Mechanism of Storage of Monoamines in Megakaryocytes and Blood Platelets; Cytochemistry and Autoradiography

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In the previous study, we found the existence of the precursors of monoamine storage organelles in the megakaryocytes (Histochem. 77:353, 1983).

This study was undertaken to clarify the capacity of storage cations and of uptake of serotonin (5-HT).

The electron probe x-ray microanalysis of freeze substituted mouse megakaryocytes demonstrated a high level of Mg and a modarate amounts of Ca in the percursory organelles.

Electron microscopic autoradiograpy defined that the uptake of ^{3}H-labeled 5-HT in the megakaryocyte was 1/80 times of the blood platelets. From light microscopic autoradiograpy, it clarified that the megakaryocytes began to store slightly 5-HT at the late stage of their maturation.

From these results it is said that the megakaryocytes are not able to store 5-HT, contrast with large amounts of cations and adenine nucleotides. Therefore, the organelles of storage of monoamines may have the capacity to accumulate amines after liberation of the platelets from megakaryotye.

The Superoxide-generating System and Localization of NADPH Oxidase in Rat Bone Marrow Neutrophils

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The localization of NADPH oxidase and the superoxide-generating system in rat bone marrow cells were investigated by applying the cytochemical method introduced by Briggs et al.

1) Under unstimulated conditions, the enzyme activity was very weak. The reaction product was found on the plasma membrane of only a few mature neutrophils. When stimulation with phorbol myristate acetate was applied, the respiratory burst was activated and resulted in an increase of the reaction-product-positive rate of mature neutrophils. Presence of the reaction product on the plasma and vacuole membranes, resulting from invagination of the former, was confirmed.

2) Regarding the generation of hydrogen peroxide, from analysis of experimental results obtained by using sulfhydryl reagents, cytoplasmic superoxide dismutase (SOD) inhibitors, and exogenous SOD, and from the pH value (7.5) of the reaction medium, it was suggested that there is a catalyst, an SOD-like substance in the plasma membrane, which participates in the generation of hydrogen peroxide from the dismutation reaction of superoxide.

Ultracytochymical Studies with Enzymic Digestive Methods for Fats on Columnar Absorptive Cells of Rat Small Intestine During Fat Absorption

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Compared from the jejunum of fasting rats, much more reaction products for phospholipase (PL) A_2-digestive method were observed under a light microscope on the columnar absorptive cells of small intestines of the adult rats killed within 2 hr after a feeding of oil. A piece of the intestines located around 10cm from pylorus was cut out of the animals, which were fastened for 24 hr and then fed on 2ml of corn oil through a stomach catheter, and decapitated 60 min after the feeding. The round slices were fixed in Karnovsky's fixative for 3 hr, washed in cacodylate buffer overnight, cut into 40μm thickness by Vibratom. Thick sections were digested by PL C, A_2 and lipase, respectively, in Tris buffer pH 7.6 containing $CaCl_2$ at 37°C for 30 min. Reaction products after PL C digestion were seen coarsely on microvilli of the absorptive cells accompanying less products within the apical vesicles, while those after PL A_2 digestion were mostly in minute fat particles within the apical vesicles and in some intercellular spaces. On the lipase-digested sections, the positivity could be seen in the fats between the microvilli and within the apical vesicles.

Ultracytochemical Observations on Alveolar Lining Layers in Rats, Especially Application of Phospholipase Digestion

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Ultrastructural localizations of pulmonary surfactants in alveolar lining layers were observed by phospholipase - digestive method and Dermer's technique for phospholipids and by ruthenium red method for mucopolysaccharides. Lungs of adult rats were perfused by a heparin-containing saline and subsequently by 1/2 Karnovsky fixative, frozen quickly in aceton - dry ice, and trimmed to minute blocks (diameter: 1mm). Floating blocks were washed in cold cacodylate buffer for 1 hr, treated with digestion medium at 37° for 40 min, immersed in 0.1% $Pb(NO_3)_2$ for 15 min, washed thoroughly, and fixed in cold 2% osmium buffer for 2 hr. The medium, pH 7.6, was composed of 0.25ml of phospholipase A_2 or C in the mixture of Tris buffer, 4ml, and 2% $CaCl_2$, 1ml. Other blocks were used to Dermer's method. Other lungs were treated with ruthenium red method. These post-fixed blocks were dehydrated by a series of aceton and embedded in Epon.

The reaction products for phospholipids were detected granularly only in the surface film of alveolar lining layers, while ruthenium red was observed abundantly in the whole layers.