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The gonadotropin FSH binds to its membrane receptor and initiates a cascade of cellular responses in target Sertoli cells. FSH-receptor couples to Gs protein and stimulates adenyl cyclase, elevates c-AMP, then activates a series of kinases and finally alters target Sertoli cell functions. Previous reports suggested that tTG covalently cross-linked FSH and receptors, and stabilized the FSH-receptor complexes. However, the existence of cross-linked FSH-receptor complexes have not yet been demonstrated.

tTG is found in various cell types and tissues. Recent data have shown that tTG has multiple functions. It also acts like a G protein when it binds with GTP and was designated as G£\h, a 50 kilodalton protein. The inhibitory effect of the tTG destabilizes/dissociates FSH-receptor binding is due to it's transglutaminase function or its G£\h function remains to be elucidated. Recent findings show that TSH couples with more than 10 different kinds of G proteins. Similarly, in addition to coupling with Gs, whether or not FSH-receptor also couples with other G proteins, such as G£\h, is not known. It is, therefore, intended to demonstrate the existence of cross-linked FSH-receptor complexes. On the other hand, efforts will also be made to trace the possible function of tTG in FSH target cells; *i. e.*, tTG may act like a G protein such as G£\h. Or, other G proteins may couple with FSH-receptor. In other words, experiments will be performed to test if FSH would activate phospholipase C-δ_1(PLC-δ_1), elevate IP_3 and DAG, or mobilize intracellular calcium etc. in the target cells.

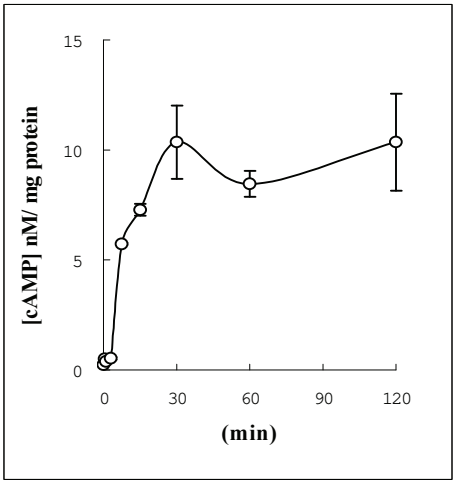
Our recent data have demonstrated the presence oftTG in cultured Sertoli cells using western blot analysis. In this study, the dose effect and time course of FSH on the elevation of intracellular cAMP in Sertoli cells were determined. The ED50 of FSH was observed between 100-300 IU/L. Thus 300 IU/L FSH was administrated to Sertoli cells in subsequent studies. The levels of intracellular cAMP level were maximal after administration of FSH for 7.5-minutes MIX, a phosphodiesterase inhibitor, pretreated Sertoli cells. On the other hand, phospholipase C-δ1 was also when the light membrane fraction of Sertoli cell after 60-min treatment of FSH was analyzed by Western blot. Furthermore, three different molecular weight protein bands of 85, 74 and 50(G£\h) kilodalton were detected by anti-tTG monoclonal Ab. Further effets be made to determine the elevation/activation sequences of cAMP, PLC-δ_1, IP_3 and DAG.

Keywords: gonadotropin, FSH, receptor, transglutaminase, G proteins, Gs, G£\h, phospholipase C-δ_1, IP_3, DAG, c-AMP, Sertoli cells.

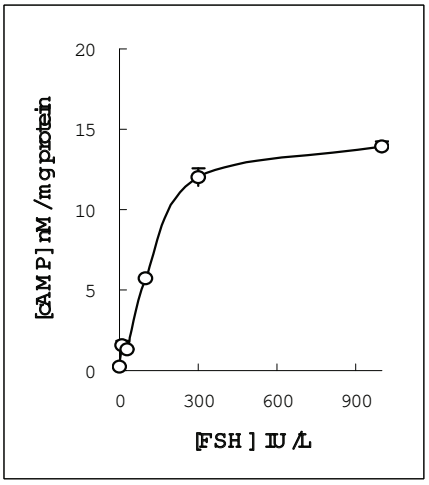
1. **<u>FSH induced intracellular cAMP accumulation in a time- and dose-dependent manner in Sertoli cells</u>**

FSH (100IU/ ml) was administered to 6 days-cultured Sertoli cells which were pretreated with 1mM of methyl-isobutyl xanthine (MIX), a phosphodiesterase inhibitor. After incubation, Sertoli cells were scrapped from culture plate in the extraction buffer and centrifuged at 20,000 x g at 4°C for 30 minutes. The supernatants were used to measure the concentration of intracellular cAMP with an WLISA kit. The data from three independent experiments were calculated with SigmaPlot software as shown in Fig. 1a and b. The levels of intracellular cAMP were maximal 7.5-minutes after administration of FSH. The ED50 of FSH was observed between 100-300 IU/ L.

a.



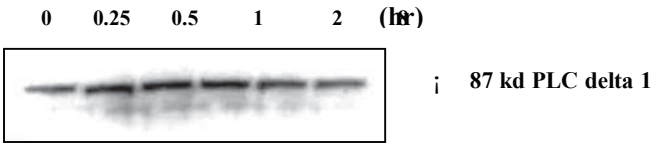
b



2. **<u>Anti-tTG antibodies recognized three different fragments of tTG in light membrane of Sertoli cells</u>**

After 6 days of culture Sertoli cells were treated with 300 IU/ L of FSH was at defined time intervals. After incubation, Sertoli cells were scrapped from culture plates in extraction buffer and the cell lysates were centrifuged 300 at xg at 4°C for 10 minutes. Furthermore, the supernatants were transferred to the new eppendorf tube and then centrifuged at 50,000 xg at 4 °C for 60 minutes. The pellets (light membrane) were resolved by western blotting analysis. The data shown that three bands of 87, 74 and 50 kD proteins were recognized by anti-tTG antibody.

3. <u>FSH stimulated the PLC δ1 activation and translocation from cytosol to membrane</u>

After 6 days of culture, Sertoli cells were treated with 300 IU/ L of FSH at the defined time intervals. FSH treated-Sertoli cells were scrapped from culture dish in the extraction buffer and the lysates were centrifuged 300 xg at 4¢J for 10 minutes. Subsequently, the supernatants were transferred to new eppendorf tube and then centrifuged at 50,000 xg at 4 ¢J for 60 minutes. The pellets (light membrane) and supernatants (cytosol) were analyzed by western blotting . The data shown that PLC £_1 was increased after 60-min FSH treatment in the light membrane of Sertoli cells. G3PDH was used as an internal control of loading protein concentration.

membrane