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The effects of phenytoin on adenosine triphosphatase activities of synaptosomes and their components

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The mechanism of the anticonvulsant action of phenytoin is often assumed to involve the sites of active translocation of sodium ions out of the cells and nerve terminals of the central nervous system. These sites are closely associated with adenosine triphosphate (ATP)-hydrolysing enzymes which are activated by various cations. The recent observations in our laboratory that the anticonvulsant ethosuximide inhibits a synaptosome sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na,K-ATPase), that the enzyme involved may be located in the external membrane of the synaptosome, and that a magnesium-activated ATPase (Mg-ATPase) located in the vesicle-containing fraction prepared from synaptosomes is also inhibited by ethosuximide, led us to investigate the effects of phenytoin on these enzymes.

Synaptosomes were prepared by homogenizing rat cerebral cortex in 0.32 M sucrose solution 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 sodium-activated, magnesium-dependent ATPase (Na-ATPase), Mg-ATPase and Na,K-ATPase activities were determined by measuring the release of inorganic phosphate from Tris-ATP as described previously (Gilbert, Scott & Wyllie, 1974).

Phenytoin (2×10^{-7}-2×10^{-4} M) did not significantly alter the Mg-ATPase activity of the synaptosomes, however the drug inhibited the Na,K-ATPase activity by approximately 80%. The synaptosomes exhibited Na-ATPase activity, in the absence of exogenous potassium ions, and this activity corresponded in magnitude to the Na,K-ATPase activity which was insensitive to phenytoin. The Na-ATPase activity was not itself altered by phenytoin.

Festoff & Appel (1968) have suggested that the effect of phenytoin on the Na,K-ATPase activity of synaptosomes depends upon the ratio of sodium : potassium in the assay medium. In their studies phenytoin inhibited the enzyme when the ratio was less than 5 : 1 but it stimulated the enzyme when the ratio exceeded 25 : 1. In the present work phenytoin inhibited the activity at all ratios tested (150 : 10, 100 : 30 and 100 : 2).

Fractions enriched in vesicles, membranes or mitochondria were prepared from disrupted synaptosomes by a method similar to that of Whittaker (1966). As in the case of the anticonvulsant ethosuximide, phenytoin inhibited only that Na,K-ATPase activity which was associated with the fractions containing the external membranes of the synaptosomes. Like ethosuximide, phenytoin also inhibited a Mg-ATPase which was present in the fractions containing vesicles.

We thank Mrs H. Anderson for technical assistance.

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