Enzyme-Gold Detection of RNA and DNA on Bufo Intermedius (2n) and Oedipus Americanus (4n)
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Imature erythrocyte cells of B. intermicus and O. americ anus obtained through induced hemolytic anemia by the phenylhydrazine were ultrastructurally analysed about the site of nuclear and cytoplasmic nucleic acids. For this we applied an enzyme-gold post embedding approach specific for ribonucleic and deoxyribonucleic acids. (Bendayan, J. Electron. Microsc. Tech J: 349-372, 1986) 3 B. intermicus presented the nuclear condensed chromatina labeled with DNA-gold particles, Mitochondria was weakly labeled. The RNA-gold labeling of this cells was present in the granules of perichromatin, dispersed chromatin midpolyenes. B. intermicus showed the ribonucleoproteins at dispersed chromatin and nucleus labeled with RNA-gold complex, indicating sites where synthesis and processing of mRNA and pre-RNA occurs. This cells display polynucous and many cytoplasmic vacuoles of different sizes filled with a fibrilar material of unknown nature, which were also labeled with RNA-gold complex. The RNA-gold labeling were observed over nuclear condensed chromatin, but cytoplasmic vacuoles and mitochondria were weakly labeled. The dispersed chromatin labeled on the nucleus of these cells is very similar to those observed in the cytoplasmic vacuoles that were labeled by DNA-gold complex, suggesting that a probable globular form of mRNA excess was synthesized for this tetraploid cells.

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Immunohistochemical Localization of Pulmonary Antioxidant Enzymes, H.P. Cihla*, D.B. Courain+, G.S. Bindley*, T.D. Oberley+, and L.W. Oberley†. University of Wisconsin Medical School, Madison, WI and University of Iowa College of Medicine, Iowa City, IA.

Manganese (Mn) and copper-zinc (CuZn) superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione S-transferase (GST) were localized at the light (LM) and electron (EM) microscopic levels in parenchymal lung tissue from normoxic rats. Tissue was fixed for 30 or 60 minutes in neutral buffered formalin (NBF), 2% gluteraldehyde, or 8% paraformaldehyde (PF). Samples were prepared for cryoultramicroscopy (cryo), or embedded in paraffin, epoxy resin, or lowicryl K4M. Sections were incubated overnight using previously characterized rabbit antisera to the antioxidant enzymes.

Labeling intensity for both LM and EM was best in NBF followed by F fixed tissue. At the EM level all antisera labeled cryo sections. As compared to cryo, K4M embedded tissue gave the best labeling for all antisera with the exception of CuZn SOD which appeared more intense in epoxy sections.

MnSOD labeled mitochondria of all cells with some staining of Type II and endothelial cell heterochromatin. Type II and II cell nuclear chromatin was heavily labeled for CuZn SOD as were lung, liver, and endothelial cell. Catalase was found in small vesicles predominantly within Type I and II. Both GPx and GST intensely labeled elastin in the septal interstitium. In general, EM localization of these enzymes demonstrated significant septal wall activities unrecognized by LM methods.


Homocysteic acid (HCA), a sulfur-containing amino acid (544, is a potent neuronal excitant that has recently emerged as a transmitter candidate in the mammalian central nervous system. Indeed, it was shown as well as other sulfur-containing amino acids such as cysteine sulfenic acid and homocystine sulfenic acid are differently released from rat brain slices upon K+-induced stimulation in a Ca2+-dependent manner. HCA release was most prominent in cortex and hippocampus (Do et al., J. Neurochem., 46:1779-786, 1986). The releasable pool of HCA can be labeled from [35S]-methionine which thus is a precursor in HCA biosynthesis. Furthermore, HCA has been proposed as endogenous agonist acting preferentially at NMDA-receptors (Do et al., J. Neurosci., 6:2226-2234, 1986).

In order to define its distribution with respect to various components of neural tissue, we attempted to localize HCA by immunohistochemistry. Polyclonal mouse antibodies to glutathione-linked HCA were developed and applied in combination with a PAP procedure and silver intensification of the DAB reaction product to stain seminath sections. HCA-immunoreactivity was in the adult and early postnatal cerebellum, in the hippocampus and in the cerebral cortex. Immunoreactivity was also seen to be localized mostly in glial elements.

Hypotheses related to this unexpected finding and previous evidence suggesting HCA as neurotransmitter will be discussed.