IS-80 GnIH directly regulates steroidogenesis in human granulosa cells

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[Objective] Current studies have shown that RF amide related peptide-3 (RFRP-3), a mammalian homolog of avian gonadotropin-Inhibitory hormone (GnIH), inhibits gonadotropin secretion via direct effects on hypothalamic GnRH neurons and pituitary gonadotrophs. In the present study, we investigated whether RFRP-3 may act directly on human reproductive tissue outside CNS. [Methods] Expression of RFRP-3 and its receptor was examined by RT-PCR and Western blotting in cultured human ovarian granulosa cells (GC) obtained from IVF-ET patients. We also analyzed the effects of RFRP-3 on steroidogenesis and intracellular cyclic AMP (cAMP) concentration in GC. This study was approved by the institutional review board. Informed consent was obtained from each patient for the use of GC. [Results] Expression of RFRP-3 and its receptor was identified in GC. Treatment with RFRP-3 reduced progesterone production and expression level of steroidogenic acute regulatory protein (StAR) stimulated by FSH and forskolin, but not those stimulated by 8-Br-cAMP. RFRP-3 inhibited FSH- and forskolin-induced cAMP accumulation. These effects were abolished by the knockdown of RFRP-3 receptor with siRNA or by the co-treatment with an antagonist of RFRP-3, RF9. [Conclusion] GnIH is expressed in human GC where it acts via its receptor to regulate steroidogenesis at the level of adenylate cyclase activation.

IS-81 Stimulatory Effect of Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) and its PACAP Type I Receptor (PAC1R) on Prolactin Synthesis in Rat Pituitary Somatolactotrophs

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[Objective] Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional peptide which stimulates cAMP accumulation. PACAP might regulate prolactin ; however, the stimulatory effect of PACAP on prolactin synthesis and release in single prolactin-producing cells is inconclusive. We investigated the role of PACAP and its PACAP type I receptor (PAC1R) on prolactin synthesis in pituitary somatolactotrophs. [Methods] Rat pituitary somatolactotroph GH3 cells were used in this study. Prolactin promoter activity was assayed by Dual-Luciferase Reporter Assay System, using a luminometer. When PAC1R expressed to GH3 cells, PAC1 receptor expressing vector (HA-tagged PAC1/pEF-BOS in pCAM17) was transfected. [Results] PACAP increased prolactin promoter activity. This increase, while significant, was less than the increase resulting from thyrotropin-releasing hormone (TRH) stimulation. By transfection of a PAC1R to the cells, the response to PACAP on prolactin promoter activity was dramatically potentiated to a degree proportional to the amount of PAC1R transfected. In the PAC1R expressing GH3 cells, TRH and PACAP alone significantly increased prolactin promoter, respectively, and combined treatment with TRH and PACAP further increased prolactin promoters. [Conclusion] PACAP functions as a stimulator of prolactin synthesis in cells expressing high levels of its receptor.

IS-82 Hypoxia induces expression of COX-2 through the homeodomain transcription factor CDX2 and orphan nuclear receptor SHP in human endometriotic stromal cells

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Objectives : Endometriosis, the presence of ectopic endometrial tissue outside the uterine cavity, is a common disease affecting women during their reproductive years. The aim of this study was to identify the molecular mechanism of transcriptional regulation of inflammatory cyclooxygenase-2 (COX-2) gene during endometriosis by hypoxia. Methods : Hypoxia induced COX-2 expression in endometrial stromal cells together with induction of the orphan nuclear receptor SHP and intestinal-specific transcription factor CDX1. HIF-1a is responsible for SHP induction mediated by hypoxia. Results : We observed that ectopic expression of CDX1 enhanced COX-2 gene expression in hypoxia-dependent fashion. Furthermore, we evaluated that induction of CDX1 by hypoxia was mediated by SHP. We also observed that expression of COX-2, CDX1, SHP and HIF-1a mRNA in hypoxia-treated human endometrial cells were significantly higher than normal regions. Conclusion : These results suggest that the SHP and CDX1 expression increased by hypoxia and also play an active role in endometriosis through regulation of inflammatory COX-2 expression.