Glycerylphosphorylcholine Phosphodiesterase in Rat Liver Plasma Membranes

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Glycerylphosphorylcholine (*sn*-glycerol-3-phosphorylcholine) was found by Dawson (1956) to be hydrolysed to glycerol-3-phosphoric acid (*sn*-glycerol-3-phosphoric acid) and choline by a soluble preparation obtained from rat liver. Fowler & de Duve (1969) have since observed glycerylphosphorylcholine phosphodiesterase activity in the particulate subcellular fractions. In contrast with other lipid-hydrolysing activities, glycerylphosphorylcholine phosphodiesterase was not lysosomal, but showed a distribution reminiscent of fragmented plasma membranes. We have therefore made more detailed studies of glycerylphosphorylcholine phosphodiesterase and of marker enzymes for other cell organelles in subcellular fractions, including purified plasma membranes.

A new assay method for glycerylphosphorylcholine phosphodiesterase has been developed, in which glycyl[³²P]phosphorylcholine is used: the radioactive products of the reaction are separated from unchanged glycerylphosphorylcholine by adsorption on Dowex 1 (formate form) anion-exchange resin.

Approx. 60% of the glycerylphosphorylcholine phosphodiesterase in the primary subcellular fractions from liver is particulate. The distribution of this particulate activity in the fractions is similar to those of 5'-nucleotidase and alkaline phosphodiesterase I, both of which are characteristic components of the plasma membrane (Coleman *et al.*, 1967; Erecińska *et al.*, 1969). Purified plasma membranes were then isolated from a crude nuclear fraction (Coleman *et al.*, 1967). Again glycerylphosphorylcholine phosphodiesterase, 5'-nucleotidase and alkaline phosphodiesterase I behaved in a largely parallel manner, and very differently from glucose 6-phosphatase, an enzyme localized in the endoplasmic reticulum. Particulate glycerylphosphorylcholine phosphodiesterase seems therefore to be localized largely in the plasma membranes of the liver cell.

Glycerylphosphorylcholine phosphodiesterase of partially purified plasma membranes had a pH optimum of 8.5. Its activity was abolished by EDTA or ethanedioxybis(ethylamine)tetra-acetate and restored almost completely by a wide range of cations at 5 mM (Mg²⁺, Ca²⁺, Mn²⁺, Co²⁺, Zn²⁺, Ni²⁺, Cu²⁺, Fe²⁺, Fe³⁺). The products of the reaction were glycerol-3-phosphoric acid and choline, both for the plasma membranes and for the soluble fraction. Glycerylphosphorylcholine phosphodiesterase and alkaline

phosphodiesterase I appear to be distinct enzymic activities.

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Phosphatidylinositol Turnover Stimulated by Acetylcholine: Subcellular Distribution in Rat Cerebral Cortex

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Cerebral cortex and sympathetic ganglia show increased phosphatidylinositol turnover during electrical stimulation or in the presence of acetylcholine. This effect is associated with the presence of synapses. Its site appears to be post-synaptic (Larrabee & Leicht, 1965), but it is not clear whether the phosphatidylinositol showing stimulated turnover is distributed throughout the post-synaptic neuron (Hokin, 1965) or is associated with an acetylcholine-receptor proteolipid that is concentrated in the synaptic junction (De Robertis, 1971; Lunt *et al.*, 1971).

The distribution of the phosphatidylinositol responsive to acetylcholine was studied in subcellular fractions (Rodriguez de Lores Arnaiz *et al.*, 1967; Kataoka & De Robertis, 1967) prepared from ³²P-labelled rat cerebral-cortex slices treated with acetylcholine (10 mM) and eserine (0.1 mM). After treatment for 2 h the specific radioactivity of the phosphatidylinositol was 206 ± 31% (mean ± S.D.) of the control value. In slices incubated with ³²P_i for 130 min, but treated with acetylcholine and eserine for only the last 10 min, the equivalent value was 138 ± 14% of the control.

Phosphatidylinositol specific radioactivities were highest in the nuclear, microsomal and synaptic-vesicle fractions and lowest in the myelin fraction, both in the controls and after acetylcholine treatment.

Measurements of marker enzymes (cytochrome *c* oxidoreductase, acetylcholinesterase) indicated that the cell fractions obtained were similar to those obtained from fresh cerebral cortex. There was, however, no clear indication that the phosphatidylinositol responsive to acetylcholine was localized in any particular subcellular fraction even after only 10 min.

These results indicate either that the increased turnover of phosphatidylinositol in rat cerebral cortex during stimulation does not occur at a single

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