*Pharmacology Biochemistry & Behavior*, Vol. 31, pp. 717–720. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

BRIEF COMMUNICATION

# LHRH Antagonizes Yawning and Genital Grooming Induced by Apomorphine 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*

Received 25 January 1988

MORA, S. AND G. DIAZ-VELIZ. *LHRH antagonizes yawning and genital grooming induced by apomorphine in rats.* PHARMACOL BIOCHEM BEHAV **31**(3) 717–720, 1988.—The effects of the pretreatment with LHRH on the behavioral effects induced by low doses of apomorphine were studied in male rats. Three doses of apomorphine (31.25, 62.50 and 125 μg/kg) were subcutaneously administered two hours after LHRH 100 μg/kg or solvent SC treatment. Apomorphine induced repeated episodes of yawning and genital grooming. Pretreatment with LHRH abolished or reduced yawning and genital grooming induced by the three doses of apomorphine, suggesting that the peptide could induce subsensitivity of DA receptors responsible for yawning and genital grooming.

LHRH Apomorphine Dopamine receptors Yawning Genital grooming

RECENT reports have postulated that LHRH is capable of modulating dopamine (DA) transmission in experimental animals. In fact, behavioral and biochemical findings support the hypothesis of an inhibitory effect of LHRH upon presynaptic synthesis and release of DA (14, 15, 17, 18, 25). Incubation of rat corpus striatum synaptosomes in the presence of LHRH decreased DA synthesis (25). Subcutaneous (14) and intracerebral (18) injection of LHRH in rats inhibited a conditioned avoidance response, which is considered as a characteristic of drugs which block DA transmission (3). In addition, LHRH is able to antagonize the stimulatory effect induced by amphetamine on motor activity and acquisition of conditioned avoidance responses (15). L-DOPA, precursor of DA, reverted the inhibitory effects of LHRH and counteracted the antagonistic effect of LHRH on the action of amphetamine (17).

The administration of small doses of apomorphine produces a behavioral syndrome characterized by hypomotility, yawning, penile erection and genital grooming. These effects are considered to be the consequence of the stimulation of presynaptic DA receptors in the CNS (5, 12, 19). The purpose of the present study was to provide further evidence for a possible interaction between LHRH and DA mechanisms. We studied the influence of LHRH on the behavioral effects induced by low doses of apomorphine. The results indicate that pretreatment with LHRH antagonized yawning and genital grooming induced by apomorphine in rats.

## METHOD

### *Animals*

A total of 60 male Sprague-Dawley rats weighing 180–200 g were used for this investigation. They were housed in groups of six under controlled lighting (8:00 to 20:00 hr) and temperature (23±2°C) and were allowed free access to standard laboratory diet and water. All animals were used only once and were always tested between 10:00 and 15:00 in a sound-attenuated and temperature-regulated room.

### *Drug Administration*

Drugs were administered subcutaneously (SC) in the dorsal part of the neck. Luteinizing hormone releasing hormone (LHRH, Sigma Chemical Co.) was dissolved in 2% benzyl alcohol and administered at the dose of 100 μg/kg. Apomorphine hydrochloride was dissolved in saline additioned with 2% sodium bisulphite and administered 120 min after LHRH pretreatment at doses of 31.25, 62.5 and 125 μg/kg. 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.

[1]This work was supported by Grant B-2707-8713 from Departamento de Investigacion y Bibliotecas, Universidad de Chile.

*Behavioral Observations*

The animals were put individually in a plastic box (30×30×30 cm) immediately after apomorphine or saline treatment. The number of yawns was scored in 5 min increments over a 30-min period. Episodes of genital grooming were also registered. The box was placed in a sound-proof chamber and the observations were made through a closed TV circuit.

*Statistics*

Student's *t*-test and two-way analysis of variance (ANOVA) followed by the Newman-Keuls Multiple Comparison Procedure were used to determine the level of significance of treatment effects. Differences were considered significant when $p$ was equal or less than 0.05.

## RESULTS

The subcutaneous administration of increased doses of apomorphine induced repeated episodes of yawning and genital grooming in the rat, the maximal effect occurred at 125 μg/kg. Figure 1 illustrates the influence of LHRH on yawning induced by apomorphine during the 30-min period. LHRH significantly inhibited the yawning produced by the three doses of apomorphine used. Figure 2 shows the time course of the interaction between LHRH and apomorphine on yawning. Statistical differences between specific pairs of means are indicated in the figure. Yawning appeared a few min after apomorphine treatment and lasted approximately 30 min. The maximal effects were observed at 10–15 min. LHRH completely blocked the effect of 31.25 μg/kg of apomorphine [Fig. 2A; treatment: $F(1,108)=76.26$, $p<0.01$] and significantly reduced yawning induced by apomorphine 62.5 μg/kg [Fig. 2B; treatment: $F(1,108)=21.13$, $p<0.01$] and 125 μg/kg [Fig. 2C; treatment: $F(1,108)=46.69$, $p<0.01$]. Table 1 summarizes the influence of LHRH on episodes of genital grooming induced by apomorphine. The effects of 62.50 and 125 μg/kg of apomorphine were significantly antagonized by LHRH pretreatment.

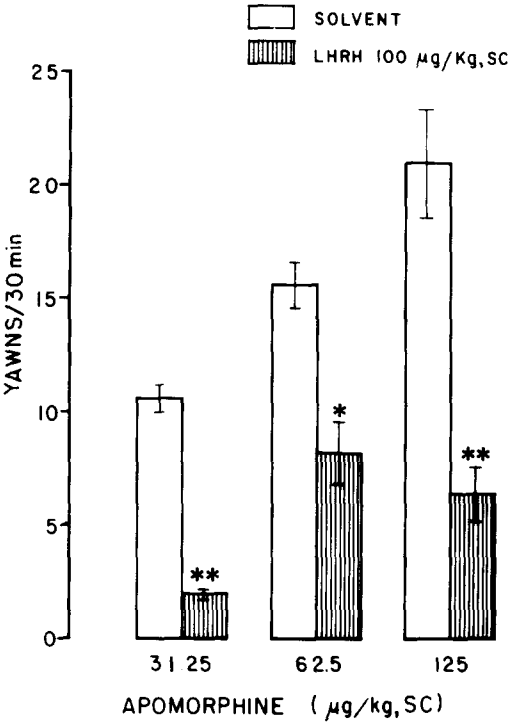


FIG. 1. Effects of LHRH 100 μg/kg or solvent on yawning induced by apomorphine (APO) in rats. Apomorphine was given SC 120 min after LHRH or solvent. The bars represent the means±SEM of yawns observed in the 30-min period. Comparisons were made by using Student's *t*-test (*$p<0.001$, **$p<0.0005$). The number of rats on each group was 10.

## DISCUSSION

The present findings indicate that exogenously administered LHRH antagonizes the behavioral effects of low doses of apomorphine, namely yawning and genital grooming. These effects, which include hypomotility, are considered to be due to an activation of DA receptors in the CNS (12). There is evidence suggesting that DA receptors mediating yawning might be identified with presynaptic DA autoreceptors (12, 19, 26). However, recent reports (23,24) postulate that DA receptors mediating yawning are a special population of postsynaptic DA receptors of the D2 type with a high affinity for DA and apomorphine. Although apomorphine is a mixed D1-D2 agonist its potency for D1 receptors is only in the micromolar range (1). Then the small doses of apomorphine that were used in this study are likely to stimulate selectively D2 receptors.

Yawning has been proposed to be a reliable behavioral sign of DA agonistic action of drugs. Furthermore, it has been suggested that yawning could be useful in the screening of drugs (13). If in a screening observation a given substance induces yawning in rats, which is blocked by DA antagonists, there is a high probability that the compound possesses DA receptor agonistic properties. Consequently, if a given drug abolishes apomorphine-induced yawning it could be classified as a DA antagonist. In fact, neuroleptics reduce yawning induced by apomorphine and other putative DA receptor agonists but do not abolish the yawning induced by cholinergic drugs (6).

Yawning behavior and genital grooming are also induced by protein hormones from the pituitary, such as ACTH, alpha-MSH and oxytocin (2, 7, 22). They have been postulated as mediators in the behavioral effects of low doses of apomorphine (22). Recently it has been reported that low doses of prolactin injected systemically induce yawning in male rats, maybe through the release of small amounts of DA from the striatum (10). On the other hand, hypophysectomy inhibits yawning, penile erection and genital grooming but not the hypomotility induced by small doses of apomorphine (20), suggesting that the lack of some pituitary hormones alters the sensitivity of DA receptors responsible for these effects, but not those responsible for the reduction of motor activity (20). Gonadal steroids seem to exert opposite effects on apomorphine-induced yawning in male rats. Whereas testosterone facilitates both yawning and penile erection (4,8), castration (4) and exogenously administered estrogens (21) antagonize them. Besides, apomorphine is less effective in inducing yawning in female than in male rats (4,21). According to Serra *et al.* (21), subsensitivity of DA receptors may explain the reduction of this behavioral response observed in males following estrogen treatment or in normal female animals.

The present results show that the hypothalamic hormone LHRH, as well as hypophysectomy and castration, prevents yawning and genital grooming induced by apomorphine. We

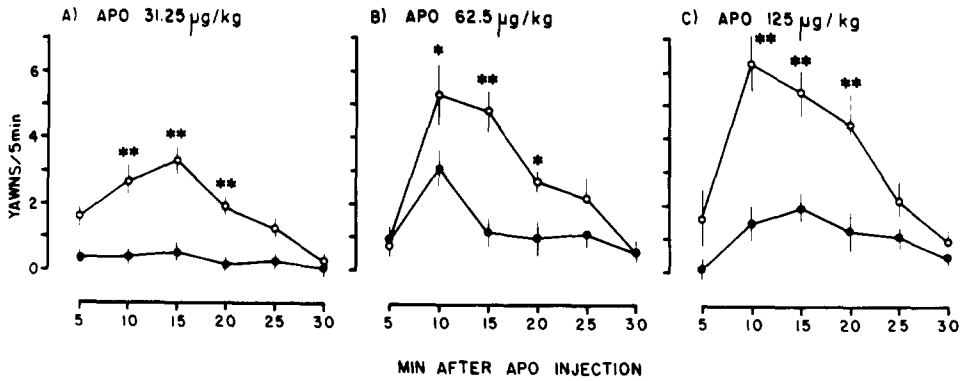


FIG. 2. Time course of the interaction between LHRH 100 µg/kg SC (filled circles) or solvent (open circles) on yawning induced by apomorphine (APO). Each point of the curves represents the mean±SEM of yawns by 5-min intervals after APO injection. Two-way ANOVA was performed on the data from each experimental condition followed by Newman-Keuls test to assess differences between specific pairs of means (*$p<0.005$, **$p<0.001$). The number of rats on each group was 10.

TABLE 1

EFFECTS OF LHRH (100 µg/kg SC) ON GENITAL GROOMING INDUCED BY APOMORPHINE IN MALE RATS

| Pretreatment | N | Apomorphine (µg/kg) | Genital Grooming Episodes in 30 Min (mean ± SEM) |
|---|---|---|---|
| Solvent | 10 | 31.25 | 2.2 ± 0.4 |
| LHRH | 10 | 31.25 | 1.4 ± 0.3 |
| Solvent | 10 | 62.50 | 1.7 ± 0.4 |
| LHRH | 10 | 62.50 | 0.7 ± 0.2 ($p<0.02$) |
| Solvent | 10 | 125 | 4.6 ± 0.5 |
| LHRH | 10 | 125 | 1.0 ± 0.3 ($p<0.0005$) |

Comparisons between groups were made by using Student's *t*-test. In parentheses are indicated differences between LHRH- and solvent-group.

N=number of rats in each group.

can speculate that LHRH is also able to influence the effect of apomorphine by inducing subsensitivity of DA receptors which mediate these behaviors. The possibility that LHRH modulates DA activity was suggested in recent reports (15,17). The peptide antagonizes the motor hyperactivity, rearing behavior and the increase in the conditioned responses induced by amphetamine (15) and this antagonism is reverted by L-DOPA (17), the precursor of DA synthesis. Besides, there is biochemical evidence of an inhibitory effect of LHRH upon DA synthesis (25). Thus, the decrease in DA transmission should exert inhibitory effects on apomorphine-induced yawning. In fact, acute depletion of brain DA by short-term reserpine (11) or alpha-methyltyrosine (9) pretreatment reduces the ability of D2 agonists to induce yawning. Longoni *et al.* (11) have postulated that D2 receptors mediating yawning appear to be functionally linked to D1 receptors in such a way that endogenous DA by stimulating D1 receptors plays a permissive role for the expression of yawning induced by postsynaptic D2 agonists.

Finally, the observation that LHRH does not appear to block the hypomotility produced by low doses of apomorphine (16) suggests that hypomotility and yawning could be mediated through the activation of different kinds of high affinity D2 receptors, which are probably located pre- and postsynaptically, respectively.

## REFERENCES

1. Alander, T.; Grawoska-Anden, M.; Anden, N. E. Physiological significance of dopamine autoreceptors as studied following their selective blockade of molindone. J. Pharm. Pharmacol. 32:780–782; 1980.
2. Argiolas, A.; Melis, M. R.; Gessa, G. L. Intraventricular oxytocin induces yawning and penile erection in male rats. Eur. J. Pharmacol. 117:395–396; 1985.
3. Arnt, J. Pharmacological specificity of conditioned avoidance response inhibition in rats: Inhibition by neuroleptics and correlation to dopamine blockade. Acta Pharmacol. Toxicol. 51:321–329; 1982.
4. Berendsen, H. H. G.; Nickolson, V. J. Androgenic influences on apomorphine-induced yawning in rats. Behav. Neural Biol. 33:123–128; 1981.
5. Carlsson, A. Receptor mediated control of dopamine metabolism. In: Usdin, E.; Bunney, W. E., eds. Pre- and postsynaptic receptors. New York: Dekker; 1975:49–63.
6. Dubuc, I.; Protais, P.; Colboc, O.; Contestin, J. Antagonism of the apomorphine-induced yawning by "atypical" neuroleptics. Neuropharmacology 21:1203–1206; 1982.
7. Gispen, W. H.; Wiegant, M.; Greven, H. M.; de Wied, D. The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure-activity studies. Life Sci. 12:633–642; 1980.
8. Holmgren, B.; Urba-Holmgren, R.; Aguiar, M.; Rodriguez, R. Sex hormone influences on yawning behavior. Acta Neurobiol. Exp. (Warsz.) 40:515–519; 1980.

9. Jackson, D. M.; Jenkins, D. F. Hypothesis: Bromocriptine lacks intrinsic dopamine receptor stimulating properties. J. Neural Transm. 62:219; 1985.
10. Laping, N. J.; Ramirez, V. D. Prolactin-induced yawning behavior requires an intact nigro-striatal dopamine system. Pharmacol. Biochem. Behav. 29:59–62; 1988.
11. Longoni, R.; Spina, L.; Di Chiara, G. Permissive role of D-1 receptor stimulation by endogenous dopamine for the expression of postsynaptic D-2-mediated behavioral responses. Yawning in rats. Eur. J. Pharmacol. 134:163–173; 1987.
12. Mogilnicka, E.; Klimek, V. Drugs affecting dopamine neurons and yawning behavior. Pharmacol. Biochem. Behav. 7:303–305; 1977.
13. Mogilnicka, E.; Boissard, C. G.; Delini-Stula, A. Effects of apomorphine, TL-99 and 3-PPP on yawning in rats. Neuropharmacology 23:19–22; 1984.
14. Mora, S.; Caro, F.; Cardenas, M. G.; Espinoza, M.; Diaz-Veliz, G. Dose-dependent and time-dependent effects of luteinizing hormone releasing hormone on active avoidance behaviour in rats. IRCS Med. Sci. 11:1108–1109; 1983.
15. Mora, S.; Diaz-Veliz, G. Influence of luteinizing hormone releasing hormone (LHRH) on the behavioral effects of amphetamine in rats. Pharmacol. Biochem. Behav. 19:157–161; 1983.
16. Mora, S.; Diaz-Veliz, G. LHRH, dopamine agonists and motor activity in rats. Arch. Biol. Med. Exp. (Santiago) 17:R134; 1984.
17. Mora, S.; Diaz-Veliz, G. Pharmacological evidence of catecholaminergic involvement in the behavioral effects of luteinizing hormone releasing hormone in rats. Pharmacol. Biochem. Behav. 24:433–438; 1986.
18. Mora, S.; Diaz-Veliz, G. Effects of the intracerebral administration of LHRH on acquisition of conditioned responses. Arch. Biol. Med. Exp. (Santiago) 19:R108; 1986.
19. Protais, P.; Dubuc, I.; Contestin, J. Pharmacological characteristics of dopamine receptors involved in the rat dual effect of dopamine agonists on yawning behavior in rats. Eur. J. Pharmacol. 94:271; 1983.
20. Serra, G.; Collu, M.; Loddo,S.; Celasco, G.; Gessa, G. L. Hypophysectomy prevents yawning and penile erection but not hypomotility induced by apomorphine. Pharmacol. Biochem. Behav. 19:917–919; 1983.
21. Serra, G.; Collu, M.; Serra, A.; Gessa, G. L. Estrogens antagonize apomorphine-induced yawning in rats. Eur. J. Pharmacol. 104:383–386; 1984.
22. Serra, G.; Collu, M.; Vargiu, L.; Gessa, G. L. Possible role of ACTH-MSH peptides in the mediation of the apomorphine-induced yawning and penile erection. In: Biggio, G.; Spano, P. F.; Toffano, G.; Gessa, G. L., eds. Advances in the bioscience. vol. 48. Neuromodulation and brain function. Oxford: Pergamon Press; 1984:211–215.
23. Serra, G.; Collu, M.; Gessa, G. L. Dopamine receptors mediating yawning: are they autoreceptors? Eur. J. Pharmacol. 120:187–192; 1986.
24. Serra, G.; Collu, M.; Gessa, G. L. Yawning is elicited by D2 dopamine agonists but is blocked by the D1 antagonist, SCH 23390. Psychopharmacology (Berlin) 91:330–333; 1987.
25. Wang, W. K.; Jenq, S.; Chiang, Y.; Chien, M. K. Inhibition of dopamine biosynthesis by gonadotropin-releasing hormone in rats. Nature 296:354; 1982.
26. Yamada, K.; Furukawa, T. Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology (Berlin) 67:39–43; 1980.