Effects of lithium on the activity of pyruvate kinase and other magnesium dependent enzymes

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It has been proposed that the many various pharmacological effects of lithium might be explained by its competition with magnesium and calcium (Birch, 1973). Many enzymes are magnesium dependent and it was decided to determine the effect of lithium on the activity of a number of those which were commercially available. Preliminary experiments were carried out on the following: pyruvate kinase, alkaline phosphatase, hexokinase, glucose-6-phosphate dehydrogenase, 3-phosphoglycerate kinase. Hexokinase was obtained from Sigma Ltd, all others from Boehringer Ltd.

The first three enzymes were inhibited by lithium concentrations of 6 mmol/l (50%), 2 mmol/l (50%) and 14 mmol/l (30%) respectively. The remaining two were not inhibited in the preliminary experiments though their investigation was not exhaustive.

Lithium inhibition of pyruvate kinase has been previously reported (Kachman & Boyer, 1953) though the concentration used (100 mmol/l) was far in excess of the plasma level (0.6-1.4 mmol/l) obtained during treatment of recurrent affective disorder (Hullin, McDonald & Allsopp, 1972). The effects of lithium in the pharmacological dose range was therefore determined by the disappearance of NADH (estimated spectrophotometrically at 340 nm) in 3 ml aliquots of the test system: pyruvate kinase (0.66 mg/l), lactate dehydrogenase (13.32 mg/l), ADP (0.5 mmol/l), $MgCl_2$ (1.5 mmol/l) and KCl (40 mmol/l). Lactate dehydrogenase was shown to be unaffected by lithium at the concentrations prevailing.

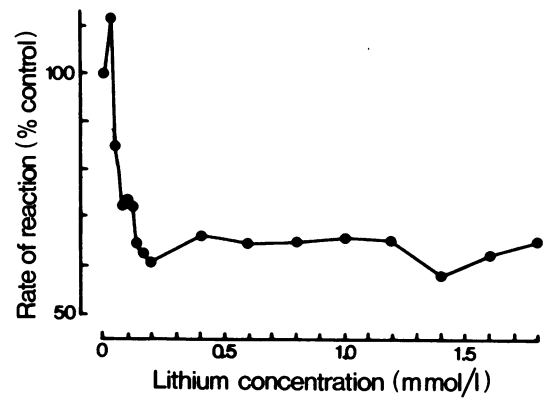

Fig. 1 The effects of various concentrations of lithium on the activity of pyruvate kinase expressed as percentage of control values.

Figure 1 shows the effect of various concentrations of lithium on the activity of pyruvate kinase. A significant inhibition occurs at normal pharmacological concentration of lithium.

We gratefully acknowledge a programme grant from the Medical Research Council.

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The effects of the anticonvulsant ethosuximide on adenosine triphosphatase activities of synaptosomes prepared from rat cerebral cortex

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The results of recent experiments in our laboratory have indicated that the anticonvulsant ethosuximide inhibits the sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na,K-ATPase) activity of nerve terminals (synaptosomes) prepared from rat cerebral cortex. The results also suggested that the synaptosomes contain a sodium-activated, magnesium-dependent ATPase (Na-ATPase) in addition to the Na,K-ATPase usually associated with the sodium pump (Gilbert, Scott & Wyllie, 1974). In contrast to the Na,K-ATPase, the Na-ATPase was not inhibited by ethosuximide (2.5×10^{-4} –

2.5×10^{-2} M); it appeared to be responsible for some 20% or more of the activity determined as Na,K-ATPase activity when both sodium and potassium ions were present in the assay medium, and it was insensitive to ouabain (1×10^{-6} – 1×10^{-3} M).

The object of the present work was to test more rigorously the hypothesis that the synaptosome contains a Na-ATPase, and to locate more precisely the site within the synaptosome where ethosuximide inhibits Na,K-ATPase activity.

Synaptosomes were prepared by homogenizing rat cerebral cortex in 0.32 M sucrose containing 1 mM EDTA and subjecting the mitochondrial fraction, obtained by differential centrifugation, to further centrifugation on a sucrose density gradient (Balfour & Gilbert, 1971). The Na- and Na,K-ATPase activities were determined by measuring the release of inorganic phosphate from Tris-ATP as described previously (Gilbert *et al.*, 1974).

Homogenization of synaptosomes with the detergent lubrol (0.2% in 0.32 M sucrose solution containing 1 mM EDTA) extracted the Na-ATPase, together with some of the Na,K-ATPase, leaving a pellet devoid of Na-ATPase activity. Ouabain (1×10^{-3} M) inhibited the Na,K-ATPase activity of the pellet almost completely (97.1 ± 0.5%) but it inhibited the lubrol-soluble Na,K-ATPase only to the extent of 66.6 ± 0.3%, the ouabain-insensitive activity corresponding approximately to the Na-ATPase activity (29.2 ± 3.0% of the apparent Na-K-ATPase activity).

Fractions enriched in vesicles, mitochondria or membranes were prepared from disrupted synaptosomes by a method similar to that of Whittaker (1966). All fractions contained ouabain-insensitive Mg-ATPase activity. However, only the Mg-ATPase of the vesicle fraction was inhibited by ethosuximide. As found by others, the enzyme was also inhibited when the medium contained sodium and potassium ions. The vesicle fraction was unique in being devoid of Na- and Na,K-ATPase activity. The mitochondrial and membrane fractions each contained Na-ATPase activity which was insensitive both to ouabain and ethosuximide. The fractions also contained ouabain-sensitive Na,K-ATPase activity, but only the membrane fraction Na,K-ATPase was inhibited by ethosuximide.

These results provide further evidence that a Na-ATPase which is insensitive both to ouabain and ethosuximide exists in the synaptosome, and they also indicate that the site of inhibition of Na,K-ATPase activity by ethosuximide may be the limiting membrane of the synaptosome.

We wish to acknowledge the financial support of Parke Davis & Co. who also supplied the ethosuximide. We thank Mrs H. Anderson for technical assistance.

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High-affinity hepatic microsomal binding of propranolol: its relationship to metabolism and to the first-pass effect

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In several species, including man (Shand & Rangno, 1972) and rat (Shand, Rango & Evans, 1972), propranolol exhibits a non-linear first-pass hepatic extraction which can be resolved into a high-affinity low capacity uptake process, and a low-affinity high-capacity mechanism. This phenomenon has been further investigated *in vitro* in the rat by examining the binding of ^{14}C-labelled propranolol to isolated liver cells, liver homogenates and subcellular fractions.

In whole cells (prepared by the method of Seglen, 1973) and whole liver homogenates (prepared in isotonic KCl buffered with 0.067 M phosphate) binding (measured by centrifugation) was non-linear and could be resolved using the method of Rosenthal (1967) into a high-affinity low-capacity site, and a low-affinity high-capacity site. The high-affinity site had a dissociation constant with an experimental range from $2\text{-}7 \times 10^{-8}$ M, and a capacity ranging from 3-8 nmol/g wet weight of liver. The only subcellular fraction possessing a high-affinity site was the microsomal fraction (100,000 *g* pellet); no high-affinity site could be demonstrated in plasma membranes, mitochondria (10,000 *g* pellet) nuclei