D101

Muscarinic Acetylcholine Receptors in the Normal, Developing and Regenerating Newt Retinas

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Newts possess the ability to regenerate a functional retina following complete removal of the original retina. We have described the time course of appearance and muturation of cholinergic system components (ChAT and AChE) in both developing and regenerating newt retinas (Dev. Brain Res. 1999). Here, we examined the appearance of the muscarinic acetylcholine receptors (mAChRs), one of postsynaptic targets for cholinergic transmission during development and regeneration. Immunoreactivity for mAChR subtypes (m2 and m4) was demonstrated in somata located in both the ganglion cell layer and inner nuclear layer. However, the labeling pattern seems to be somewhat different between the two, the anti-m2 antibody labeled ganglion cells intensively and the inner plexiform layer (IPL), while the anti-m4 antibody labeled amacrine cells mainly and did not label the IPL. The m2 mAChR was first detected in somata located at the most proximal level of the retina well before cholinergic neurons could be detected in both developing and regenerating retinas. The m4 mAChR was first detected in the retina at the beginning of the morphological development of the IPL in both developing and regenerating retinas. The fact that the time course of appearance of the cholinergic system components during regeneration was similar to that observed during development suggests that common mechanisms may control the neuronal differentiation during both the development and regeneration. To invesigate whether the mAChRs expressed well before cholinergic neurons appeared responds to ACh, we made living slice preparations from regenerating retinas and examined the cytoplasmic Ca^{2+} concentration changes to application of exogenous ACh.

D102

Observation on the Surface Structure of Oocyte in Korean Brown Frog, *Rana dybowskii.*

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The structural change of oocyte surface during oocyte maturation was investigated using scanning electron microscopy in Korean brown frog, *Rana dybowskii.* The follicular wall surrounding the Rana oocyte is composed of the follicle cell layers (thecal and granulosa cell) and an acellular space (the vitelline envelope). Intact follicles consist of the thecal layer, granulosa cell, vitelline envelope and oocyte. The density of granulosa cell is 330-350 no./mm^2 and total number of this cell is about 6700 - 7200 per oocyte. Granulosa cells connect with each others and associate with oocyte through microvilli. Granulosa cell microvilli penetrate into vitelline membrane via hole of vitelline envelope and associated oocyte microvilli. During progesterone and hCG induced oocyte maturation, this association between granulosa cell and oocyte was disappeared. After manually discarding vitelline envelope by forcep, oocyte microvilli were observed on the surface of devitellined oocyte. In the position where the white spot of germinal vesicle (GV) is observed, oocyte microvilli formed clusters partially. Moreover, the length of oocyte microvilli become more short and the density of that is decreased during maturation. The length of oocyte microvilli is 1.5 mM in the immature oocyte,

whereas that is 0.6 mM in the mature oocyte. These results demonstrate that a role of intercellular association between granulosa cell and oocyte with microvilli during oocyte maturation.

D103

Involvement of PI3-kinase in the Progesterone-Induced Oocyte Maturation in *Rana dybowskii.*

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Previously, we have shown that the $p70^{s6k}$ play an essential role during the early phase of oocyte maturation in *Rana dybowskii*. To investigate further the early signal transduction components involved in this process, the possible role of phosphatidylinositol-3 kinase (PI3 kinase) during oocyte maturation was examined. Progesterone-induced oocyte maturation was significantly inhibited by wortmannin and LY294002, specific inhibitors of PI3 kinase. Protein synthesis was also significantly suppressed by wortmannin treatment during oocyte maturation. Moreover, PI3 kinase inhibitor suppressed progesterone induced phosphorylation of S6 kinase in a dose dependent manner. Likewise, PI3 kinase inhibitors significantly inhibit the phosphorylation of mitogen activated protein (MAP) kinase which was increased during oocyte maturation. Finally, progesterone induced H1 kinase activity was also inhibited by PI3 kinase inhibitors in a dose dependent manner. Taken together, these results suggest that PI3 kinase play as an initial component of signal transduction pathway which is resided in the upstream of $p70^{s6k}$, MAP kinase, and MPF production during progesterone mediated amphibian oocyte maturation.

D104

Effects of Endocrine Disrupters, Tributyltin and Triphenyltin on Progesterone-Induced Oocyte Maturation in *Rana dybowskii.*

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The purpose of the present study is to investigate the effect of endocrine disrupters on oocyte maturation in amphibian. We hypothesized that the organotin compounds, tri-n-butyltin (TBT; anti-fouling agent) and tri-phenyltin (TPT), may disrupt progesterone-induced oocyte maturation in Korean brown frog *Rana dybowskii*. Exposure of TBT or TPT was sufficient to inhibit progesterone-induced oocyte maturation in Rana. This inhibitory activity was specific to TBT and TPT; other organotin compounds (Mono-butyltin; MBT, Di-butyltin; DBT) did not show any specific effect on oocyte maturation. When TBT was pretreated for 1hr before P4 treatment, this inhibitory effect of TBT was reached maximum level. The progesterone-induced protein synthesis was significantly decreased by TBT and TPT. Moreover, TBT and TPT also inhibited the activation of MAPK during P4-induced oocyte maturation. However, the inhibition of TBT to oocyte maturation was not observed during spontaneous maturation. We propose that TBT and TPT could inhibit P4-induced oocyte maturation through a step involving inhibition of protein synthesis and MAPK signal pathway.

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Gametogenesis and Reproductive Cycle of the Turban Shell, *Lunella coronata coreensis* (Gastropoda: