PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 142–144 (1974)

Estrogen Influence on the Hypothalamic Enzymes Involved in the Formation of Melanocyte-Stimulating Hormone Release-Inhibiting Factor (MSH-R-IF) (37764)

MARIA ESTER CELIS AND S. TALEISNIK
(Introduced by S. M. McCann)
Instituto de Investigación Médica Mercedes y Martín Ferreyra,[1] *Córdoba, Argentina*

Melanocyte-stimulating hormone (MSH) secretion is known to be under the control of the nervous system; MSH-R-IF, an agent present in hypothalamic extracts, inhibits the release of MSH. This substance is formed by enzymatic degradation of oxytocin (1) and the enzyme is located in the hypothalamus and attached to the microsomal fraction. The oxytocin fragment L-prolyl-L-leucyl glycinamide, was identified as the product capable of inhibiting the release of MSH (2), and this tripeptide was also found to be present in hypothalamic extracts (3). The influence of estradiol benzoate (EB) on the activity of this enzyme is now reported since previous work had shown that estrogen affects MSH secretion (4). These results have been previously presented in abstract form (5).

Material and Methods. Male and female rats from our own colony were used as a source of hypothalamic tissue. The animals were maintained under controlled lighting (lights on from 5:00 AM to 7 PM and provided food and water *ad libitum*.

Stalk-median eminence (SME) was obtained from animals in the following conditions: (a) Intact female rats in estrus. (b) Rats ovariectomized at 1 month of age were used 2–3 weeks after the operation. (c) Ovariectomized rats injected subcutaneously with a single dose of 10 μg of estradiol benzoate (EB) and killed 24 hr later. (d) Ovariectomized EB-primed rats treated with cycloheximide (70 μg/100 g body weight ip) or with actinomycin D (25 μg/100 g body weight ip) 1 hr before the administration of EB and killed 24 hr later. (e) Male rats treated with cycloheximide or actinomycin D. The SME's were homogenized in 0.25 *M* sucrose (0.2 ml per SME) and centrifuged at 17,000*g*. Different amounts of supernatant were incubated with 150 mU of oxytocin in phosphate buffer at pH 7.0 during 2.5 hr. The reaction was stopped by heat treatment for 5 min. The MSH-R-IF formed during the incubation was assayed by the capacity of the incubation mixture to block the depletion of pituitary MSH induced by the injection of an acidic extract of SME from male rats. Consequently, the incubates were mixed with an acidic extract of SME from male rat equivalent to two hypothalami and an aliquot was injected into the jugular vein in each of two male rats. The animals were killed 20 min later and the pituitaries removed, pooled, weighed, and homogenized in distilled water (5 mg/ml). The MSH content in the pooled glands was measured by an *in vitro* assay (6). The activity of MSH in each group injected with the extracts was compared with that of a standard of two pooled glands from male rats injected with the solvent. Potency and confidence limits were calculated according to accepted statistical methods (7).

Results. SME extracts from female rats at estrus when incubated with oxytocin yielded MSH-R-IF in an amount sufficient to inhibit the depletion of pituitary MSH content in-

[1] Supported by the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

Synthetic oxytocin was kindly provided by Sandoz A. G., Basel.

TABLE I. Effect of Estrogen on the Activity of the Hypothalamic Enzyme Which Forms MSH-R-IF.

Source of SME[a] incubated	Decrease in pituitary MSH concentration in recipient rats (percent of control)[b]
Rats at estrous	1.9 (−12.2–7.8)[c]
Ovariectomized rats	46.5 (31.3–59.2)[d]
Ovariectomized–EB-treated rats	11.9 (4.2–23.7)[c]
— MSH-RF	45.0 (35.–52.7)

[a]An equivalent of three SME was incubated in each group.

[b]Means (95% limits) of three experiments.

[c]Significantly different from MSH-RF group.

duced by the injection of MSH-releasing factor (MSH-RF) (Table I). On the contrary, extracts of SME obtained from ovariectomized rats failed to affect this depletion. EB (10 μg) was able to reverse the effect of ovariectomy on the activity of the SME extracts.

To quantify the increase in activity of SME extracts after EB treatment, different amounts of SME were incubated with oxytocin and the the product tested for MSH-R-IF. Although incubation of the equivalent of five SME from ovariectomized rats formed no detectable MSH-R-IF, an equivalent of three SME from the EB-treated animals produced enough MSH-R-IF to block completely the effect of MSH-RF. Equivalents of one or one-half SME, although not as active as three SME, also yielded MSH-R-IF which was effectively detected by the assay method (Fig. 1).

Cycloheximide prevented the increase in enzyme activity resulting from EB injection since the activity in three SME was insufficient to produce detectable amounts of MSH-R-IF (Table II). Actinomycin D had a similar effect although it was less active than cycloheximide since the amount of enzyme present in three SME from treated rats was still enough to produce MSH-R-IF, but that in two SME was insufficient.

Three SME of male rats incubated with oxytocin formed sufficient amounts of MSH-R-IF to prevent the depletion of pituitary MSH by the injection of MSH-RF, but three SME from animals treated 24 hr before with cycloheximide or actinomycin D failed to produce this effect (Table II).

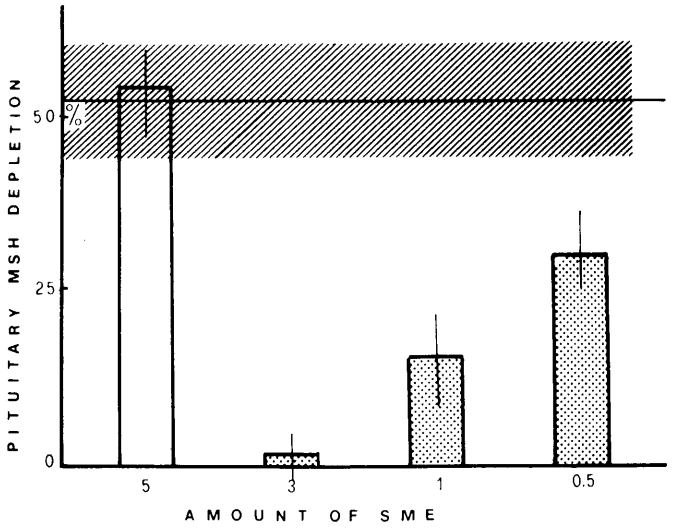

FIG. 1. Difference in the SME content of the enzyme which forms MSH-R-IF between ovariectomized and ovariectomized-EB-treated rats. The horizontal line indicates depletion of pituitary MSH induced by the injection of MSH-RF and the striped area is the 95% limits. The bars are the mean values of two experiments showing inhibition of the MSH-RF effect by the MSH-R-IF formed upon the incubation of different amounts of SME equivalents from ovariectomized (white bar) or ovariectomized–EB-primed rats (dotted bars) with 150 mU oxytocin. Vertical lines are 95% confidence limits.

TABLE II. Effect of Inhibitors of Protein Synthesis on the Enzymes That Form MSH-R-IF.

Source of incubated SME	Number of SME incubated	Decrease of pituitary MSH concentration in recipient rats (percent of control)[a]
Ovariectomized estrogen primed		
Nontreated	3	−8.4 (−21.3–3.0)[b]
Cycloheximide treated	3	53.3 (40.2–61.7)
Actinomicin D treated	3	13.2 (−4.2–27.9)[b]
	2	46.7 (38.7–53.3)
— MSH-RF		59.9 (41.1–69.9)
Male rats		
Nontreated	3	−8.2 (−19.4–9.4)[b]
Cycloheximide treated	3	47.2 (41.1–54.9)
Actinomicin D treated	3	50.0 (41.7–55.6)
MSH-RF		58.3 (46.6–65.3)

[a,b]Same footnotes as in Table I.

Discussion. The presence of an enzyme which yielded MSH-R-IF when incubated with oxytocin has been demonstrated in hypothalamic tissue of male rats (1). The present study shows that hypothalamic tissue from female rats at estrus possesses similar enzymatic activity; however, that from ovariectomized rats was inactive even when a pool of five hypothalami was used for the reaction. Estrogen administration to spayed rats resulted 24 hr later in a restoration of the action suppressed by the removal of the ovaries. These findings provide evidence that the enzyme which catalyzes the formation of MSH-R-IF is estrogen dependent. Several other enzymes were found to have a similar behavior (8–9). After EB treatment an equivalent of one and even of one-half hypothalamus showed enough enzymatic activity to form MSH-R-IF when incubated with oxytocin. On the contrary, up to five hypothalami from ovariectomized rats failed to show any effect. Therefore, it could be inferred that the activity of the enzyme had increased at least 10-fold by the treatment

The effect of estrogen on the enzyme activity was found to be blocked by previous treatment with cycloheximide or actinomycin D, two agents known to inhibit protein synthesis (10–11). The present experiments seem to indicate that cycloheximide exerted a more intense inhibition. These results would suggest that the increase in the enzyme activity after EB treatment is due to the synthesis of new enzyme.

Summary. The activity of the enzyme which yielded MSH-R-IF upon incubation of hypothalamic extracts with oxytocin was found to be estrogen sensitive. It was absent in ovariectomized rats and increased after estrogen treatment. A dose of 10 μg EB induced a 10-fold increase in the hypothalamic activity of the enzyme. Cycloheximide and actinomycin D inhibited the effect of estrogen suggesting that estrogen promotes the formation of new enzyme.

1. Celis, M. E., and Taleisnik, S., Int. J. Neurosci. 1, 223 (1971).

2. Celis, M. E., Taleisnik, S., and Walter, R., Proc. Nat. Acad. Sci. USA 68, 1428 (1971).

3. Nair, R. M. G., Kastin, A. J., and Schally, A. V., Biochem. Biophys. Res. Commun. 43, 1376 (1971).

4. Taleisnik, S., and Tomatis, M. E., Neuroendocrinology 5, 24 (1969).

5. Celis, M. E., and Taleisnik, S., Abstr. IV Int. Congr. Endocrinol. Washington, 229 (1972).

6. Taleisnik, S., and Orías, R., Amer. J. Physiol. 208, 293 (1965).

7. Bliss, C. I., "The Statistics of Bioassay." Academic Press, New York (1952).

8. Singhal, R. L., and Veladares, J. R. E., Amer. J. Physiol. 218, 321 (1970).

9. Hooper, K. C., Biochem. J. 110, 151 (1968).

10. Reich, E., Cancer Res. 23, 1428 (1963).

11. Siegel, M. R., and Sisler, H. D., Nature (London) 200, 675 (1964).

Received Sept. 4, 1973. P.S.E.B.M., 1974, Vol. 145.