

Effect of LH-RH and Hypothalamic Extracts on 3'-5'-Cyclic-AMP Pituitary Levels of Immature and Adult Female Rats *in vivo*

B. N. Díaz-Chico, J. C. Díaz-Chico and M. E. Díaz-Díaz

Colegio Universitario de Las Palmas
Departamento de Fisiología y Bioquímica
P.O. Box. 550. Las Palmas de Gran Canaria (Islas Canarias)

(Received on May 9, 1979)

B. N. DIAZ-CHICO, J. C. DIAZ-CHICO and M. E. DIAZ-DIAZ. *Effect of LH-RH and Hypothalamic Extracts on 3'-5'-Cyclic-AMP Pituitary Levels of Immature and Adult Female Rats in vivo*. Rev. esp. Fisiol., 36, 53-56. 1980.

Adult female rat hypothalamic extract and LH-RH increased the 3'-5'-cyclic-AMP (cAMP) level of immature pituitary *in vivo*, while immature female rat hypothalamic extract had the opposite effect. LH-RH and immature female rat hypothalamic extract did not change the cAMP pituitary levels 60 min after injection to adult female rats.

It has been accepted that cAMP (3'-5'-cyclic-AMP) is the second messenger of intracellular effector of several hormones (2, 3, 13). In the field of hypothalamic hormones, ZOR *et al.* (16) have shown that the presence of hypothalamic extracts in the culture medium of rat pituitary increased the cAMP tissular levels. LABRIE *et al.* (6, 7) and recently other authors (4, 5, 9), have demonstrated that cAMP is the mediator in the synthetic LH-RH and pituitary interaction *in vitro*.

In previous experiments in our laboratory (1, 2), we found out that there is a difference in the response to synthetic LH-RH between adult and immature female rat pituitaries *in vitro*. The present study tries to verify *in vivo* the conclusions obtained *in vitro* for the cAMP and

LH-RH interrelation in the pituitary tissue.

Materials and Methods

21 day old immature female Wistar rats, and adult female Wistar rats between 180 g and 200 g b.wt. were used. They were housed in our animal installations, and subjected to cycles of 14h. light and 10h. darkness, they received water and standard food *ad libitum*. An hypothalamic extract from adult female rats (Adult FRHE), containing 4 hypothalami per ml (430 mg w.w. tissue/ml), and an hypothalamic extract from immature female (Immature FRHE) containing 8 hypothalami per ml (522 mg w.w. tissue/ml), were prepared by the method of

McCANN (8) and kept at pH 7.4 by sodium bicarbonate (14). A solution of synthetic LH-RH (Beckman Instruments Inc. Palo Alto, Cal.), 10 nM/ml in saline, was prepared.

The animals were injected into the jugular vein with 0.5 ml/100 g of b.wt. of FRHE, or LH-RH solution, under light ether anesthesia. The control groups were injected with saline, 60 min after injection, the animals were killed by decapitation. The pituitaries were isolated, rapidly weighed and homogenized in a Tris-HCl 0.5 M buffer containing 4mM EDTA- Na_2 (pH 7.5) in order to inhibit the c-AMP phosphodiesterase, then heated for 3 min in boiling water and centrifuged.

The cAMP tissular levels were determined by a competitive binding radioassay, by a kit purchased from the Radiochemical Centre (Amersham, England).

Results and Discussion

Table I shows the cAMP levels found in the female rat pituitary 60 min after injection of the different stimuli investigated. The cAMP levels of adult female rat pituitaries were not significantly al-

tered by the injection with either immature FRHE or synthetic LH-RH. The cAMP levels found in these experiments agree with the results obtained by RATNER *et al.* (10) and YOSHIDA *et al.* (15) in female rat pituitary.

The cAMP concentration found in immature female rat pituitary are significantly greater than of the adult ($p < 0.001$). The adult FRHE or LH-RH increased cAMP levels in immature female rat pituitaries, 60 min after intravenous injection. However, the immature FRHE produced the opposite effect. The findings agree with our previous results obtained with proteins and RNA biosynthesis stimulation in immature pituitaries *in vitro* (2, 8).

The increase in cAMP synthesis in immature female rat pituitary after inoculation with LH-RH or adult FRHE could be substantiated by the existence of specific receptors for LH-RH associated with adenyl-cyclase activity on pituitary cell membranes, as described by SPONA (11, 12).

From the present results it is not possible to explain the decrease in cAMP concentration in immature female rat after immature FRHE injection, and further investigations are required.

Table I. Effect of hypothalamic extracts or synthetic LH-RH inoculation on cAMP female rat pituitary levels, *in vivo*.

Number of animals per group are in parentheses. Results are expressed $\bar{x} \pm \text{SE}$. FRHE = Female rat hypothalamic extract. Comparisons with controls were effectuated by the Student t-test. N.S. = non significant.

Inoculation		pM cAMP/mg ww tissue	Comparisons with controls
IMMATURE FEMALE RATS			
Control (saline)	(10)	10.9 ± 0.4	—
Immature FRHE	(8)	8.8 ± 0.4	$p < 0.001$
Adult FRHE	(6)	16.3 ± 2.5	$p < 0.05$
LH-RH	(8)	15.7 ± 1.1	$p < 0.01$
ADULT FEMALE RATS			
Control (saline)	(7)	6.4 ± 0.3	—
Immature FRHE	(6)	5.8 ± 0.3	N.S.
LH-RH	(6)	6.0 ± 0.3	N.S.

Although in adult female rat pituitary membrane specific LH-RH receptors associated with adenylyl-cyclase activity have been described (11, 12), significant differences have not been found 60 min after LH-RH injection to adult female rat. This apparent absence of stimulation on cAMP synthesis could be explained as a return to normal cAMP concentration at this time, after a previous acute increase and decrease in cAMP levels, as described by YOSHIDA *et al.* (15).

The lack of immature FRHE for cAMP synthesis stimulation in both adult and immature female rat pituitaries, in contrast with adult FRHE or LH-RH, and, on the other hand, the different responses of adult and immature female rat pituitaries to assayed stimuli, reveal that the hypothalamic content and/or its relation with the pituitary adenylyl-cyclase system are greatly affected by sexual maturation.

Acknowledgement

The authors are grateful to D. J. Rivero Báez for his technical assistance, and to Da. M. Bartolomé for her help in manuscript redaction.

Resumen

Se estudia el efecto de la LH-RH y los extractos hipotalámicos sobre la concentración de AMP-cíclico en hipófisis de ratas *in vivo*, 60 minutos después de la inyección intravenosa del estímulo.

La inyección de extracto hipotalámico de rata adulta o de LH-RH aumenta la concentración de AMP-cíclico en hipófisis de rata inmadura, mientras que el extracto de hipotálamo inmaduro la disminuye.

La LH-RH y los extractos de hipotálamo de rata inmadura no modificaron la concentración de AMP-cíclico en hipófisis de rata adulta.

References

1. DÍAZ CHICO, B. N. and DÍAZ CHICO, J. C.: *Rev. esp. Fisiol.*, **33**, 291-296, 1977.
2. DÍAZ CHICO, B. N. and DÍAZ DÍAZ, M. E.: *Rev. esp. Fisiol.*, **34**, 127-131, 1978.
3. HARLAND, J. C., ROBINSON, G. A. and SUTHERLAND, E. W.: *Ann. Rev. Physiol.*, **33**, 311-336, 1971.
4. JUSTISZ, M.: In «Mechanismes d'action intracellulaires des hormones» (R. Vokaer, ed.). Masson et Cie, Paris, 1970, p. 93.
5. KANEKO, T., SAITO, S., OKA, H., ODA, T. and YANAIHARA, N.: *Metabolism*, **22**, 77-80, 1973.
6. LABRIE, F., PELLETIER, G., LEMAY, A., BORGEAT, P., BARDEN, N., DUPONT, A., SAVARAY, M., COTÉ, J. and BOUCHER, R.: In «Karolinska Symp. Res. Meth. Reprod. Endocr.» (E. Diczfalussy, ed.). Periodica, Copenhagen, 1973, p. 301.
7. LABRIE, F., BORGEAT, P., BARDEN, N., BEAULIEU, M., FERLAND, J., DROUIN, J., DE LEAN, A. and MORIN, O.: In «Subcellular mechanisms in reproductive neuroendocrinology» (F. Naftolin, K. J. Ryan and J. Davies, eds.). Elsevier Scientific Publishing Co., Amsterdam, 1976, p. 391.
8. MC. CANN, S. M. and PORTER, J. C.: *Physiol. Rev.*, **49**, 240-284, 1969.
9. MAKINO, T.: *Am. J. Obstet. Gynec.*, **115**, 606-611, 1973.
10. RATNER, A., WILSON, M. C., SRIVASTAVA, L. and PEAKE, G. T.: *Neuroendocrinology*, **20**, 35-44, 1976.
11. SPONA, J.: *FEBS Lett.*, **34**, 24-26, 1973.
12. SPONA, J.: *FEBS Lett.*, **39**, 221-224, 1974.
13. SUTHERLAND, E. W., ROBINSON, G. A. and BUTCHER, R. W.: *Circulation*, **37**, 279-306, 1968.
14. YORK, D. H., BAKER, F. L. and KRAISER, L.: *Neuroendocrinology*, **11**, 212-219, 1973.
15. YOSHIDA, T., HATTORI, Y., HOSISAI, H., HIRANO, M., TAKAHASHI, K. and SUZUKI, M.: *Acta Endocrinologica (Kbh)*, **79**, 658-666, 1975.
16. ZOR, U., KANEKO, T., SCHNEIDER, H. P. G., MC. CANN, S. M., LOWE, I. P., BLOOM, G., BORLAND, B. and FIELD, J.: *J. Biochem.*, **63**, 918-925, 1969.

