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 identity 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 foskolin, an adenylyl cyclase activator, induced NGFI-B mRNA, implying the role of adenylate 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 Adenylate 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 adenylate cyclase-activating polypeptide (PACAP) has also been suggested to play a role in ovulation during

the periovulatory period. In the present study, gonadotropin and GnRH regulation of PACAP gene expression were examined in PMSG/hCG-treated immature rat ovaries and cultured preovulatory follicles. The major cell types expressing PACAP mRNA were granulosa cells of preovulatory follicles and some theca/interstitial cells. In preovulatory follicles cultured in serum-free medium, PACAP transcripts were transiently induced by LH, reaching a maximum 6~9 h after stimulation. Treatment of preovulatory follicles with LH stimulated GnRH receptor gene expression within 3 h. Interestingly, cotreatment with GnRH antagonist suppressed the LH-stimulated PACAP gene expression in a dose-dependent manner, suggesting that GnRH mediates the LH action on induction of PACAP gene expression in preovulatory follicles. Furthermore, treatment of preovulatory follicles with GnRH agonist also stimulated PACAP gene expression in a time- and dose-dependent manner. This GnRH-induced PACAP gene expression was inhibited by an inhibitor of PLA2, but not by an inhibitor of adenylate cyclase, or an inhibitor of protein kinase C, implying the role of PLA2 activation in GnRH-stimulated PACAP gene expression. Addition of PACAP-38 or -27 antagonist in culture of preovulatory follicles inhibited GnRH-stimulated progesterone production at 6~9 h, suggesting the role of endogenously produced PACAP in GnRH-stimulated progesterone production. Lastly, GnRH could also stimulate the PACAP promoter activity. Taken together, the present study demonstrates that GnRH plays a role in regulating PACAP gene expression and thus may act as a local regulator during periovulatory period.

O-7 Vero Cell과 Cumulus Cell을 이용한 공동배양 체계에서 IL-1β와 IL-6의 분비 및 Mouse 배아의 발생에 관한 연구

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인간의 난자를 체외에서 포배기 배아로 발생시키는데 있어서 defined culture system을 도입하려는 많은 연구가 있음에도 불구하고, 각종 체세포를 이용한 공동배양 체계에서 높은 발생율을 보이고 있다. 공동배양 체계에서는 배아의 발생과 착상에 영향을 미치는 여러 성장인자들과 cytokine들이 분비되는 것으로 알려져 있다.

본 연구의 목적은 vero cell이나 cumulus cell을 배양하였을 때, 분비되는 interleukine-1β (IL-1β)와 interleukine-6 (IL-6)를 분석하고, 이들이 mouse 배아의 체외발생에 어떠한 영향이 있는지를 조사한 것이다.

기본 배양액으로 20% 인간난포액이 첨가된 YS 배양액을 사용하였으며, IL-1β와 IL-6는 vero cell이나 cumulus cell을 각각 혹은 혼합 배양한 24시간 후에 ELISA와 western blotting에 의해 분석하였다. mouse는 C_{57}BL과 CBA의 F1 (국제실험동물)을 공시하였다. 본 실험에 사용한 배아는 과배란과 체외수정을 통하여 확보하였으며, 96시간 및 120시간 동안 배양하면서 feeder layer와 배아발생과의 관계를 조사하였다.

cumulus cell을 배양하였을 때 IL-1β는 187.4 pg/ml가 분비되었으나 IL-6는 2 pg/ml 수준에서 검출되지 않았다. 이와는 반대로 vero cell에서는 IL-1β는 검출되지 않은 반면, IL-6는 2,200 pg/ml이 분비되었다. 또한 cumulus cell과 vero cell을 혼합배양하였을 때, IL-1β는 198.1 pg/ml