NON-LINEARITY OF HUMAN RENAL ($Na^+ + K^+$)-ATPase: ELIMINATION BY PURIFICATION AND CORRELATION WITH CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY

J. Mark Braughler and Clinton N. Corder

University of Pittsburgh
School of Medicine
Departments of Pharmacology and Medicine
Pittsburgh, PA. 15261

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SUMMARY

Human renal ($Na^+ + K^+$)-ATPase exhibits a non-linear reaction rate which can be eliminated by purification of the enzyme. The activity in the microsomes and the first several stages of purification is nearly zero after 8 minutes of assay. The reaction of the purified enzyme, however, is linear with assay time. This change to a linear reaction rate correlates with the loss of cyclic AMP-dependent protein kinase activity from the ($Na^+ + K^+$)-ATPase preparation.

INTRODUCTION

Non-linearity of the ($Na^+ + K^+$)-ATPase reaction has been reported for microsomal preparations of the enzyme from the rat kidney (1-2), human kidney (3-4), and human myocardium (5). This non-linearity may be mediated by factors which are separate from the ATPase itself (1-2). An extensive purification of human renal ($Na^+ + K^+$)-ATPase[1] demonstrated that non-linearity was not a problem in highly purified preparations of the enzyme. This suggested that the cause of the non-linearity was eliminated during the purification. Preliminary evidence is presented here that the causative factor may be a cyclic AMP-dependent protein kinase.

[1] Braughler, J.M. and Corder, C.N., manuscript in preparation.

METHODS

Collection of tissue: Kidney was obtained at autopsy from patients (ages 32-38 years) with normal renal function (serum creatinine $\leq$ 1.5 mg%) who had received no cardiac glycoside or diuretic therapy.

Preparation of microsomes: The whole kidney was minced with scissors and homogenized 1:5 with 100 mM tris, pH 7.4, and 0.33 M sucrose (tris-sucrose). The suspension was then centrifuged at 10,000 x g for 20 min. The resultant supernatant was centrifuged at 100,000 x g for 1 h. The 100,000 x g pellets were then suspended in 2 volumes of tris-sucrose and centrifuged again at 100,000 x g for 1 h. These final pellets were resuspended in 25 mM imidazole pH 7.0, 1 mM disodium EDTA, at an approximate protein concentration of 10-14 mg/ml and frozen at -90°C.

Purification of $(Na^+ + K^+)$-ATPase from the microsomes: The microsomes were first treated with desoxycholate (DOC) according to the procedure of Kyte (6). This was followed by NaI treatment, DOC solubilization, glycerol precipitation and ammonium sulfate $[(NH_4)_2SO_4]$ fractionation (7).

Assays: The ATPase assay was at 37°C with 0.07 mg protein/ml in 100 mM imidazole pH 7.4, 6 mM ATP, 6 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl and .02% BSA. The reaction was started by addition of enzyme into 1 ml of the prewarmed assay mix. At various time intervals, aliquots were deproteinized and inorganic phosphate was determined (8). Total ATPase was determined in the absence of ouabain. Mg^{2+}-ATPase was determined in the presence of 1 mM ouabain. $(Na^+ + K^+)$-ATPase was the difference between the total and Mg^{2+}-ATPase. Purified preparations contained less than 1% Mg^{2+}-ATPase.

Membrane bound protein kinase activity was determined by the procedure of Kinne et. al. (9).

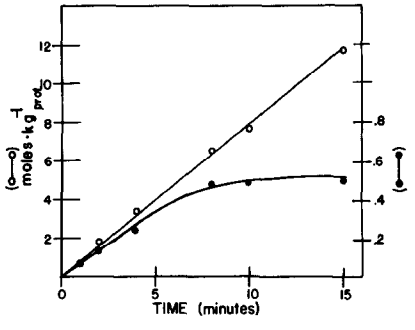

Figure 1: $(Na^+ + K^+)$-ATPase activity was determined as described in "Methods". Activity is represented as moles of liberated phosphate per kilogram of protein (moles $\cdot$ kg^{-1} prot). $(Na^+ + K^+)$-ATPase activity in the microsomes (●—●). $(Na^+ + K^+)$-ATPase activity of the purified enzyme (o—o).

RESULTS & DISCUSSION

Figure 1 shows the $(Na^+ + K^+)$-ATPase activity as a function of assay time in the microsomes and the purified enzyme. The activity was non-linear in all microsomal preparations as well as the first stages of purification (DOC and NaI treated enzymes, not shown) such that the rate of hydrolysis of ATP was zero after 8 min of incubation. The enzymatic activity was linear in the purified enzyme (Figure 1). That purified preparations of $(Na^+ + K^+)$-ATPase exhibit a linear reaction rate suggests that some factor or factors responsible for the non-linearity can be eliminated by purification.

It has been suggested that cyclic AMP-dependent protein kinase may alter $(Na^+ + K^+)$-ATPase activity (10-12). Table I shows the relationship between $(Na^+ + K^+)$-ATPase and membrane bound cyclic AMP-dependent protein kinase activities at various stages of purification of $(Na^+ + K^+)$-ATPase. In purified preparations which exhibit linear reaction rates cyclic AMP-dependent protein kinase activity is minimal or absent. Less purified, non-linear $(Na^+ + K^+)$-ATPase preparations (microsomal, DOC treated, and NaI enzymes) are associated with higher levels of cyclic AMP-dependent protein kinase.

Recently, Kinne et. al. (9) showed that cyclic AMP-dependent protein kinase and $(Na^+ + K^+)$-ATPase levels were inversely related in brush border microvilli and basal-lateral membrane fractions from the rat renal cortex. In view of the present findings it seems likely that a cyclic AMP-dependent protein kinase may serve as a modulator of $(Na^+ + K^+)$-ATPase activity *in vivo* and *in vitro*. A detailed study of this hypothesis is currently underway.

TABLE I

Purification Step	Reaction* Character	$(Na^+ + K^+)$-ATPase** moles per kg per H	cyclic AMP-dependent protein kinase p moles ^{32}P incorporated per mg per H
microsomes	non-linear	4	46.5
DOC treated microsomes	non-linear	22	42.5
NaI enzyme	non-linear	35	42.5
glycerol precipitated enzyme	linear	70	2.86
$(NH_4)_2SO_4$ enzyme	linear	72	0

*Non-linear, enzymatic rate zero by 8 min; Linear, enzymatic rate linear with time for at least 15 min. (See figure 1).

**The specific activity in the non-linear preparations was determined by the early part of the reaction which was the closest approximation of the initial enzymatic rate.

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