Glycerylphosphorylcholine Phosphodiesterase in Rat Liver Plasma Membranes

By KATHERINE A. LLOYD-DAVIES,* R. H. MICHELL and R. COLEMAN (*Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.*)

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.

This work was supported by a grant from the Medical Research Council.

Coleman, R., Michell, R. H., Finean, J. B. & Hawthorne, J. N. (1967) *Biochim. Biophys. Acta* **135**, 573
Dawson, R. M. C. (1956) *Biochem. J.* **62**, 689
Erecińska, M., Sierakowska, H. & Shugar, D. (1969) *Eur. J. Biochem.* **11**, 465
Fowler, S. & de Duve, C. (1969) *J. Biol. Chem.* **244**, 471

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

By E. G. LAPETINA and R. H. MICHELL (*Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.*)

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

* Present address: Department of Biochemistry, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

intracellular site or that the phosphatidylinositol synthesized during this process equilibrates very rapidly between the different membrane structures within the cell.

E. G. L. was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina. We are grateful to Dr. G. G. Lunt for his help.

De Robertis, E. (1971) *Science* **171**, 963

Hokin, L. E. (1965) *Proc. Nat. Acad. Sci. U.S.A.* **53**, 3169

Kataoka, K. & De Robertis, E. (1967) *J. Pharmacol. Exp. Ther.* **156**, 114

Larrabee, M. G. & Leicht, W. S. (1965) *J. Neurochem.* **12**, 1

Lunt, G. G., Canessa, O. & De Robertis, E. (1971) *Nature (London)* **230**, 187

Rodriguez de Lores Arnaiz, G., Alberici, N. & De Robertis, E. (1967) *J. Neurochem.* **14**, 215

Effects of Electrical Stimulation, Tetrodotoxin and Cinchocaine on Phosphoinositide Metabolism in Isolated Rabbit Vagus Nerve

By J. G. SALWAY and I. E. HUGHES (*Departments of Biochemistry and Pharmacology, School of Medicine, University of Leeds, Leeds LS2 9NL, U.K.*)

The rapid metabolism of phosphoinositide P in nervous tissue has been known for some time, but the physiological significance of this is not understood. Hawthorne & Kai (1970) have suggested that phosphoinositide metabolism may be associated with the molecular mechanisms involved in regulating the ionic fluxes associated with axonal depolarization and hence the action potential. This theory has been investigated in the present work by studying the effect of electrical stimulation, tetrodotoxin and cinchocaine (these two drugs block action potentials) on the incorporation of [32 P]P_i into the phosphoinositides of isolated rabbit vagus nerves.

The cervical portion of rabbit vagus nerve was removed, desheathed, attached to stimulating and recording electrodes, placed in an organ bath containing physiological saline (0.9% NaCl) and tested for viability by eliciting and recording action potentials. The nerves were allocated randomly to one of four treatment groups as follows: unstimulated, stimulated, tetrodotoxin-treated, cinchocaine-treated. The last two groups were incubated with the appropriate drug for 10 min at a concentration sufficient to abolish the action potential. All groups were incubated with [32 P]P_i for 1 h and then stimulated at 3 Hz for 2 min (except for the unstimulated group). Monophosphoinositide, diphosphoinositide and triphosphoinositide were extracted and their radioactivities measured as described by Yagihara *et al.* (1969).

Of the [32 P]P_i incorporated into lipid, 75–85%

was in the phosphoinositides and 15–25% was in the quantitatively much larger group of remaining phospholipids. Electrical stimulation produced a small increase (26%; $P = 0.05$) of monophosphoinositide labelling, but had no significant effect on diphosphoinositide and triphosphoinositide labelling. Tetrodotoxin had no significant effect on phosphoinositide labelling. However, cinchocaine caused a 343% increase of [32 P]P_i incorporation into monophosphoinositide and a 42% ($P = 0.05$) increase into diphosphoinositide. These findings were unexpected and as yet are unexplained.

The theory referred to would predict an increased incorporation of [32 P]P_i into diphosphoinositide and triphosphoinositide after electrical stimulation that would be absent from the nerves treated with tetrodotoxin and cinchocaine. The results do not support this theory, which implicates phosphoinositide metabolism with the regulation of depolarization.

The authors thank Dr. R. P. Hullin for his support and the Medical Research Council for financial assistance.

Hawthorne, J. N. & Kai, M. (1970) in *Handbook of Neurochemistry* (Lajtha, A., ed.), vol. 3, p. 491, Plenum Press, New York and London

Yagihara, Y., Salway, J. G. & Hawthorne, J. N. (1969) *J. Neurochem.* **16**, 1133

Is the Liver Cytochrome P-450-Linked Biotransformation System Lecithin-Dependent?

By B. R. CATER, VALERIE WALKDEN and T. HALLINAN (*Department of Biochemistry, Royal Free Hospital School of Medicine, Hunter Street, London WC1N 1BP, U.K.*)

It has often been suggested that lipids play an essential role in maintaining the function of the liver cytochrome P-450 system, and recent experiments, namely deoxycholate solubilization of the system and phospholipase C treatment, indicate that lecithin (phosphatidylcholine) is the essential lipid required. The former results demonstrated that deoxycholate-solubilized cytochrome P-450, when recombined with NADPH-cytochrome P-450 reductase, was inactive unless lecithin was added (Strobel *et al.*, 1970; Lu *et al.*, 1970, 1971). Phospholipase C treatment of the system showed that on hydrolysis of most of the microsomal lecithin, type I metabolism was extensively inhibited. Surprisingly type II metabolism was either unimpaired (Eling & Di Augustine, 1971) or only slightly inhibited (Chaplin & Mannering, 1970) by this treatment.

Our investigations suggest alternative interpretations of both lines of evidence for lecithin-dependence. We find that deoxycholate itself inhibits type I metabolism (aminopyrine demethylase) and that