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RENAL EXCRETION OF Na^+ AND K^+ IN CADMIUM-INTOXICATED RATS

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ABSTRACT: *Changes in urinary Na^+ and K^+ excretions, renal cortical microsomal Na^+-K^+-ATPase activity, cortical tissue electrolyte content and plasma aldosterone level were studied in rats treated with $CdCl_2$ (2 mg Cd/kg/day, s.c. injection) for 7-14 days. After 7 days of cadmium exposure, urinary excretion of Na^+ was markedly reduced. This change was accompanied by an increase in Na^+-K^+-ATPase activity, a fall in tissue Na^+ content, a rise in tissue K^+ content and an elevation of plasma aldosterone level. After 14 days of cadmium exposure, however, Na^+ excretion returned to normal range. K^+ excretion increased significantly, Na^+-K^+-ATPase activity decreased, tissue Na^+ content increased, K^+ content slightly decreased and aldosterone level returned to normal range. These results suggest that a short-term exposure to cadmium produced Na^+ retension and a long-term exposure caused a K^+ loss, possibly due to alterations of sodium pump capacity in the proximal renal tubule.*

Key words: *Cadmium-intoxication, Electrolyte excretion, Na^+-K^+-ATPase, Aldosterone*

INTRODUCTION

Exposure to cadmium results in various renal functional changes. It has been observed in cadmium-intoxicated humans and animals that renal excretions of glucose (Kazantzis *et al.*, 1963; Adams *et al.*, 1969; Nomiyama *et al.*, 1975), amino acids (Kazantzis *et al.*, 1963; Adams *et al.*, 1969; Goyer, 1972; Gieske *et al.*, 1974; Nomiyama, 1975; Bernard *et al.*, 1979), phosphate (Adams *et al.*, 1969; Iwao *et al.*, 1980) and calcium (Scott *et al.*, 1976) are markedly increased. Since under normal conditions these substances are mostly reabsorbed in the proximal tubule by processes associated with Na^+ reabsorption (Lassiter *et al.*, 1963; Dennis *et al.*, 1982; Ng *et al.*, 1982; Ullrich, 1983), an increase in their urinary excretions may imply a decrease in proximal tubular Na^+ reabsorption. Several studies in cadmium-exposed animals (Perry *et al.*, 1971; Nishiyama *et al.*, 1984; Kim *et al.*, 1988), however, indicated that urinary excretion of Na^+ did not change or sometimes decreased rather than increas-

ed.

The present study was, therefore, undertaken to evaluate systematically the effect of cadmium exposure on the renal handling of Na^+ and K^+ in rats. During a course of cadmium treatment urinary excretions of various solutes, renal cortical Na^+-K^+-ATPase activity, tissue electrolyte content and plasma level of aldosterone were studied.

MATERIALS AND METHODS

Animals

Sprague-Dawley male rats (250-300 g) were maintained unless otherwise mandated by experimental protocol, under standard laboratory conditons with *ad libitum* access to food and water. After 4 days of baseline period, the experimental group received s.c. dosage of 2 mg Cd ($CdCl_2$ dissolved in saline)/kg body wt. once a day for 14 days. The cotrol group received the same volume of nomal saline.

Urinalysis

Renal functions were determined at 7 day intervals. Animals were kept in metabolic cages and were fasted for 24 hours. Urine was collected under a film of mineral oil in a glass cylinder to prevent evaporation. The urine samples were analyzed for Na^+ and K^+ (Radiometer Flame Photometer, Model FLM 3), creatinine (Wako Technical Bulletin No. 271-10509, Wako Pure Chem. Ind., Osaka, Japan), glucose (Sigma Diagnostics No. 315, Sigma Chem. Co., St. Louis, MO U.S.A.), protein (Lowry *et al.*, 1951), and phosphate (Fiske and SubbaRow, 1925).

Na^+-K^+-ATPase Assay

Na^+-K^+-ATPase was assayed in renal cortical microsomal preparations. Microsomes were prepared by a method similar to that described by Jørgensen and Skou (1971). Slices of kidney cortex were homogenized in 10 volumes of imidazole-sucrose buffer (0.03 M imidazole, 0.25 M sucrose, pH 7.6 at 25°C). 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 imidazole-sucrose buffer to a concentration of approximately 2 mg protein per m*l* and stored at -60°C. Protein concentration of this suspension was determined by the method of Lowry *et al.* (1951). Prior to ATPase assay, aliquots of microsomal preparations were treated with deoxycholate by incubating them in a solution containing 0.6 mg/m*l* deoxycholate, 2 mM EDTA and 25 mM imidazole (pH 7.6 at 25°C) for 30 min, and the mixture was adjusted to contain 0.2 mg protein per m*l*. The ATPase activity was assayed by measuring inorganic phosphate (Pi) liberated by ATP hydrolysis during 10 min incubation of deoxycholate-treated microsomes with 1 m*l* of appropriate medium containing Na_2-ATP as the substrate. The total ATPase activity was determined in the presence of Na^+, K^+ and Mg^{2+}, and the Mg^{2+}-ATPase in the absence of K^+ and presence of ouabain in the incubation medium. The difference bet-

ween the total- and Mg^{2+}-ATPase activity was taken as the measure of Na^+-K^+-ATPase activity. Unless otherwise stated, concentrations of Na^+, K^+, Mg^{2+}, and ATP in the incubation medium were 150, 20, 3, and 2 mM, respectively. The medium pH was adjusted to 7.4 at 37°C with imidazole-HCl. After 10 min preincubation at 37°C 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 centrifuged at 3,500 × g for 15 min. Inorganic phosphate in the supernatant was measured according to Fiske and SubbaRow (1925). The enzyme activity was expressed as u moles Pi/mg of microsomal protein per hour.

Determination of Tissue Electrolyte Content

The Na^+ and K^+ contents in renal cortex were determined. Renal cortical slices of approximately 0.5 mm thick were cut using a Stadie-Riggs tissue slicer. Immediately, slices were blotted on the filter paper, weighed and dried at 105°C for 16 hr to a constant weight. The dried tissues were then weighed and extracted with 0.1 N nitric acid for 48 hr. The concentration of Na^+ and K^+ in the extract was determined using a Flame Photometer (Radiometer Flame Photometer, Model FLM 3). From these measurements, tissue contents of water, Na^+ and K^+ were calculated.

Plasma Aldosterone Assay

Blood samples were collected by heart puncture using heparinized syringes. Plasma was immediately separated and stored in the refrigerator. Aldosterone level in the plasma sample was determined by a method of radioimmunoassay (Coat-A-Count. Aldosterone, Diagnostic Products Corp., Los Angeles, CA).

Statistical Analysis

Statistical evaluation of the data was done using the Student's t-test (unpaired comparison) and all results were presented as the mean ± SE.

RESULTS

Table 1 compares urinary excretions of water creatinine, glucose, protein and phosphate in control and cadmium-treated groups. In both groups, creatinine excretion did not change significantly during the 14 days of treatment period. However, the excretions of water, glucose, protein and phosphate increased markedly in cadmium group after 14 days of treatment. During the early phase of cadmium treatment, the excretions remained relatively unchanged. These results are exactly comparable to those observed in an earlier study (Kim *et al.*, 1988).

Fig. 1 depicts the effect of cadmium treatment on the urinary excretions of Na^+ and K^+. The Na^+ excretion (Fig. 1A) decreased markedly after 7 days of cadmium treatment. The average amount of Na^+ excretion in the cadmium group (0.44 ± 0.17 meq/kg·day, $N = 14$) was less than 20% of that observed in the control group (2.15 ± 0.24, $N = 11$). During the later phase (14th day) of cadmium treatment, however, the Na^+ excretion returned to the control level.

Table 1. Effects of cadmium treatment on urinary excretion of various substances

	Days of Treatment	Saline (N = 11)	Cadmium (N = 14)	*p*
Water	0	22.2 ± 2.0	23.2 ± 1.6	NS
(ml/kg·day)	7	24.7 ± 2.1	22.6 ± 1.3	NS
	14	26.0 ± 2.3	47.7 ± 4.7	<0.01
Creatinine	0	30.3 ± 1.9	35.5 ± 1.8	NS
(mg/kg·day)	7	29.9 ± 3.0	31.3 ± 2.3	NS
	14	30.6 ± 3.1	36.6 ± 6.4	NS
Glucose	0	2.7 ± 0.3	3.4 ± 0.3	NS
(mg/kg·day)	7	4.3 ± 1.1	3.4 ± 0.5	NS
	14	3.4 ± 0.4	12.6 ± 2.4	<0.01
Protein	0	210 ± 14	247 ± 17	NS
(mg/kg·day)	7	213 ± 23	207 ± 23	NS
	14	209 ± 24	518 ± 92	<0.01
Phosphate	0	0.73 ± 0.09	0.94 ± 0.11	NS
(m moles/kg·day)	7	0.84 ± 0.14	1.28 ± 0.17	NS
	14	0.60 ± 0.11	1.64 ± 0.22	<0.01

Values are mean ± SE.

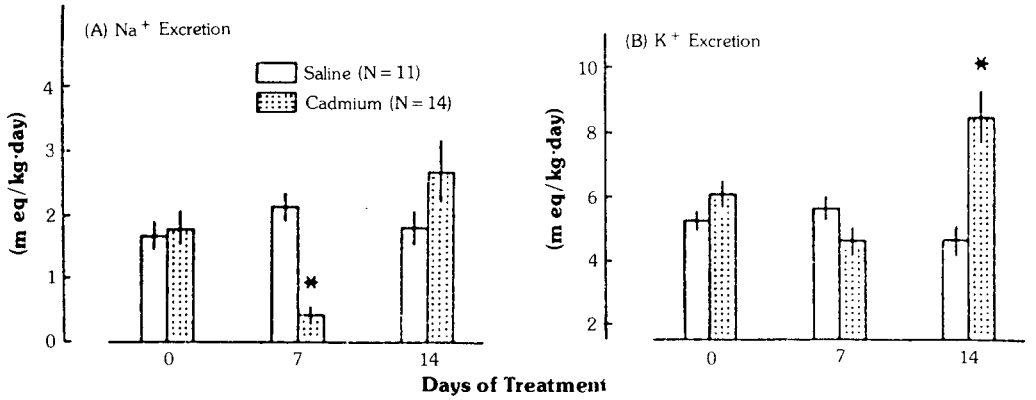

Fig. 1. Effect of cadmium treatment (2 mg Cd/kg·day, s.c. injection) on urinary excretions of Na^+ (A) and K^+ (B). Each bar and vertical line represent the mean ± S.E.
*Significantly different ($p < 0.05$) from the matched control value.

The urinary excretion of K^+ (Fig. 1B) did not change significantly after 7 days of cadmium treatment, but it increased markedly thereafter. The level of K^+ excretion after 14 days of cadmium treatment (8.46 ± 0.83 meg/kg·day, N = 14) was approximately 84% greater than the control value (4.61 ± 0.49, N = 11).

In order to elucidate a possible mechanism(s) with which cadmium altered the renal electrolyte excretion, changes in Na^+-K^+-ATPase activity was measured in renal cortical microsomal frations. As summarized in Table 2, the enzyme activity of the cadmium group increased somewhat (15%) after 7 days of treatment, but it significantly (18%) decreased after 14 days of treatment, as compared with the corresponding con-

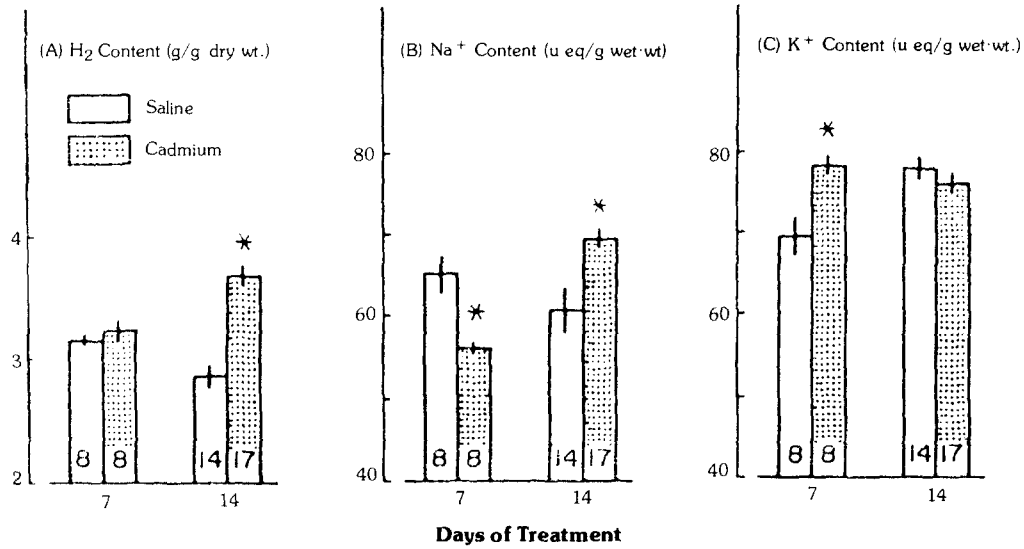

Fig. 2. Effect of cadmium treatment (2 mg Cd/kg·day, s.c. injection) on water (A), Na^+ (B) and K^+ (C) contents of renal cortex. Each bar and vertical line represent the mean ± SE. Figure inside each bar represents the number of determination.
*Significantly different (p 0.05) from the matched control value.

trol.

Fig. 2 shows the Na^+, K^+ and water contents in renal cortical tissues of saline- and cadmium-treated animals. The tissue water content (Fig. 2A) of cadmium group was not different from the control value after 7 day treatment, but it was approximately 28% higher than the control after 14 day treatment, indicating that the renal tissue underwent a marked swelling during the later phase of cadmium treatment. Therefore, tissue electrolyte contents were evaluated on wet weight, instead of dry weight, basis.

As shown in Fig. 2B, the tissue Na^+ content of cadmium group was significantly lower (by 14%) than the control level after 7 days, and it was significantly higher than the control value after 14 days of treatment. In the case of tissue K^+ content (Fig. 2C), cadmium group showed a slightly higher value after 7 days, and an equivalent value after 14 days, as compared with the control group.

As another approach to elucidate the mechanism underlying the cadmium-induced alterations in electrolyte excretion, the plasma level of aldosterone was measured. The results summarized in Table 3 indicated that the hormone secretion increased markedly during the early phase (7th day) of cadmium exposure, but it returned to the control level during the later phase (14th day).

DISCUSSION

Na^+ Excretion

Na^+ excretion in the cadmium group decreased drastically during the early phase of cadmium treatment and it returned to the control level during the later phase (Fig. 1A).

Table 2. Effect of cadmium treatment on renal cortical Na^+-K^+-ATPase activity (μmoles Pi/mg protein·hr)

Days of Treatment	Saline	Cadmium	p
7	50.11 ± 0.87(8)	57.64 ± 1.78 (8)	<0.01
14	54.46 ± 1.38(7)	44.73 ± 1.72 (7)	<0.01

Values are mean ± SE.
( ) number of determination

Table 3. Effect of cadmium on plasma concentration of aldosterone (mg/dl)

Days of Treatment	Saline	Cadmium	p
7	16 ± 7 (6)	1710 ± 749 (6)	<0.05
14	27 ± 12 (10)	28 ± 9 (10)	NS

Values are mean ± SE.
( ) number of rats

These results are exactly comparable to those observed in a previous study (Kim *et al.*, 1988).

The early reduction of Na^+ excretion was probably due to enhanced Na^+ reabsorption in both proximal and distal tubules. The Na^+-K^+-ATPase activity measured in renal cortical microsomes after 7 days of cadmium exposure was significantly higher than that in the control preparation (Table 2). Since the enzyme system is an integral part of sodium pump (Skou, 1975), such as increase of the enzyme activity would indicate that the capacity for active Na^+ transport was increased. Analysis of electrolyte content of the renal cortical tissue also indicated that the sodium pump activity was increased in 7 day cadmium-treated animals, i.e., the tissue Na^+ content was significantly reduced and the K^+ content was significantly elevated, as compared with the corresponding control values (Fig. 2). Although the renal cortex contains distal tubules as well as proximal tubule, the total mass of proximal segments is far greater than that of distal segments (Sperber, 1944). Furthermore, accumulation of cadmium and consequent morphological changes are known to be confined to proximal tubular cells (Kawai, 1978; Kjellstrom, 1986). Thus, the changes in the Na^+-K^+-ATPase activity and electrolyte composition observed in cadmium-treated animals would reflect alterations of the sodium pump activity in the proximal tubule. The drastic increase in plasma aldosterone in 7 day cadmium-treated rats (Table 3) suggests that the distal tubular Na^+ reabsorption was also enhanced. A similar conclusion has been made by Nishiyama and Nakamura (1984), who observed a marked increase in plasma aldosterone in rats during 7 days of cadmium treatment (2 mg Cd/kg·day). Regardless of interpretation, the present results suggest that the Na^+ retention and hypertension observed in short-term cadmium exposed animals (Perry *et al.*, 1971; Doyle *et al.*, 1975; Nishiyama and Nakamura, 1984) might be due to increased Na^+ reabsorption in the proximal and distal nephrons.

Return of Na^+ excretion to the control level during the late phase of cadmium treatment (Fig. 1A) is difficult to interprete. After 14 days of cadmium exposure, renal cor-

tical Na^+-K^+-ATPase activity was significantly reduced (Table 2), tissue Na^+ content was significantly increased (Fig. 2B), and tissue K^+ content was slightly decreased, although statistically not significant (Fig. 2C). These results may suggest that the sodium pump activity in the proximal tubule was reduced. Moreover, urinary excretion of glucose increased markedly (Table 1), indicating that the Na^+ transport coupled with organic compounds were reduced. Despite these facts, urinary excretion of Na^+ was not significantly different from the control level (Fig. 1A). Therefore, it is suspected that Na^+ reabsorption from the distal tubule was increased, thereby compensating for the reduction of Na^+ reabsorption in the proximal tubule. Plasma aldosterone level at this period was not elevated (Table 3). Thus, the increase in distal tubular Na^+ reabsorption, if it occurred, must have been simply due to the increased Na^+ delivery to the distal nephron. Direct analysis of the Na^+ reabsorption in various segments of nephron using micropuncture technique would verify this notion.

K^+ Excretion

The K^+excretion in the cadmium group did not change significantly in the early phase (7th day) of cadmium treatment, but it increased significantly in the later phase (14th day) (Fig. 1B). Such changes in K^+ excretion may be due to variations in the proximal tubular transport of Na^+ and other solutes. It is known that the urinary excretion of K^+ is largely determined by the amount of K^+ secretion in the distal tubule, which, in turn, depends on the Na^+ delivery to the distal tubule and the distal tubular flow rate as well as peritubular uptake of K^+ (Giebisch, 1983). As described above, the Na^+ reabsorption from the proximal tubule was probably increased in the early phase of cadmium treatment due to activation of sodium pump. A subsequent reduction of Na^+ delivery to the distal nephron would decrease the amount of distal tubular Na^+ reabsorption, and hence the luminal negative potential, a driving force for K^+ secretion (Giebisch, 1983). As a consequence, the K^+ secretion could not be increased in the face of an increase in aldosterone secretion (Table 3).

In the late phase, however, the proximal tubular reabsorption of Na^+ and other solutes were probably reduced, as judged by the changes in Na^+-K^+-ATPase activity, tissue electrolyte content, and glucose, phosphate and protein excretions (see above). This would lead to an increase in Na^+ and fluid deliveries to the distal nephron. In fact, the urine flow of the cadmium group at this time was 80% higher than that of the control group (Table 1). Under this condition, driving force for K^+ secretion in the distal tubule could be increased by an increased Na^+ reabsorption, which enhances Na^+-K^+ exchange in the basolateral membrane, and by facilitated washout of K^+ from the lumen (Giebisch, 1983). Consequently, the urinary excretion of K^+ could be increased. Another reason for the rise in K^+ excretion may be an increased excretion of impermeable anions, such as phosphate (Table 1). A large amount of phophate in the distal tubular lumen would facilitate K^+ secretion by changing electrical potential gradient across the luminal membrane (Giebisch, 1983).

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