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# Effect of LH-RH, Gonadotrophins or Sex Hormones on Protein Synthesis in Hypothalamus and Pituitary of Immature Female Rat *in vitro*

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Protein synthesis in cerebral cortex, hypothalamus and pituitary of immature female rats was studied after treatment with 5 doses of LH-RH, gonadotrophins or sex hormones. None of the hormonal doses affected protein synthesis in cortex. Sex hormones and gonadotrophins increased protein synthesis in hypothalamus. These effects are attributed to positive feed-backs of these hormones on immature female rat hypothalamus. All treatments increased protein synthesis in pituitary. The direct effect of LH-RH on pituitary and the feed-back effect of sex hormones or gonadotrophins are compared and discussed.

It is commonly accepted that the hypothalamic control of pituitary function begins at the perinatal period in the rat (3, 10). This mechanism undergoes several changes from birth to puberty which affect the gonadotrophins and sex hormones concentrations in blood (7, 9, 13, 16). These changes have been imputed to a maturation process in sex-hormones feed-back mechanisms (4, 5, 12, 17, 21) but many aspects of this hypothesis remain unknown.

Since pituitary hormones are proteins or glucoproteins, and hypothalamic hormones are small peptides, the use of protein synthesis as a method to study the hypothalamic and pituitary functions (1, 11, 14) is correct. In this work, protein synthesis has been used to examine the relations between LH-RH and the feed-backs provoked by gonadotrophins or sex hormones injection on pituitary and hypothalamus of immature female rats.

## Materials and Methods

Female Wistar rats, 21 days old, housed in our animal installations were utilized. Subjected to cycles of 14 hours light

Table I. *Effect of treatment to 21 days old female rats with LH-RH, PMSG, HCG, FSH-LH, Oestradiol or Progesterone, on $^{14}$C-Aminoacids incorporation in cerebral cortex, hypothalamus and pituitary* in vitro.

Results expressed in mean ± S.E.M.; number of animals in parentheses. Comparisons with controls were effectuated by Student t test. [(a) = means $p < 0.05$; (b) = $p < 0.01$; (c) = $p < 0.001$; otherwise no significant difference with controls appears.]

| | Cerebral cortex | Hypothalamus | Pituitary |
|---|---|---|---|
| | A) *Results in DPM/mg wet weight tissue.* | | |
| Controls | (7) 20.51 ± 0.8 | (12) 32.50 ± 0.51 | (10) 586.9 ± 49.2 |
| LH-RH | (8) 20.88 ± 1.96 | (7) 34.55 ± 1.60 | (8) 857.0 ± 80 [b] |
| PMSG | (7) 22.69 ± 1.98 | (7) 63.53 ± 3.39[c] | (7) 1,145.8 ± 122[c] |
| HCG | (7) 24.63 ± 2.95 | (8) 58.88 ± 8.99[b] | (7) 851.4 ± 104[a] |
| FSH-LH | (8) 20.51 ± 1.08 | (8) 48.00 ± 1.63[c] | (7) 1,391.2 ± 145[c] |
| Oestradiol | (6) 23.49 ± 1.74 | (10) 97.34 ± 5.85[c] | (9) 1,609.4 ± 88 [c] |
| Progesterone | (8) 26.36 ± 1.62 | (9) 92.20 ± 6.76[c] | (9) 1,500.7 ± 138[c] |
| Analysis of Variance | | | |
| F. Ratio | 1.60 | 30.36 | 18.09 |
| P. Value | N.S. | < 0.01 | < 0.01 |
| | B) *Results in DPM/μg protein.* | | |
| Controls | (10) 1.28 ± 0.15 | (9) 2.73 ± 0.35 | (10) 32.05 ± 2.83 |
| LH-RH | (6) 1.65 ± 0.33 | (6) 3.19 ± 0.36 | (8) 48.24 ± 8.56[a] |
| PMSG | (7) 1.53 ± 0.09 | (9) 2.90 ± 0.38 | (7) 39.9 ± 5.50 |
| HCG | (8) 1.87 ± 0.34 | (9) 4.94 ± 0.31[c] | — |
| FSH-LH | (6) 1.75 ± 0.20 | (8) 3.19 ± 0.27 | (7) 60.38 ± 7.28[b] |
| Oestradiol | (6) 1.60 ± 0.16 | (7) 5.56 ± 0.58[c] | (9) 46.82 ± 11.53 |
| Progesterone | (8) 1.73 ± 0.18 | (9) 5.35 ± 0.55[b] | (9) 51.87 ± 10,4 |
| Analysis of Variance | | | |
| F. Ratio | 0.89 | 9.04 | 1.38 |
| P. Value | N.S. | < 0.01 | N.S. |

and 10 hours darkness, suckled by their mothers until the first injection, and then maintained with water and food *ad libitum* until sacrificed. Groups of 10 animals were injected subcutaneously during 5 days, with a daily single dose of any of the following hormones and doses: 10 $\mu$g of LH-RH (Beckman); 60 U.I. of PMSG (Leo); 50 U.I. of HCG (Leo); 5 U.I. of FSH + 5.6 U.I. of LH (Farma-Lepori); 5 mg of progesterone (Schering) or 250 $\mu$g of oestradiol benzoate (Schering) in 0.25 ml saline or corn oil.

Animals were decapitated 24 h after the last injection, and hypothalamus, pituitary and part of frontal cortex were immediately dissected, weighed and introduced in assay tubes containing ice cold isotonic medium which consisted of NaCl 120 mM; KCl 5 mM; $MgSO_4$ 1.3 mM; $CaCl_2$ 0.5 mM; phosphate buffer 10.3 mM and glucose 7.7 mM (pH 7.4).

The tubes were incubated in a thermostatic bath (37 ± 1° C), continously oxygenated and shaked during 15 min. Then 1 $\mu$Ci of a $^{14}$C-aminoacids mixture ($^{14}$C-Aa) (The Radiochemical Centre, Specific activity 45 mCi/milliatom of carbon) dissolved in isotonic medium was added in each tube. Incubations were performed during 90 min, and then 1 ml of 20% TCA was added to each tube.

Te tissue samples were collected by centrifugation, pouring off the supernatant solution. The tissues were homogenized by a teflon pestle in 2 ml of 10% TCA solution containing a non labelled aminoacids mixture (0.1 M) and centrifuged. The precipitate was washed twice with 2 ml of 10% TCA solution, once with chloroform methanol (2:1 V/V) and once with dietyl ether. All the supernatants were poured off. The last precipitate was air dried and dissolved in 1 ml of 1 M KOH. Half ml of this solution was placed in a counting glass vial and neutralized with 50 $\mu$l of 10 M HCl. Then dissolved in a scintillation cocktail containing 0.55% Permablend III (91% PPO and 9% Bis-MSB, Packard) in toluene, with 20% of Triton x-100. Radioactivity of samples was measured with a Nuclear Chicago (ISOCAP 300) scintillation counter. DPM were calculated by channels ratio method.

Protein concentration was determined by the method of LOWRY *et al.* (15), using serum albumine as standard.

Radioactivity is expressed in DPM/mg tissue and DPM/$\mu$g protein. All results are presented as the means + S.E.M. and analyzed for variance according to SNEDECOR (19). Significance was determined by the Student T-test.

## Results

Table I shows the results obtained expressed as DPM/mg wet tissue weight (A) or as DPM/$\mu$g protein (B).

*Cerebral Cortex.* None of the assayed hormonal treatments altered significantly the protein synthesis in frontal cortex of immature female rats.

*Hypothalamus.* The synthetic LH-RH did not bring about alterations in hypothalamic protein synthesis. Gonadotrophins increased significantly with regard to the control results. The treatment with oestradiol benzoate or progesterone increased very much (about 3 fold) the hypothalamic protein synthesis.

*Pituitary.* All hormonal treatments assayed increased the pituitary protein synthesis. The activity order, at assayed doses, were: LH-RH < gonadotrophins < sexhormones.

## Discussion

The hypothalamic or pituitary capacity for protein synthesis in prepuberal female rat after treatment with LH-RH, gonadotrophins or sexhormones during 5 days is a previous approach to the new stage

of these organs after their accelerated evolution imposed by the assayed hormones.

Table I shows that prepuberal female rat pituitary increases its protein synthesis capacity after 5 days treatment with LH-RH. It has already been reported that pituitaries incubated with LH-RH *in vitro* increase their protein synthesis (5) and that LH-RH increases the FSH and LH synthesis in tissue culture (14). According to these authors it is possible to infer from our results that LH-RH treatment to immature female rats increases the gonadotrophin synthesis capacity of its pituitaries also *in vivo*. This hypothesis is also supported by the finding that LH-RH treatment increases both RNA and protein synthesis in the immature ovary (6 and DÍAZ-CHICO unpublished results) which could only be explained by an increase in gonadotrophin release.

The increase in protein synthesis capacity of immature female rat hypothalamus after oestradiol treatment is in agreement with the results obtained by FAIGON and MOGUILEWSKY (11) *in vitro*. Their results are interpreted to be a consequence of a positive feedback loop to the hypothalamus mediated by oestradiol, as CALIGARIS *et al.* pointed out (2). Our results seem to confirm *in vivo* the existence of this positive feed-back loop to the hypothalamus in the immature female rat, which is also induced by a high dose of oestradiol.

The effect of progesterone treatment on hypothalamus resembles that of the oestradiol. This is why it seems to be possible that a positive feed-back for progesterone takes place in the immature female rat.

Gonadotrophin treatments (PMSG, HCG or PSH + LH) increase the hypothalamus capacity for protein synthesis. This effect could be interpreted either as a positive feed-back of these hormones to the hypothalamus, according to OJEDA and RAMÍREZ (18), or as an indirect effect through the positive feed-back for the sexhormones, which ovarian synthesis and liberation is stimulated by gonadotrophin treatment to immature female rat.

The pituitary protein synthesis stimulation by FSH + LH treatment is difficult to interpret, but apparently an indirect effect through hypothalamus takes place, either as a direct action of gonadotrophins on hypothalamus or as an indirect action through sexhormones.

Oestradiol and progesterone treatments increase the protein synthesis capacity of immature female rats pituitary more than gonadotrophins or LH-RH at assayed doses. From these findings it is possible to infer that the sex-hormones treatments to immature female rat affect not only the hypothalamus, by the above described positive feed-back, but also the pituitary, perhaps raising the hypothalamic hormones action on hypothalamus. An interference of oestradiol with LH-RH receptors of adult rat pituitaries has been described by SPONA (20), but the true significance of this priming action of sex-hormones on immature female rat pituitaries in relation with the sexual maturation requires further experiments.

## Resumen

Se estudia la síntesis proteica en corteza cerebral, hipotálamo e hipófisis de rata inmadura, después del tratamiento con 5 dosis de LH-RH, gonadotrofinas u hormonas sexuales. Ninguna de las dosis hormonales ensayadas afecta la síntesis proteica en la corteza. Las hormonas sexuales y las gonadotrofinas aumentan la síntesis proteica en hipotálamo. Estos efectos son atribuidos a *feed-backs* positivos de dichas hormonas sobre el hipotálamo de rata hembra inmadura. Todos los tratamientos ensayados aumentan la síntesis proteica en la hipófisis. Se comparan y discuten el efecto directo de la LH-RH y los indirectos a través de efectos *feed-backs* de hormonas sexuales y gonadotrofinas sobre la hipófisis.

## References


1. BAULIEU, E. E. and ROCHEFORT, H.: In «Mechanismes d'actions intracellulaires des hormones» (R. Vokaer, ed.). Masson et Cie., Paris, 1970, pp. 53-72.
2. CALIGARIS, L., ASTRADA, J. J. and TALEISNIK, S.: *J. Endocr.*, **55**, 97-105, 1972.
3. CHOWDHURY, M. and STEINBERGER, E.: *J. Endocr.*, **69**, 381-384, 1976.
4. DEBELJUK, L., ARIMURA, A. and SCHALLY, A. V.: *Endocrinology*, **90**, 1499-1052, 1972.
5. DÍAZ-CHICO, B. N. and DÍAZ-CHICO, J. C.: *Rev. esp. Fisiol.*, **33**, 291-296, 1977.
6. DÍAZ-DÍAZ, M. E. and DÍAZ-CHICO, B. N.: *Rev. esp. Fisiol.*, **34**, 127-130, 1978.
7. DÖLHER, K. D. and WUTTKE, W.: *Endocrinology*, **94**, 1003-1008, 1974.
8. DÖLHER, K. D. and WUTTKE, W.: *Endocrinology*, **97**, 898-907, 1975.
9. DÖLHER, K. D. and WUTTKE, W.: *Acta Endocrinologica (Kbh)*, **85**, 718-728, 1977.
10. EGUCHI, Y., SAKAMOTO, Y., ARISHIMA, K. and MORIKAWA, Y.: *Endocrinology*, **96**, 504-507, 1975.
11. FAIGON, M. R. and MOGUILEWSKY, J. A.: *Experientia*, **32**, 392-394, 1976.
12. GROTJAN, H. E. and JHONSON, D. C.: *Acta Endocrinologica (Kbh)*, **84**, 254-267, 1977.
13. KRAGTH, C. L. and DAHLGREN, J.: *Neuroendocrinology*, **9**, 30, 1972.
14. LABRIE, F., PELLETIER, G., LEMAY, A., BORGEAT, P., BARDEN, N., DUPONT, A., SARAVAY, M., COTÉ, J. and BOUCHER, R.: In «Karolinska Symposium on research methods in reproductive endocrinology» (E. Diczfalusy, ed.). Periodica, Copenhaguen, 1973, pp. 301-340.
15. LOWRY, O. H., ROSENBROUGH, J. N., FARR, A. L. and RANDALL, J. R.: *J. Biol. Chem.*, **193**, 265-274, 1951.
16. MEIJS-ROELOFS, H. M. A., UILENBROEK, J. Th. J., DE JONG, F. H. and WELSCHEN, R.: *J. Endocr.*, **59**, 295-304, 1973.
17. NEGRO-VILAR, A., OJEDA, S. R. and MCCANN, S. M.: *Endocrinology*, **93**, 729-735, 1973.
18. OJEDA, S. R. and RAMÍREZ, V. D.: *Endocrinology*, **84**, 786-793, 1969.
19. SNEDECOR, G. W. and COCHRAN, W. G.: «Métodos estadísticos», CECSA, España, 1971.
20. SPONA, J.: *FEBS Lett.*, **39**, 221-224, 1974.
21. STEELE, R. E. and WEISZ, J.: *Endocrinology*, **95**, 513-520, 1974.