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OUABAIN-INSENSITIVITY OF HIGHLY ACTIVE Na^++K^+-DEPENDENT ADENOSINETRIPHOSPHATASE FROM RAT KIDNEY

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Summary: Highly purified preparations of Na^++K^+-dependent adenosinetriphosphatase were isolated from rat kidney by two different procedures. The I_{50} values for ouabain inhibition of the rat kidney enzyme at various stages of purification were determined to be essentially the same for all fractions tested (0.7 to 1.0 x 10^{-4}M). These results suggest that the marked insensitivity of the rat enzyme to inhibition by cardiac glycosides is due to the primary structure of the enzyme, and not to some other component in the tissue.

Introduction: The Na^++K^+-dependent adenosinetriphosphatase (Na^+,K^+-ATPase; EC. 3.6.1.3.) is the enzyme that performs the energy-dependent transport of Na^+ and K^+ across the plasma membrane; and it is also the receptor for some, if not all, of the pharmacologic effects of cardiac glycosides (1-3). It has been known for some time that there are species and tissue differences amongst the sensitivities of Na^+,K^+-ATPase preparations to inhibition by cardiac glycosides, and that the sensitivities of the isolated enzymes correlate well with the sensitivities of the source tissues and species to cardiac glycosides. The concentrations of ouabain required to induce a positive inotropy in dog heart or a diuresis in dog kidney are 10^2 to 10^3-fold lower than those needed to elicit these responses in rat heart and kidney (4-7). The I_{50} values for ouabain inhibition of dog heart and kidney Na^+,K^+-ATPase preparations are also approximately 10^3-fold lower than those of the highly insensitive enzyme preparations isolated from rat heart and kidney (8,9). It is not known whether these variations in the sensitivities of enzyme preparations from

Abbreviation: SDS, sodium dodecyl sulfate.

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different sources to cardiac glycoside inhibition are due to alterations in the structure of the enzyme or due to the presence (or absence) of tissue components that affect the enzyme-inhibitor interaction. The identification of the determinants of this variable sensitivity is important because it will undoubtedly contribute to a better understanding of the nature of cardiac glycoside interaction with Na^+,K^+-ATPase.

Yoda and Hokin (10) and Wallick et al. (11) have suggested that the insensitivity of the rat Na^+,K^+-ATPase to ouabain may be due to the absence or alteration of binding sites on the enzyme for the sugar moiety or for the unsaturated lactone ring of the cardiac glycoside, respectively. More recently, Choi and Akera (12) reported that the apparent tissue-dependent differences they observed in the ouabain sensitivities of Na^+,K^+-ATPase preparations isolated from dog heart, brain, and kidney were artifacts of the purification procedure.

In view of the above observations, and the fact that earlier studies on the interaction of cardiac glycosides with rat Na^+,K^+-ATPase were conducted with very impure preparations, we have (a) attempted to purify the rat kidney Na^+,K^+-ATPase by two different procedures, and (b) determined the ouabain sensitivity of the rat kidney Na^+,K^+-ATPase at different stages of purification.

Methods: Frozen rat kidneys were obtained from Pel Freez Biologicals (Rogers, Arkansas). Na^+,K^+-ATPase was isolated from the outer medulla of rat kidney by a modification of Jørgensen's SDS-gradient procedure (13), and from whole rat kidney using the deoxycholate-solubilization method of Lane et al. (14).

Enzyme activity was measured at 37° by either the spectrophotometric coupled-enzyme assay (8), or by the colorimetric determination of released orthophosphate (15). The concentration of ATP was 5 mM in the former, and 3 mM in the latter assay. In both assay procedures, 100 mM Na^+ and 10 mM K^+ were used.

Phosphorylation of the enzyme by (γ-^{32}P)ATP, and SDS-polyacrylamide gel electrophoresis were done by conventional procedures (16-18).

Results and Discussion: The results of the application of the deoxycholate-solubilization procedure of Lane et al. (14) are shown in Table 1. It is clear that the extent of purification achieved here for the

Table 1. Isolation of Na^+,K^+-ATPase from Rat Kidney by Solubilization with Deoxycholate. Na^+,K^+-ATPase was prepared from whole rat kidney as described previously (14), except that the ratio of sodium deoxycholate to protein was 0.25 mg/mg at the detergent-wash step and 1.0 mg/mg at the detergent-extraction step.

Fraction	Na^+,K^+-ATPase Activity (μmol Pi/mg/hr)
Microsomes	33
NaI-treated Microsomes	76
Glycerol Precipitate	470

Na^+,K^+-ATPase from whole rat kidney is not nearly as great as that obtained when the same procedure is applied to the outer medulla of dog or lamb kidney (14,16). In view of this, the "rapid" version of the SDS-gradient procedure of Jørgensen (13) using rat kidney outer medulla was attempted. The optimal concentration of SDS for the treatment of the rat kidney microsomes was determined experimentally, as illustrated in Fig. 1. Comparing the sharp peak of activity obtained here with increasing SDS concentration, to the plateau of activity reported by Jørgensen (Fig. 10 of ref 13) with rabbit kidney, suggests that the rat kidney Na^+,K^+-ATPase is more sensitive than the rabbit enzyme to the inactivating effect of SDS. This is consistent with the observation that both the optimal concentration of SDS and the maximum specific activity of the rat kidney enzyme (1,250 μmol Pi/mg/hr) are lower than those reported for the enzyme from rabbit kidney (13).

SDS-polyacrylamide gel electrophoresis of the enzymes isolated from rat kidney indicated that neither of these preparations is pure. In addition to the α and β subunits present in the purified Na^+,K^+-ATPase fractions from dog, rabbit and lamb kidney (13,14,16), the SDS-gradient enzyme from rat kidney medulla contained a small amount (approximately 6% of the total protein) of a higher molecular weight protein. The deoxycholate-solubilized rat enzyme also contained a smaller amount of a fourth protein that migrated just ahead of the α subunit. Although a functional significance of the higher molecular

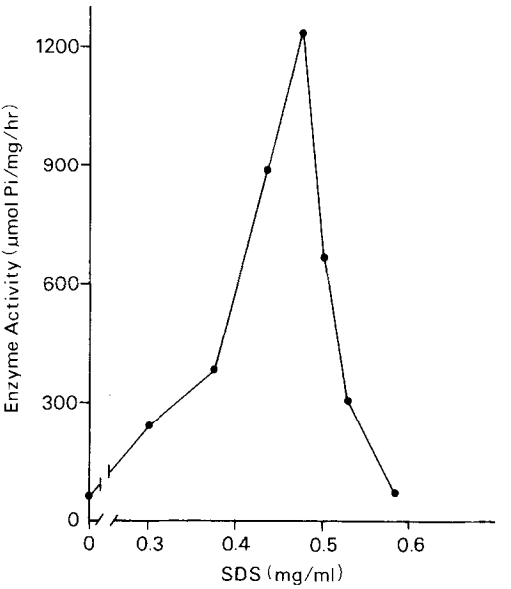

Fig. 1. Purification of Na^+,K^+-ATPase from Rat Kidney Outer Medulla. Microsomal suspensions (1.4 mg protein/ml) were treated with indicated SDS concentrations in the presence of 3 mM ATP, incubated, and centrifuged as described by Jørgensen (13). The indicated activities are those of the final sediments. Specific activity of the starting material, crude microsomes, was 37 μmol Pi/mg/hr.

weight protein can not be ruled out at the present time, we suspect that it is a contaminant.

Similar to the Na^+,K^+-ATPase preparations from other sources, the rat kidney enzyme was phosphorylated from (γ-^{32}P)ATP in the presence of Na^+ and Mg^{2+}; and all of the incorporated phosphate was found in the α subunit.

The ability of ouabain to inhibit the hydrolytic activity of the following rat kidney Na^+,K^+-ATPase fractions was measured: 1. The microsomes of outer medulla (Fig. 1). 2. The NaI-treated microsomes of Table 1. 3. The deoxycholate-solubilized and then glycerol-precipitated fraction of Table 1. 4. The SDS-treated gradient enzyme with a specific activity of 1,250 μmol Pi/mg/hr. Consistent with the previous report of Allen and Schwartz (8), the inhibition of these rat Na^+,K^+-ATPase preparations by ouabain was not time dependent. The maximal inhibition, at all concentrations of ouabain used, was obtained within 1-3 min after the exposure of the enzyme to ouabain. As

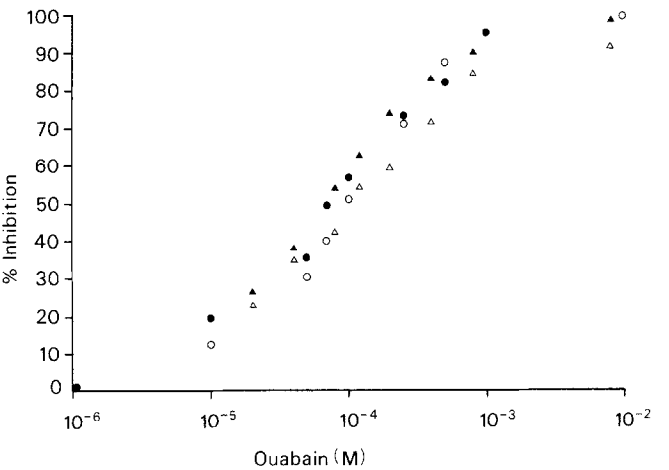

Fig. 2. Inhibitory Effects of Varying Concentrations of Ouabain on Na^+,K^+-ATPase Activities of Four Different Enzyme Preparations from Rat Kidney. ●, crude microsomes of outer medulla; ○, SDS-treated gradient enzyme; ▲, NaI-treated microsomes; △, glycerol precipitate.

shown in Fig. 2, the I_{50} values for ouabain inhibition of these four enzyme fractions are essentially the same (0.7 to 1.0 x 10^{-4}M). These values are also consistent with the I_{50} values of 1.0 x 10^{-4}M reported previously (8) for crude rat heart and kidney fractions that had been prepared with a technique different from the two procedures used in the present study.

These results suggest that the marked insensitivity of rat Na^+,K^+-ATPase to inhibition by cardiac glycosides is not due to an artifact of isolation. Further, in view of the variety of purification steps used to prepare these rat kidney enzymes, it appears unlikely that the insensitivity is due to the presence of a non-ATPase component in the tissue. It does appear likely that the determinant of the cardiac glycoside insensitivity of the rat kidney enzyme is contained within the primary structure of the Na^+,K^+-ATPase protein.

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