Release of Immuno-Reactive and Biologically Active LH from Fetal Mouse Pituitary in Response to Synthetic Gonadotropin Releasing Factor (LRF)

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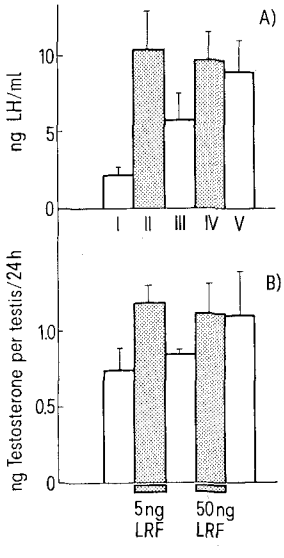
Summary. In an incubation system, LRF stimulated significantly the release of LH from 18-day-old mouse fetal pituitary. This LRF-induced LH release, measured by RIA in the incubation medium was able to increase the testosterone production by age-matched fetal testes. This data suggests that the hypothalamo-hypophyseal-testicular axis is functional at the end of mouse prenatal life.

It is now well established that in adult mammals both synthesis and release of gonadotropins by the pituitary are stimulated by the hypothalamic hormone LRF[4-6]. Recent studies have demonstrated that LRF could increase the secretion of immuno-reactive LH by the fetal pituitary in several species including rat[7], sheep[8] and human[9]. In the present paper we attempt to determine whether the fetal mouse pituitary is able to respond to synthetic LRF by increasing LH release, and whether this response produces a biological effect.

Experimental protocol for incubation of 20 fetal pituitaries and measure of LH release

Experiment	Time (min)	Incubation media	
	−30	Eagle's medium	Discarded
I	0	Eagle's medium	
II	30	5 ng LRF	RIA of LH
III	60	Eagle's medium	Bioassay of LH
IV	90	50 ng LRF	
V	120	Eagle's medium	



A) Immuno-reactive LH release from 18-day-old fetal mouse pituitary during each 30 min incubation period with (black columns) or without (white columns) synthetic LRF. B) Testosterone production by 18-day-old fetal testis cultivated during 24 h in the respective pituitary incubation media. Each bar represents the mean ± SD of quadruplicate pituitary incubations.

Material and methods. Albino swiss mice were studied. On day 18 of pregnancy (± 0.5 day), the females were sacrified by cervical dislocation, the uteri were removed and immersed into isotonic tyrode: Eagle's medium (50:50 v/v).

Fetal pituitaries were excised and pooled within 20 min. 20 fetal pituitaries from different litters were placed in 2 ml of minimum essential Eagle's medium containing 10% chick embryo serum (v/v), 5% glutamine (w/v) and 2% penicillin-streptomycin (w/v), then incubated in a metabolic shaker at 37 °C, under O_2:CO_2 (95:5). After a 30 min period, the pituitaries were removed from the medium and placed in fresh medium. This was considered as time 0 of the incubation.

Then the medium was aspirated at 30 min intervals and replaced by fresh medium containing or not synthetic LRF (Laboratoire Roussel, Paris): at time 0, 60 min and 120 min, the medium added was the basal Eagle's Medium; at time 30 min the medium contained 5 ng LRF; at time 90 min it contained 50 ng LRF (Table).

On each of the 5 pituitary incubation media (I–V according to Table) LH assay was performed: LH was measured (in quadruplicate) by radioimmunoassay according to the method described by Blake and al.[10], with a mouse standard[11]. Our results are expressed as NIAMD rat RP 1. A bioassay of LH was also performed on the same media, using the following procedure: in 0.6 ml of medium, 6 age-matched fetal testes were cultivated during 24 h. Then the testosterone content of the medium was measured as described previously[12,13]. The paired student's test was used for statistical analysis.

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[3] The expert technical assistance of Mrs M. T. Latreille and A. L'Héritier are gratefully acknowledged.
[4] A. V. Schally and C. Y. Bowers, Endocrinology *75*, 312 (1964).
[5] V. L. Gay, G. D. Niswender and A. R. Midgley, jr, Endocrinology *86*, 1305 (1970).
[6] T. W. Redding, A. V. Schally, A. Arimura and H. Matsuo, Endocrinology *90*, 764 (1972).
[7] S. J. Schafer and W. H. McShan, Neuroendocrinology *16*, 332 (1974).
[8] D. L. Foster, T. A. Cruz, G. L. Jackson, B. Cook and A. V. Nalbandov, Endocrinology *90*, 673 (1972).
[9] G. V. Groom, M. A. Groom, I. D. Cooke and A. R. Boyns, J. Endocr. *49*, 335 (1971).
[10] C. A. Blake, R. I. Weiner, R. A. Gorski and C. H. Sawyer, Endocrinology *90*, 855 (1972).
[11] A. Hus-Citharel, unpublished data.
[12] J. A. Mahoudeau and H. Bricaire, Ann. Biol. Clin. *30*, 559 (1972).
[13] G. Pointis and J. A. Mahoudeau, C. r. Acad. Sci., Paris *279*, 1197 (1974).

Results and discussion. The Figure shows the results of radioimmunoassays (A) and bioassays (B) of LH released from fetal mouse pituitaries into the incubation media, with or without LRF. The LH release measured by RIA was clearly stimulated by addition of 5 or 50 ng LRF to the incubation system. The decrease in LH content following removal of LRF was significant in medium III ($p<0.02$), but not in medium V, and did not reach the control level. The high LH level in these media as compared with the control medium (I) may be due to the presence of LRF remaining in the incubation flask and/or to a long-term stimulating effect of LRF upon these pituitaries.

It is noteworthy that the LH stimulation after 50 ng LRF (media IV) was not greater than after 5 ng (media II). This might indicate that a maximal response is obtained by addition of 5 ng LRF. However, an exhaustion phenomenon cannot be excluded.

The bioassays of LH are in good agreement with RIA. This reinforces the reliability of our present and previous results[14]. Moreover, the highly significant increase in biologically assayable LH under LRF suggests that hypothalamic stimulation of fetal pituitary may produce a detectable effect on the fetal testis. There is good evidence in humans that the biological activity of LH arizes from its β-subunit which seems to be synthetized under hypothalamic influence[15,16]. If this is the case in mouse, we could tentatively speculate that the hypothalamo-gonadotropin axis is already functional in mouse at the end of intra-uterine life, as it was suggested for other pituitary hormones, including TSH, GH and ACTH[17]. At least we can conclude that mouse fetal pituitary is responsive to LRF.

[14] G. POINTIS and J. A. MAHOUDEAU, Acta endocr., capenh. in press (1976).
[15] P. FRANCHIMONT and J. L. PASTEELS, C. r. Acad. Sci., Paris *275*, 1799 (1972).
[16] C. HAGEN and A. S. MCNEILLY, J. Endocr. *67*, 49 (1975).
[17] A. JOST, J. P. DUPOUY and M. RIEUTORT, Recent Progr. Horm. Res. *41*, 209 (1974).

Effects of Injury on the Concentration of α_1-Macroglobulin and α_2-Macroglobulin in the Plasmas of Male and Female Rats

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Summary. The effects of injury on the concentration of α_1-macroglobulin and α_2-macroglobulin in the plasmas of male and female rats has been investigated. At 5 days after injury to the male rats the α_1-macroglobulin concentration increased to 131% of its preinjury value. The α_2-macroglobulin concentration increased more rapidly to a maximum of 86 times its initial value. In the female rats α_2-macroglobulin increased only slightly and α_1-macroglobulin not at all.

The two rather similar α-macroglobulins of the rat have been referred to as: α_1-macroglobulin (α_1M), or slow α_1-globulin[3]; α_2-macroglobulin (α_2M), or slow α_2-globulin[4]; α_2-glycoprotein (GP)[5]; α_2 (acute phase)-globulin[6]; α-2-GP[7].

An increased concentration of α_2M in the plasmas of rats has often been employed as an index of tissue damage. Although present at high concentration in foetal rats α_2M is present at less than 50 μg/ml in the plasmas of normal male laboratory rats[8]. After injury its concentration may rise as much as 40 times. However after adrenalectomy injury no longer leads to this increase[9,10]. During recent work aimed at purification of rat α_2M lower concentrations of this protein were found in the plasmas of injured female as compared to injured male rats of the same strain. In order to confirm and extend this finding the concentration of α_2M in the plasmas of male and female rats has been estimated at various times after injury. For purposes of comparison the concentration of α_1M in the same plasmas was also estimated.

In a separate experiment the plasma haptoglobin concentration of injured male and female rats was estimated.

Materials and methods. Male and female hooded rats of the strain maintained at NIMR (weighing 170–190 g) were used throughout the experiments. All the rats received an i.m. injection of 2.5 mg cortisone (Cortisone Acetate Injection BP) into a hind thigh and 0.4 ml purified turpentine oil s.c. divided equally between both flanks, and then after 1–12 days the various groups were bled. The injections and bleedings which were by cardiac puncture under light anaesthesia were always done between 14.00 and 15.00 h. After storage of the sera for up to 7 days at 2 °C, the α_1M and α_2M concentrations were measured by radial immunodiffusion[11] using monospecific rabbit antisera against α_1M and α_2M and purified samples of the two proteins as standards. When very low concentrations of α_2M were to be estimated, the very faint rings were intensified by soaking the gel in 0.5% phosphotungstic acid. Haptoglobin was estimated by its peroxidase activity when complexed with methaemoglobin[12]. All values in the text are expressed as means $\pm$ SD.

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[3] C. H. W. HORNE and J. FERGUSON, J. Endocr. *54*, 47 (1972).
[4] G. H. BEATON, A. E. SELBY, M. J. VEEN and A. M. WRIGHT, J. biol. Chem. *236*, 2005 (1961).
[5] A. E. BOGDEN, G. A. NEVILLE, W. E. WOODWARD and M. GRAY, Proc. Am. Ass. Cancer Res. *5*, 6 (1964).
[6] E. J. SARCIONE, in *Plasma Protein Metabolism* (Eds. M. A. ROTHSCHILD and T. WALDMANN; Academic Press, New York 1970). chapter 23, p. 369.
[7] A. E. BOGDEN and J. H. GRAY, Endocrinology *82*, 1077 (1968).
[8] K. GANROT, Biochim. biophys. Acta *295*, 245 (1973).
[9] W. G. HEIM and S. R. ELLENSON, Nature, Lond. *213*, 1260 (1967).
[10] H. E. WEIMER and V. COGGSHALL, Can. J. Physiol. Pharm. *45*, 767 (1967).
[11] G. MANCINI, A. O. CARBONARA and J. F. HEREMANS, Immunochemistry *2*, 235 (1965).
[12] J. A. OWEN, F. C. BETTER and J. HOBAN, J. clin. Path. *13*, 163 (1963).