Demonstration of electron-dense material in clear synaptic vesicles using cationic ferrocenyl compounds

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Summary. Electron-dense material in clear synaptic vesicles in rat cerebral cortex and neuromuscular junctions of frog cutaneous pectoris muscle was demonstrated by using ferrocenyl cationics. Electron-dense spots were usually attached to the inner surface of the vesicular membrane. Control experiments (treatment with Triton X-100 or cetylpyridinium chloride; enzyme digestion with trypsin, hyaluronidase, neuraminidase, sulfatase and \(\beta\)-glucuronidase) suggested that the electron-dense material is a glycoprotein.

Introduction

Ferrocene compounds are widely used in histochemistry, including immunohistochemistry, for the labelling of proteins (Franz 1967, 1968; Franz and Mohr 1969; Mann 1967; Peterlik 1967; Falk et al. 1969) and the staining of glycogen and thiol groups (Allen and Perrin 1974). Franz (1969) has synthesized a ferrocene-containing invert soap, methyl-octyl-dimethyl-ammonium chloride (a cationic detergent, referred to as "ferrocene cationic"), which may be used for the demonstration of acidic mucopolysaccharides. Subsequently, other ferrocene cationics have been prepared and used for this purpose (Franz et al. 1977, 1978). The applicability of these compounds as cytochemical reagents for the visualization of acidic groups is based on the following principles:

1. Quaternary (positively charged) alkylammonium groups bind to acidic groups of the tissue.
2. The iron atom in the ferrocene part gives the electron density.
3. The hydrophobic properties of the long-chain alkyl groups render the reaction product insoluble.

For the study of biological membranes and membrane-bound structures, including synapses, we employed the already mentioned chemical compounds. Investigations were performed on clear synaptic vesicles, where Ovtscharoff (1978) has demonstrated proteinaceous material.

Materials and methods

Forteen adult Wistar rats and ten adult frogs (\(Rana temporaria\)) of both sexes were used. After thiopental narcosis, the rats were perfused via the aorta with 2.5% glutaraldehyde in 0.1 m cacodylate buffer, pH 7.2. Small pieces of the parietal cortex and cerebel-

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Fig. 2. Electron-dense spots in the axon terminals of rat cerebral cortex. $\times 92,000$

Fig. 3. Electron-dense granules in synaptic vesicles of different forms and sizes. Cerebral cortex, rat. $\times 140,000$

Fig. 4. Most of the electron-dense granules are attached to the inner surface of the vesicular membrane. Cerebral cortex, rat. $\times 168,000$

Fig. 5. Electron-dense material in the synaptic vesicles of the frog neuromuscular junction. $\times 65,000$

Fig. 6. Pinocytotic vesicles in the endothelium. Cutaneous pectoris muscle, frog. $\times 160,000$