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Identification of Ca^{2+}-channel(s) in the Mouse Follicular Oocyte and Preimplantational Embryos

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In the present studies, it was aimed to find out that what type of Ca^{2+}-channel(s) is present in the ooctye and preimplantational embryos of the mouse, and how Ca^{2+}-channel(s) changes depending on the developmental progresses. Three types of voltage-dependent Ca^{2+}-channels (① P/Q-type ② N-type ③ L-type) were identified in the follicular oocytes of the mouse, and the 3 types Ca^{2+}-channels were also present in all type of oocyte, zygote, 2-cell embryo, 4-cell embryo and 8-cell embryos but it was found that depending on the developmental progresses, the distributional changes of three type Ca^{2+}-channels were found to be very variable. In 4-cell and early 8-cell embryos some of blastomeres have no specific Ca^{2+}-channel(s) and some of the embryo even have no Ca^{2+}-channel(s) at all. Assuming 8-cell embryo as the first step of differentiation distributional change pattern of $Ca2^{2+}$-channels must be prepared in 4-cell embryos in advance. It is assumed that this early distributional changes of Ca^{2+}-channel be the basis of differentiation.

In addition, two types of Ca^{2+}-leak channels were also identified in the present studies, in which two of the leak channel showed quite different Ca^{2+}-influx. Further studies are needed for the investigation of other types Ca^{2+}-channel and Ca^{2+}-leak channel.

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Inhibition of Voltage-Dependent Ca^{2+}-Channels by Ca^{2+}/Calmodulin Dependent Protein Kinase II

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Calcium/calmodulin dependent protein kinase II (CaM KII) is intracellular multifunctional serine /threonine protein kinase. CaM KII activation is dependent calcium and calmodulin. It takes part in various processes in cellular metabolisms. We tested CaM KII role in calcium channel activation in metaphase II arrested mouse egg. In the present study, using confocal laser scanning microscope and fluo-3AM (intracellular Ca^{2+} indicator), the change of intracellular Ca^{2+}-concentration ($[Ca^{2+}]i$) was investigated. CaM KII ihibitor, KN-93 50uM and 100uM treatment group showed lower $[Ca^{2+}]i$ than the control group. Exposure to KN-93 (50uM), W-7 (calmodulin antagonist, 50uM) decreased whole cell Ca^{2+} currents in whole cell patch clamp method. But control and KN-92 (inactive analogue of KN-92) treated group did not show any change in the whole cell Ca^{2+} current. These results indicate CaM KII is involved in the activation of calcium channel of mature mouse egg.

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Neuronal Differentiation of HiB5 Hippocampal Progenitor Cells by Activation of cAMP-dependent Protein Kinase A

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cyclic AMP-dependent protein kinase (PKA) signaling has been shown to be a critical regulator for neuronal or astroglial differentiation in the developing brain and neuronal cell lines. However, the involvement of PKA signaling in hippocampal neuronal development and differentiation is poorly understood. In the present study, we investigated whether stimulation of the cAMP-dependent protein kinase (PKA) signal pathway regulates differentiation of hippocampal progenitor, HiB5 cells. Treatment of HiB5 cells with 0.5mM dibutyryl-cAMP (dbcAMP) caused dramatic morphological changes with neurite out-growth within 24hr and inhibition of proliferation at 39C in N2 medium condition. To characterize the dbcAMP-induced differentiation of HiB5 cells, the expressions of several marker genes such as nestin, vimentin, GFAP, NFH and NFm were investigated. Nestin (a marker for neural preucusor cells) and GFAP (a marker for astrocyte) decreased and NFH and NFm increased 24hr after dbcAMP treatment. NF200 (a maker for differentiating neurons) was localized at the dendrites making a large complex in the dbcAMP-induced differentiating HiB5 cells. MAP2 (a post-mitotic neuronal marker) increased only when astrocyte-conditioned media was added following dbcAMP treatment. To study the direct role of active PKA, PKA catalytic subunit alpha was fused to green fluorescence protein (GFP) for the cell-based approach. We found that overexpression of the PKA catalytic unit alpha in HiB5 cells caused neuritogenesis and neurite-outgrowth (60%). In this process, PKA activity increased and augmented phosphorylation of cyclic AMP responsive element binding protein (CREB). Altogether, these results suggest that PKA is evidently involved in neuronal differentiation of HiB5 cells.

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Cyclooxygenase (COX) -2 Is Regulated by Progesterone and Estrogen in the Rat Uterine Cell Lines

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Cyclooxygenase (COX) is the rate-limiting enzyme in the synthesis of prostaglandins (PGs), which plays an important role in regulation of uterine implantation process. Although PGs in uterine is known to be regulated by ovarian steroid hormones, such as estrogen (E) and progesterone (P), the mechanism by which ovarian steroids regulate COX-2 is not known. To examine the differential action of E and P on the expression of COX-2, we used two immortalized uterine cell lines, such as CUS-V2 and CUE-P. Uterine stromal cell line, CUS-V2 was incubated for 24 hours with P (100nM) or vehicle, then the media were transferred to uterine epithelial cell line, CUE-P to examine the stromal-epithelial cell interactions. CUE-P was treated with E (10nM) or vehicle. Protein levels of COX-2 in the CUE-P were determined by western blot analysis. When P-treated CUS-V2 media were added into the CUE-P culture media, COX-2 protein level was induced 3.5 fold at 6 hours after estrogen treatment, and the levels were maintained during the next 24 hours. This COX-2 expression was not induced significantly in absence of P treatment in CUS-V2 cells. The COX-2 expression was blocked by P antagonist, Ru486 (500nM) and/or E antagonist, tamoxifen (50nM), respectively. No significant effect was observed in media transfer from other cell lines, indicating tissue specific expression of COX-2 in CUE-P cells. These results suggest that COX-2 expression in epithelial cells is regulated by E, as well as putative paracrine factor regulated by P from stromal cells.