Changes of Glutathione peroxidase mRNA in hyperplastic nodule induced in rat livers by a hepatocellular carcinogen, 3'-methyl-4-dimethylaminoazobenzene. Non-isotope labeling in situ hybridization and immunohistochemistry. S. Sato, S. Yoshimura, N. Lertprasertsuke, T. Koji[1], K. Watanabe, PK. Nakane[1], A. Nakamura[2], Tokai Univ., Kanagawa, [1]Nagasaki Univ., Nagasaki, [2]Kitasato Univ., Kanagawa, JAPAN

Glutathione peroxidase (GSH-PO) is known to be a "selenium (Se) dependent enzyme" and one of the most effective biological lipid peroxides scavenger. The enzyme is widely distributed in almost all important organs, especially liver. "Hyperplastic nodules (HNs)" recognized as precancerous lesions and hepatocellular carcinoma (HC) are produced in rat liver by feeding animals with 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB), however failed to show the localization of GSH-PO. The present study was thus designed to investigate whether this marked decrease of GSH-PO in HN and HC is due to impairment of transcription or of translation. Because HNs are too minute to obtain sufficient amount to carry "northern" blot tests which was successfully done with fairly large HC tissues, the in situ hybridyzation was prefered. MATERIALS & METHODS: Livers of rats fed 1)control diet, 2)3'-Me-DAB for 12 weeks, and 3)diet lacking selenium (Se(-)) were fixed by 4% paraformaldehyde and a GSH-PO cDNA probe labeled with digoxigenin was used for in situ hybridyzation technique. Direct immunoperoxidase technique was also employed for the locaization of GSH-PO. RESULTS & DISCUSSION: In the liver of control rats, immunoreactivity and mRNA signal for GSH-PO were localized to hepatocytes throughout the hepatic lobule with zonal distribution. In the liver of Se(-) rats, both were negative suggesting that selenium deficiency affects not only translational process but also transcriptional synthesis of mRNA. In the liver of rats fed 3'-Me-DAB, immunoreactivity and mRNA signal were negative in hepatocytes of HNs. Whereas both were preserved in remaining hepatocytes surrounding the nodule. The result suggests that the decrease of GSH-PO in HNs is due to decreased synthesis of mRNA at transcriptional level.

Glucocorticoid (GC) Regulation of Vasopressin (VP) Expression in Cultured Magnocellular Neurons. K. Schilling*, P. Oeding*1, H. Schmale*1, and Ch. Pilgrim. Abt. Anatomie u. Zellbiologie, Univ. Ulm; 1 Inst. für Zellbiochemie u. Klin. Neurobiologie, Univ. of Hamburg, Federal Rep. of Germany.

In the intact animal, adrenalectomy increases VP expression in parvocellular but not in magnocellular neurons. In order to investigate whether this discrepancy is due to differences in intracellular signal transduction or synaptic input, the effect of GC on VP expression was tested in cultures of 14 day old fetal rat diencephalon containing magnocellular but not parvocellular VP neurons.

Selective neutralization of GC present in the serum-supplemented culture medium by the drug RU 38 486 resulted in an increase of the numbers of magnocellular VP-immunoreactive cells and a parallel increase of the levels of VP mRNA. This effect was specific for VP cells, as neither the expression of general neuronal marker proteins nor of oxytocin was affected by RU. RU was not mitogenic for VP neurons. Furthermore, RU did not enhance the effects of protein kinase C or of adenylate cyclase activation on VP expression. RU increased VP expression also in cells that were synaptically isolated by growth in 14 mM Mg++, suggesting that its effects were not transmitted transsynaptically.

These results show that GC can regulate VP expression also in magnocellular neurons. The absence of such an effect in vivo may best be explained by the assumption of a synaptic input in vivo that interferes with the effect of GC on magnocellular neurons.

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Immunoelectron Microscopy of Laminin and Type-IV Collagen in Reduced Ameloblasts and Junctional Epithelial Cells in Rat Molars. T. Sawada, T. Yanagisawa, S. Takuma, H. Hasegawa, and K. Watanabe. Tokyo Dent. Coll., Chiba; Tokai U. Sch. of Med., Isehara, Japan.

After perfusion fixation, upper and lower rat jaws were dissected out, demineralized with EDTA, and prepared into frozen sections for demonstration of laminin and type-IV collagen by means of indirect immuno-peroxidase methods. A basement-membrane-like structure appearing between reduced ameloblasts and the enamel surface reacted positively for laminin. Occasional occurrence of reaction products in rough endoplasmic reticula of reduced ameloblasts indicated laminin production. But no reaction products for type-IV collagen occurred in the basement-membrane-like structure. The basement membrane of capillaries and the papillary layer located above the reduced ameloblasts reacted positively for both laminin and type-IV collagen. In the junctional epithelium, intense laminin reaction products occurred in both the basement-membrane-like structure (internal basal lamina) on the enamel and basement membrane demarcating the gingival connective tissue from the junctional epithelium (external basal lamina). Reaction products were frequently observed in the rough endoplasmic reticula of junctional epithelium cells. No type-IV collagen reaction products were seen in the internal basal lamina. The external basal lamina showed reaction products for type-IV collagen. These results indicate that the basement-membrane-like structure of reduced ameloblasts is similar in chemical composition to that of the junctional epithelial cells.

A subpopulation of motoneurons contains glutaminase-like immunoreactivity. E. Senba,* T. Kaneko,* N.Mizuno, and M. Tohyama. Osaka Univ. Medical School, Osaka, and Kyoto Univ. Medical School, Kyoto, Japan.

Excitatory neurotransmitter glutamate is mainly derived from glutamine, and phosphate activated glutaminase (PAG), which converts glutamine into glutamate, has been considered as a possible marker of glutamatergic neurons. Immunoytochemistry conbined with a fluorescent dye tracer (Fluoro Gold) method revealed that a subpopulation of somatic, branchial and visceral motoneurons in the brainstem and spinal cord of adult male Wistar rats (200-250g body weight) contain a high concentration of PAG. Among these motoneurons, neurons in the dorsal motor nucleus of the vagus nerve (dmnX), sympathetic and parasympathetic preganglionic neurons in the spinal cord and urethral sphincter motoneurons (L6) were most intensely immunostained. PAG-like immunostaining was also observed in endplates of urethral sphincters. Trigeminal, facial and hypoglossal motoneurons showed moderate immunoreactivities. However, motoneurons in the ventral horn of the spinal cord, except urethral sphincter motoneurons, were not immunostained. In addition, some of these PAG-like immunoreactive motoneurons contained choline acetyltransferase, calcitonin gene-related peptide or galanin. PAG-like immunostaining in dmnX motoneurons was decreased after axotomy. In young animals of postnatal day 5, almost all the motoneurons, including those in the ventral horn of the spinal cord, showed strong PAG-like immunoreactivity. The possible functional roles of this enzyme and glutamate in motoneurons are discussed.