**SUPPLEMENTARY MATERIAL**

**Luteinizing Hormone Reduces the Activity of the NPR2 Guanylyl Cyclase in Mouse Ovarian Follicles, Contributing to the Cyclic GMP Decrease that Promotes Resumption of Meiosis in Oocytes.** Jerid W. Robinson, Meijia Zhang, Leia C. Shuhaibar, Rachael P. Norris, Andreas Geerts, Frank Wunder, John J. Eppig, Lincoln R. Potter, and Laurinda A. Jaffe

**TABLE S1. Primers and fluorescent probes used for qRT-PCR.**

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| --- | --- | --- | --- |
| target | forward primer, 5’-3’ | probe, 5’-3’ | reverse primer, 5’-3’ |
| *Npr1* | GCACTCGAGGCTGACCTACT | CCCTGCTATTCCTTGTCACCTCCCC | GTGTCATTGCTGGCACAGG |
| *Npr2* | GGAAAAGGAAAAATGCGAACT | ACTGGCTCTTGGGAGAGCAAAAGGG | TTTACAGGAGTCCGGGAGGT |
| *Gucy2c* | CCGCTGGAAGTTATGGATTG | AAGGAAACCCTAACCAGGATCCTGC | ATCAGCTTCCTGGCTGGAG |
| *Gucy2d* | CTGGCCCTGGACATCCTTAG | TATGCAGGCAACTTTCGGATGAGGC | CCTGACACGGATGGGTACAT |
| *Gucy2e* | GGAGATTCCCCCAGAGAGAC | CAGGCCAGGCCAGTTTACTGGGAAG | CTGTCCCCAGTAGAGCTTCA |
| *Gucy2f* | CTTGCAACCAGCAGAGATTG | TTCCAAAGGAGAAAAGCAGAGAGGC | GGCTTGTTTCGCACCAACT |
| *Gucy2g* | AAAGGTGATGGAAGGATTGTG | CCCTAGTGAGGGAGTGCTGGGATGA | GAAGGGAAGATGGGCCTTAG |
| *Gucy1a3* | GACTGTCCTGGCTTTGTGTTC | CCCCGAGATCAAGGGAGGAGCT | TCACTAGGGAAGTTTGGTGGA |
| *Gucy1a2* | CCAGACAACTTTCCGAAGGA | TTCCTGGGGTCTGCTATTTCCTGGA | GGCTTTGGGTCAGTCCTTAAC |
| *Gucy1b3* | CTCGGATCCACTGTTCCATT | AGAGGCCCAGTGTCTATGAAGGGCA | ACCAGACTTGCATTGGTTCC |
| *Gucy1b2* | CAAGAAGCCCCGTGCTGT | CTGAGCAACATGGACCACCACCAG | CGTCTGCTGGATCACTGTTG |
| *Npr3* | ACCATTGAGAGGCGAAATCA | AAGAGGAAAGCAACATCGGGAAGCA | TGGAATCTTCTCGCAGCTCT |
| *Rpl32* | TTCATCAGGCACCAGTCAGA | TGTGAAAATTAAGCGAAACTGGCGG | TTGTCAATGCCTCTGGGTTT |

Probes were labeled with FAM (5’) and TAMRA (3’).



**FIGURE S1.** A simplified model to estimate how much the decrease in NPR2 activity in the follicle, to ~50% of the basal level at 20 minutes after LH application, could decrease the concentration of cGMP in the follicle. Starting from the pre-LH cGMP concentration of ~3 M (Norris et al., 2010), the figure shows the concentration of cGMP that would be attained after a decrease in NPR2 activity to 50% of the pre-LH level, as a function of the Km of the cGMP phosphodiesterase in these cells. The calculation used to make the graph is described below.

Before LH is applied, cGMP is being degraded at a rate that is equal to the rate at which it is being produced. After LH signaling decreases guanylyl cyclase activity and the concentration of cGMP begins to decrease, cGMP phosphodiesterase activity will also decrease, due to the decrease in its substrate. A new equilibrium cGMP concentration will be reached when the cGMP phosphodiesterase activity falls to 50% of its original value, such that rates of production and degradation of cGMP will again be equal. If the activity of the phosphodiesterase behaves as described by the Michaelis-Menten equation,

V/Vmax = [cGMP]/(Km + [cGMP]),

we can calculate the initial V/Vmax (before LH) using the Km value and the known initial concentration of cGMP, which is 3 M. For example, if Km = 1 M,

Vo/Vmax = 3/( Km + 3) = 3/(1 + 3) = .75

Using this value for Vo/Vmax, we can then calculate the concentration of cGMP when V/Vmax falls to 50% of Vo/Vmax:

V/Vmax = .375 = [cGMP]/(1 + [cGMP])

.375 + .375 [cGMP] = [cGMP]

.375 = [cGMP] - .375[cGMP]

.375 = .625 [cGMP]

[cGMP] = .375/.625 = .6 M

Fig. S1 was generated using similar calculations to determine the equilibrium cGMP concentration for the particular Km's shown. The graph shows that if the phosphodiesterase has a higher affinity for cGMP (lower Km), it is capable of reducing the cGMP to a lower concentration.

Based on its sensitivity to sildenafil and tadalafil, ~75% of the cGMP phosphodiesterase activity in the non-LH-treated follicle is likely to be due to PDE5 (Vaccari et al., 2009), so we asked what would be predicted by this model if all of the cGMP phosphodiesterase activity was assumed to be due to PDE5. We disregarded factors that are not taken into account by the Michaelis-Menten equation (such as regulation of PDE5 by cGMP binding to allosteric sites and cGMP-dependent phosphorylation; see Francis et al., 2011). Although some of the values for the Km of PDE5 made recombinantly are higher (Loughney et al., 1998; Corbin et al., 2000; Lin et al., 2000; Wang et al., 2006; Zoraghi et al., 2006), almost all of the values for the Km of native PDE5 purified from mammalian tissues (aorta, platelets, trachea, lung) are in the range of ~0.2 to ~2 M (e.g., Lugnier et al., 1986; Thomas et al., 1990; Robichon, 1991; Rousseau et al., 1994; Chulia et al., 1997; Kotera et al., 2000; Kameni-Tcheudji et al., 2007). PDE5 isolated from mammalian tissues is likely to be a better predictor of biological activity since its post-translational processing would have occurred normally.

With Km values ranging from 0.2 to 2 M, a decrease to 50% of the basal guanylyl cyclase activity is calculated to result in equilibrium cGMP concentrations of 0.17 to 0.85 M. Thus, a 50% reduction in NPR2 activity could potentially account for the measured decrease in the follicle cGMP concentration from ~3 M before LH treatment to ~0.5 M 20 minutes later.

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