

O-5 Identification of Nuclear Receptors in Rat Ovarian Follicles: Induction of NGFI-B Gene by Gonadotropins

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Although expression of steroid/thyroid hormone receptor genes has been extensively studied in other tissues, little information is available in the ovary. The present study was therefore designed to identify gonadotropin-inducible nuclear receptor genes in rat ovarian preovulatory follicles. Preovulatory follicles, obtained from ovaries of PMSG-primed immature rats, were cultured in serum-free medium in the presence of LH for 6 hr. Total RNA extracted from cultured follicles was used for reverse transcription and polymerase chain reaction with degenerate oligonucleotide primers corresponding to the DNA binding domain of nuclear receptor, which is highly conserved among the steroid/thyroid receptor family. We identified 61 positive clones using nested primers. Nucleotide sequence analysis revealed six distinct nuclear receptors including retinoic x receptor α and β , chicken ovalbumin upstream promoter-transcription factor and RIP15, TR4, THR and NGFI-B. Among six cloned receptors, NGFI-B gene expression was inducible by gonadotropins. Northern blot analysis revealed that hCG treatment caused a rapid and transient induction of NGFI-B gene expression, reaching a maximum at 1 hr. Similarly, treatment of cultured preovulatory follicles with LH resulted in transient expression of NGFI-B gene. The induction of NGFI-B mRNA by LH was similar between granulosa and thecal-interstitial cells of cultured preovulatory follicles. Treatment with forskolin, an adenylyl cyclase activator, induced NGFI-B mRNA, implying the role of adenylyl cyclase activation. In contrast, treatment with TPA, a protein kinase C activator, had no effect. Moreover, treatment of preovulatory follicles with epidermal growth factor and gonadotropin-releasing hormone also resulted in the induction of NGFI-B gene expression. Taken together, the present study indicates that degenerate primers corresponding DNA binding domain of nuclear receptors could be useful for cloning genes expressed in the ovary. Among the cloned receptors, NGFI-B gene was rapidly and transiently induced by gonadotropins, suggesting the possible role of NGFI-B in the ovulatory process.

O-6 Regulation of Pituitary Adenylyl Cyclase-Activating Polypeptide Gene Expression by Gonadotropin-releasing Hormone in Rat Ovarian Preovulatory Follicles

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It is well known that GnRH acts as a local ovarian regulator. Recently, pituitary adenylyl cyclase-activating polypeptide (PACAP) has also been suggested to play a role in ovulation during