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.