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Influence of Luteinizing Hormone Releasing Hormone (LHRH) on the Behavioral Effects of Amphetamine in Rats[1]

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MORA, S. AND G. DÍAZ-VÉLIZ. *Influence of luteinizing hormone releasing hormone (LHRH) on the behavioral effects of amphetamine in rats.* PHARMACOL BIOCHEM BEHAV **19**(2) 157–161, 1983.—The influence of luteinizing hormone releasing hormone (LHRH) on the behavioral effects induced by several doses of D-amphetamine (0.25, 0.5, 1.0 and 2.0 mg/kg IP) was studied. A dose response relation was previously established for the effects of LHRH (50, 100 and 200 μg/kg SC) on acquisition and retention of conditioned avoidance responses (CARs). The neuropeptide impaired acquisition and improved retention of CARs, without modifying spontaneous motor activity. Pretreatment with 100 μg/kg of LHRH antagonizes the enhancement in acquisition of CARs due to D-amphetamine 0.5, 1.0 and 2.0 mg/kg, the impairment in retention induced by amphetamine 1.0 and 2.0 mg/kg, and the hypermotility and the increased rearing behavior induced by amphetamine 1.0 and 2.0 mg/kg. These results suggest that brain catecholamines, particularly dopamine, could play a role in the behavioral effects of LHRH. Interactions between LHRH and central dopaminergic mechanisms are discussed.

LHRH Amphetamine Avoidance behavior Dopamine Motor activity

A SUBSTANTIAL amount of evidence indicates that hypothalamic releasing hormones may exert brain effects in addition to their actions on the anterior pituitary gland. These brain effects may have behavioral or neurologic consequences. Investigation of luteinizing hormone releasing hormone (LHRH) has offered the most direct evidence of a central action for this peptide. LHRH injected either subcutaneously or into a cerebral ventricle potentiates mating behavior in estrogen-primed ovariectomized and hypophysectomized female rats [13,19] as well as testosterone-primed castrated male rats [14]. It has been suggested that LHRH neurons of the preoptic-hypothalamic regions may exert an influence on the brain by a system of collateral fibers that could project to various hypothalamic and extrahypothalamic areas where LHRH could modulate sexual performance, either directly or indirectly, through a catecholamine system [15]. Other behavioral effects of LHRH have also been described. In fact, an extensive study has shown that LHRH potentiates the behavioral effects of DOPA both in normal intact as well as hypophysectomized mice treated with pargyline [20]. LHRH also enhances the stimulant properties of 5-HTP, a serotonin precursor, in pargyline treated mice [20]. Central administration of LHRH markedly reduces barbiturate-induced sleeping time [1]. After subcutaneous administration this hormone has demonstrated to be as potent as $ACTH_{4-7}$ in inhibiting the extinction of a pole-jumping avoidance response [2]. Recently, we have demonstrated that subcutaneous administration of LHRH (100 μg/kg) impairs the acquisition of a conditioned avoidance response in intact normal as well as castrated male rats [11]. In addition, LHRH antagonizes the dose related impairment in acquisition and retention performance induced by testosterone in castrated animals [12].

Microelectrophoresis of hypothalamic hormones has revealed both excitatory and inhibitory effects of TRH, LHRH and somatostatin on hypothalamic [4] as well as extrahypothalamic neurons [21]. This ability of the hypothalamic peptides to alter neuronal excitability has suggested transmitter or modulator roles for these neuropeptides. The postulate that neuropeptides act as neuromodulators includes the premise that their effects might be the consequence of interactions with "classical" neurotransmitter systems. The results of several experiments clearly indicate that some neuropeptides indeed exert at least some of their effects on

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brain function via an interaction with catecholamine neurotransmission in discrete brain regions [3].

This work was carried out in order to establish a dose-response relationship for the behavioral effects of LHRH and elucidate a possible role for catecholamines in these effects. We studied the interaction between LHRH and amphetamine administered in several dose levels. The mechanism of action of amphetamine is fairly clearly defined. This drug enhances motor activity and both acquisition and retention of several conditioning tasks in rodents [18]. These behavioral changes have been correlated with an increased release of brain catecholamines, dopamine and norepinephrine [5].

METHOD

Animals

A total of 218 male Sprague Dawley rats weighing 220±10 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. All 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) was dissolved in 2% benzyl alcohol and D-amphetamine sulphate in saline, to appropiate concentrations, so that in all cases the doses to be injected were in a volume of 0.1 ml/100 g of body weight. In order to establish dose-response relationship, LHRH was administered subcutaneously (SC) in doses of 50, 100 or 200 μg/kg. Control animals received 2% benzyl alcohol. Interaction between LHRH and amphetamine was studied using LHRH 100 μg/kg or 2% benzyl alcohol injected 60 min before intraperitoneal (IP) administration of D-amphetamine sulphate (0.25, 0.5, 1.0 or 2.0 mg/kg) or saline. LHRH (HRF^R) was kindly donated by Laboratorios Ayerst, Brazil.

Spontaneous Motor Activity

The animals were individually placed in an Activity Platform (Lafayette Instrument Co.) connected to an electromechanical counter and spontaneous motor activity was recorded for 15 min beginning 5 min after placing the animal in the platform. Simultaneously the following responses were recorded: number of rearings, head shaking and the time (seconds) spent in grooming behavior.

Active Avoidance Conditioning

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

Each animal was submitted to two sessions of shuttle avoidance conditioning with an interval of seven 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 which after 5 sec was overlapped with a foot-shock until the animal escaped to the opposite chamber. A conditioned response (CAR) was defined as a crossing within 5 sec. Intertone interval was 30 sec. Animals were considered conditioned when they fullfilled the following criterion: at least 5 CARs in the first 30 trials and at least 10 CARs in the last 20 trials. "Retention" was considered as the difference in the same animal's performance between the two sessions.

Schedule of Drug Administration

In the study of the dose-response relationship for LHRH each animal was injected SC with LHRH or 2% benzyl alcohol. After 90 min its spontaneous motor activity was measured and 30 min later it was submitted to the acquisition session. When interaction LHRH × amphetamine was studied each animal was also injected with amphetamine or saline IP 60 min after LHRH treatment.

Data Analysis and Statistics

One-way and two-way analysis of variance for groups of unequal size were used to determine the level of significance of treatment effects. Significant differences between groups were determined by the Newman-Keuls Multiple Comparison Procedure. Comparisons between treated groups and their controls were assessed by the Dunnett's test. The use of additional statistical tests is indicated in context. In all cases differences were considered to be significant when p was equal to or less than 0.05.

RESULTS

Study of the Dose-Relationship for LHRH

(a) Spontaneous motor activity and other behavioral responses. No significant modification in spontaneous motor activity, rearing behavior, grooming nor head shaking was observed after LHRH administration.

(b) Acquisition and retention of CARs. One-way analysis of variance indicated that LHRH caused a significant impairment in the percent of CARs performed in the acquisition session, $F(3,50)=3.4424$, $p<0.025$ (Fig. 1A). Dunnett's test for comparison of the treated groups with the control revealed that the effects of LHRH 100 and 200 μg/kg were significant. There was a good correlation between the dose of LHRH and the impairment in the acquisition ($r=-0.9638$, $p<0.001$). There was also a significant dose-response decrease ($r=-0.9840$, $p<0.01$) in the number of animals achieving the conditioning criterion (Table 1). Chi-square analysis demonstrated significant depressant effects of LHRH 100 and 200 μg/kg.

Retention of CARs was not significantly influenced by LHRH 50 or 100 μg/kg but a significant improvement was observed in the animals treated with LHRH 200 μg/kg (Fig. 1B).

Interaction Between LHRH 100 μg/kg and Amphetamine

(a) Spontaneous motor activity and other behavioral responses. The influence of pretreatment with LHRH 100 μg/kg on the hypermotility and rearing behavior induced by different doses of amphetamine is presented in Fig. 2. Two-way analysis of variance indicated significant main effects of amphetamine and LHRH on spontaneous motor activity, $F(4,137)=6.7528$, $p<0.0001$ and $F(1,137)=8.6002$, $p<0.005$, respectively, and rearing behavior, $F(4,136)=12.1203$, $p<0.001$ and $F(1,136)=9.3768$, $p<0.005$. Newman-Keuls

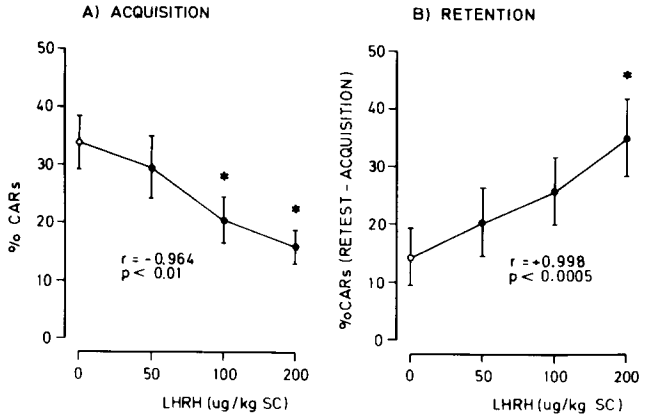

FIG. 1. Effect of LHRH SC on the acquisition and retention of conditioned avoidance behavior. Each point represents the mean±SE of 13–14 animals in each group. Comparisons with controls were assessed by Dunnett's test (*$p<0.05$).

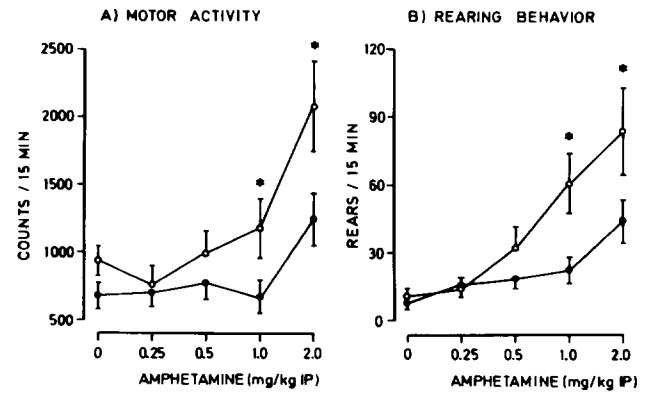

FIG. 2. Effects of the pretreatment with LHRH 100 μg/kg SC (●) or solvent (2% benzyl alcohol) (○) on the motor activity and rearing behavior induced by increasing doses of amphetamine. Each point represents the mean±SE of 12–16 animals in each group. Comparisons between specific pairs of means was performed using Newman-Keuls Procedure (*$p<0.05$).

comparison procedure revealed that no significant change was induced by LHRH in absence of amphetamine. Nevertheless, this hormone was effective in reducing the stimulation induced by the higher doses of amphetamine (1.0 and 2.0 mg/kg). LHRH was unable to modify the amphetamine-induced effects on grooming behavior and head shakings.

(b) Acquisition and retention of CARs. Fig. 3A shows the effects of LHRH and amphetamine in the acquisition of CARs. A two-way analysis of variance of the acquisition data indicated significant effects of amphetamine, $F(4,127)=19.1896$, $p<0.0001$ and LHRH, $F(1,127)=46.3453$, $p<0.0001$, and an interaction between both drugs, $F(4,127)=3.3016$, $p<0.02$. Newman-Keuls comparison confirmed that LHRH 100 μg/kg induced a significant impairment in the acquisition of the control animals, and counteracted the improving effects of almost all doses of amphetamine administered.

Both amphetamine and LHRH showed significant main

TABLE 1

EFFECTS OF LHRH ON THE NUMBER OF ANIMALS ACHIEVING THE CONDITIONING CRITERION

Treatment (SC)	Number per total rats studied	Chi-square test*
Solvent	10/14	—
LHRH 50 μg/kg	6/13	n.s.
LHRH 100 μg/kg	3/14	$p<0.005$
LHRH 200 μg/kg	1/13	$p<0.0005$

*Compared with solvent group.

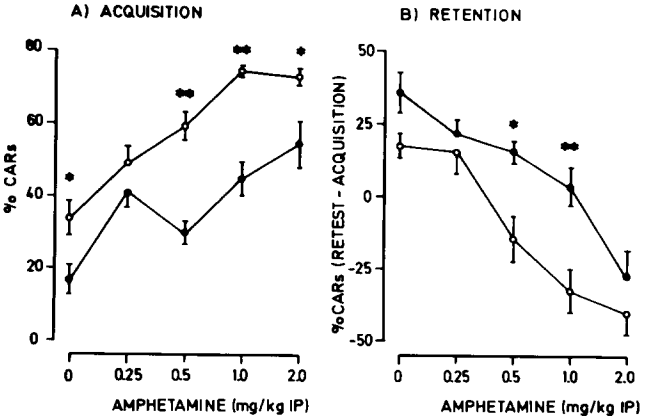

FIG. 3. Influence of the pretreatment with LHRH 100 μg/kg SC (●) or solvent (2% benzyl alcohol) (○) on the amphetamine-induced effects on acquisition and retention of CARs. Each point represents the mean±SE of 12–16 animals in each group. Comparisons between specific pairs of means was evaluated by Newman-Keuls Procedure (*$p<0.05$ and **$p<0.001$).

effects on the retention of CARs, assessed by two-way analysis of variance. In fact, retention was severely impaired in the animals treated with higher doses of amphetamine, $F(4,129)=25.2885$, $p<0.0001$. This effect was attenuated by the pretreatment with LHRH, $F(1,129)=19.0865$, $p<0.0001$. Newman-Keuls procedure indicated that LHRH antagonized the effects of amphetamine 0.5 and 1.0 mg/kg.

DISCUSSION

The present study demonstrates that subcutaneous administration of LHRH is able to induce behavioral effects in male rats. These pharmacological effects seem to be much more evident when the animals are also treated with amphetamine, suggesting an interaction between both drugs.

LHRH administered in high doses induced a dose-dependent impairment in the acquisition and an apparent improvement in the retention of CARs, without modifying significantly the spontaneous motor activity or other behavioral responses. This rules out the possibility that LHRH-induced changes on conditioning behavior are a consequence of effects on rat motor activity. Nevertheless, when injected before amphetamine, the neuropeptide acts apparently as a buffer against behavioral effects of increasing dosages of amphetamine. Pretreatment with LHRH antagonized

both the enhancement in the acquisition and the impairment in the retention of CARs due to amphetamine. Besides, LHRH modified amphetamine-induced effects on spontaneous motor activity and rearing behavior. This interaction between LHRH and amphetamine is significant with the higher doses of amphetamine used in this study.

Dose response relations for the behavioral effects of LHRH have not been widely explored. It is known that LHRH is active in picomole concentrations for pituitary effects; but doses thousand times higher are needed to induce behavioral changes. With neuropeptides the administered dose may seem excessively high, but because of their rapid metabolism the concentration in the brain may still be in physiological range. Furthermore, the blood-brain barrier difficults access of the most potent peptides to the brain tissues and their effects may appear later. The induction of lordosis behavior in ovariectomized-hypophysectomized estrone primed female rats requires small quantities of LHRH (150 to 500 ng SC) [16]. On the other hand, huge doses of LHRH (4 to 8 mg/kg IP) are necessary to potentiate the stimulant properties of L-DOPA and 5-HTP in pargyline pretreated mice [20]. The dose response relation, found by us, indicates that moderate doses of LHRH are needed to modify acquisition of CARs, and facilitates further pharmacological studies. LHRH must be extremely active to be able to induce behavioral effects even after subcutaneous administration. Although LHRH half life in plasma is only 4 min [7], its behavioral effects are present longer than two hours after injection. This is in agreement with the time course for inducing enhancement in lordotic response [13] which appears 2 or 3 hours following SC administration and it is observed for approximately 8 hours. It has been demonstrated that plasma levels of LHRH become indetectable before either endocrine or behavioral effects are evident. The peptide may be quickly destroyed, but perhaps initiates a sustained train of events that gradually reach threshold for expression. This supports the idea that LHRH exerts an indirect effect on nervous tissue.

In view of the evidence that the central action of amphetamine requires the synthesis of catecholamines, the interaction LHRH × amphetamine suggests that central catecholamines could play an important role in the behavioral effects of LHRH. The spontaneous motor activity stimulating action of amphetamine is presumably mediated by release of dopamine (DA) and norepinephrine (NE) from central neurons. The increase in rearing behavior, a component of stereotyped behavior, induced by amphetamine is probably a consequence of release of DA from DA nerve terminals. Both DA and NE appears to be involved in the stimulation of conditioned behaviors observed after amphetamine administration. Even low doses of this drug, that did not modify motor activity induced significant improvement in the acquisition of CARs. In our experimental conditions, this enhancement in acquisition was not followed by a similar increase in the retention of the response. In fact, an amnesic effect was observed in the animals treated with amphetamine 1.0 to 2.0 mg/kg.

The ability to inhibit conditioned avoidance responding, without affecting escape responding [17] and antagonize the stimulating effects of amphetamine, have been considered as a characteristic action of almost all drugs which block central DA receptors [6]. Our results show that LHRH does not fully antagonizes, but only attenuates the stimulation produced by amphetamine. It is not possible to conclude if the site of action of the neuropeptide is presynaptic or postsynaptic.

There are several data supporting the idea of an interaction between LHRH and DA in the rat brain. There is morphological evidence of a precise location of DA varicosities and LHRH terminals within the same regions of the median eminence (ME), supporting the concept of a potential regulatory mechanism of DA on LHRH release [9]. DA has been found to stimulate the release of LHRH from the palisade zone of the ME, as well as from synaptosomal fractions of the medial basal hypothalamus (MBH) [22]. Recent biochemical findings have shown that LHRH can itself suppress DA synthesis in rat corpus striatum, suggesting that LHRH exerts a negative feedback action on DA neurons. That is, LHRH inhibits its own release by inhibiting DA synthesis [23]. This action could also have behavioral consequences and explain, at least in part, the interaction LHRH × amphetamine related in this paper. It is interesting that there is a close similitude between this interaction and the interaction α-methyl-p-tirosine × amphetamine, in the acquisition of CARs. In fact, it has been demonstrated [8] that the enhancement in acquisition induced by amphetamine (1.0 and 2.0 mg/kg) is blocked by the inhibitor of the catecholamine synthesis. A dopaminergic mechanism seems to be involved also in the increase of lordotic behavior induced by LHRH, since amphetamine has been shown to inhibit the lordosis response in a dose-dependent fashion [10]. More specific neurochemical studies are obviously needed to clarify whether the antagonism by LHRH on amphetamine-induced behaviors is due to the blockade by LHRH of amphetamine induced changes in DA metabolism and/or synthesis.

In conclusion, the present findings would suggest that LHRH possess the ability to reduce DA activity in the brain. Through this mechanism LHRH by itself could influence learning and memory processes or modulate the effects of DA activity stimulators, such as amphetamine. These effects may eventually have relevancy in clinical conditions, since a compound antagonizing the effects of amphetamine might find application in the treatment of schizophrenia or other mental diseases.

REFERENCES

1. Bisette, G., C. B. Nemeroff, P. T. Loosen, A. J. Prange, Jr. and M. A. Lipton. Comparison of the analeptic potency of TRH, $ACTH_{4-10}$, LHRH and related peptides. *Pharmacol Biochem Behav* **5:** Suppl 1, 135–138, 1976.
2. de Wied, D., A. Witter and H. M. Greven. Behaviourally active ACTH analogues. *Biochem Pharmacol* **24:** 1463–1468, 1975.
3. de Wied, D. and D. H. G. Versteeg. Neurohypophyseal principles and memory. *Fed Proc* **38:** 2348–2354, 1979.
4. Dyer, R. G. and R. E. J. Dyball. Evidence for a direct effect of LRF and TRF on single unit activity in the rostral hypothalamus. *Nature* **252:** 486–488, 1974.
5. Glowinski, J. and J. Axelrod. Effect of drugs on the uptake, release and metabolism of H^3-norepinephrine in the rat brain. *J Pharmacol Exp Ther* **149:** 43–49, 1965.
6. Janssen, P. A., C. J. E. Niemegeers and K. H. L. Shellekens. It is possible to predict the clinical effects of neuropletic drugs (major tranquillizers) from animal data. *Arzneimitelforsch* **15:** 1196–1206, 1965.
7. Mauk, M. D., G. A. Olson, A. J. Kastin and R. D. Olson. Behavioral effects of LHRH. *Neurosci Biobehav Rev* **4:** 1–8, 1980.

8. Mc Gaugh, J. L. Drug facilitation of learning and memory. *Annu Rev Pharmacol* **13:** 229–241, 1973.
9. Mc Neill, T. H. and J. R. Sladek, Jr. Fluorescence-immunocytochemistry: Simultaneous localization of catecholamines and gonadotropin-releasing hormone. *Science* **200:** 72–74, 1978.
10. Michanek, A. and B. J. Meyerson. Influence of estrogen and progesterone on behavioral effects of apomorphine and amphetamine. *Pharmacol Biochem Behav* **16:** 875–879, 1982.
11. Mora, S., A. G. Nasello and M. Mandelilopes. Luteinizing hormone releasing hormone (LHRH): Depressant effects on rat conditioned avoidance behavior. *IRCS Med Sci* **8:** 933, 1980.
12. Mora, S., A. G. Nasello, M. Mandelli-Lopes and G. Díaz-Véliz. LHRH and rat avoidance behavior: Influence of castration and testosterone. *Physiol Behav* **30:** 19–22, 1983.
13. Moss, R. L. and S. M. Mc Cann. Induction of mating behavior in rats by luteinizing hormone releasing factor. *Science* **181:** 177–179, 1973.
14. Moss, R. L., S. M. Mc Cann and C. A. Dudley. Releasing factors and sexual behavior. *Prog Brain Res* **42:** 37–46, 1975.
15. Moss, R. L., R. Riskind and C. A. Dudley. Effects of LHRH on sexual activities in animal and man. In: *Central Nervous System Effects of Hypothalamic Hormones and Other Peptides*, edited by R. Collu, A. Barbeau, J. R. Ducharme and J. G. Rochefort. New York: Raven Press, 1979, pp. 345–366.
16. Moss, R. L. Actions of hypothalamic hypophysiotropic hormones on the brain. *Annu Rev Physiol* **41:** 617–631, 1979.
17. Niemegeers, D. J. E., F. J. Verbruggen and P. A. J. Janssen. The influence of various neuropletic drugs on shock avoidance responding in rats. I. Nondiscriminated Sidman avoidance procedure. *Psychopharmacologia* **16:** 161–174, 1969.
18. Orsingher, O. A. and S. Fulginiti. Effect of alpha methyl tyrosine and adrenergic blocking agents on the facilitating action of amphetamine and nicotine on learning in rats. *Psychopharmacologia* **19:** 231–240, 1971.
19. Pfaff, D. W. Luteinizing hormone releasing factor potentiates lordosis behavior in hypophysectomized ovariectomized female rats. *Science* **182:** 1148–1149, 1973.
20. Plotnikoff, N. P. and A. J. Kastin. Neuropharmacological review of hypothalamic releasing factors. In: *Neuropeptide Influences on the Brain and Behavior*, edited by L. H. Miller, C. A. Sandman and A. J. Kastin. New York: Raven Press, 1977, pp. 81–107.
21. Renaud, L. P., J. B. Martin and P. Brazeau. Depressant action of TRH, LHRH and somatostatin on activity of central neurons. *Nature* **255:** 233–235, 1975.
22. Rotsztejn, W. H., S. V. Drouva, E. Pattou and C. Kordon. Met-enkephalin inhibits in vitro dopamine-induced LHRH release from mediobasal hypothalamus of male rats. *Nature* **274:** 281–282, 1978.
23. Wang, W. K., L. S. Jenq, Y. Chiang and M. K. Chien. Inhibition of dopamine biosynthesis by gonadotropin-releasing hormone in rat. *Nature* **296:** 354, 1982.