The circadian regulator REVERBα downregulates the LH surge-induced expression of cyclooxygenase-2 (Cox2) in mouse granulosa cells

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Background: Expression and the function of genes generating circadian rhythms in the ovary have not been fully examined from proestrous stage to estrous stage in spite of the interest for the involvement of the circadian clock on ovulation. Here, the circadian clock network and the involvement of one of its regulators, RevErba, in the expression of Cox2, the inducer of ovulation in ovaries, were examined. Results: Coordinated, rhythmic expression was found for main circadian regulatory genes, suggesting that the ovarian circadian clock is functional from proestrous to estrous stage. Because the expression level of RevErba was the lowest at the time of occurrence of LH surge but increased immediately after that, we hypothesized that the duration of the transient rise of Cox2 expression induced by LH surge is adjusted by the suppressive effect of REVERBa on Cox2 expression. Using cultured granulosa cells and a linker-scan mutagenesis screen of the potential regulatory region of Cox2, we identified REVERBa binding sites (RORE). Luciferase reporter vectors containing the promoter region of Cox2 with and without RORE (pGLR and pGLdR) were transfected into cultured mouse granulosa cells. Agreeing with our hypothesis, dose-dependent expression of RevErba into the culture cells suppressed the induction of pGLR by forskolin, which mimicks the LH surge in vitro. Contrarily, over-expression of RevErba did not affect expression of pGLdR. Finally, binding of REVERBa was detected to this regulatory region in phase of the beginning repression of endogenous Cox2. Conclusion: Our results suggest that RevErba is involved in fine-tuning of Cox2 expression, which is important for the efficiency of ovulation.