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Net Activity of Phospholipase A2 in Brain and the Lack of Stimulation of the Phospholipase A2-Acylation System

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Certain observations reported previously from this laboratory have not proved reproducible. These are (1) the relatively rapid hydrolysis of added phosphatidylcholine by phospholipase A2 of tissue from the cerebral cortex of the guinea pig and (2) the stimulation by 10μM-noradrenaline and by 1.0nM-cyclic AMP of the phospholipase A2-acylation system of isolated synaptic membranes.

It has been reported previously from this laboratory that the phospholipase A2-acylation system in synaptic membranes, isolated from the cerebral cortex of the guinea pig, can be stimulated by biogenic amines and cyclic nucleotides [R. J. Gullis & C. E. Rowe (1973) *Biochem. Soc. Trans.* **1**, 849; (1975*a*) *Biochem. J.* **148**, 197–208; (1975*b*) *Biochem. J.* **148**, 557–565; (1975*c*) *Biochem. J.* **148**, 567–581; (1976*a*) *J. Neurochem.* **26**, 1217–1230]. Some of this work has been summarized briefly [C. E. Rowe & R. J. Gullis (1976) in *Function and Metabolism of Phospholipids in the Central and Peripheral Nervous Systems* (Porcellati, G., Amaducci, L. & Galli, C., eds.), pp. 211–225, Plenum Press, New York and London].

Since these publications, Dr. S. K. Fisher and I have been unable to confirm some of the key observations reported. In particular we have failed to observe (1) the relatively high values of net activity of phospholipase A2, reported to be detectable after 1min, in homogenate, crude mitochondrial fraction, synaptosomal fraction and synaptic membranes of cerebral cortex of the guinea pig, (2) stimulation of net activity of phospholipase A2 in homogenate by 10μM-noradrenaline, and of net activity in synaptic membranes by 10μM-noradrenaline and 1.0nM-cyclic AMP, and (3) stimulation of the incorporation of oleate into choline glycerophospholipid of synaptic membranes by 10μM-noradrenaline and 1.0nM-cyclic AMP.

Considerable effort has been made to reproduce the reported relatively high values of phospholipid hydrolysis after 1min by homogenate and by subcellular fractions. In our hands, using the procedures described previously, there was little detectable activity in the period 0–3min after addition of substrate. After 60min 0.1–0.5nmol of fatty acid was released from added phospholipid per mg of tissue protein. Pretreatment of phospholipid substrate by different procedures, including purification by chromatography on a column of silicic acid (Robertson & Lands, 1962), purification by t.l.c. on Kieselgel G or washing with 0.10M-$CaCl_2$, 0.10M-sodium EGTA, 0.10M-potassium EGTA or 0.10M-Tris EGTA, had little effect on subsequent hydrolysis observable 3min after addition of substrate to the mixture of tissue and cofactors. Changing the order of addition of cofactors or substitution of Tris ATP by disodium ATP had little effect. Since silica gel has been shown to activate phospholipase A2 of snake venom (Goerke *et al.*, 1971), it seemed possible that Kieselgel G, which was usually used in the purification of substrate, might be essential for activation of tissue phospholipase. Although some changes in net activity of phospholipase A2, attributable to Kieselgel G, were sometimes observed, high rates of phospholipid hydrolysis during the first minute were not obtained. It is hoped to publish elsewhere some of the results obtained during these re-investigations.

Clearly it would be time-consuming to repeat all the experiments described in the extensive work cited in the publications given in the first paragraph, but failure to reproduce key observations must inevitably cast doubt on other aspects of the work. I have not attempted to repeat the previously reported effects of neurotransmitters, cyclic nucleotides and other reagents on the concentration of arachidonic acid in synaptosomes and synaptic membranes [R. J. Gullis & C. E. Rowe (1976*b*) *FEBS Lett.* **67**, 256–259]. It should be noted, however, that the changes in concentrations of arachidonate were reported as being due to procedures identical with those reported to stimulate the phospholipase A2-acylation system. This stimulation is not reproducible in our hands.

Since the completion of these re-investigations Dr. R. J. Gullis has informed me that data presented in the publications by Gullis & Rowe (1973, 1975*a*,*b*,*c*, 1976*a*,*b*) were not the *bona fide* results of the many experiments performed.

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Note from the Chairman of the Editorial Board

The manuscript of the above paper by C. E. Rowe was accompanied by a signed statement from R. J. Gullis agreeing to its publication in full [see also R. J. Gullis (1977) *Nature* (*London*) **265**, 764].