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

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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

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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.

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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?

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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

added lecithin can substantially relieve this inhibition. As the reconstituted cytochrome *P*-450 system still contains deoxycholate (Lu *et al.*, 1969), lecithin may be essential here to overcome similar deoxycholate inhibition.

Treatment of microsomal fraction with phospholipase C (*Clostridium welchii*) exerts several effects on cytochrome *P*-450 system activities. At 5° or 37°C (about 95% lecithin hydrolysis) aminopyrine demethylase is inhibited by 50–75%. At 37°C phospholipase C inhibits aniline hydroxylase by about 30%, but this inhibition is not due to lecithin depletion because defatted serum albumin prevents inhibition. Further, at 5°C phospholipase C does not inhibit aniline hydroxylase but slightly enhances it, though 95% lecithin hydrolysis can still be obtained.

Recent investigations may explain these findings. When microsomal fraction is treated with phospholipase C, a highly active endogenous acylhydrolase generates fatty acids from the diglyceride products even at 5°C (Cater & Hallinan, 1971). Fatty acids are high-affinity substrates for the cytochrome *P*-450 system and at low concentration selectively inhibit other type I metabolism (confirmed in this investigation) without inhibiting type II (aniline) metabolism (Orrenius & Thor, 1969; Di Augustine & Fouts, 1969).

Finally, added sonicated lecithin does not completely restore the aminopyrine demethylase activity lost by 5°C-phospholipase C-treated microsomal fraction. Phospholipids reactivate many other phospholipid-depleted enzyme systems reportedly phospholipid-dependent, and Fleischer *et al.* (1962) maintain that reactivation is an essential criterion that must be met before an enzyme can be considered to be phospholipid-dependent.

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- Cater, B. R. & Hallinan, T. (1971) *FEBS Lett.* **16**, 137
 Chaplin, M. D. & Mannering, G. J. (1970) *Mol. Pharmacol.* **6**, 631
 Di Augustine, R. P. & Fouts, J. R. (1969) *Biochem. J.* **115**, 547
 Eling, T. E. & Di Augustine, R. P. (1971) *Biochem. J.* **123**, 539
 Fleischer, S., Brierley, G., Klouwen, H. & Slautterback, D. J. (1962) *J. Biol. Chem.* **237**, 3264
 Lu, A. Y. H., Junk, K. W. & Coon, M. J. (1969) *J. Biol. Chem.* **244**, 3714
 Lu, A. Y. H., Strobel, H. W. & Coon, M. J. (1970) *Mol. Pharmacol.* **6**, 213
 Lu, A. Y. H., Kuntzman, R., West, S. & Conney, A. H. (1971) *Biochem. Biophys. Res. Commun.* **42**, 1200
 Orrenius, S. & Thor, H. (1969) *Eur. J. Biochem.* **9**, 415

Strobel, H. W., Lu, A. Y. H., Heidema, J. & Coon, M. J. (1970) *J. Biol. Chem.* **245**, 4851

The Interaction of Glutamate Dehydrogenase and Malate Dehydrogenase with Phospholipid Membranes

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The phenomenon of allotopy has been clearly described for the F_1 coupling factor (Racker, 1967). The present report describes a search for allotropic effects involving the NAD-linked dehydrogenases of the mitochondrion. Such effects would complement the solution variables that are known to affect structural transitions in some of these enzymes (Dodd & Radda, 1969). The interaction between sonicated dispersions of purified phospholipids, corresponding to those found in the inner mitochondrial membrane, and the enzymes were studied by kinetic and spectroscopic methods.

With glutamate dehydrogenase the charge type of the lipid head group determines the extent of lipid-protein complexing. Phosphatidylcholine did not significantly alter the activity of the enzyme at lipid concentrations up to 1 mM, and the n.m.r. spectrum of the lipid (1%, w/v) was not altered in the presence of enzyme (up to 2%, w/v). Phosphatidylethanolamine did not affect the activity in the oxidation of glutamate, but interfered with the assay in the reverse direction, possibly owing to Schiff-base formation with 2-oxoglutarate. The negatively charged cardiolipin, which is found uniquely in the inner mitochondrial membrane, strongly and reversibly inhibited the enzyme (50% inhibition with 0.1–20 μ M-lipid, depending on conditions). Another anionic phospholipid, phosphatidylserine (which does not occur in mitochondria), also inhibited the enzyme, but less effectively (50% inhibition at about 8 μ M). The lipid-enzyme interaction decreases with increasing ionic strength, indicating an electrostatic contribution to the binding. N.m.r. studies suggest that there is also an apolar interaction involving the lipid chains.

The studies with malate dehydrogenase concentrated on detecting possible stabilization of this enzyme through interaction with lipids. A clear-cut stabilization was found with lysophosphatidylcholine. Under conditions such that the enzyme alone lost 50% of the initial activity in about 2 h, lysophosphatidylcholine gave a slight (20%) activation and this activity remained constant for several hours. N.m.r.

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