OSMOREGULATORY MECHANISM IN THE EMBRYOS AND LARVAE OF MEDAKA (*ORYZIAS LATIPES*)

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Medaka, a freshwater(FW)-dwelling teleost, adapts to seawater(SW) with successful reproduction. Medaka eggs survive a direct transfer from FW to SW, while adult Medaka does not. We examined osmoregulatory strategies of Medaka throughout its life cycle. The dechorionated embryos, fertilized in FW and SW, were stained with a vital florescent dye DASPEI to detect the activity of chloride cells(CLC). DASPEI-positive CLC appeared on the yolk-sack membrane of the day two embryos. As development proceeds, the CLC-occupied area was gradually changed from the embryonic body surface to the gill epithelia. At any developmental stage, CLC of the SW-acclimated individuals showed higher activity than that of FW ones. Lacking osmoregulatory organs, the embryos would have a similar osmoregulatory mechanism to adults. Paraffin section of the day 0 embryos, however, showed no CLC. CLC-mediated osmoregulation is possible to initiate one day after fertilization. It is interesting to note how the much earlier embryos without CLC, as well as unfertilized eggs, control their interior osmotic environments. We may consider different phases of homeostatic strategies in the Medaka life cycle.

ESTABLISHMENT AND CHARACTERIZATION OF IMMORTALIZED FETAL BOVINE BRAIN CELLS BY SV40T ANTIGEN

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As a possible *in vitro* cellular model for BSE prion studies, we established immortalized bovine brain cell lines by transfecting SV40T antigen. Cells were dissociated from fetal bovine brain tissues which had been kept in liquid nitrogen for eight years. After two days in primary culture with DME/F12 medium supplemented with 10% fetal bovine serum, EGF, basic FGF and other supplements, cells were transfected with pSV3neo construct. After selection with G418, four novel immortalized cell lines were established. Three of them showed a cobblestone-like morphology, and the rest had a spindle shape morphology. All the cell lines exhibited stable proliferative activity up to at least hundred population doublings. These cell lines were immunostained with antibodies against vimentin, alpha-smooth muscle actin or von Wilebrand factor. In addition, these cells formed capillary-like structures when cultured on Matrigel. Furthermore, these cells incorporated fluorescent dye-labeled low density lipoprotein. These results suggest that these immortalized fetal bovine brain cell lines retained endothelial-like properties.

EXTENSION AND REPULSION OF NEURITES: INVOLVEMENT OF HEPARIN-BINDING FACTORS IN CO-CULTURE SYSTEM OF NG108 CELLS

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Previously, we reported that the extension and repulsion of neurites were found in the co-culture of NG108 and SM3 cells. The involvement of neuropilin-semaphorin was suggested by DNA array and immunocytochemical analysis. In the present study, we report the results of co-culture with other cell lines, and the involvement of heparin-binding factors.

ACTION OF ACETYLCHOLINE AGONIST CARBACHOL ON HYPERPROLIFERATION OF HEPATOCYTES CO-CULTURED WITH ENDOTHELIAL AND SMOOTH MUSCLE CELLS

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The ventromedial hypothalamic-lesioned animal is considered that vagus hyperactivity rather than overeating contributes to the abnormal cell proliferation in peripheral tissues and the obesity. In previous study, hepatocyte proliferation occurred in rats with mimicked vagus hyperactivity by administration of muscarinic acetylcholine agonist carbachol. In vitro using the cell line, we found that hepatocyte proliferation occurred by mixed culture with vascular endothelial cell and vascular smooth muscle cells although it was not shown in alone culture of hepatocytes.

LOCALIZATION OF NEUROTROPIN RECEPTORS TrkA IN PC12 CELLS:3D-STRUCTURAL ANALYSIS BY HIGH VOLTAGE ELECTRON MICROSCOPE

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Nerve growth factor (NGF) is essential for the development, survival and differentiation of neural cells. High affinity NGF receptor TrkA mediates the signal transduction of NGF. It has been reported that TrkA receptors are associated with caveolae, which are small invaginated pits on the plasma membrane, but little is known about the subcellular localization of them. Our previous study revealed that TrkA was localized mainly in many clusters of invaginated pits of the plasma membrane. The aim of this present study was to clarify whether or not these structures are localized in caveolae. For immunocytochemistry, PC12 cells were reacted with TrkA or caveolin-1, caveola-specific protein, or clathrin antibody and analyzed by high-voltage electron microscopy and IMOD (3D reconstruction software). Our results indicated that caveolin-1 immunoreactivity was found at the invaginated pits on the plasma membrane and these structures were resembled TrkA's pits. However, these invaginated pits by caveolin-1 immunoreactivity were different from caveolae. In addition to both of them were formed a mesh or web-like structure in the cytoplasmic.

ASCORBIC ACID INDUCE ENDOTHELIAL CELL DEATH SPECIFIC FOR NEW BLOOD VESSEL

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Ascorbic acid is essential for vein maintenance. However, high concentration of ascorbic acid induced cell death had been observed in several cell lines *in vitro*. But in the confluent condition, ascorbic acid did not induce cell death. In the present research, ascorbic acid induced new blood vessel cell death *in vivo*. These results suggest that endothelial cells with tube formation induced by collagen or *in vivo* are specifically induced cell death by ascorbic acid.

SPHINGOSINE-1-PHOSPHATE ACTIVATES QUIESCENT SATELLITE CELLS

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The regenerative potential of adult skeletal muscle is maintained by the presence of muscle satellite cells, which are skeletal muscle-resident stem cells. Satellite cells are normally quiescent but are activated in response to various stimuli such as injury, overload and denervation. Activated satellite cells then enter the cell cycle to produce large number of myogenic progenitor cells. To maintain functional skeletal muscle for life, the activation of satellite cell must be tightly regulated by the certain mechanisms. However, the molecular mechanisms involved in satellite cell activation are largely unknow. We have focused on the roles of sphingolipids in the event, and found that sphingosine-1-phosphate derived from sphingomyelin at the inner leaflet of the plasma membrane promotes satellite cells into the cell cycle.

BT-IgSF, A BRAIN- AND TESTIS-SPECIFIC IMMUNOGLOBULIN SUPERFAMILY IS INVOLVED IN NEURONAL ADHESION IN THE CENTRAL NERVOUS SYSTEM

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BT-IgSF is one of the immunoglobulin superfamily members. We have reported that the messages of BT-IgSF were exclusively expressed in both brain and testis and the recombinant BT-IgSF showed a homophilic binding activity in transfected cells, while we have not yet detected the endogenous molecule in vitro and in vivo. In the present paper, we have raised several antibodies to examine localization and function of BT-IgSF in neuronal cells and tissues from the central nervous system (CNS). Primary cultured neurons from mouse hippocampus were strongly stained for BT-IgSF. When neurons were cultured either for a longer period, some of the BT-IgSF molecules were relocalized in their neurites as well as somatic membranes. A histochemical analysis revealed that neurons in both CA 1 to 3 of hippocampus and dentate gyrus were stained positively. BT-IgSF promoted not only neuronal attachment but also neurite outgrowth. Therefore, these data indicate that BT-IgSF plays some roles in neuronal adhesion in the CNS through modifying cell-cell contact properly.