on the LHRH-associated side of the preference box before (open bars) and after (filled bars) conditioning. Represented are mean time and SEM of five experimental groups (n = 12) with different presession intervals.

tistical tendency, $F(4, 55) = 2.25$, $p = 0.075$. In addition, a significant effect was found for PI × conditioning × period: $F(12, 165) = 1.89$, $p < 0.05$.

Further analysis (ANOVA for separate PIs with only the within-subjects factors conditioning and period) did not reveal any significant effect with PIs of 0, 75, or 120 min. With a PI of 15 min, significant effects of conditioning and conditioning × period were found, $F(1, 11) = 14.85$ and $F(3, 33) = 4.55$, respectively, both $p < 0.01$, and a similar conditioning effect was seen with the 45-min PI, $F(1, 11) = 9.79$, $p < 0.01$. This effect was again period dependent: conditioning × period, $F(3, 33) = 3.01$, $p < 0.05$. To illustrate the conditioning × period effects, Fig. 2 depicts per period of 15 min the difference scores of time spent on the LHRH-associated side during the preference test minus time spent on this LHRH-associated side of the box during the adaptation session. Positive values indicate a shift in preference toward the LHRH-associated side.

The conditioning × period interaction was further analyzed with t-test comparisons between data obtained during the adaptation session and preference test for each 15-min period separately. For the 15-min PI, significant preference shifts were noted for all four periods with $t(11)$ values of -2.34, -2.92, -2.96, and -4.31, all $p < 0.05$, respectively, for periods 1–4. For the 45-min PI, a trend for a preference shift was seen for the first two periods, $t(11) = -1.88$ and -2.10, with $p = 0.087$ and $p = 0.060$, respectively. The last two periods showed a significant difference with $t(11) = -3.09$, $p < 0.01$, for the third and $t(11) = 2.28$, $p < 0.05$, for the fourth period. In the 0-, 75- and 120-min PI groups, not a single period was found with a significant shift in preference (Fig. 2).

Number of entrances of the two compartments of the preference box (taken as index for locomotor activity) were analyzed for the 60-min preference test with an ANOVA without the within-subjects factor conditioning. This analysis revealed a difference between groups in number of entrances: PI, $F(4, 55) = 8.97$, $p < 0.001$. The mean number of entrances was 81. The least activity was noted in the 75-min PI group and most active were animals in the 45-min PI group. The number of entrances significantly decreased during the 60-min preference test: period, $F(3, 165) = 194.27$, $p < 0.001$. This decline of locomotor activity was found in all groups; no significant interaction effect of PI × period was found.

DISCUSSION

The present results demonstrate that the LHRH-induced conditioned place-preference in male rats, as assessed in earlier experiments (5,6), is time dependent. For this peptide, a clear relationship was found between presession interval and expression of CPP. Both the onset as well as the offset of the stimulus effect were established in this study. When animals were injected immediately prior to, or 75 or 120 min prior to 30-min exposure to distinct environmental cues, CPP did not develop. Only the intermediate presession intervals of 15 and 45 min were effective in producing a CPP effect.

There is evidence to suggest that induction of CPP requires a substantial overlap in time between the appetitive effects of the drug and the exposure to the distinct environmental stimuli (2,3,9,11,22,27). This overlap hypothesis is, for instance, supported by the results of Bardo and Neisewander (3), who showed that CPP developed with an IV injection of morphine just prior to a single 30-min conditioning session. Interestingly, this effect was not blocked by an IV naloxone injection immediately after the conditioning session. This indicates that effects of morphine present during, and not after, the conditioning sessions are of relevance here. If, indeed, overlap between stimulus effect of a drug and exposure to particular environmental stimuli is a prerequisite for CPP, then the lack of effect with a PI of 0 min indicates that the stimulus properties of 5μg/kg LHRH, injected IP, were not sufficiently present within about 30 min postinjection time. The failure to find CPP with PIs of 75 and 120 min, together with the effectiveness of both the 15- and 45-min PI, suggests that after onset the stimulus effect remained present for at least about 15 min

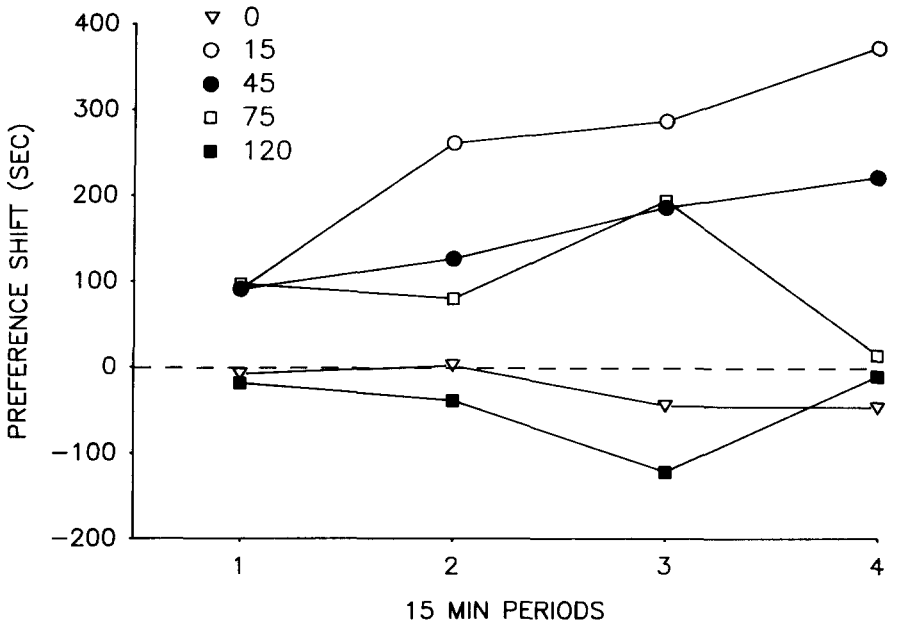

FIG. 2. Differences in time spent on the LHRH-associated side of the preference box before and after conditioning for four subsequent periods of 15 min. Represented are mean preference shifts of five experimental groups with different presession intervals (n = 12). Positive and negative values on the ordinate denote an increase and a decrease, respectively, in time spent on the LHRH-associated side after conditioning.

and at the utmost for about 45 min. The offset of the stimulus effect of LHRH thus took place somewhere between 45 and 75 min after injection.

However, it should be realized that unambiguous evidence, supporting the "substantial overlap" condition as a prerequisite for the development of CPP, is still scarce. The empirical finding that CPP can only be established by injecting a drug prior to, or during, the association sessions and not when injected after the sessions could still be explained in terms of delay (forward) conditioning. The relevant appetitive aspects of drug administration (i.e., those interoceptive effects likely to be associated with exteroceptive stimuli) may have a long delay of onset and be manifested only after the conditioning sessions, even when the drug is injected prior to these sessions. A lack of CPP seen with lengthening of the PI may then be due to ineffective simultaneous or backward conditioning (26,31). In line with this, injecting the drug after the association sessions would lead to a CS–US interval that is simply too long to be effective (26,31). The fact that in drug discrimination procedures relatively short PIs are usually found to be effective does not necessarily refute this "long delay of effect" hypothesis since it has been suggested that one should dissociate for a given drug the discriminative stimulus effects from the affective stimulus effects (16,17).

Aside from the question whether the development of CPP requires overlap or delay of effect, it can be observed that the onset of the appetitive stimulus effect of LHRH as measured with CPP is relatively slow as compared with various drugs from different pharmacological classes. For example, the opiate morphine, injected IP immediately before conditioning sessions of 20-min duration, was found to induce CPP (27), and similar effects were found with the psychostimulants nicotine and amphetamine injected SC just prior to sessions of 20 min (9–11). In addition, the IP-injected peptide substance P showed also CPP with a short PI of 1 min and a session duration of 15 min (23). It is not clear why there is such a long latency of appetitive effect of LHRH, but it has been reported to be typical for peptides in general that behavioral effects are only evident at some considerable time after administration (14,15,19,24). This lag of time between injection of the peptide and the onset of action has been found for most peptides thus far studied for behavioral effects [see also (32)], for example, corticotropin (ACTH), endorphins, enkephalins, α-MSH, neuropeptide Y, oxytocin, somatostatin, and vasopressin. Furthermore, the delayed onset of effect was observed for a variety of behavioral parameters, including, for example, aggressive and sexual behavior, explorative and locomotor activity, (conditioned) avoidance behavior, startle response, several (operant) learning tasks, and memory (14, 15,19,24,32). For LHRH in particular, administered either peripherally or centrally, relatively long latencies of behavioral effects have been reported (14,15,18,19,24). After systemic injection, levels of circulating LHRH often become undetectable before substantial behavioral effects can be observed. In rats, the half-life of disappearance from the plasma is approximately 7.5 min when radioactivity of tritiated LHRH is measured between 3 and 10 min after IV injection (7), whereas it is not unusual that behavioral effects of LHRH are only apparent after more than an hour has elapsed since the administration. For example, systemic injections have been shown to facilitate various aspects of sexual behavior, in both male and female rats, after about 1.5–2 h. These effects persisted up to 6–8 h after injection (14,15,18,19,24).

The longest PI effective in producing CPP was 45 min, which is not exceptional according to the literature. Although PIs of 0–20 min are most often used in CPP experiments, longer PIs have been reported to be effective, even as long as 120 min with IP amphetamine (28). The lack of effect with

the 75- and 120-min PIs may have been due to the ineffectiveness of backward conditioning, where US presentation (LHRH effect) occurs some time before CS presentation (environmental cues). There is some controversy as to whether or not conditioning can be established with backward conditioning, but normally conditioning is very poor, if not completely absent (4,26,31).

The mechanisms by which the behavioral effects of LHRH, including the CPP effect, are exerted after systemic injections remain to be clarified. There are, however, indications that LHRH is acting as a neuromodulator in the CNS of rodents. Results obtained with the Everett potentiation test (8) revealed that IP-injected LHRH enhances behavioral effects of IP-administered L-DOPA and serotonin in mice (24). In addition, studies investigating the effects of serotonergic, dopaminergic, α-, and β-adrenergic receptor blockers on the LHRH-induced facilitation of lordotic behavior of female rats supported the view that LHRH modulates behavior indirectly by interacting with these neurotransmitter systems (19). With regard to the CPP effects of LHRH, the possible interaction with dopamine is especially of interest because results from a large number of studies suggest that this neurotransmitter has an important role in the acquisition and expression of CPP [see (13)]. Future research should thus be aimed at investigating the involvement of dopaminergic neurotransmission in the LHRH-induced CPP. However, although numerous studies indicate that systemic injections of LHRH may indeed influence various aspects of behavior by affecting CNS activity (14,15,18, 24,32), nothing is at present known about the mechanism responsible for the appetitive effects of LHRH. It therefore remains open whether or not the LHRH-induced CPP is actually dependent upon binding of LHRH to specific receptors located within the CNS. The possibility exists that peripheral mechanisms are involved in the produced conditioning, as it is well known that binding sites for LHRH are present in peripheral tissues as, for example, the anterior pituitary (1,25).

With respect to the mechanism of action of LHRH, an additional research question may be whether or not LHRH induces CPP when injected after the conditioning sessions. General depressants of CNS activity such as diazepam and morphine, for which the temporal characteristics of affective stimulus effects have been studied, only induced CPP when injected prior to (or during) the conditioning sessions (which has become the standard CPP procedure) and had no effect when injected after the sessions (27,29). On the other hand, general stimulants of CNS activity such as nicotine and amphetamine produced CPP when injected before, but conditioned place aversion when injected after, exposure to a distinct environment (10,11). It might be worthwhile to be able to classify LHRH as a depressant or a stimulant dependent upon whether this peptide shows a lack of CPP effect or whether it induces CPA, respectively, when injected after the association sessions. However, one should be cautious about such a classification. First, only a limited number of drugs have been investigated for their temporal stimulus characteristics in a CPP procedure and generalizations are therefore at this moment precarious (10,11,27,29). Second, the temporal characteristics of the CPP stimulus effect are only one aspect of the action of a drug. There are other parameters (e.g., dose–response function) of the stimulus effect, possibly leading to another classification when between-drug comparisons are made. Furthermore, the possibility exists that LHRH injections immediately after the conditioning sessions still induce CPP, in which case the interpretation of the effect becomes problematic.

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