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RENAL CORTICAL Na-K-ATPase IN CADMIUM-INTOXICATED RATS

Yung Kyu Kim and Yang Saeng Park

Department of Physiology,
Kosin Medical College, Pusan 602-030, Korea

= Abstract =

Changes in sodium, potassium-activated adenosinetriphosphatase (Na-K-ATPase) activity was studied in renal cortical microsomes of cadmium-intoxicated rats. Cadmium-intoxication was induced by subcutaneous injections of $CdCl_2$ dissolved in saline at a dose of 2 mg Cd/kg·day for 16 days. The Na-K-ATPase activity was determined at the end of 4th and 16th day of treatment and 20 days after treatment ceased, and the values were compared with those observed in matched control (saline-treated) animals. The enzyme activity was not significantly altered after 4 days of Cd-treatment, but it was markedly reduced after 16 days of the treatment (35.4±3.2(SE) m moles Pi/g protein per hr as opposed to 56.7±3.0 in the control). During 20 days of recovery period the enzyme activity was considerably reverted. These results are discussed in relation to changes in renal transport of Na^+, glucose, and p-aminohippurate in cadmium-intoxicated animals.

INTRODUCTION

The sodium, potassium-activated adenosinetriphosphatase (Na-K-ATPase) is believed to be involved in a variety of renal transport processes. It has been shown that reabsorptions of sodium[7,15,17] and water[14] in the kidney are intimately related to the activity of the Na-K-ATPase. The enzyme system may also play a role in the proximal tubular reabsorption of organic solutes, such as glucose and amino acids, which depend on sodium transport.[24] According to "the sodium gradient hypothesis",[3,21] transport of these substances across the brushborder membrane is coupled with sodium transport (cotransport). The inward-directed sodium electrochemcal gradient, established by the activity of the

sodium pump in the basolateral membrane, provides the driving force for the cotransport. Since the Na-K-ATPase is an integral part of the sodium pump,[22] the solute reabsorption by the cotransport mechanism will ultimately be determined by the activity of Na-K-ATPase. The secretion of various organic anions in the proximal tubule is another transport process which may require the Na-K-ATPase activity. Several studies in isolated renal cortical tissues[16,23] have shown parallel changes in active p-aminohippurate (PAH) transport and Na-K-ATPase activity under various experimental conditions.

As described elsewhere,[10,11] renal transports of Na^+, glucose, and PAH are markedly altered in cadmium-intoxicated rats. The present paper describes changes in renal cortical Na-K-ATPase activity in relation to the above changes.

MATERIALS AND METHODS

Sprague-Dawley male rats (250~300g) were maintained under standard laboratory conditions with *ad libitum* access to food and water. Cadmium intoxication was induced by daily subcutaneous injections of $CdCl_2$ at a dose of 2 mg Cd/kg body weight per day for up to 16 days. The control group recieved the same volume of saline.

At appropriate intervals animals were sacrificed for assay of Na-K-ATPase in the kidney. Renal cortical microsomes were prepared by a method similar to that described by Jϕrgensen and Skou.[6] Slices of kidney cortex prepared using a Stadie-Riggs microtome were homogenized in 10 volumes of imidazole-sucrose buffer (0.03 M imidazole, 0.25 M sucrose, pH 7.6 at 25℃). The homogenates were then centrifuged at 1,300×g for 10 min in a refrigerated centrifuge (Sorvall, Model RC-5B). The supernatant was centrifuged at 9,500×g for 15 min and the resulting supernatant was centrifuged again at 25,000×g for 30 min. The pellet was suspended in 2 ml of imidazole-sucrose buffer to a concentration of approximately 2 mg protein per ml and stored at -60℃. Protein concentration of this suspension was determined by the method of Lowry et al.[13]. Prior to ATPase assay, aliquots of microsomal preparations were treated with deoxycholate by incubating them in a solution containing 60 mg% deoxycholate, 2 mM EDTA and 25 mM imidazole (pH 7.6 at 25℃) for 30 min at 25℃, and the mixture was adjusted to contain 0.25 mg protein per ml. The ATPase activity was assayed by measuring inorganic phosphate (Pi) liberated by ATP hydrolysis during 10 min incubation of deoxycholate-treated microsomes with 1 ml of appropriate medium containing Na_2-ATP as the substrate. The total ATPase activity was determined in the presence of Na^+, K^+ and Mg^{++}, and the Mg-ATPase in the absence of K^+, and presence of ouabain (1 mM) in the incubation medium. The difference between the total-and Mg-ATPase activity was taken as the measure of Na-K-ATPase activity. Unless otherwise stated, concentrations of Na^+, K^+, Mg^{++}, and ATP in the incubation medium were 130~150, 20, 3, and 1 mM, respectively. When the ATP concentration was varied, the Mg/ATP ratio was maintained at 3/1. The medium pH was adjusted to 7.4 at 37℃ with imidazole-HCl. After 10 min preincubation at 37℃ the reaction was initiated by adding ATP stock solution. The reaction was terminated by adding 0.2 ml of ice-cold 6% perchloric acid, and the mixture was centri-

fuged at 3,500×g for 15 min. Inorganic phosphate in the supernatant was measured according to Fiske and SubbaRow[4]. The enzyme activity was expressed as m moles Pi/g of microsomal protein per hr.

Statistical evaluation of the data was done using the Student's t-test and all results are presented as the mean±SE.

RESULTS

Fig. 1 depicts the effect of Cd treatment (2 mg/kg·day, s.c. injection) on the Na-K-ATPase activity in renal cortical microsomes. The enzyme activity in 16-day Cd-treated animals (35.4±3.2 m moles Pi/g protein per hr) was significantly lower than that observed in saline-treated (control) animals (56.7±3.0). During early phase of treatment period, there was no apparent alteration in the enzyme activity. The value of the enzyme activity in 4-day Cd-treated animals (49.1±1.7) was exactly comparable to the corresponding control value (50.3±0.5). The enzyme activity measured 20 days after cessation of cadmium injection (44.8±1.0) was significantly higher than that in 16-day Cd-treated animals, but it was still somewhat lower than that in matched control animals (52.7).

Fig. 2 shows kinetic analysis of Na-K-ATPase activity at the end of 16 days of treatment period. In both saline-and Cd-treated animals the velocity of enzyme reaction increased curvilinearly with the ATP concentration (Fig. 2A). From the Hofstee plot[5] of the data (Fig. 2B), it was found that the Km (0.4±0.1 and 0.5±0.1 mM in the control and Cd-group, respectively) was not changed, but the Vmax (77.6±8.1 and 53.9±6.6 m moles Pi/g protein per hr in the control and Cd-group, respectively) was significantly ($p < 0.05$) reduced in Cd-treated animals.

DISCUSSION

The primary purpose of the present study was to evaluate that changes in renal transport of Na^+, glucose, and organic anions in Cd-treated animals are associated with alterations of Na-K-ATPase system in the proximal tubule.

The Na-K-ATPase activity of renal cortical microsomes was markedly attenuated in rats treated with cadmium (2 mg Cd/kg·day) for 16 day (Fig. 1). Kinetic anlysis indicated that the change was due to reduction in Vmax and not to alteration in Km (Fig. 2). These results suggest that the capacity of sodium pump in the renal cortex was reduced by cadmium treatment. Although the renal cortex contains distal tubules as well as proximal tubules, the total mass of the proximal segments is far greater than that of the distal segments[24]. Furthermore, Cd accumulation and consequent morphological changes in the kidney of Cd-treated animals are known to be confined mainly to proximal tubular cells[8,12]. Thus, the change in enzyme activity observed in the present study may reflect alteration of sodium pump in the proximal tubule.

As we have reported in detail elsewhere[10,11], treatment of rats with cadmium, as in the present study, resulted in alterations of renal transport systems. Table 1 summarizes some of these changes. The urinary excretion of Na^+ was drastically reduced after 4 days of Cd treatment, indicating that tubular Na^+ reabsorption was increased. This change may have not involved alterations of proximal tubular sodium pump capacity, since the renal

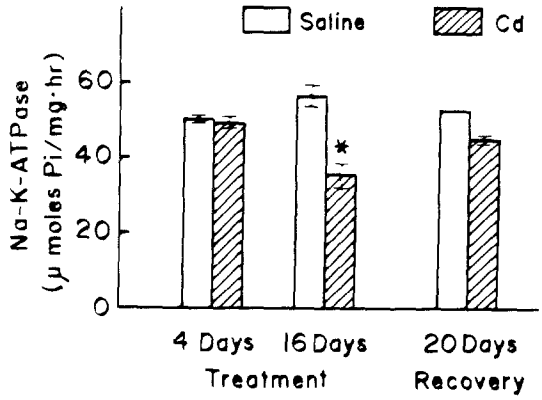

Fig. 1. Na-K-ATPase activity of renal cortical microsomes of rats treated with saline or cadmium (2 mg/kg·day). The enzyme activity was measured in the presence of 1mM ATP. Values represent mean±SE of 2~6 animals.

*Significantly different ($p<0.05$) from the control (saline-treated) animal.

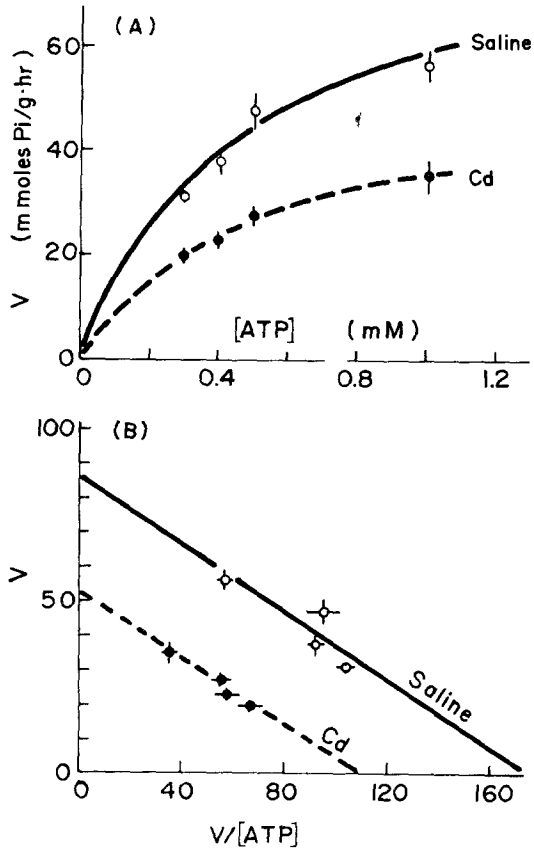

Fig. 2. A) Velocity of renal cortical microsomal Na-K-ATPase reaction as a function of ATP concentration in rats treated with saline or Cd (2 mg/kg·day) for 16 days. Data represent the mean±SE of 6 determinations in each group. B) Hofstee plot of the data shown in Fig. 2A. In this plot, the intercept of the line with the Y-axis represents V_{max}, and the slope represents $-K_m$.

cortical Na-K-ATPase activity was not apparently changed during this period (Fig. 1). The mechanism with which Na^+ reabsorption was facilitated is not clearly understood. However, in view of the fact that aldosterone secretion increases markedly following Cd treatment[18], we speculate that Na^+ reabsorption was facilitated in the distal tubules. In 16-day Cd-treated animlas, Na^+ excretion was not significantly increased (Table 1), even though the Na-K-ATPase activity was substantially reduced (Fig. 1). This may be due to combined effects of Cd treatment on the proximal and distal tubular Na^+ transport systems. In other words, during the later phase of Cd treatment the proximal tubular Na^+ reabsorption became impaired due to reduction of sodium pump capacity, such that the effect of distal tubular transport process was completely masked. This possibility is now being studied.

The urinary excretion of glucose was not changed by Cd after 4 days of treatment, but it was drastically increased after 16 days of treatment (Table 1). These results may also imply that proximal tubular Na^+ reabsorption was reduced in the latter period of Cd treatment. Since glucose is reabsorbed exclusively in the proximal tubule mostly by Na-glucose

Table 1. Urinary Sodium and Glucose Excretions and Active PAH Transport in Renal Cortical Slices in Saline- and Cadmium-treated Rats

	Pre-Treatment	Treatment 4 days	Treatment 16 days	Recovery 20 days
Na^+ Exc. (m Equiv./kg·day)				
Saline (7)	1.4±0.1	1.4±0.2	1.4±0.3	1.5±0.3
Cd (7)	1.3±0.3	0.1±0.1*	2.4±0.8	1.4±0.3
Glucose Exc. (mg/kg·day)				
Saline (7)	2.7±0.5	3.1±0.7	3.2±0.6	4.2±0.2
Cd (6)	4.2±0.6	5.3±0.2	24.7±12.3*	9.1±1.7*
PAH Influx (m moles/kg·15 min)†				
Saline (3)		1.26±0.05	1.27±0.14	1.15±0.40
Cd (3)		0.77±0.15*	0.25±0.05*	0.33±0.12*

Cd group received subcutaneous injections of $CdCl_2$ at a dose of 2mg Cd/kg·day.

† Maximal influx rate (Vmax) of active PAH influx in isolated renal cortical slices.

* Significantly ($p<0.05$) different from the matched control (saline-treated).

Data represent the mean ± SE.

cotransport system[24], an increase in urinary glucose would indicate that proximal tubular Na^+ transport is inhibited. It is therefore possible that the Cd-induced glycosuria observed in the present and other studies[1,19] is accounted for, at least in part, by a reduction in sodium pump capacity in the proximal tubule.

The active transport of PAH in renal cortical slices was significantly attenuated in both 4-day and 16-day Cd-treated animals, and the defect was not recovered during 20 days after exposure ceased(Table 1). Oxygen consumption of the renal tissue was not altered by Cd treatment[10], thus, the energy producing catabolism linked to active PAH transport was probably not impaired. Although the PAH transport in the kidney slice has been shown to be reduced under certain conditions which restrain the Na-K-ATPase activity[2,16,20,23], the impaired PAH transport in Cd-treated animals as observed in the present study may not be associated with Na-K-ATPase inhibition. As shown in Fig. 1, the enzyme activity was not reduced after 4 days of Cd-treatment, at which time the PAH transport was substantially declined. Moreover, during 20 days of recovery period the enzyme actvity was significantly reverted, but the PAH transport was not. Evidently, the organic anion transport system in the proximal tubular basolateral membrane was directly affected by Cd treatment. In the kinetic analysis of PAH transport in renal cortical slices of Cd-treated rats[10] the Vmax of active influx was markedly reduced with no change in the Km, and both the passive influx and efflux were not apparently changed. We, therefore, believe that the number of effective carriers in the basolateral membrane of proximal tubules was decreased in Cd-treated animals. The mechanism with which Cd in-

duces such a change remains to be identified.

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