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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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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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