Phosphorylation of the cGMP phosphodiesterase PDE5 is required for the LH-induced increase in cGMP-hydrolytic activity in mouse ovarian follicles

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Background

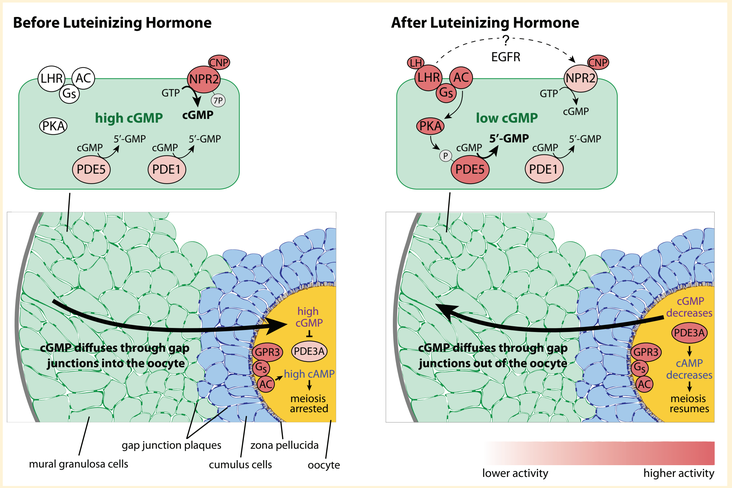

Figure 1: Model of cGMP-mediated meiotic arrest and the rapid LH-induced cGMP decrease that leads to meiotic resumption. cGMP is produced in mural granulosa and cumulus cells by the membrane guanylyl cylcase natriuretic peptide receptor 2 (NPR2)[1], which requires phosphorylation on up to 7 juxtamembrane S/T residues for full activity[2]. A steady-state level of cGMP in rat granulosa cells is maintained by basal phosphodiesterase activity of PDE5 and PDE1[3]. cGMP maintains meiotic arrest by diffusing through gap junctions into the oocyte where it inhibits the phosphodiesterase PDE3A, keeping intra-oocyte cAMP high and preventing the activation of meiotic targets[4].
LH rapidly reduces cGMP levels by inactivating NPR2[5] through dephosphorylation[6]. How LH signaling leads to NPR2 dephosphorylation is not fully understood, but involves EGFR activation, at least in part[7]. The LH-induced cGMP decrease in the mural cells reverses the concentration gradient, such that cGMP now diffuses out of the oocyte[8], allowing PDE3A to hydrolyze cAMP and thus relieve meiotic inhibition. However, if NPR2 dephosphorylation is prevented, there is still a substantial decrease in follicle cGMP levels in response to LH[6,7], suggesting that other mechanisms also contribute to this process. Recently, in rats, we found that LH signaling rapidly phosphorylates PDE5, which is associated with an ~70% increase in its cGMP-hydrolytic activity[3]. Though it has been reported that complete inhibition of PDE5 activity delays LH-induced meiotic resumption[9], it is not known whether the phosphorylation and *increase* in PDE5 activity contributes to the regulation of cGMP levels and meiotic resumption.

Questions

1) Does LH signaling cause PDE5 phosphorylation in mouse follicles, as we previously reported for rat follicles?

2) Is PDE5 phosphorylation required for the LH-induced increase in cGMP-hydrolytic PDE5 activity?

3) Is the phosphorylation and activation of PDE5 required for the LH-induced decrease in follicle cGMP and meiotic resumption?

Rationale and Approach

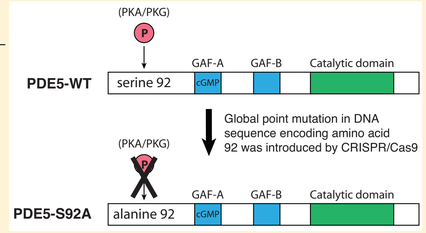

Figure 2: Regulation of PDE5 activity, and mutation to prevent LH-induced phosphorylation. Binding of cGMP to the GAF-A domain of PDE5 increases its cGMP-hydrolytic activity[10]. In wild-type PDE5 (PDE5-WT), phosphorylation of serine 92 by PKA/PKG further increases PDE5 activity, probably by stabilizing the binding of cGMP to the GAF-A domain[10]. Using CRISPR/Cas9, we generated mice with a global mutation in PDE5 that replaced serine 92 with an alanine (PDE5-S92A), such that PDE5 cannot be phosphorylated[10] in response to LH.

Results

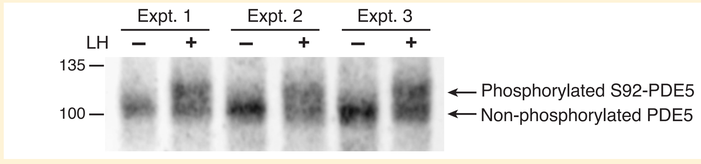

Figure 3: PDE5 is phosphorylated in response to LH in mouse follicles. Mouse follicles were treated +/- 10 ug/ml ovine LH for 30 min. Lysates were run on a gel containing Phos-tag, which differentially slows the migration of phosphorylated proteins, and immunoblotted with a total PDE5 antibody as previously described[3,6].

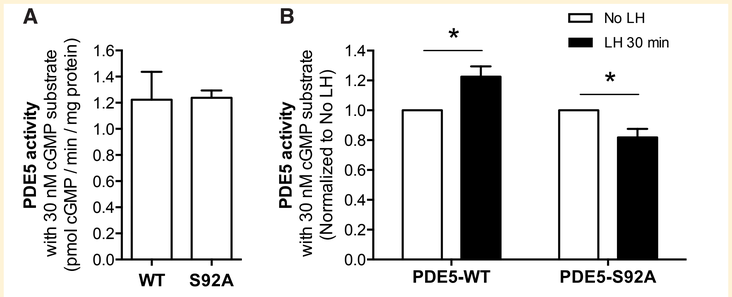

Figure 4: PDE5 phosphorylation is not required for basal PDE5 cGMP-hydrolytic activity, but is required for the LH-induced increase in activity. **A**. PDE5 activity was not different in lysates of untreated follicles from mice with either wild-type PDE5 or mutated PDE5 that cannot be phosphorylated. **B**. In wild-type mouse follicles, PDE5 activity increases by ~20% in response to 30 min of LH treatment. However, this increase is prevented when PDE5 cannot be phosphorylated. The reason for the decrease in PDE5 activity in mutant follicles after LH treatment is not clear, but may involve altered cGMP binding to the GAF-A domain as follicle cGMP levels decrease[10]. PDE5 activity was measured by subtracting the activity remaining in the presence of 100 nM sildenafil from the total cGMP-hydrolytic activity[3]. Bars represent the mean +/- sem of 5 experiments for each genotype. Asterisk indicates $p < 0.05$ for paired t-test on non-normalized data.

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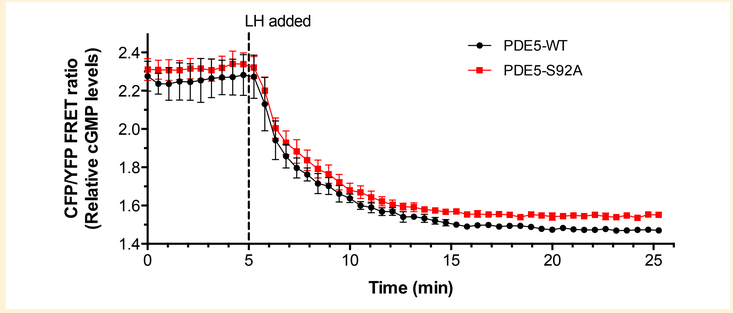

Figure 5: PDE5 phosphorylation is not required for the rapid decrease in follicle cGMP levels in response to LH. The rate of decrease in, and the equilibrium level of, cGMP in mural granulosa cells after LH treatment are not different in follicles from PDE5-WT and PDE5-S92A mice that also express the cGi500 FRET sensor for cGMP. This indicates that activation of PDE5 is not required to achieve low follicle cGMP levels in response to LH. Methods are decribed in ref. (8). Briefly, follicles were mounted in a perfusion slide filled with culture medium and imaged every 30 sec before and after perfusing with LH. Changes in the CFP/YFP ratio of the cGi500 FRET sensor, which reflects cGMP levels, were measured in the mural granulosa region of 2 PDE5-WT and 5 PDE5-S92A follicles. Mean +/- sem plotted.

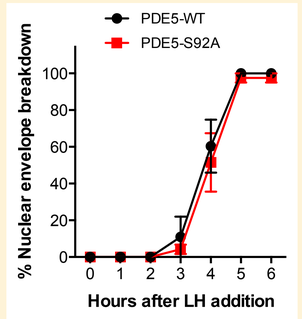

Figure 6: Preventing the phosphorylation of PDE5 does not inhibit or delay meiotic resumption in response to LH. Breakdown of the oocyte nuclear envelope is the first visual sign of meiotic resumption. The timing of LH-induced meiotic resumption is identical in follicle-enclosed oocytes from wild-type and PDE5-S92A mice. Though LH-induced meiotic resumption is delayed if PDE5 activity is completely inhibited[9], these data suggest that the *increase* in PDE5 activity is not required for meiotic resumption. Data are the mean +/- sem of 7 total experiments and 97 follicles.

Conclusions and Future Directions

- **Using mutant mice, we show that LH signaling increases the cGMP-hydrolytic activity of PDE5 by phosphorylation of serine 92.**
- **However, phosphorylation of PDE5 is not required for the rapid LH-induced decrease in follicle cGMP levels or for meiotic resumption.**
- **Studies are underway to identify other mechanisms that lower cGMP after LH, focusing on PDE1 activity, which contributes to meiotic resumption in rats[3].**

References

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