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Physiological role of α-melanocyte-stimulating hormone in modulating the secretion of prolactin and luteinizing hormone in the female rat

(stress/pulsatile secretion/α-melanocyte-stimulating hormone antiserum)

OMID KHORRAM, JOAO C. BEDRAN DE CASTRO, AND SAMUEL M. MCCANN

Department of Physiology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235

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ABSTRACT **Long-term ovariectomized (OVX) rats were injected in the third cerebral ventricle with 5 μl of the globulin fraction of an antiserum raised against α-melanocyte-stimulating hormone (α-MSH) or an equal volume of the globulin fraction of normal rabbit serum (NRS). Immunoneutralization of brain α-MSH produced an increase in the area under the secretion curve of prolactin (Prl), the amplitude of Prl pulses, and mean plasma Prl ($P < 0.01$). In animals that had received two injections of NRS or anti-MSH and were subjected to a 2-min ether stress, Prl levels significantly increased within 5 minutes in the NRS-injected rats, whereas Prl levels in the antiserum-injected rats did not increase any further from the initially high baseline levels. The administration of antibodies against α-MSH produced a small increase ($P < 0.05$) in the area under the secretion of luteinizing hormone (LH) and mean plasma LH; however, the number of LH pulses was unaffected. We conclude that endogenous α-MSH of central origin is a physiological neuromodulator of release of Prl and LH in the OXV rat and is involved in the stress-induced release of Prl.**

α-Melanocyte-stimulating hormone (α-MSH) is a tridecapeptide that has a clear effect on pigmentation and adaptive color changes in lower vertebrates. Although many functions have been attributed to this peptide in mammals, only its behavioral (1) and developmental (2, 3) effects appear to be important physiologically.

Recently, α-MSH has been found extensively throughout the brain, with high concentrations detected in axons and terminals of the hypophysiotropic area of the hypothalamus (4, 5). The cell bodies containing this peptide have been localized in the arcuate nucleus (5) and more recently in the dorsolateral region of the hypothalamus (6). Based on the distribution of this peptide and its detection in the hypophysial portal blood by a specific radioimmunoassay (RIA) (7), we evaluated the effect of this peptide on the release of anterior pituitary hormones.

In recent studies we found that injection of α-MSH into the third ventricle of the brain of ovariectomized (OVX), freely moving rats produced a dose-dependent inhibition of basal and stress-induced release of prolactin (Prl) (8) and inhibited pulsatile release of luteinizing hormone (LH) (9). These effects could be blocked by prior administration of the inhibitor of catecholamine synthesis α-methyl-*p*-tyrosine or the dopamine receptor blocker spiroperidol (8, 9). α-MSH did not alter Prl and LH release from hemipituitaries and dispersed anterior pituitary cells incubated *in vitro*, which indicates that this peptide exerts its effect on Prl and LH release via an action in the hypothalamus to stimulate dopamine release, which, in turn, inhibits Prl and LH release (8, 9). To determine whether the inhibitory effect of α-MSH may be physiologically relevant in the regulation of these hormones, we passively immunized rats with antisera to α-MSH.

MATERIALS AND METHODS

Adult female Sprague–Dawley rats of the Holtzman strain were employed. Females were OVX to eliminate the influence of ovarian steroids and elevate gonadotropin levels. The animals were used 3–6 weeks after ovariectomy. Antiserum was delivered to the brain via a cannula implanted in the third brain ventricle (10). A Silastic cannula implanted 24 hr before the experiment in the right external jugular vein (11) was used for withdrawal of blood samples (0.25 ml). After withdrawal of every sample, the blood volume was restored by an injection of an equal volume of heparinized (20 units/ml), physiological saline. After every four samples, erythrocytes (0.5 ml) prepared previously were injected. The cells were obtained from OVX rats the day prior to the experiment and suspended in 0.9% saline to a hematocrit of 45%.

The antiserum used (KDM-1) has been fully characterized (12). Briefly, this antiserum cross-reacts on a molar basis 0.029% with corticotropin (ACTH)-(1–24), 0.004% with ACTH-(1–39), 0.001% with ACTH-(1–10), and 36% with desacetyl-α-MSH and does not cross-react at a 100 molar excess with β-endorphin, β-MSH, γ_3-MSH, and corticotropin-like intermediate lobe peptide. This antiserum is used in RIA at an initial dilution of 1:10,000 and has an affinity constant of 5.8×10^{10} liters·mol^{-1}, as determined from a Michaelis–Menten association fit (13). The gamma globulins from the antiserum or normal rabbit serum (NRS) were purified by ammonium sulfate precipitation. An equal volume of 80% ammonium sulfate solution was added dropwise to the serum and the mixture was allowed to stand for 1 hr at 4°C. This mixture was centrifuged for 1 hr at 8000 × *g* in a J2-21 Beckman centrifuge. The pellet containing the gamma globulins was washed two times with 40% ammonium sulfate. The pellet was suspended in 0.9% NaCl solution (a volume equivalent to the starting volume of antiserum). Five microliters of the globulin fraction of the antiserum or this fraction from NRS was injected intraventricularly 24 hr before the initial blood sampling. On the day of the experiment one blood sample was withdrawn and the animals were injected a second time with 5 μl of the globulin fractions of the antiserum or NRS, and blood samples were withdrawn every 10 min for 3 hr.

The effect of passive immunization of the animals on the stress-induced release of Prl was determined by injecting the animals with 5 μl of either anti-MSH or NRS (globulin fraction of each) 24 hr before the experiment and on the day of

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Abbreviations: MSH, melanocyte-stimulating hormone; OVX, ovariectomized; LH, luteinizing hormone; Prl, prolactin; NRS, normal rabbit serum.

the experiment. Twenty-five minutes after the second injection of the globulin fraction the animals were subjected to 1.5 min of ether stress and blood samples were withdrawn 5, 15, 30, 60, 90, and 120 min after exposure to ether. Ether stress was administered by placing the rat in a small jar that contained cotton saturated with ether vapor at the bottom.

Data Analysis. For analysis of pulsatile release of Prl, a pulse of hormone was identified as proposed earlier (14). A pulse of Prl was a hormone level that was greater than two times the coefficient of variation of the assay, utilizing the lowest preceding Prl value as the reference point. Pulse amplitude was the difference between the peak and the nadir hormone levels.

Since the pulses of LH were more uniform in amplitude as compared to the Prl pulses and thus satisfied the prerequisites of the cycle detector program developed by Clifton and Steiner (15), this program was used for analysis of the data.

The areas under the curve were calculated by planimetry.

Statistical Analysis. The results of the effect of injection of anti-MSH on pulsatile release of Prl and LH were analyzed by Student's *t* test. The results of the stress experiment were analyzed by analysis of variance with repeated measure followed by the Student–Newman–Keuls multiple comparison test.

RESULTS

Fig. 1 illustrates plasma Prl concentrations 24 hr after intraventricular anti-MSH administration. Plasma Prl levels were signficantly ($P < 0.05$) elevated 24 hr after the injection of anti-MSH on comparison with the values in the NRS-injected rats. Representative hormonal profiles after the second injection of the anti-MSH or NRS are shown in Fig. 2, and the summary of the analysis of a large number of such profiles is presented in Fig. 3. As is evident from the individual patterns of Prl pulses after the intraventricular injection of anti-MSH, the Prl pulse amplitude ($P < 0.05$), mean plasma Prl during the 3-hr sampling period ($P < 0.01$), and the area

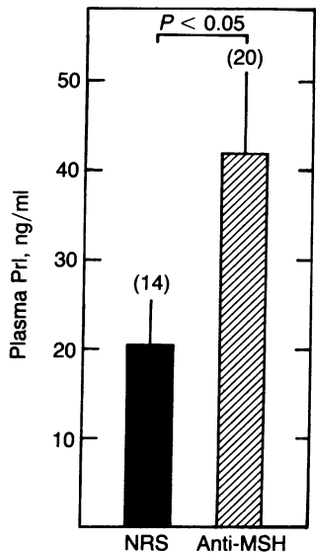

FIG. 1. Effect of intraventricular injection of 5 μl of globulins prepared from NRS and KDM-1 serum, an antiserum to α-MSH, on plasma levels of Prl in OVX, freely moving rats 24 hr after the injection. In this and subsequent figures, numbers on the bars indicate the number of animals in the group.

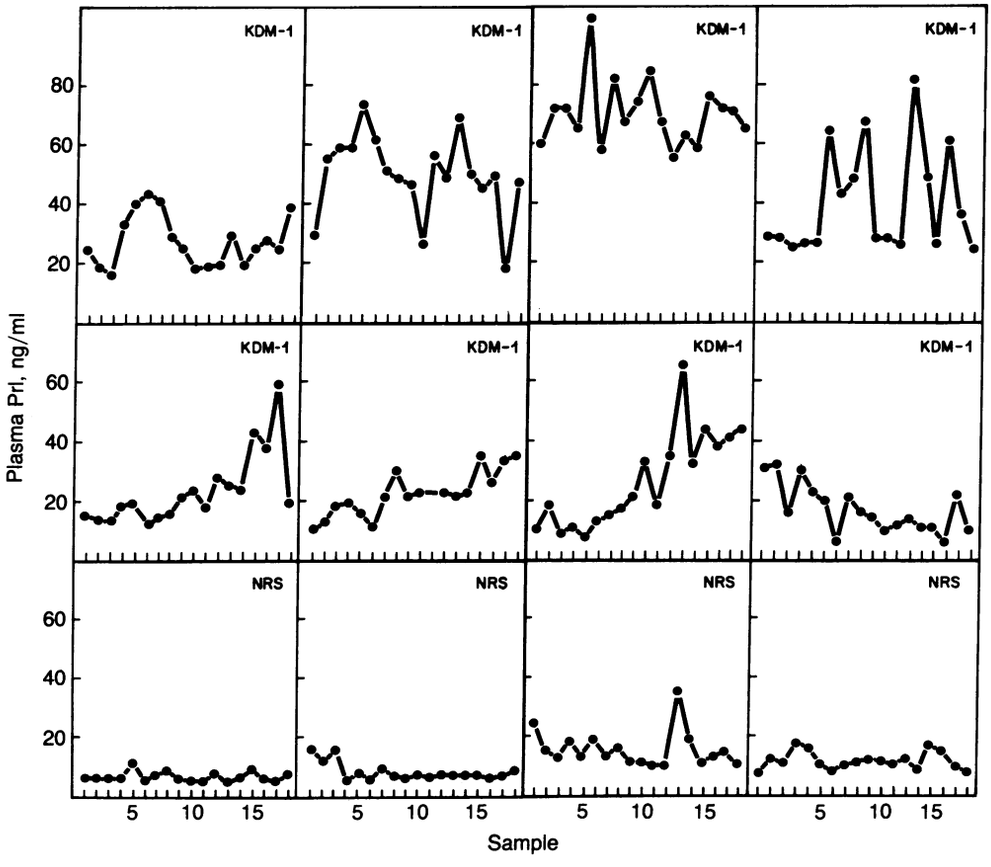

FIG. 2. Representative 3-hr hormonal profiles of the same animals shown in Fig. 1 after a second intraventricular injection of 5 μl of NRS or antiserum (KDM-1). Blood samples were withdrawn every 10 min starting 20 min after the second intraventricular injection of anti-MSH or NRS.

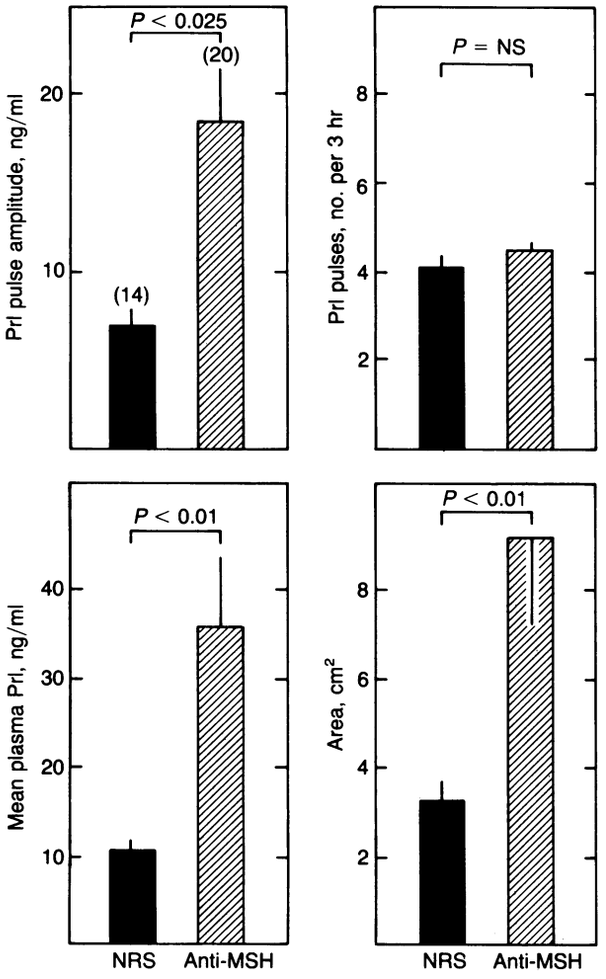

FIG. 3. Summary of various parameters of pulsatile release of Prl represented in Fig. 2. NS, not significant.

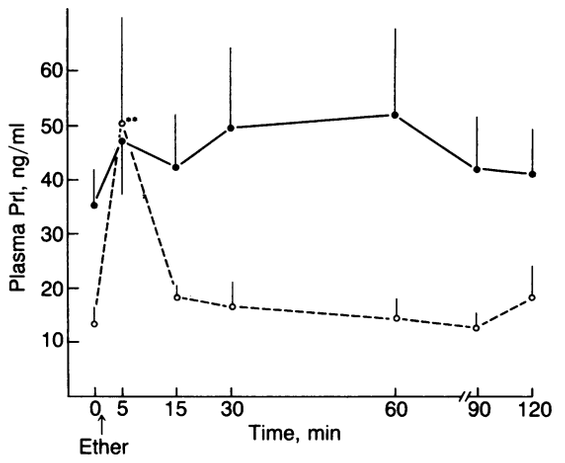

FIG. 4. Effect of anti-MSH (●, $n = 11$) or NRS (○, $n = 7$) on the ether-induced Prl release. OVX rats were injected in the ventricle with 5 μl of KDM-1 or NRS 24 hr prior to ether exposure. On the day of the experiment a blood sample was taken and animals were injected a second time with the same volume of anti-MSH or NRS. Twenty minutes later they were exposed to 1.5 min of ether, and blood samples were taken at various times after ether exposure. **: $P < 0.025$ relative to time 0.

under the secretion curve ($P < 0.01$) were all higher in the antiserum-injected than in the NRS-injected rats. However, the number of Prl pulses was unaffected by the antiserum.

The results of passive immunization with anti-MSH on stress-induced Prl release are shown in Fig. 4. As in the previous experiment, a single injection of anti-MSH significantly ($P < 0.025$) elevated plasma Prl levels 24 hr later, when compared to the values in the NRS-injected rats. Ether exposure significantly ($P < 0.025$) elevated plasma Prl levels within 5 min in the NRS-injected rats but had no effect in the antiserum-injected animals. By 15 min, plasma Prl levels had returned to baseline values in the NRS-injected rats, whereas the initially high basal levels of Prl in the antiserum-injected rats were maintained. Plasma Prl levels in the antiserum-

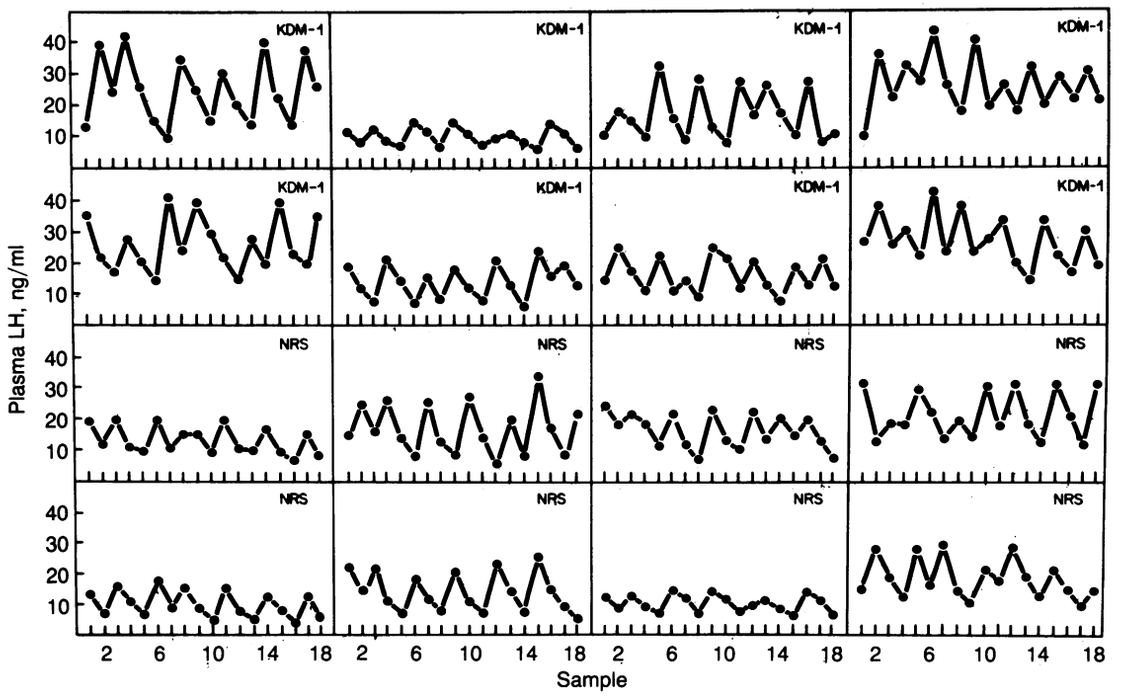

FIG. 5. Effect of anti-MSH or NRS on the 3-hr plasma profiles of LH in OVX, freely moving rats.

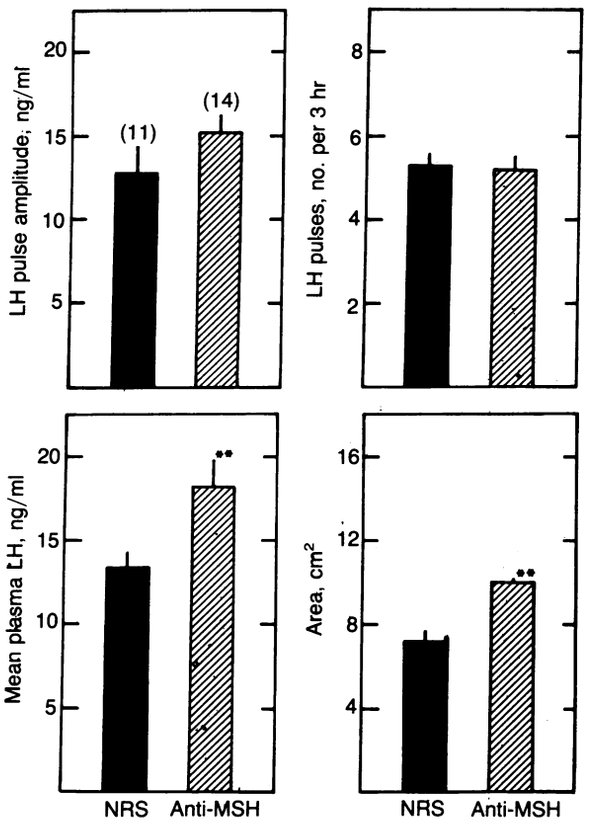

FIG. 6. Summary of various parameters of LH pulses shown in Fig. 4.

treated rats were significantly higher than the corresponding hormone levels in the NRS-injected rats at 15, 30, 60 ($P < 0.05$), 90 ($P < 0.025$), and 120 min ($P < 0.05$).

The effect of injection of anti-MSH on the 3-hr plasma LH profiles is depicted in Fig. 5 and the summary of these data is shown in Fig. 6. There was variation in the response of the animals to the passive immunization, with some of the NRS-treated animals displaying LH profiles similar to those of the anti-MSH-injected animals. The statistical analysis of the LH profiles indicated that KDM-1 increased significantly ($P < 0.05$) mean plasma LH and the area under the curve. Pulse amplitude was slightly higher in the anti-MSH-injected rats as compared to the controls, although this change did not reach statistical significance ($P < 0.06$). The frequency of the LH pulses was unaffected by anti-MSH.

DISCUSSION

The results of this study indicate that α-MSH is a physiological inhibitor of basal and stress-induced Prl secretion and has a minor effect on the secretion of LH in the OVX rat. The effect of the antiserum on the secretion of Prl and LH is specific since gamma globulins prepared from NRS were without effect. As an extra control, the antiserum was run on an affinity column with α-MSH attached to it. The eluate which lacked any antibodies against α-MSH, as determined by RIA and immunocytochemistry, was then purified by ammonium sulfate precipitation in an identical manner as NRS or KDM-1 serum. When microinjected into the third ventricle as in the experiments with anti-MSH serum, the gamma globulin fraction of this control serum had no effect on Prl levels 24 hr later and had no effect on the pulses of either hormone (data not shown).

The effect of anti-MSH serum on Prl secretion is intriguing since β-endorphin, a related peptide that is derived from the same precursor molecule (pro-opiomelanocortin) as α-MSH (16), has a physiologically significant (17) stimulatory effect on the secretion of Prl (18). Furthermore, the effect of β-endorphin on Prl secretion in contrast to that of α-MSH is via inhibition of the activity of the tuberoinfundibular dopaminergic neurons (19). Thus, the question that is raised is how two related peptides, which are found in the same neurons and are presumably released at the same time, could produce opposite effects on the secretion of Prl. One possible explanation is that the α-MSH neurons involved in modulating the release of Prl have cell bodies in the dorsolateral hypothalamus. The α-MSH cell bodies in this region have been demonstrated to contain exclusively α-MSH and not β-endorphin or β-lipotropin (6, 20). This would then allow for selective release of α-MSH without β-endorphin. Alternatively, it may be postulated that α-MSH is an endogenous antagonist of the opioid peptide, β-endorphin. α-MSH by opposing the action of the β-endorphin could prevent a hyperprolactinemic state, which would be the case if the effect of β-endorphin went unopposed.

In an earlier study (8) we demonstrated that intraventricular injection α-MSH was more effective in lowering plasma Prl in animals that had been stressed initially, suggesting that α-MSH released under stressful conditions may be involved in restoring the high levels of Prl in stress to baseline levels. The results of this study support this hypothesis. When brain α-MSH was immunoneutralized, ether stress failed to alter the elevated plasma Prl.

The effect of KDM-1 on pulsatile release of LH was not as clear and dramatic as the effects on Prl. The regulation of pulsatile secretion of LH is complex and involves a number of neurotransmitters. Dopamine is thought by some investigators (21) to stimulate the secretion of LH, whereas others have provided evidence supporting an inhibitory effect (22, 23). Gallo (22) has provided evidence that dopamine decreases the frequency of LH pulses. If α-MSH-induced inhibition of LH is mediated by dopamine, as postulated earlier by us (9), it is unclear why the intraventricular injection of anti-MSH did not alter the frequency of LH pulses. It is possible that the relatively small effects of the antiserum on LH may reflect incomplete immunoneutralization of endogenous α-MSH.

We conclude that endogenous brain α-MSH is involved in regulating the amplitude of Prl pulses but not the number of Prl pulses, which is thought to be under noradrenergic control (24), and that α-MSH plays a role in the stress-induced release of Prl. α-MSH appears to play a minor role under these conditions of hyperstimulation of LH secretion in regulating the release of LH, possibly by a common mechanism as involved in the release of Prl. The Prl and LH release-inhibiting effects of α-MSH can now be added to the list of proposed physiological effects of α-MSH in the mammalian brain.

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