The Effect of Synthetic Luteinizing Hormone Releasing Hormone (LH-RH) on the Protein Synthesis of Different Hypothalamic Areas and Anterior Pituitary Gland[1] (37299)

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Several evidences indicate that pituitary hormones may control their own secretion by regulating at the hypothalamic level the synthesis and/or release of their respective hypothalamic hormones (1). The existence of this mechanism of regulation generally regarded as "short feedback," has been demonstrated by using different experimental techniques. For instance, the implantation of small amounts of LH in the median eminence of normal or castrated rats of both sexes results in a decrease of pituitary and plasma LH levels (2, 3).

On the other hand, LH has an inhibitory effect *in vitro* on the metabolic activity of the hypothalamic area supposedly involved in the synthesis of its regulatory principle (4). Finally it has been recently proposed that hypothalamic hormones can also affect their own synthesis and/or release by direct action on the hypothalamus, a mechanism of control which seems to be of a negative type (1, 5).

Considering that changes in protein synthesis by the hypothalamus could in some way reflect modifications in the hypothalamic synthesis of peptides involved in the control of the anterior pituitary gland, the present experiments were conducted in order to explore the possibility that synthetic luteinizing hormone releasing hormone (LH-RH) could affect the *in vitro* incorporation of labeled amino acids into proteins of different hypothalamic areas.

These studies were extended to the anterior pituitary gland with the aim of determining the action of this hypothalamic factor on protein synthesis of this portion of the gland.

Material and Methods. A [^{3}H] L-amino acid mixture (sp act, 1 mCi/0.064 mg) was obtained from New England Nuclear Corp. This mixture contained (in terms of μCi/mCi): alanine, 80: arginine, 70; aspartic acid, 80: glutamic acid, 125; glycine, 40; histidine, 15: isoleucine, 50; leucine, 140; lysine, 60; phenylalanine, 80; proline, 50; serine, 40; threonine, 50; tyrosine, 40; valine, 80. One microcurie of this mixture contained a total of 0.424 nmole. Unlabeled amino acids were obtained from Merck Darmstadt. Experiments were performed on male rats, weighing 150–170*g*, from the strain of the Instituto de Fisiología. They were housed under constant temperature (23 $\pm$ 2°) and lighting (12 hr light and 12 hr darkness), and fed *ad libitum* with a standard diet and water. The animals were killed by decapitation and the whole hypothalamus was dissected according to the limits given by De Groot (6). The sample was placed in its dorsal surface and cut under a dissecting microscope into three portions by two frontal sections; the first section was made through the optic chiasma and the second immediately behind the infundibulum. These sections divided the hypothalamus into the following three areas: a prechiasmatic portion, namely the anterior hypothalamus (including the preoptic and anterior hypothalamic areas and the paraventricular and suprachiasmatic nucleus): a retroinfundibular portion, namely the posterior hypothalamus (including the mamillaris and posterior hypothalamic nucleus), and between the two sections, the medial hypothalamus (including the median

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eminence and the arcuate, ventromedial and dorsomedial nuclei).

Each hypothalamic area was divided by a sagittal section into two symmetrical portions: one was used as control and the other for the experimental procedures. A slice of cerebral cortex was taken from each frontal lobe. Anterior pituitary glands were separated from the infundibular process and divided sagittally under a dissection microscope.

Four hemipituitaries were used in each experiment and were paired to permit exposure of each pituitary to both experimental and control conditions.

The samples were gently blotted on filter paper, weighed on a torsion balance and incubated in 1 ml of isotonic medium containing (μmoles): NaCl, 120; KCl, 5; K_2HPO_4, 1.3; $MgSO_4$ 1.3; $CaCl_2$, 1; Tris–HCl (pH 7–4), 3.3; glucose, 10; and 0.5 μCi of the [^{3}H] L-amino acid mixture. Experimental tubes contained in addition 4 ng of synthetic LH-RH (Pyro-GLU-HIS-TRP-SER-TYR-GLY-LEU-ARG-PRO-GLY-NH_2).

Incubation was performed for 60 min at 37° in a Dubnoff metabolic shaker under a O_2:CO_2 (95:5, v/v) atmosphere. After incubation the tubes were rapidly cooled in crushed ice, the tissue was washed twice with saline, centrifuged in a refrigerated centrifuge, and homogenized in 2 ml of 10% trichloroacetic acid (TCA) containing a mixture of unlabeled amino acids (the same amino acids as those present in the labeled mixture in a final concentration of 0.1%). The homogenate was centrifuged in refrigerated centrifuge at 6500*g* for 15 min, and the TCA-insoluble residue washed twice with 5% TCA, twice with chloroform: methanol (1:1, v/v), and once with 2 ml ether; the precipitate was separated by centrifugation in the cold. The residue was then resuspended in 2 ml of 5% TCA and heated at 90° for 15 min; after cooling and centrifuging, the protein residue was dissolved in 0.5 ml of NaOH and aliquots were taken to determine the protein content by the method of Lowry *et al.* (7), using crystalline bovine serum albumin as standard.

Protein recovered was 46–60 μg/mg fresh tissue in the nervous system and 50–60 μg/ml fresh tissue in the anterior pituitary gland. In another aliquot, radioactivity was determined in a Packard Tri-Carb liquid scintillation counter, where each sample was counted long enough to give a standard error of less than 3%. Counts were corrected to 100% efficiency by the channels ratio method. The radioactivity incorporated into proteins was expressed as specific activity, i.e., dpm/μg of protein.

All results are shown as means ± SEM and were compared statistically by means of Student's *t* test. Differences were considered significant at a *p* value lower than .05.

Results. The time-course of amino acid incorporation was studied in preliminary experiments. The incorporation of labeled amino acids into proteins of the anterior, middle and posterior hypothalamus, cerebral cortex and anterior pituitary gland was linear during the first 90 min of incubation (Figs. 1 and 2); therefore, subsequent incubation lasted 60 min.

Table I shows that LH-RH decreased protein synthesis in the anterior hypothalamus while the synthetic activity in the middle and posterior hypothalamus as well as in the cerebral cortex was unchanged. The incorporation of labeled amino acids into proteins of the anterior pituitary gland *in vitro* was significantly increased by the addition of LH-RH to the medium.

Discussion. These results demonstrated that LH-RH specifically depresses the incorporation of labeled amino acids into proteins of the anterior hypothalamus *in vitro*. It has been previously demonstrated that changes in the secretion of gonadotropins result in modifications of the oxidative and protein metabolism in various areas of the hypothalamus (8, 9). Because luteinizing hormone (LH) decreases the metabolic activity of the anterior hypothalamus, it was proposed that such an effect could reflect an inhibitory action of this pituitary hormone on the metabolic processes involved in the synthesis of LH-RH (4). This hypothesis has been recently confirmed by Docke and Glasser (10) who showed that LH implants in the anterior hypothalamus inhibited LH secretion, probably by inhibiting the synthesis of LH-RH.

The possibility that hypothalamic products

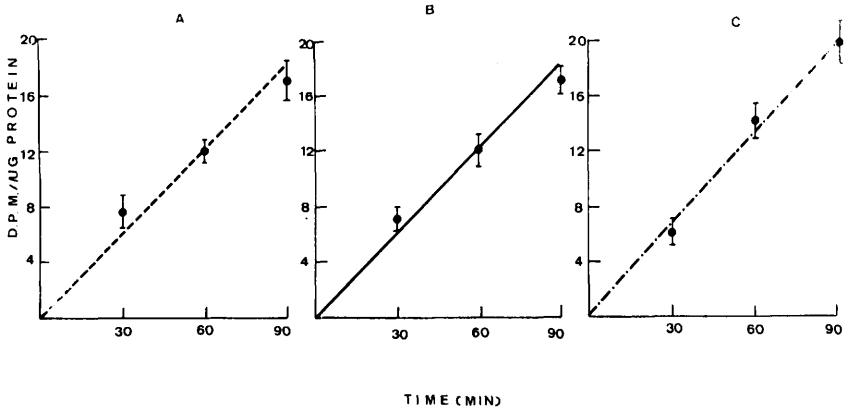

FIG. 1. Time-course of incorporation of a ^{3}H-L-amino acid mixture into proteins of: (A) anterior, (B) middle and (C) posterior hypothalamus of rats (six separate determinations in male rats). Means ± SEM corrected for zero time radioactivity.

may also influence hypothalamic function has received experimental support. For instance, it has been previously demonstrated that synthetic thyrotropic (TRH) is able to inhibit the protein synthesis in the middle hypothalamus (5). On this basis it has been postulated that TRH has at hypothalamic level an inhibitory influence on its own secretion. On the other hand it has been also showed that treatment of gonadectomized estrogen–progesterone treated rats which typically show increased stores of follicle-stimulating hormone releasing hormone (FSH-RH), with hypothalamic extracts brings them back to their normal levels (1). The present experiments on the action of LH-RH on different areas of the central nervous system indicate that the anterior hypothalamus is the only region in which there is a modification in protein synthesis.

Changes in the *in vitro* incorporation of amino acids into proteins could not represent parallel modifications in the protein synthesis, nevertheless, it seems reasonable to assume that the *in vitro* incorporation of amino acids into hypothalamic proteins could reflect some of the metabolic processes implicated in the synthesis of the hypothalamic hormones. If this is true, and accepting that these factors can affect their own secretion it could be postulated that the specific sensitivity of the anterior hypothalamus to LH-RH reflects a

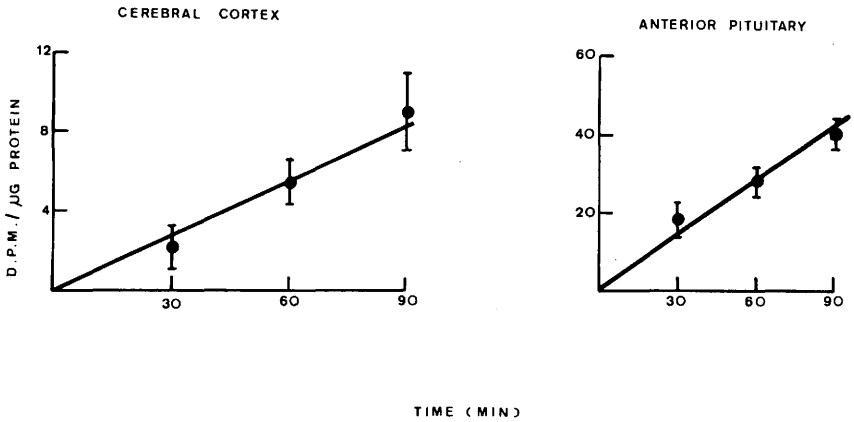

FIG. 2. Time-course of incorporation of a ^{3}H-L-amino acid mixture into proteins of cerebral cortex and anterior pituitary gland of rats (three separate determinations in male rats). Means ± SEM corrected for zero time radioactivity.

TABLE I. Effect of Synthetic LH-RH on Protein Synthesis of the Hypothalamus and Anterior Pituitary Gland.[a]

	Dpm/μg/protein				
	Hypothalamus			Cerebral cortex	Anterior pituitary
	Anterior	Middle	Posterior		
Control	13.93 ± 1.12[b] (14)	8.54 ± 1.09 (16)	10.92 ± 1.95 (17)	12.25 ± 5.12 (9)	19.40 ± 1.60 (13)
LH-RH	9.64 ± 1.01 (14)	8.59 ± 1.30 (16)	10.95 ± 1.42 (17)	11.24 ± 2.63 (9)	25.39 ± 1.12 (13)
p value	<.005	NS	NS	NS	<.0025

[a] Numbers in parentheses are number of determinations. NS = not significant.
[b] Mean ± standard error.

direct participation of such hypothalamic area in the synthesis of LH-RH.

In this respect our results confirm previous publications in which it has been demonstrated that the anterior hypothalamic area is involved in the synthesis of LH-RH (11).

Finally it may be possible that the inhibitory effect of LH-RH on the anterior hypothalamus may be connected with an unknown mechanism by which LH-RH has at that level an inhibitory influence on its own secretion. More experimental evidence is necessary to clarify this aspect.

The results of the present study also showed that LH-RH is able to increase the amino acid incorporation into proteins of anterior pituitary gland. It has been clearly demonstrated that synthetic LH-RH produces LH and FSH release and synthesis by the anterior pituitary (12–14).

On this basis it can be postulated that the enhanced incorporation of labeled amino acids into proteins of pituitary produced by LH-RH *in vitro* reflect increased synthesis of gonadotropins. The fact that many experimental evidences indicate (12–14) that hypothalamic releasing hormones not only release pituitary hormones but also stimulate their synthesis, further supports this hypothesis.

Summary. The effect of synthetic luteinizing hormone releasing hormone (LH-RH) on the *in vitro* incorporation of labeled amino acid into proteins of different hypothalamic areas, cerebral cortex and anterior pituitary gland was studied.

LH-RH specifically decreased the incorporation of labeled amino acids into proteins of the anterior hypothalamus whereas the anterior pituitary gland showed enhanced protein synthesis.

No modifications were found in the middle and posterior hypothalamus and in the cerebral cortex.

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