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.

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

J. McEWEN*, D.G. SHAND & G.R. WILKINSON
(introduced by I.H. STEVENSON)

Department of Pharmacology and Therapeutics, University of Dundee and Division of Clinical Pharmacology, Vanderbilt University, Nashville, Tennesee, U.S.A.

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

200 *g* pellet) or in the 100,000 *g* supernatant (this latter measured by equilibrium dialysis).

Further studies of high-affinity binding with parallel metabolism experiments indicated a relationship between high-affinity binding and metabolism. Each was potentiated by oxygen and suppressed by carbon monoxide: drugs which displaced propranolol from high-affinity binding inhibited propranolol metabolism (SKF 525-A, chlorpromazine, imipramine and lignocaine): changes in high-affinity binding with sex and age correlated with changes in metabolism.

This approach may provide another possible method of examining drug-cytochrome P-450 complex, analogous to microsomal difference spectra with the added advantage of providing a capacity term. Although there are some quantitative difficulties in directly correlating this microsomal high-affinity capacity with the threshold of the first-pass phenomenon, it may be the case that such high-affinity subcellular binding is a common property of drugs which undergo first-pass hepatic extraction.

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Changes in renal function following chronic phenobarbitone administration

E.E. OHNHAUS* & H. SIEGL

Department of Medicine, University of Bern, Switzerland

Following chronic administration of phenobarbitone (30 mg kg^{-1} day^{-1} i.p.) to rats increases in the urinary excretion of unchanged chlorothiazide were found (Ohnhaus, 1972). As the drug/creatinine clearance ratio for chlorothiazide is similar to that for *p*-amino hippurate (PAH) and therefore to renal plasma flow (Beyer, 1958), phenobarbitone might have an influence on glomerular filtration rate (GFR) or renal plasma flow (RPF).

SRF-male rats were treated intraperitoneally with phenobarbitone (30 mg kg^{-1} day^{-1}) or sodium chloride respectively for 4 days. The latter rats formed a control group. Endogenous creatinine clearance in conscious rats and inulin clearance without induced diuresis under inactin anaesthesia were measured 24 h following the last dose of phenobarbitone. In an additional group of pretreated rats diuresis was induced by infusing 0.9% NaCl + 2% mannitol i.v. for 30 min at 5.0 ml 100 g^{-1} h^{-1} and inulin- and PAH-clearance were measured under the same experimental conditions.

Following 4 days treatment with phenobarbitone endogenous creatinine clearance and inulin-clearance with and without induced diuresis were not significantly different in control and phenobarbitone treated animals; changes in urine volume were not found. In contrast PAH-clearance was significantly increased in the phenobarbitone treated in comparison to the controls from 2.87 ml min^{-1} 100 g^{-1} body weight to 4.84 ml min^{-1} 100 g^{-1} body weight ($P < 0.001$).

These results indicate no changes in the glomerular filtration rate following chronic phenobarbitone administration, whereas the renal plasma flow is increased by about 69%. The increased excretion of chlorothiazide following chronic administration of phenobarbitone can therefore be attributed to changes in renal hemodynamics.

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