

## D101

### Expression of Phospholipase C $\beta 1$ and $\gamma 1$ during Mouse Oocyte Maturation and Early Preimplantation Embryo Development as determined by competitive RT-PCR

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It is well known that phospholipase C (PLC) plays an important role in the intracellular signaling in a variety of cell types. However, the involvement of PLC in mouse oocyte maturation and early preimplantation embryo development remains unknown. In the present study, we examined the expression patterns of PLC  $\beta 1$  and  $\gamma 1$  during mouse oocyte maturation and preimplantation embryo development. Total RNAs prepared from a single mouse oocyte and preimplantation embryo were subjected to competitive reverse transcription-polymerase chain reaction (RT-PCR procedure). PLC  $\gamma 1$  mRNA level was easily detected in germinal vesicle (GV)-stage oocyte and decreased as meiotic resumption proceeded, while PLC  $\beta 1$  mRNA was scarcely detected in all stages of meiotic maturation. After fertilization, both PLC  $\beta 1$  &  $\gamma 1$  mRNA levels began to increase at morula stage and were more prominent in blastocyst-stage embryos. To elucidate the possible involvement of PLC via protein kinase C (PKC) pathway during oocyte maturation, we examined the effect of sphingosine, a potent PKC inhibitor. A treatment of sphingosine (100 $\mu$ M) facilitated the meiotic resumption by 10-20% over the control within 1 hr, as judged by germinal vesicle breakdown (GVBD), indicating that PLC-PKC pathway may function as an inhibitory signal of meiotic maturation. In summary, the present study shows that PLC  $\beta 1$  and  $\gamma 1$  are expressed in a developmental stage-specific manner during mouse oocyte maturation and preimplantation embryo development.

## D102

### Regulation of GnRH gene expression by NMDA and Nitric oxide in immortalized GT1-1 neuronal cells

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Growing evidence indicates that N-methyl-D-aspartic acid (NMDA) plays an important role in the neural/endocrine control, however, its involvement in gene expression and signaling pathway in gonadotropin-releasing hormone (GnRH) neurons are poorly understood. In the present study, we examined whether GnRH gene expression is regulated by NMDA in immortalized hypothalamic GnRH neurons (GT1-1 cells) *in vitro*. NMDA was found to stimulate GnRH gene expression in dose- and time- dependent manners. NMDA-induced GnRH mRNA levels were blocked by MK-801 or AP-5 (NMDA receptor antagonists), but not by CNQX (a non-NMDA receptor antagonist). Because NMDA receptors exist as either homodimer or heterodimer, we analyzed transcripts of NMDA receptor subunits using reverse transcription polymerase chain reaction (RT-PCR). In GT1-1 cells,  $\zeta 1$ ,  $\epsilon 1$ ,  $\epsilon 2$ ,  $\epsilon 4$  subunits transcripts were expressed, while  $\epsilon 3$  subunit mRNA was undetectable, indicating that the action of NMDA may be mediated through  $\zeta 1/\zeta 1$  receptor homodimer, or  $\zeta 1/\epsilon 1$ ,  $\zeta 1/\epsilon 2$ ,  $\zeta 1/\epsilon 4$  receptor heterodimer. Since NMDA neural signal is propagate into the target cell through nitric oxide (NO)/cGMP pathway in many neurons, we examined the possibility that NMDA-induced GnRH gene expression is mediated through NO/cGMP pathway. Neuronal NO synthase (NOS) was expressed in GT1-1 cells as judged by RT-PCR. Application of GT1-1 cells with NOS inhibitors (L-NAME, D-NAME, NoArg, methylene blue) prior to NMDA treatment, inhibited NMDA-induced GnRH gene expression. Moreover, treatment of GT1-1 cells with NO donor (sodium nitroprusside) or 8-Br-cGMP slightly increased GnRH gene expression. Taken together, this study indicates that NMDA regulates GnRH gene expression in GT1-1 cells, probably through NMDA/NO/cGMP signal transduction pathway.