Signaling between the LH Receptor and the NPR2 Guanylyl Cyclase in Mouse and Rat Preovulatory Follicles

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Activation of the luteinizing hormone (LH) receptor in the granulosa cells of mouse and rat preovulatory follicles causes dephosphorylation of regulatory sites on the membrane guanylyl cyclase, natriuretic peptide receptor 2 (NPR2), decreasing production of cGMP and promoting resumption of meiosis in the oocyte. However, it is unknown how LH regulates dephosphorylation of NPR2. Since NPR2 dephosphorylation occurs rapidly, within 10 minutes, we hypothesize that it is triggered by LH-induced changes in protein phosphorylation that either increase the activity of a phosphatase or decrease the activity of a kinase in the presence of constant phosphatase activity. LH stimulation rapidly increases the phosphorylation of the phosphatase subunits PPP1R12A and PPP2R5D on serines whose phosphorylation is known to increase phosphatase activity. However, LH-induced dephosphorylation of NPR2 was not prevented in follicles from mice in which serine 507 of PPP1R12A and serines 53, 81, 82, and 566 of PPP2R5D were all changed to alanines, thus opposing activation of these phosphatases. These findings indicate that LH does not reduce NPR2 phosphorylation by way of these PPP family phosphatase regulatory subunits.

Alternatively, LH signaling could reduce NPR2 phosphorylation by inhibiting an active kinase. Although the kinases responsible for maintaining the basal phosphorylation of the regulatory serines and threonines of NPR2 are unknown, one candidate that stands out is glycogen synthase kinase-3 (GSK3), which is encoded by 2 similar genes, *Gsk3a* and *Gsk3b*. Phosphorylation of GSK3A and GSK3B, on serines 21 and 9 respectively, inhibits their kinase activity. Previous research has shown that mouse and rat preovulatory follicles express both GSK3A and GSK3B, and both GSK3A-serine 21 and GSK3B-serine 9 are rapidly phosphorylated in response to LH signaling, by way of protein kinase A (PKA). If GSK3 phosphorylates NPR2, it could be responsible for maintaining NPR2 activity and meiotic arrest prior to LH signaling and could link LH-induced PKA activation to NPR2 dephosphorylation, in the pathway by which LH reinitiates meiosis. In support of this hypothesis, incubation of mouse follicles with a selective GSK3 inhibitor (CHIR-99021, 10 μM) decreased NPR2 phosphorylation. Consistent with a reduction in NPR2 dephosphorylation, this inhibitor also caused nuclear envelope breakdown in follicle-enclosed oocytes. CHIR-99021 did not cause nuclear envelope breakdown in isolated oocytes that were maintained in meiotic arrest with the PDE3 inhibitor milrinone, indicating that the GSK3 inhibitor caused meiotic resumption by acting on the granulosa cells, rather than directly on the oocyte. Mice with genetic modifications of *Gsk3a* and *Gsk3b* will facilitate future studies of the possible function of GSK3 kinases in regulating meiotic progression in preovulatory follicles.

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