Relationship between metastin/kisspeptin, neurokinin B (NKB) and dynorphinin mRNA expression in the granulosa cells of rats

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Kisspeptin, NKB and dynorphin are co-expressed in KNDy neurons in the hypothalamus. We recently reported that granulosa cells are also KNDy cells and the expression of those peptides is stimulated by preovulatory LH surge, but relationship between these peptides is unknown. In the present study, mutual effect of these peptides on respective mRNA expression was investigated. Granulosa cells were prepared from eCG treated immature female rats. Cells were treated with hCG 0.01 IU/ml and/or kiss-10 (10^-11M), NKB (10^-6M) or dynorphin (10^-6M) after 24 hours of preincubation. 1) The expression of GnRH, kisspeptin, dynorphin and NKB were all augmented by hCG. 2) Dynorphin and hCG additively augmented both NKB and kisspeptin expression. Kiss-10 with hCG also stimulated both dynorphin and NKB expression, while NKB inhibited hCG stimulated kisspeptin and dynorphin expression. 3) Kiss-10 showed no effect on GnRH expression, while GnRH stimulated annexin A5 expression. Surprisingly GnRH agonist enhanced kisspeptin expression. 4) In vivo gene expression showed acute increase of kisspeptin and dynorphin after hCG injection, while NKB expression was delayed and sustained. 5) hCG treatment was demonstrated to proceed luteinization by p21, p27, LH receptor and FOXO1 expression and progesterone production. These data demonstrate that kisspeptin and dynorphin are stimulated their expression by hCG and augment each other in granulosa cells. NKB seems to cease the enhancement of kisspeptin and dynorphin by LH. The novel sequence of events found in this study induced by hCG/LH is hypothesized to be beneficial for luteinization of granulosa cells.