Cytochemical Investigation of Hydrogen Peroxide Producing Oxidases in the Mongolian gerbil Salivaly Glands

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Recently the ultracytochemical localization of several hydrogen peroxide producing oxidases have been demonstrated by a cerous chloride method, and suggested the association with hydrogen peroxide resolving enzymes. But in salivaly gland which contains peroxidase it is not known of the existence of hydrogen peroxide producing oxidases. Therefore we investigated the cytochemical localization of them in Mongolian gerbil salivaly gland.

In case cerous chloride method was used, reaction products were always recognized, whether substrates of hydrogen peroxide producing oxidases were present or not. Reaction sites were found on the intercellular canalicular plasma membrane, the luminal plasma membrane and the lateral cell membrane or at the periphery of the mitochondria. Only in the acinar cells of submandibular gland, NADH emphasized their reactions. pCMB and kojate inhibited their reactions, but NaN_3 did not. These results suggested that the reaction sites are the localization of hydrogen peroxide produced by some facter, and we have a greate interest in association with hydrogen peroxide producing oxidases.

Ontogenetic Studies of ^{3}H-substance P Binding Sites in the Rat Brain

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It has been reported that substance P positive structures appear at a very early ontogenetical stage and continue to increase in number and in density until the stage between postnatal days 5 and 15 and then decrease progressively in number as the rat grows. In this paper, the ontogeny of specific ^{3}H-substance P binding sites in the rat brain was investigated with use of in vitro macro autoradiographic technique. In the septal nucleus, amygdala and dorsomedial and periventricular nuclei of the thalamus, ^{3}H-substance P binding sites existed at adult level at 1 day after birth. In the ventral nucleus of the thalamus, ^{3}H-substance P binding sites were richly observed at the postnatal stage of 1 and 7 days, although they were observed at a negligible level in this brain area of adult rats. In the striatum, olfactory tubercle and preoptic area, ^{3}H-substance P binding sites were weakly observed at 1 day after birth and reached adult level at 7 days after birth. It seems that the differentiation of ^{3}H-substance P binding sites starts at an early stage of the ontogeny, compared with that of Ca channel antagonist binding sites, which was investigated by us through the same experimental technique. The role of the substance P neuron system in the ontogeny is under further investigations.

Nicotinamide Adenine Denucleotide Phosphate (NADPH)-Diaphorase Histochemistry in the Cat and Human Brains

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We modified a direct method of NADPH-Diaphorase histochemistry in order to study of NADPH-diaphorase positive cells in the fixed cat and autopsied human brains.

This NADPH-Diaphorase histochemistry allows rapid and stable visualization of the paticular neuron populations.

We perfused with 4% paraformaldehyde and 0.4% glutaraldehyde and postfixed with 4% paraformaldehyde for 12 hours. The tissues were cut at 30-50 um by Vibratome and stained for NADPH-diaphorase activity by incubating free floating sections in 10 ml Tris HCl buffer solution containing 10 mg NADPH and 2.5 mg Nitro Blue Tetrazolium (NBT) at 37°C for 1 hour. The NBT was reduced in the presence of NADPH to an insoluble blue end products, formazan, staining cells.

The heavily positive stained neurons which had prominent processes were observed in the neocortex,striatum,white matter, amygdalla,dorsolateral pontine tegmentum and reticular formation. The moderate stained neurons which did not have prominent cell processes were observed in the nucleus tractus diagonalis Broca, the nucleus basalis of Meynert, entopeduncular nucleus and nucleus subthalamics.

Immunohistochemical Demonstration of Fibronectin in Rat Brains Lesioned with 5,6-dihydroxytryptamine (5,6DHT).

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The role of fibronectin(FN) in neuronal regeneration of the central nervous system(CNS) has been studied immunohistochemically by using antibody against FN. Highly purified FN of dog plasma was obtained by single application of gelatin-sepharose affinity chromatography. The anti-FN serum was generated in a rabbit by immunizing with the purified FN. The antiserum showed highly specific for FN, and had a good titer enough to use in immunohistochemistry. For chemical lesioning of CNS, serotonin(5HT) neurotoxin 5,6DHT was injected into the lateral ventricle of the rat brain. Two weeks after the injection, immunoreactive nerve fibers detected by using anti-5HTserum indicated that 5HT fibers in such regions as the hippocampus, habenular nuclei and fornix were suffered from the neurotoxin. There were numerous dot-like positive punctate probably indicating regenerating 5HT fibers. When comparable sections were stained with anti-FN serum, similar dot-like structures were observed in identical regions. Since no specific staining for FN is observed in intact animals, the result suggests that FN is associated with regeneration of nerve fibers.