Mechanism of Human chorionic gonadotropin (HCG) Secretion in Accelerated BeWo cells

Hiroshi SUEMIZU, Yoshiyuki OSAMURA and Keiichi WATANABE

Department of Pathology, Tokai University, School of Medicine

This immunoelectron microscopic study is aimed at to elucidate the secretory mechanisum in accelerated secretion induced by cholera toxin (CT) and TPA. The involvement of microtubules is also investigated. (MATERIALS AND METHODS) BeWo cells, human choriocarcinoma cell line, were treated with (1)cholera toxin (CT) which activates protein kinase A, or (2)TPA which is protein kinase C activator. CT or TPA treated BeWo cells were fixed with 4% PFA and the localization of HCG was observed at light and electron microscopic levels (pre-embedding method). BeWo cells were also treated with colcemid. (RESULTS AND DISCUSSION) HCG was observed mainly in perinuclear space (PNS) and in rough endoplasmic reticula (RER) in untreated BeWo cells. In TPA treated BeWo cells, HCG was localized in PNS and small vesicles, and HCG secretion in this condition was inhibited by colcemid. In CT treated BeWo cells, HCG was observed in PNS and prominent and irregular RER, and the secretion was not affected by colcemid. These date suggested that accelerated HCG secretion induced by TPA was transported via small vesicles and was partialy dependent on the cytoskeltal systems.

Chelate Staining(XXXVI)The Release of Cadmium from Cadmium-thionein in Tissues by Oxidation Using Hydrogen Peroxide

Yawara SUMI, Masayuki K. HARA, Tomomi ISHIGE, Takeshi MURAKI and Takuro SUZUKI

First Department of Anatomy, St. Marianna University School of Medicine Kawasaki

We have histochemically succeeded in staining of cadmium bound to metallothionein in tissue. The cadmium has been regarded as one of the masked metals, which are difficult to stain in histochemical metal staining for long time. The pretreatment of tissue sections using oxidizing agents is useful for visualization of the cadmium in tissue containing cadmium-thionein.

Rats(8 wk old) were injected sc with cadmium chloride at a dose of 3.0 mg Cd/kg body weight as 0.1 ml solution/rat 4 times a week for 2, 3 and 4 wk. The liver and kidneys excised were fixed in formalin and sectioned 6 μm. The tissue sections were treated in a 1% hydrogen peroxide solution buffered with 0.04M borate(pH 8) for 30 min at room temperature, and then stained with benzothiazolylazo-beta-naphthol for 30 min. Coloration in bluish purple was observed from the pretreated sections, but with the untreated ones, no the coloration was observed. The results obtained from spectrophotometrical experiments on the release of cadmium from the section in a test-tube also indicated that hydrogen peroxide clearly contributed to the release of the cadmium.

Immunohistochemical Study of Galactosyltransferase Using a Monoclonal Antibody

Tatsuo SUGANUMA[1,2], Kaori IHIDA[1], Shinichiro TSUYAMA[1], Fusayoshi MURATA[1]

Departments of Anatomy[1] and Biochemistry[2], Faculty of Medicine, Kagoshima University, Kagoshima

Previous immunohistochemical observations of galactosyltransferase have to be reevaluated, since the occurence of blood type substance recognizing components in the polyclonal antibody has been confirmed. We have chromatographically purified N-acetylglucosaminide β1-4 galactosyltransferase about 1,700 fold from F9 embryonal carcinoma cells after solubilization with Triton X-100, using N-acetylglucosamine as the acceptor. The enzymological properties including behavior toward α-lactalbumin were very similar to those of the enzyme from other sources. A monoclonal rat IgM antibody (GTF-4) raised against the purified galactosyltransferase from F9 cells was obtained. After confirming the specificities of the antibody to the enzyme, immunoelectron cytochemical study was performed using preembedding staining method with cryostat sections. GTF-4 clearly labeled Golgi apparatus of various cells such as intestinal absorptive cells, Paneth cells, spermatids and epithelial cells of epididymis. Apical surface of intestinal goblet cells was moderately labeled, while the brush border of intestinal absorptive cells was virtually unstained.

Morphological Analysis of Photoreceptor Membranes of Drosophila Visual Mutants

Emiko SUZUKI, Kazushige HIROSAWA and Tomoko SHIRAISHI

Department of Fine Morphology, Institute of Medical Science, University of Tokyo, Tokyo

A visual mutant of Drosophila (rdgA) degenerates the rhabdomeric membranes spontaneously after eclosion. The rhabdomeric micrivilli of rdgA are much shorter than those of wild type (canton-S) even at birth. We have reported that a membranous structure (subrhabdomeric cistern) is absent in the rdgA photoreceptors (Cell Tissue Res 252:293, 1988).

Circumstantial evidences, small amount of lysosomes and abundance of rough ER, suggest that rdgA photoreceptors lack ability to transport membrane components from Golgi areas to the rhabdomeric membranes. Also, freeze-fracture method revealed the mutant membranes contained less amount of intramembranous particles.

To see if there is a difference in the membrane metabolism between rdgA and wild type, autoradiography was done with tritiated amino acids and mannose/glucosamine. Their incorporation was evidently different; high in wild type and low in rdgA. Time course experiments showed that the radioactivity decreased much faster in the mutant.

A monoclonal antibody to opsin was applied to sections of visual cells. The rhabdomeric membranes of rdgA did not bind with the antibody.

Not only the metabolism but also opsin molecules are different in the mutant fly. Correlation between the morphology and genetic defect is not clear at present.