

Involvement of short-lived proteins in the regulation of expression of the LH receptor

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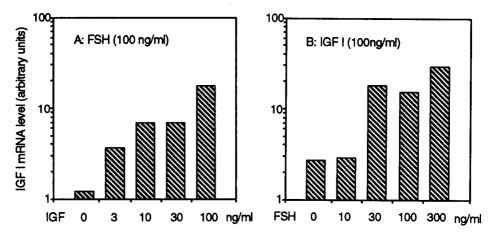


Fig 1. Effect of increasing doses of IGF I (A) or FSH (B) on IGF I mRNA levels. Pig granulosa cells were cultivated *in vitro* in the presence of 100 ng/ml FSH and increasing doses of IGF I (A), or with 100 ng/ml IGF I and increasing doses of FSH (B). Total RNA of these cells was analyzed by Northern blotting, hybridization with an IGF I cDNA probe and densitometric scanning of the autoradiograms. The results presented are from a single experiment representative of 2 similar experiments.

Involvement of short-lived proteins in the regulation of expression of the LH receptor. B Goxe, R Salesse (Unité d'Ingénierie des Protéines, INRA-Biotechnologies, 78352 Jouv-en-Josas Cedex, France)

The expression of the LH receptor can be induced in porcine granulosa cells in primary culture (Goxe et al (1992) J Mol Endocrinol 8, 119-129). As depicted in figure 1A, cells exhibited a transient over-expression of the LH receptor upon treatment with cycloheximide, but only af-

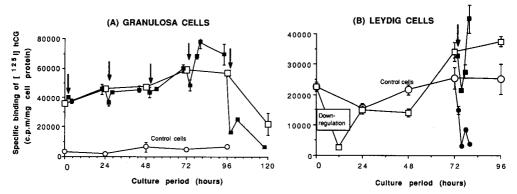


Fig 1. Effect of cycloheximide on LH receptor on granulosa and Leydig cells cultured in DMEM-HAM F12 (1:1 medium). (A) Granulosa cells: after confluence, cells were cultured in medium (O: control cells) or stimulated with hFSH (0.6 nM) for 120 h (□). Cycloheximide (10 μg/ml) was added (arrows) for 2 h. Then the medium was changed and cells subcultured for 2, 4, (6 for 72 h) and 24 h (n) in their previous medium. (B) Leydig cells: cells were cultured in medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml) and cAMP (10⁻⁵ M) for 96 h. The LH receptor was down-regulated (□) or not (O: control cells) by 25 ng/ml hCG for 5 h. At 72 h cycloheximide was added (arrow) for 2 h and cells subcultured for 2, 4, 6 and 8 h (■ and ●).

ter 72 h of FSH stimulation. At this time, the expression of the receptor itself seemed to reach a maximum (fig 1A) while the amount of its messenger RNA already decreased after having reached a maximum at 48 h (data not shown).

In porcine Leydig cells in primary culture, the expression of the LH receptor appeared as constitutive (fig 1B). In control cells, a cycloheximide treatment after 72 h of culture induced a decreased of the LH receptor level. However, in

cells first down-regulated by hCG, cycloheximide at 72 h elicited an early decrease, and then an increase of LH receptor.

In both cells, the half-life of the receptor itself was more than 24 h (unpublished results). Thus, in appropriately stimulated granulosa and Leydig cells, transient inhibition of protein synthesis by cycloheximide, indicated that short-lived proteins could be involved in the control of LH receptor expression.