Stimulation of mitogen-activated protein kinase by gonadotropin-releasing hormone in human granulosa-luteal cells

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摘要

Abstract

The present study investigated the activation of mitogen-activated protein kinases (MAPKs) by a GnRH agonist (GnRHa) in human granulosa-luteal cells (hGLCs). The phosphorylation state of p44 and p42 MAPK was examined using antibodies that distinguish phospho-p44/42 MAPK (Thr(202)/Tyr(204)) from total p44/42 MAPK (activated plus inactivated). Activation of MAPK by GnRHa was observed within 5 min and was sustained for 60 min after treatment. GnRHa stimulated MAPK activation in a dose-dependent manner, with maximum stimulation (6.7-fold over basal levels) at 10(-7) M. Pretreatment with a protein kinase C (PKC) inhibitor, GF109203X, completely blocked GnRHa-induced MAPK activation. In addition, pretreatment with a PKC activator, phorbol-12-myristate 13-acetate, potentiated GnRH-induced MAPK activation. These results indicate that GnRHa stimulates MAPK activation through a PKC-dependent pathway in hGLCs, possibly coupled to G(q)alpha protein. MAPK activation was also observed in response to 8-bromo-cAMP or cholera toxin, but not pertussis toxin. Forskolin (50 microM) substantially stimulated a rapid cAMP accumulation, whereas GnRHa (10(-7) M) or pertussis toxin (100 mg/ml) did not affect basal intracellular cAMP levels. Cotreatment of GnRHa (10(-7) M) did not attenuate forskolin- or hCG-stimulated cAMP accumulation. These results suggest that the GnRH receptor is probably not coupled to G(s)alpha or G(i)alpha in hGLCs. Finally, GnRHa (10(-7) M) stimulated a significant increase in Elk-1 phosphorylation and c-fos messenger RNA expression, as revealed by an in vitro kinase assay and Northern blot analysis, respectively. These results clearly demonstrate that GnRH activates the MAPK cascade through a PKC-dependent pathway in the human ovary.