

Signaling pathways activated by the LH receptor in Leydig cells.

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Results from a number of laboratories have shown that the agonist-engaged LHR activates several families of G proteins including G_s , $G_{i/o}$ and $G_{q/11}$. Although it has been known for many years that the activation of steroidogenesis involves the G_s /adenylyl cyclase/cAMP/PKA pathway more recent studies from several laboratories have shown that this pathway is also responsible for the activation of the ERK1/2 cascade. In MA-10 cells we have recently shown that PKA (directly or indirectly) activates Ras and that this GTPase is an intermediary in the cAMP/PKA-mediated activation of the ERK1/2 cascade. In MA-10 cells the activation of $G_{q/11}$ leads to the activation of a phospholipase C resulting in the activation of the inositol phosphate/diacylglycerol pathway. The functional consequences of the activation of this pathway are still unclear, however. Lastly, no information is available about the functional consequences of the LHR-induced activation of the $G_{i/o}$ family.

Using antiphosphotyrosine blots of whole cell lysates we have recently found that activation of the LHR in MA-10 cells leads to the tyrosine phosphorylation of an abundant 120 kDa protein that was subsequently identified (using a proteomic approach) as the focal adhesion kinase (FAK). The use of phosphospecific antibodies reveal that activation of the LHR results in the phosphorylation of Tyr⁵⁷⁶ which is one of the two FAK residues phosphorylated by the Src family of tyrosine kinases. Further studies showed that MA-10 cells do not express Src but do express Fyn and Yes (two other members of the Src family of kinases). We also showed that activation of the LHR results in an increase in the enzymatic activity of these two tyrosine kinases and that dominant-negative mutants of Fyn and Yes inhibit the LHR-induced phosphorylation of FAK. Using a variety of pharmacological inhibitors, dominant negative mutants, selective activators or constitutively active mutants we were able to exclude the involvement of G_s , $G_{i/o}$ and the beta arrestins as mediators of the LHR-induced phosphorylation of FAK and we were able to show that this LHR effect is mediated by the activation of $G_{q/11}$. Furthermore our current data suggests that the LHR-induced phosphorylation of FAK is not a result of the $G_{q/11}$ -mediated activation of phospholipase C but is instead mediated by a member of the Rho family of GTPases, which is directly or indirectly activated by $G_{\alpha_{q/11}}$.

These studies identify a new $G_{q/11}$ -sensitive pathway that is activated by the LHR and leads to the phosphorylation of FAK in Leydig cells. Since FAK is an integrator of signals that promote cell migration our data also suggest that the LHR modulates the migration of Leydig cells.