DIPHENYLHYDANTOIN AND HUMAN MYOCARDIAL MICROSOMAL (Na^+, K^+) - ATPase

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ABSTRACT

Diphenylhydantoin has no effect on the activity of human myocardial microsomal (Na^+, K^+) - ATPase or on the inhibitory action of ouabain.

There is now a great deal of evidence that microsomal (Na^+, K^+) - ATPase supports the activity of the sodium pump (1). In vitro, digitalis glycosides cause a characteristic inhibition of the activity of the enzyme in microsomal preparations taken from a wide variety of tissues including the myocardium (2 - 6). Toxic doses of digitalis have been shown to cause a loss of potassium from the heart in man and animals (7 - 9) an action which is consistent with the effects of inhibiting the sodium pump.

Clinically, diphenylhydantoin has been found to be of value in the treatment of dysrhythmias caused by digitalis intoxication (10 - 13) and, more recently, Helfant et al. (14) have shown that in dogs the reversion of the dysrhythmias induced by digitalis is accompanied by a reversal of the myocardial loss of potassium. These authors have suggested that the diphenylhydantoin acts on the myocardial cell by reversing the inhibition of (Na^+, K^+) - ATPase caused by digitalis.

We have tested this hypothesis by studying the effects of diphenylhydantoin sodium on human myocardial microsomal (Na^+, K^+) - ATPase in vitro.

EXPERIMENTAL

The enzyme preparation has been made from post mortem material using a technique similar to that published previously (15). The assay mixture contained: 3m M Mg Cl_2; 100m M NaCl; 10m M KCl; 2m M ATP (di sodium salt adjusted to pH 7.4 with Tris) and 50m M Tris - HCl buffer pH 7.4 in a total volume of 1 ml. The difference between the activity found in the above system and one containing 10^{-3}M ouabain was taken as the (Na^+, K^+) - ATPase activity. Both reactions were started after 5 minutes pre-incubation at 37°C with 0.08 mg microsomal protein. The reaction was stopped with 0.2 ml perchloric acid after 30 minutes incubation at 37°C. The release of inorganic phosphate was measured by a modified method of Berenblum and Chain (16). Protein was determined by the method of Lowry et al. (17).

The results are expressed as μ moles inorganic phosphate liberated/hour/mg protein.

RESULTS

The figures shown in the tables are the means of three parallel experiments. Table 1

TABLE 1.

Effects of various concentrations of Diphenylhydantoin on Human Myocardial microsomal (Na^+, K^+) - ATPase and Mg^{++} - ATPase

Results expressed as μ moles inorganic phosphate liberated/hour/mg protein

Concentration of diphenylhydantoin	(Na^+, K^+) - ATPase	Mg^{++} - ATPase
0	3.95	1.38
10^{-4}M	3.92	1.51
10^{-3}M	4.05	1.44
10^{-2}M	3.86	1.43

shows the Mg^{++} and (Na^+, K^+) activity of the preparation with and without diphenylhydantoin. Diphenylhydantoin had no effect on (Na^+, K^+) or Mg^{++} ATPase activity. Table 2 shows the inhibitory effects of ouabain in various concentrations on the (Na^+, K^+) - ATPase activity of the preparation with and without diphenylhydantoin. Diphenylhydantoin has no influence on the inhibitory action of ouabain.

TABLE 2.

The effect of Diphenylhydantoin on the inhibition of
Human Myocardial microsomal (Na^+, K^+) - ATPase by Ouabain
Results expressed as μ moles inorganic phosphate liberated/hour/mg protein

Ouabain concentration	Activity without diphenylhydantoin	Activity with 10^{-2}M diphenylhydantoin
0	1.48	1.48
10^{-6}M	0.94	0.96
10^{-5}M	0.79	0.80
10^{-4}M	0.00	0.00

DISCUSSION

We conclude from these experiments that the action of diphenylhydantoin on the cardiac dysrhythmias and loss of potassium caused by digitalis glycosides is not mediated by any influence on myocardial microsomal (Na^+, K^+) - ATPase.

Similar studies (18) have recently shown that diphenylhydantoin has an inhibitory action on microsomal (Na^+, K^+) - ATPase prepared from rat and guinea pig brain, an action which may be related to the anticonvulsant properties of the drug. The present

studies indicate that such an effect does not necessarily apply to tissues other than brain.

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