Korean J Physiol Pharmacol
Vol 6: 41–46, February, 2002

Renal Tubular Acidosis in Cadmium-Intoxicated Rats

Do Whan Ahn, Kyoung Ryong Kim, Jang Kyu Choi, and Yang Saeng Park

Department of Physiology, Kosin University College of Medicine, Busan 602-703, Korea

Effect of cadmium (Cd) intoxication on renal acid-base regulation was studied in adult male Sprague-Dawley rats. Cd intoxication was induced by subcutaneous injections of $CdCl_2$ at a dose of 2 mg Cd/kg/day for 3~4 weeks. In Cd-intoxicated animals, arterial pH, PCO_2 and plasma bicarbonate concentration decreased, showing a metabolic acidosis. Urine pH and urinary bicarbonate excretion increased and titratable acid excretion decreased with no change in ammonium excretion. In renal cortical brush-border membrane vesicles derived from Cd-exposed animals, the Na^+/H^+ antiporter activity was significantly attenuated. These results indicate that chronic exposures to Cd impair the proximal tubular mechanism for H^+ secretion (i.e., Na^+/H^+ antiport), leading to a metabolic acidosis.

Key Words: Cadmium, Kidney, Acid-base

INTRODUCTION

Kidneys play a major role in the regulation of H^+ balance by maintaining normal body store of bicarbonate (HCO_3^-) and by excreting the H^+ that is derived from the daily production of fixed acid (Valtin, 1983). These tasks of the kidney are accomplished through almost complete reabsorption of filtered HCO_3^- and excretion of titratable acids (TA) and ammonium salts, the processes critically dependent on the activities of renal carbonic anhydrases, Na^+/H^+ antiporter in the luminal membrane, and Na^+-K^+-ATPase pump in the basolateral membrane (Rose, 1989).

Previous studies on chronic Cd-exposed animals have shown that the renal carbonic anhydrase activity is attenuated (Ogawa et al, 1973), Na^+-K^+-ATPase activity is impaired (Kim et al, 1988), urine pH is increased (Sato & Nagai, 1982), and the HCO_3^- excretion is enhanced (Sato & Nagai, 1982). Such results may suggest that Cd intoxication impairs the renal ability to regulate acid-base balance and thus leads to a metabolic acidosis.

The present study was, therefore, undertaken to systematically evaluate effects of Cd exposure on the blood acid-base status and the renal handling of HCO_3^-, TA and ammonium in experimental animals.

METHODS

Animals

Adult male Sprague-Dawley rats weighing 200~300 g were used. Animals were maintained under standard laboratory conditions with *ad libitum* access to food and water, unless otherwise mandated by experimental protocol. Cadmium intoxication was induced by subcutaneous injections of $CdCl_2$ at a dose of 2 mg Cd per kg body weight per day for 3~4 weeks. If animals showed a significant polyuria, they were considered to be cadmium intoxicated (Kim et al, 1988).

Urinalysis

At one-week intervals, animals were kept in metabolic cages and were denied food and water for 24 hours. Urine was collected under mineral oil using thymol as preservative. Urine samples were analyzed for Na^+/K^+ (Radiometer flame photometer, FLM-3, Copenhagen, Denmark), creatinine (Wako Technical Bulletin No. 271-10509, Wako Pure Chemical Ind., Osaka, Japan), phosphate (Fiske & SubbaRow, 1925), pH/PCO_2 (Radiometer Acid-Base Analyzer ABL-30, Copenhagen, Denmark), ammonium and titratable acidity (Radiometer Acid-Base Titrator, TTT85, Titrator ABU-80 Autoburette, Radiometer Copenhagen). Urine HCO_3^- concentration was calculated from pH and PCO_2 using Henderson-Hasselbalch equation with a solubility factor of 0.0306 (Gambino et al, 1966). The pK was calculated from the following formula: $6.33-0.5\ ([Na]+[K])^{1/2}$ (Hastings & Sendroy, 1925). In the measurement of titratable acidity, 2 ml urine sample was titrated with 0.1 N NaOH to pH 7.4. Urinary ammonium concentration was estimated on the same sample by adding 2 ml of 8% formaldehyde (adjusted to pH 7.4 with Na_2HPO_4) and titrating with 0.1 N NaOH (Richterich & Colombo, 1981). Formaldehyde liberates H^+ from NH_4^+ according to the following equation: $4NH_4^+ + 6CH_2O \rightarrow (CH_2)_6N_4 + 4H^+ + 6H_2O$. The quantity of H^+ is estimated by back titration of the sample to pH 7.4 with NaOH. In some animals, blood was collected from abdominal aorta or directly from heart and analyzed for plasma creatinine. Glomerular filtration rate (GFR) was estimated by creatinine clearance. Other parameters were calculated by conventional methods.

Corresponding to: Yang Saeng Park, Department of Physiology, Kosin Medical College, 34 Amnam-dong, Suh-gu, Busan 602-703, Korea. (Tel) 82-51-990-6413, (Fax) 82-51-241-5458, (E-mail) yspark@ns.kosinmed.or.kr

ABBREVIATIONS: BBM, brush-border membrane; BBMV, brush-border membrane vesicle; BLM, basolateral membrane; TA, titratable acid.

Determination of blood buffer line

Rats were lightly anesthetized with intraperitoneal injections of pentobarbital (30 mg/kg body wt) and blood samples were collected from abdominal aorta using heparinized syringes. Each blood sample was divided into four portions. One portion was used for pH, hematocrit and hemoglobin determinations. Each of the three other portions was equilibrated in a tonometer with a gas mixture of 5, 10 or 15% CO_2 and at least 70% O_2 to insure full oxygenation. After 10-min equilibration at 37°C, blood buffer line was determined. Plasma HCO_3^- was calculated from the pH and PCO_2 using the Henderson-Hasselbalch equation with a solubility factor of 0.0306 (Gambino et al, 1966). The pK value was corrected according to the formula $\triangle pK/\triangle pH=0.047$ (Stinebaugh & Austin, 1967).

Determination of Na^+/H^+ exchange in renal brush-border membrane vesicles

Renal cortical brush-border membrane vesicles (BBMV) were prepared by Percoll gradient centrifugation as described by Kinsella et al (1979) & Scalera et al (1981). Brush-border membrane (BBM) fractions were purified by the Mg-aggregation method of Booth & Kenny (1974). Purity of the membrane preparations was routinely monitored using the marker enzyme alkaline phosphatase (BBM) and Na^+-K^+-ATPase (basolateral membrane, BLM). Details of the procedures for membrane preparation and marker enzyme assay were described previously (Lee et al, 1990).

The activity of the Na^+/H^+ exchanger in the BBMV was determined in two ways: 1) the ^{22}Na uptake driven by an outwardly-directed H^+ gradient and 2) the proton efflux driven by an inwardly-directed Na^+ gradient. The ^{22}Na uptake into vesicles was measured at 25°C by a rapid filtration technique (Harris et al, 1984). An aliquot of 10 μl BBMV (0.1 mg protein), loaded with pH 6.0 buffer containing 220 mM mannitol, 20 mM KCl, 40 mM 2-(N-Morpholino)-ethanesulfonic acid (MES) and 40 mM Hepes, were incubated in 90 μl of pH 7.4 (or 6.0) buffer containing 270 mM mannitol, 40 mM Hepes/KOH and 1 mM NaCl. At specified intervals following the addition of the isotope (0.2 μCi), incubations were terminated by addition of 2 ml ice-cold incubation medium containing 0.1 mM amiloride, followed by prompt filtration through a prewetted 0.45 micron filter (HAWP; Millipore Corp., Bedford, MA, USA). The filter was washed with an additional 4 ml of the incubation medium and dissolved in 6 ml Luma gel (Lumac, the Netherlands). Radioactivity was determined by liquid scintillation counting (Packard, Tri-carb 2500 TR). Nonspecific retention of radioactivity to the filters was subtracted from total counts of each sample.

The proton efflux rate was assessed by the use of acridine orange absorbance change as a measure of transmembrane pH gradient (Kinsella et al, 1984) employing an Hewlett Packard dual beam wavelength spectrophotometer (HP8452 A) with 494 nm as the absorbing wavelength and 546 nm as the reference wavelength. An aliquot of 20 μl (150~300 μg protein) vesicles, pH 6.0, was diluted in 2 ml of pH 7.4 buffer containing 270 mM mannitol, 20 mM KCl, 40 mM Hepes/KOH, and 6 μM acridine orange. The addition of vesicles resulted in a rapid reduction of absorbance, followed by a slow recovery. The rate of intravesicular pH dissipation was assessed by the method of Zelikovic et al (1991). The steady-state absorbance minus the absorbance at varied times was plotted as a logarithmic function vs. time. The negative slope of this line represents the rate constant of pH gradient dissipation.

To insure identical assay conditions for control and experimental groups, measurements were made in vesicles prepared on the same day from control and experimental animals, using the same solutions.

Chemicals

^{22}Na was obtained from New England Nuclear (Boston, MA, USA). Amiloride was purchased from Merk, Sharp, and Dohme, Inc. (West point, PA, USA), and acridine orange was from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Statistics

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

RESULTS

Urine flow, urine pH and urinary solute excretions

Table 1 summarizes the effects of Cd exposure on urine flow, urine pH and urinary acid-base excretions in rats. Subcutaneous injections of Cd at a dose of 2 mg/kg·day for 3~4 weeks resulted in a marked polyuria, a characteristic of Cd nephrotoxicity (Kim et al, 1988). The urine pH and the bicarbonate (HCO_3^-) excretion increased markedly, titratable acid (TA) excretion decreased slightly, but the ammonium (NH_4^+) excretion did not change significantly in Cd-exposed animals.

Fig. 1 illustrates the relationship between HCO_3^- excretion and urine pH in the control and Cd-exposed animals.

Table 1. Urine flow, urine pH, and urinary excretions of bicarbonate (HCO_3^-), titratable acid (TA) and ammonium (NH_4^+) in control and Cd-exposed rats

	Treatment	Control	Cadmium
Urine Flow	pre	20.2±2.3	21.4±1.9
ml/kg · day	post	15.4±0.9	31.7±2.3*
Urine pH	pre	6.16±0.03	6.17±0.04
	post	6.18±0.12	6.76±0.18*
HCO_3^- Excretion	pre	54.4±18.2	52.1±10.9
μEq/kg · day	post	27.3±8.6	370.1±165.4*
TA Excretion	pre	0.98±0.13	1.06±0.14
mmole/kg · day	post	1.08±0.13	0.78±0.17
NH_4^+ Excretion	pre	2.19±0.12	2.20±0.11
mmole/kg · day	post	2.36±0.17	2.17±0.21

Cd group animals were subcutaneously injected with 2 mg Cd/kg · day for 3 to 4 weeks. The values represent the mean±SE of 10 rats in each group. *$p<0.05$ compared with the matched control value.

The individual HCO_3^- excretion rates are plotted against the urine pH on a semilog scale. In general, there was a positive correlation between the two variables. The data for the Cd-exposed animals followed the same general pattern as the controls, however, the absolute value was generally higher than in controls. These indicate that the rise in urine pH in the Cd-exposed animals was associated with an increased HCO_3^- excretion.

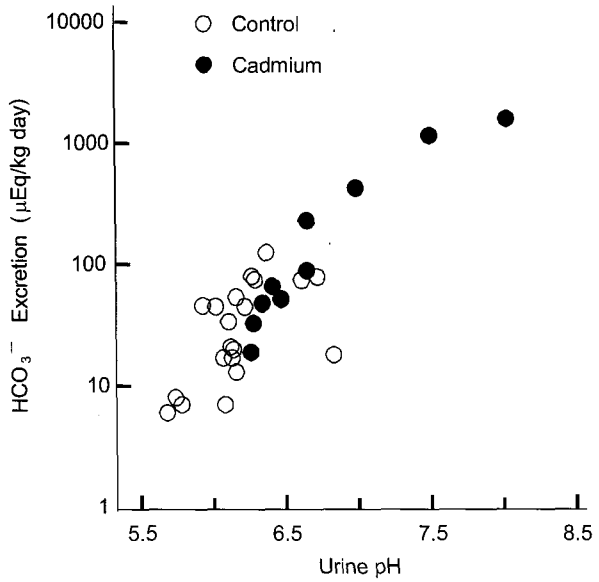

Fig. 1. Bicarbonate (HCO_3^-) excretion plotted against urine pH in control and Cd-exposed rats.

Fig. 2 relates the TA excretion and the urine pH. As expected, the TA excretion was inversely proportional to the urine pH both in the control and Cd-exposed animals. The graph also shows that the reduction in TA excretion in the latter group of animals was attributed to the increased urine pH.

These results indicate that the Cd exposure enhanced urinary HCO_3^- excretion, increasing urine pH, and the TA excretion was consequently decreased, but the NH_4^+ excretion was not significantly changed.

Blood acid-base status

The rise in renal HCO_3^- output in Cd-exposed animals would lead to a metabolic acidosis. In order to confirm this, we evaluated the acid-base status of the blood in the next series of experiment.

Fig. 3 depicts average blood buffer lines determined in 6 control and 6 Cd-exposed rats. The buffer line for the Cd-exposed animals appeared to be shifted to the lower left of the control line. The average buffer slope ($\triangle HCO_3^-/\triangle$ pH) was -19.8 ± 1.2 mEq/l/pH unit for the controls and -16.8 ± 0.9 for the Cd group ($p<0.10$). The *in vivo* pH value determined under pentobarbital anesthesia averaged 7.42 ± 0.02 in the control and 7.36 ± 0.04 in the Cd group ($p< 0.10$, Table 2). Whether these represent true *in vivo* arterial pH is not certain. At normal pH of 7.4 the buffer lines indicated an arterialized PCO_2 of 39.6 ± 0.7 mmHg and a plasma HCO_3^- concentration of 26.5 ± 0.4 mEq/l for the controls and PCO_2 of 34.2 ± 0.6 and plasma HCO_3^- of 22.8 ± 0.4 for the Cd-exposed animals ($p<0.01$ in both cases). The hematocrit and hemoglobin concentration of the blood were significantly ($p<0.05$) reduced in the Cd-exposed animals (Table 2). These data indicate that the Cd exposure resulted in a metabolic acidosis and anemia.

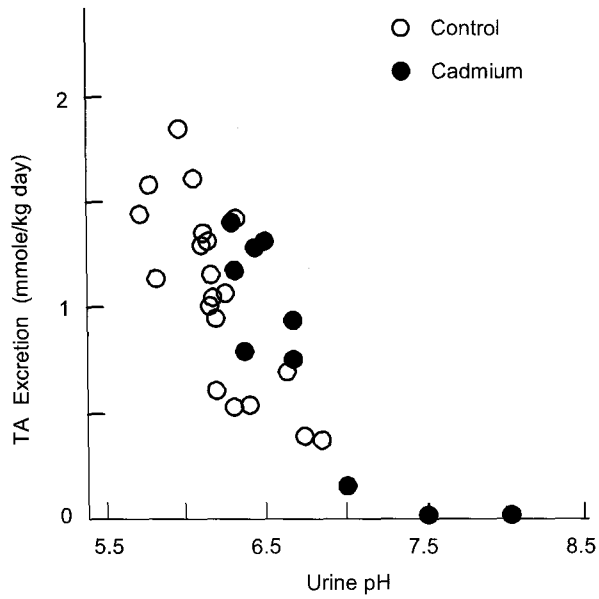

Fig. 2. Titratable acid (TA) excretion as a function of urine pH in control and Cd-exposed rats.

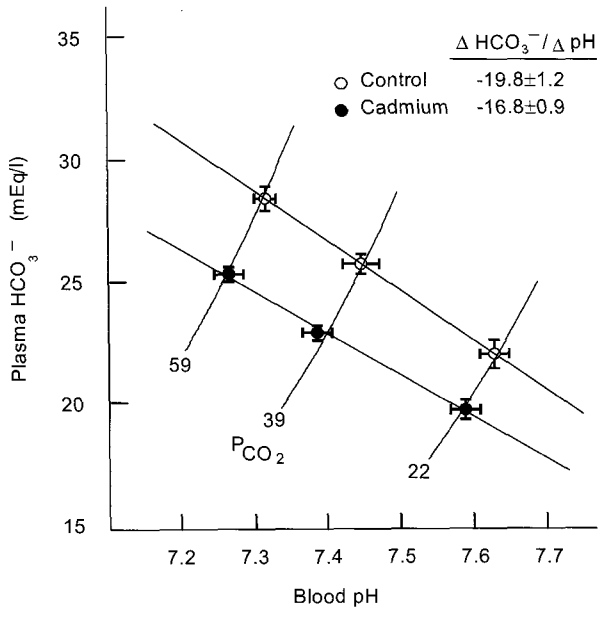

Fig. 3. Blood buffer lines of control and Cd-exposed rats. Values represent the mean±SE of 6 rats in each group.

Table 2. Blood pH, hematocrit, and hemoglobin concentration in control and Cd-exposed rats

	Control	Cadmium	p
Arterial pH	7.42±0.02	7.36±0.04	<0.10
Hematocrit, %	40.1±0.5	31.0±1.3	<0.001
Hemoglobin, *g/dl*	14.6±0.4	11.2±0.3	<0.05

The Cd-group animals were subcutaneously injected with 2 mg Cd/kg/day for 3~4 weeks. The values represent the mean±SE of 6 rats in each group.

Na^+/H^+ exchange in renal cortical brush-border membrane vesicles

Reabsorption of HCO_3^- and formation of TA and NH_4^+ all involve H^+ secretion (Valtin, 1983). The H^+ secretion in the proximal tubule is mediated by the Na^+/H^+ antiporter in the luminal membrane (Kinsella & Aronson, 1980; Goldfarb & Nord, 1987; Preisig et al, 1987). To determine the effect of Cd intoxication on the proximal tubular Na^+/H^+ antiporter, we evaluated Na^+/H^+ exchanges in renal cortical BBMV derived from Cd-exposed rats.

Fig. 4 illustrates the time courses of Na^+ uptake by BBMV of the control and Cd- exposed rats. When vesicles were preloaded with pH 6.0 buffer and then the Na^+ uptake was assayed by incubation in pH 7.4 buffer ($H^+_i > H^+_o$) containing Na^+ (1 mEq/l), a marked stimulation of Na^+ uptake was observed compared to Na^+ uptake in the absence of proton gradient ($pH_i = pH_o = 6.0$). Indeed, in the control vesicles, imposition of a $H^+_i > H^+_o$ gradient induced an uphill accumulation ("overshoot") of Na^+ to a level above the equilibrium value, which could be nearly abolished by 1 mM amiloride, an inhibitor of Na^+/H^+ exchanger (Kinsella & Aronson, 1981; Benos, 1982; Taylor et al, 1987) (data not illustrated). In the Cd group vesicles, however, the proton-gradient dependent Na^+ uptake was markedly attenuated and the overshoot phenomenon was not apparent. The proton-gradient independent uptake was comparable between the two groups, indicating that the Na^+ permeability of the membrane was not changed by Cd exposure. These results indicate that the suppression of proton-gradient dependent Na^+ uptake in the Cd group vesicles was due to reduction of Na^+/H^+ exchange activity.

In order to reaffirm the above notion, we also studied dissipation of pH gradient in the presence and absence of Na^+ (1 mEq/l) in the extravesicular medium using acridine orange, a weak basic dye. Acridine orange (final concentration of 6 μM) was added to the cuvette containing 2 ml of pH 7.4 buffer with or without Na^+. When BBMV equilibrated with pH 6.0 buffer lacking Na^+ were added, the absorbance reflecting acridine orange concentration in the extravesicular medium decreased abruptly due to movement of the dye into the acidic vesicle interior and trapping as a nonpermeable protonated form. Subsequently, the absorbance rose gradually, the rate of rise being greater in the Na^+-containing medium than in the Na^+-free medium (data not shown). The gradual rise in the absorbance occurs as the pH gradient is dissipated, allowing the exit of the nonprotonated (permeable) form of the dye from vesicles; thus it reflects the rate of dissipation of the imposed pH gradient due to proton efflux (Zelikovic et al, 1991). Therefore, the acceleration of the absorbance change

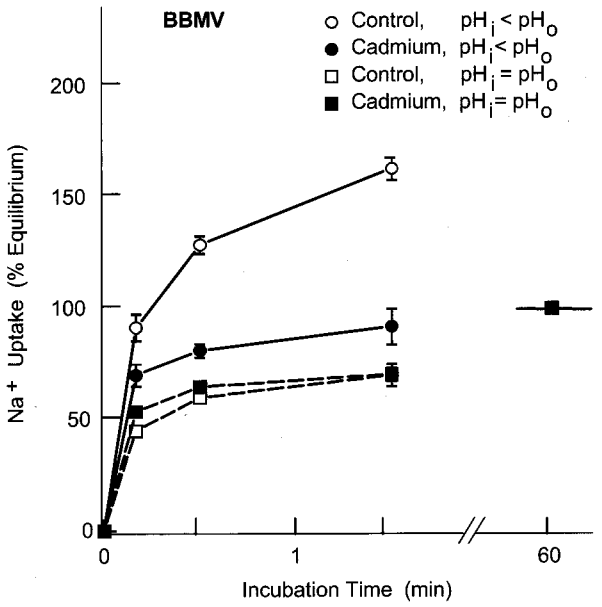

Fig. 4. Time courses of Na^+ uptake by renal cortical brush-border membrane vesicles of control and cadmium exposed rats in the presence and absences of a pH gradient. Data represent the mean± SE of 3 determinations in each group.

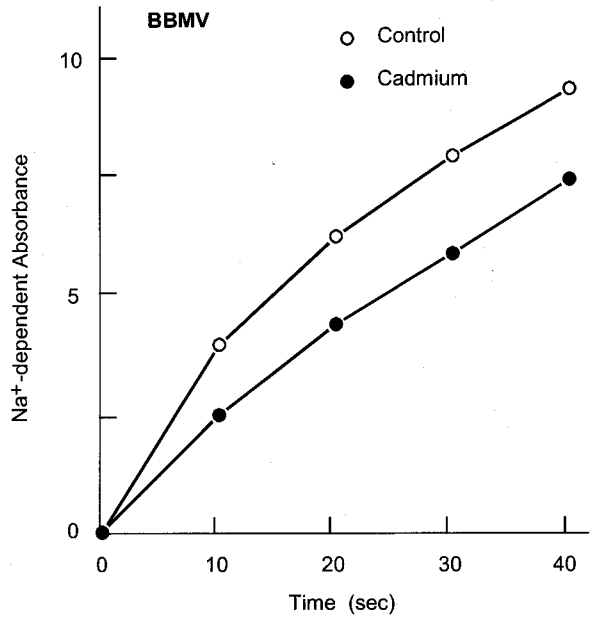

Fig. 5. Time courses of Na^+-dependent pH-gradient dissipation in renal cortical brush-border membrane vesicles of control and Cd-exposed rats. The Na^+-dependent component was calculated by subtracting the acridine orange absorbance in the Na^+-free medium from that in the Na^+-containing medium.

in the Na^+-containing medium represents a facilitated efflux of H^+ by Na^+/H^+ exchange driven by an inwardly-directed Na^+ gradient.

Fig. 5 compares the time courses of Na^+-dependent absorbance change between the control and Cd group vesicles.

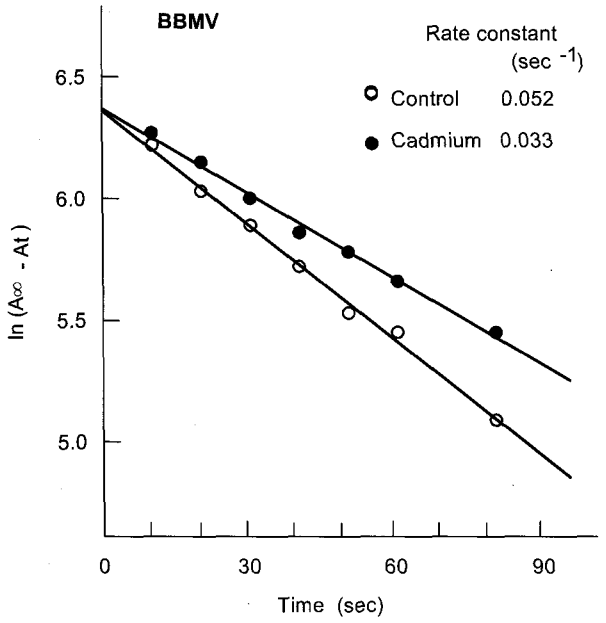

Fig. 6. Rate of pH-gradient dissipation in the presence of extravesicular Na^+ (1 mEq/l) in renal cortical brush-border membrane vesicles of control and Cd-exposed rats. The steady-state absorbance minus the absorbance at varied times was plotted as a logarithmic function vs. time. The negative slope of this line represents the rate constant of pH gradient dissipation.

The Na^+-dependent component was calculated by subtracting the absorbance observed in the Na^+-free medium from that in the Na^+-containing medium. Evidently, the rate of pH gradient dissipation was significantly retarded in the Cd group as compared with the controls. This retardation could not be accounted for by a decreased H^+permeability, since the pH gradient dissipation in the absence of Na^+ was identical in the two groups (data not shown). The rate constant of the pH gradient dissipation in the presence of extravesicular Na^+ averaged 0.052/sec in the control and 0.033/sec in the Cd group (Fig. 6). These results clearly indicate that the Na^+/H^+ antiporter in the proximal tubular luminal membrane was impaired in the Cd-intoxicated rats.

DISCUSSION

The present study demonstrated that chronic exposures to Cd induced metabolic acidosis in rats. In animals showing clear signs of Cd intoxication, the HCO_3^- excretion increased, and this was accompanied by a rise in urine pH and a fall in TA excretion (Table 1 and Figs. 1 and 2). Such changes in renal acid-base excretion would lead to a depression of HCO_3^- store and an accumulation of hydrogen ion in the body. Indeed, the pH-HCO_3^- diagram of the blood (Fig. 3) indicates that the plasma HCO_3^- concentration was markedly reduced in Cd-exposed animals. Their arterial pH (7.36 ± 0.04) determined under pentobarbital anesthesia was slightly ($p < 0.10$) lower than that of the controls (7.42 ± 0.02). The estimated PCO_2 at pH 7.4 was significantly ($p < 0.05$) reduced in the Cd group (34.2 ± 0.7) as compared with the control (39.6 ± 0.6). Thus, the Cd-exposed rats disclosed a low arterial pH, a low arterial PCO_2 (probably due to compensatory hyperventilation), and a decreased plasma HCO_3^- level, characteristics of metabolic acidosis (Rose, 1989).

This metabolic acidosis was most likely developed as a consequence of impaired ability of renal tubule to reabsorb the filtered HCO_3^-. Under normal conditions, approximately 90% of the filtered load of HCO_3^- is reabsorbed in the very early segment of proximal tubule (Liu & Cogan, 1984; 1987; Maddox & Gennari, 1987) by a process of acid secretion, which is mediated primarily by the Na^+/H^+ antiporter in the luminal membrane (Kinsella & Aronson, 1980; Aronson, 1983; Goldfarb & Nord, 1987; Preisig et al, 1987). The present study indicates that the activity of the Na^+/H^+ antiporter was impaired by Cd. In renal cortical BBMV of the Cd-exposed rats, both the Na^+ uptake driven by an outwardly-directed H^+ gradient and the H^+ efflux driven by an inwardly-directed Na^+ gradient were significantly attenuated (Figs. 4 and 5). These attenuations were not due to alterations in Na^+ or H^+ permeability, since neither the H^+-gradient independent Na^+ uptake nor the Na^+-gradient independent H^+ efflux appeared to be changed. The molecular mechanism by which the Na^+/H^+ antiporter was affected by Cd is yet to be determined.

Reduction of TA excretion in Cd-exposed rats (Table 1 and Fig. 2) must be attributed to the increased urine pH. Calculations of H^+ buffering using the Henderson-Hasselbalch equation for the $HPO_4^{2-} - H_2PO_4^-$ system ($pH = 6.8 + \log (HPO_4^{2-}/H_2PO_4^-)$) and the average value of phosphate excretion in the present study (1.9 mmoles/kg.day), indicated that the amount of H^+ buffered by HPO_4^{2-} was 1.3 mmoles/kg·day at urine pH 6.0, 0.9 at pH 6.5, 0.4 at pH 7.0 and 0 at pH 7.4, similar to the TA excretion depicted in Fig. 2. Thus, the data in Fig. 2 essentially represent the amount of H^+ excreted in combination with phosphate. It is, therefore, apparent that the decrease in TA excretion in the Cd-exposed rats was a direct consequence of the increased urine pH, and secondary to the proximal tubular rejection of HCO_3^-.

In summary, the results of the present study indicated that chronic exposures to inorganic cadmium induced metabolic acidosis, characterized by a high urine pH and a high HCO_3^- excretion. This acidosis was primarily associated with an impairment of the proximal tubular mechanism for HCO_3^- reabsorption (Na^+/H^+ antiport), thus can be classified as a proximal type of renal tubular acidosis (proximal RTA). The molecular mechanism with which Cd alters the Na^+/H^+ antiport remains to be elucidated.

REFERENCES

Aronson PS. Mechanism of active H^+ secretion in the proximal tubule. *Am J Physiol* 245: F647–F659, 1983

Benos D. Amiloride: A molar probe of sodium transport in tissues and cells. *Am J Physiol* 242: C131–C145, 1982

Booth AG, Kenny AJ. A rapid method for the preparation of microvilli from rabbit kidney. *Biochem J* 142: 575–581, 1974

Fiske CH, SubbaRow Y. The colorimetric determination of phosphorus. *J Biol Chem* 66: 375–400, 1925

Gambino SR, Astrupt P, Bates RG, Campbell EMJ, Chinard FP, Nahas GG, Siggard-Anderson O, Winters R. Report of the ad hoc committee on methodology in current concepts of acid-base measurement. *Ann NY Acad Scie* 133: 259–267, 1966

Goldfarb D, Nord EP. Asymmetric affinity of Na^+-H^+ antiporter for Na^+ at the cytoplasmic versus external transport site. *Am*

J Physiol 253: F950–F968, 1987

Harris RC, Seifter JL, Brenner BM. Adaptation of Na^+-H^+ exchange in renal microvillus membrane vesicles. Roles of dietary protein and uninephrectomy. *J Clin Invest* 74: 1979–1987, 1984

Hastings AB, Sendroy J Jr. The effect of variations in ionic strength on the apparent first and second dissociation constants of carbonic acid. *J Biol Chem* 65: 445–455, 1925

Kim YK, Choi JK, Kim JS, Park YS. Changes in renal function in cadmium-intoxicated rats. *Pharmacol Toxicol* 63: 342–350, 1988

Kinsella JL, Aronson PS. Properties of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am J Physiol* 238: F461–F469, 1980

Kinsella JL, Aronson PS. Amiloride inhibition of Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am J Physiol* 241: F374–F382, 1981

Kinsella J, Cujdik T, Sacktor B. Na^+-H^+ exchange activity in renal brush border membrane vesicles in response to metabolic acidosis: The role of glucocorticoids. *Proc Natl Acad Sci* 81: 630–634, 1984

Kinsella JL, Holohan PD, Pessah NI, Ross CR. Isolation of luminal and antiluminal membranes from dog kidney cortex. *Biochim Biophys Acta* 552: 468–477, 1979

Lee HY, Kim KR, Woo JS, Kim YK, Park YS. Transport of organic compounds in renal plasma membrane vesicles of cadmium intoxicated rats. *Kidney Int* 37: 727–735, 1990

Liu F-Y, Cogan MG. Axial heterogeneity in the rat proximal convoluted tubule. I. Bicarbonate, chloride, and water transport. *Am J Physiol* 247: F816–F821, 1984

Liu F-Y, Cogan MG. Kinetics of bicarbonate transport in early proximal convoluted tubule. *Am J Physiol* 253: F912–F916, 1987

Maddox DA, Gennari JF. The early proximal tubule: A high-capacity delivery-responsive reabsorptive site. *Am J Physiol* 252: F573–F584, 1987

Ogawa E, Suzuki S, Tsuzuki H, Kawajin M. Basal study on early diagnosis of cadmium poisoning: Change in carbonic anhydrase activity. *Japan J Pharmacol* 23: 97–105, 1973

Preisig PA, Ives HE, Cragoe EJ Jr, Alpern RJ, Rector FC Jr. Role of the Na^+/H^+ antiporter in rat proximal tubule bicarbonate absorption. *J Clin Invest* 80: 970–978, 1987

Richterich R, Colombo JP. *Clinical Chemistry*. John Wiley & Sons, New York, p 698–703, 1981

Rose BD. *Clinical Physiology of Acid-Base and Electrolyte Disorders*. 3rd ed. McGraw-Mill, New York, p 286–322, 1989

Sato M, Nagai Y. Renal damage and cadmium in subcellular fractions. In: Foulkes E ed, *Biological Roles of Metallothionein*. Elsevier North-Holland, New York, p 163–179, 1982

Scalera V, Huang YK, Hildermann B, Murer H. Simple isolation method for basal lateral plasma membranes from rat kidney cortex. *Membr Biochem* 4: 49–61, 1981

Stinebaugh BJ, Austin WH. Acid-base balance-common sense approach. *Arch Internal Med* 119: 182–188, 1967

Taylor Z, Yang W-C, Shuffield J, Sack E, Arruda JAL. Chronic hypercapnia changes the V-max of Na-H antiporter. *Am J Physiol* 253: F394–F400, 1987

Valtin H. *Renal Function, Mechanisms Preserving Fluid and Salt Balance in Health*. 2nd ed. Little, Brown and Company, Toronto, p 177–196, 1983

Zelikovic I, Stejskal E, Lohstroh P, Budreau A, Chesney RW. Developmental maturation of Na^+-H^+ exchange in rat renal tubular brush-border membrane. *Am J Physiol* 261: F1017–F1025, 1991