Korean J Physiol Pharmacol
Vol 6: 21–26, February, 2002

Transport of Tetraethylammonium in Renal Cortical Endosomes of Cadmium-Intoxicated Rats

Hee Seok Park, Kyoung Ryong Kim, and Yang Saeng Park

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

Effects of cadmium (Cd) intoxication on renal endosomal accumulation of organic cations (OC^+) were studied in rats using ^{14}C-tetraethylammnium (TEA) as a substrate. Cd intoxication was induced by s.c. injections of 2 mg Cd/kg/day for 2~3 weeks. Renal cortical endosomes were isolated and the endosomal acidification (acridine orange fluorescence change) and TEA uptake (Millipore filtration technique) were assessed. The TEA uptake was an uphill transport mediated by H^+/OC^+ antiporter driven by the pH gradient established by H^+-ATPase. In endosomes of Cd-intoxicated rats, the ATP-dependent TEA uptake was markedly attenuated due to inhibition of endosomal acidification as well as H^+/TEA antiport. In kinetic analysis of H^+/TEA antiport, Vmax was reduced and Km was increased in the Cd group. Inhibition of H^+/TEA antiport was also observed in normal endosomes directly exposed to free Cd (but not Cd-metallothionein complex, CdMt) *in vitro*. These data suggest that during chronic Cd exposure, free Cd ions liberated by lysosomal degradation of CdMt in proximal tubule cells may impair the endosomal accumulation of OC^+ by directly inhibiting the H^+/OC^+ antiporter activity and indirectly by reducing the intravesicular acidification, the driving force for H^+/OC^+ exchange.

Key Words: Cadmium, Kidney, Endosome, Organic cation, H^+-ATPase, H^+/TEA antiport

INTRODUCTION

Cadmium (Cd) is an important occupational and environmental pollutant generated in zinc and lead mines, metallurgical and plating industries, and manufacturing processes of various pigments and compounds (Schroeder, 1965; Flick et al, 1971; Tsuchiya, 1978). Humans are exposed to Cd via food, water, air, and dust. Chronic exposures to cadmium may result in various renal functional impairments (Friberg, 1950; Kazantzis et al, 1963; Axelsson & Piscator, 1966; Piscator, 1966; Adams et al, 1969; Goyer et al, 1972; Nordberg & Piscator, 1972; Nomiyama et al, 1973; Gieske & Foulkes, 1974; Nomiyama et al, 1975; Bernard et al, 1979; Iwao et al, 1980; Bernard et al, 1981; Nomiyama et al, 1982; Kim et al, 1988), largely due to inhibition of specific transport systems in the renal proximal tubule (Kim et al, 1988; Kim et al, 1990; Lee et al, 1990; Lee et al, 1991; Ahn & Park, 1995; Kim & Park, 1995; Herak-Kramberger et al, 1996; Bluementhal et al, 1998; Kim et al, 1998).

Recent studies on rats (Herak-Kramberger et al, 1998; Kim et al, 2000) have shown that acidification of proximal tubular endosomes is seriously impaired by Cd exposure. Such change would not only disturb the process of renal protein endocytosis (Choi et al, 1999) but also affect other transport functions mediated by the endosomal acidification. During the process of renal secretion, organic cations (OC^+) are sequestered into proximal tubular endosomes, and this is achieved by H^+/OC^+ antiport driven by the pH gradient across the endosomal membrane (Pritchard et al, 1994). Since Cd impairs the endosomal acidification, the driving force of H^+/OC^+ antiport, one would expect that the endosomal accumulation of OC^+ could be inhibited by Cd exposure.

The present study was undertaken to examine this possibility, using renal cortical endosomes isolated from Cd-intoxicated rats. Tetraethylammonium (TEA) was used as a model substrate. The results demonstrated that the TEA accumulation was significantly attenuated in endosomes derived from Cd-exposed animals, and this was due to an inhibition of H^+/TEA antiporter activity as well as the alteration of endosomal acidification.

METHODS

Animals

Male Sprague Dawley rats (200~300 g) were maintained under standard laboratory conditions and food and water *ad libitum*. Cd intoxication was induced by daily subcutaneous injections of $CdCl_2$ at a dose of 2 mg Cd/kg body weight per day for 2~3 weeks, as described previously (Kim et al, 1988). Saline was injected into the control animals.

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: TEA, tetraethylammonium; NEM, N-ethylmaleimide; NIG, nlgerlcin; Mt, metallothionein; OC^+, organic cation.

Isolation of renal cortical endosomes

Rat renal cortical endosomes were isolated by the method of Sabolic and Burkhardt (1990). Animals were sacrificed by cervical dislocation. Kidneys were taken out and immediately immersed in ice-cold Ringer's solution. After removing the capsule, cortical slices of ~0.3 mm thick were cut off by a razor blade. The pooled tissue (2~2.5 g wet) was put into 35 ml of homogenizing buffer (300 mM mannitol, 12 mM HEPES/Tris, pH 7.4) and homogenized with 20 strokes in a loose-fitting glass/Teflon Potter homogenizer (1,200 rpm). After adding another 35 ml of homogenizing buffer, the suspension was centrifuged at 2,500 *g* for 15 min. The pellet (P_1) was discarded and the supernatant (S_1) was centrifuged at 20,000 *g* for 20 min. Most of the resulting supernatant (S_2) was decanted and saved. The rest of the supernatant (about 2 ml) was used to disperse the fluffy upper part of the pellet (P_{2a}) by careful swirling of the tube. Care was taken not to disturb the hard, yellowish brown mitochondrial pellet (P_{2b}). Supernatant S_2 and the dispersed fluffy pellet P_{2a} were combined and centrifuged at 48,000 *g* for 30 min. The resulting supernatant (S_3) was siphoned off and discarded. The pellet (P_3) contained crude plasma membranes and endosomes.

Endosomes were separated from other membranous components on a Percoll density gradient. Fraction P_3 was resuspended in 30 ml homogenizing buffer by 10 strokes (1,200 rpm) in a tight-fitting glass/Teflon Potter homogenizer. Undiluted Percoll (6.1 g) was added to 32 g of vesicle suspension (16%, w/w, Percoll). The suspension was well mixed and centrifuged at 48,000 *g* for 30 min. The resulting self-orienting gradient was fractionated from top to bottom by pumping a 60% sucrose solution onto the bottom of the centrifuge tube via a steel cannula. The last 5 ml of the gradient containing the highest activity of proton pump was pooled (P_4) and diluted with 30 ml of cold potassium buffer (300 mM mannitol, 100 mM KCl, 5 mM $MgSO_4$, 5 mM HEPES/Tris, pH 7.0). The sample was kept on ice for 30 min and then centrifuged at 48,000 *g* for 30 min. The clear supernatant (S_5) was completely removed by suction. The fluffy white membrane pellet (P_5), which overlayed the hard glassy pellet of Percoll, was transferred into the Eppendorf tube and dispersed by vigorous vortexing in 1 ml of potassium buffer. The sample was centrifuged (by using a tube adapter) at 2,500 *g* for 15 min. The slightly opaque supernatant (S_6) was removed completely by careful suction using a syringe with a needle. The white-yellow pellet on the bottom of the tube (P_6) containing endocytotic vesicles was dispersed in 50 μl of potassium buffer and immediately transferred to a new Eppendorf tube. Care was taken not to re-suspend the membranes adherent to the walls of Eppendorf containing a high amount of brush-border membranes. The protein concentration in the final vesicle preparation was adjusted to be ~10 mg/ml in a total volume of ~60 μl. The isolated endosomes were immediately used or kept frozen in liquid nitrogen and used within 2 weeks. The deposition of vesicles at 4°C overnight or longer is known to result in a time-dependent drop in ATPase activity and increase in proton and potassium conductance in the membranes.

Determination of proton pump activity

The proton pump activity in the endosomal membrane was determined by measuring the ATP-dependent intravesicular acidification using a pH gradient ($\triangle$pH)-sensitive fluorescent dye acridine orange (Sabolic et al, 1985; Sabolic & Burkhardt, 1986; 1990). The weak base acridine orange accumulates in vesicles whenever intravesicular pH is acidic relative to the pH of the extravesicular medium. The fluorescence of accumulated dye is quenched. Thus, the degree of fluorescence quenching (decrease of absorption) is a measure of the magnitude of the pH gradient across the vesicular membrane.

In a typical experiment, 100~120 μg of endosome were added to 2 ml of potassium buffer containing 6 μM acridine orange and 2.5 μM valinomycin (to prevent formation of potential gradients). Acridine orange fluorescence was measured at 25°C on a SLM 4800 C spectrofluorometer with excitation at 510 nm and emission at 548 nm. When ATP (0.2 or 2 mM) was added, fluorescence fell rapidly. Addition of a K^+/H^+ exchanger nigericin (10 μM) restored the initial level of fluorescence within 20~30 s.

Determination of TEA uptake

Uptake of ^{14}C-TEA (usually 100 μM) was determined using a rapid filtration method (Hopfer et al, 1973). An aliquot of endosomes (2.5 mg/ml) was incubated in 9 volumes of incubation medium (in mM: 100 mannitol, 100 KCl, 1 $MgSO_4$, and 20 HEPES/Tris, pH 7.4) containing 100 μM ^{14}C-TEA (0.05 μCi), and ATP and other agents where indicated at 25°C. At predetermined times, a 100- μl aliquot was removed and quickly filtered through Millipore filter (HAWP, 0.45 μm), which was soaked overnight in distilled water prior to use. The filter was washed with 5 ml of ice-cold incubation medium. ^{14}C-TEA in the filter was dissolved in 5 ml of Lumagel (Lumac, AC Landggaf, The Netherlands) and the ^{14}C activity was counted on liquid scintillation counter (Packard Tricarb 4530 C). Nonspecific binding of ^{14}C-TEA to the membrane was determined by incubating endosomes in distilled water containing 0.1% deoxycholate and ^{14}C-TEA.

For the uptakes of TEA by imposed pH gradient, endosomes were suspended in vesicle buffer (in mM: 100 mannitol, 100 KCl, 1 $MgSO_4$, and 20 MES/Tris, pH 5.4), and were then centrifuged at 25,000 g for 15 min. After removing the supernatant, the pellet on the bottom of the tube was dispersed in the same buffer. TEA uptakes by imposed pH gradients were determined as described above.

Chemicals

[^{14}C] TEA (specific activity, 3.7 μCi/μmol) was obtained from New England Nuclear. Tetraethylammonium (TEA) bromide, acridine orange, N-ethylmaleimide (NEM), nigericin, $CdCl_2$, Tris and HEPES were purchased from Sigma Chemicals (St. Louis, MO, USA). Percoll was from Pharmacia Fine Chemicals (Sweden). All other chemicals used were of analytical grade.

Statistical analysis

Statistical evaluation of data was done using Student's t-test (unpaired comparison) or Analysis of covariance.

RESULTS

Endosomal acidification and TEA uptake

Fig. 1A shows time courses of intravesicular acidification of rat renal cortical endosomes under various conditions. Addition of ATP to the extravesiclualr medium induced a rapid quenching of fluorescence, reflecting intravesicular acidification. The quenching was prevented by addition of nigericin (10 μM), a K^+/H^+ exchanger. Both the initial rate and the maximal degree of quenching were changed with the ATP concentration. At a high level of ATP (2 mM), the quenching sustained rather long (more than 20 min), but at a low level of ATP (0.2 mM) it reverted quickly (within 20 min) to the initial level, reflecting consumption of available ATP and decreased proton pump (H^+-ATPase) activity.

Fig. 1B presents time courses of TEA uptake by endosomes from the same batch used for the acidification study. Addition of ATP to the extravesicular medium induced an uphill transport (accumulation above the equilibrium level) of TEA into the vesicle interior, which was abolished by nigericin (10 μM). The time course of TEA uptake at various ATP levels was similar to that for the fluorescence quenching as shown in Fig. 1A. The uphill accumulation was maintained rather long (more than 20 min) at 2 mM ATP, but only transiently at 0.2 mM ATP, consistent with the changes in pH gradient at these ATP concentrations.

These data are compatible with the notion that TEA accumulation by renal cortical endosomes is mediated by H^+/TEA antiport driven by the proton gradient generated by H^+-ATPase in the vesicle-limiting membrane (Pritchard et al, 1994).

Fig. 2 shows changes in intravesicular acidification (Fig. 2A) and TEA uptake (Fig. 2B) in renal cortical endosomes derived from Cd-intoxicated rats. Both the ATP-dependent-fluorescence quenching and -TEA uptake were markedly attenuated in the Cd group.

Endosomal uptake of TEA by imposed pH gradient

In order to test the possibility that Cd intoxication also affects the H^+/TEA antiport system directly, we measured the TEA uptake driven by an imposed pH gradient in the absence of ATP. Endosomes from control and Cd-intoxicated animals were homogenaized in either pH 7.4 or 5.4 buffer, and their uptake of TEA from pH 7.4 buffer was measured at various time intervals (Fig. 3). In both the control and Cd group, an outwardly-directed H^+ gradient (pHi/pHo = 5.4/7.4, $[H^+]i/[H^+]o$ = 100/1) markedly stimulated TEA uptake over that in the absence of H^+ gra-

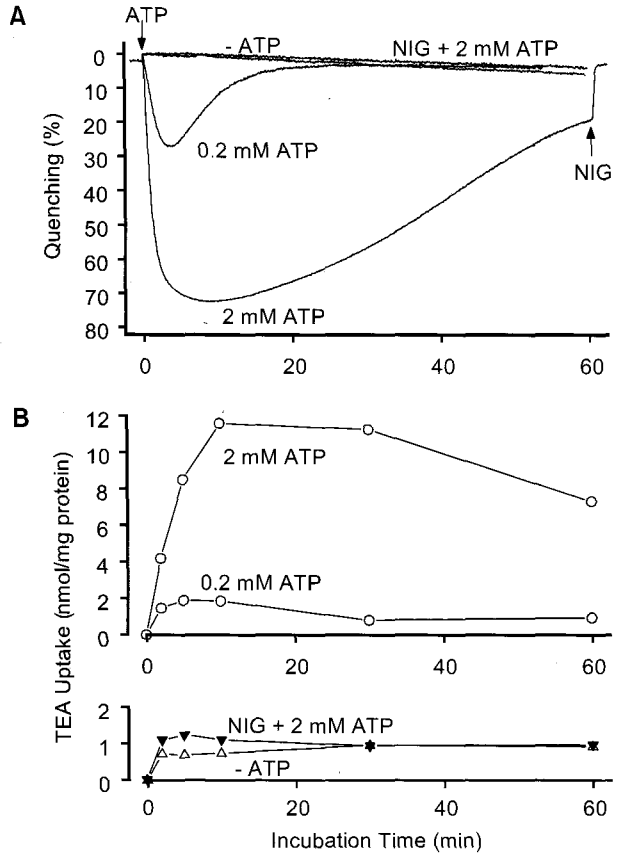

Fig. 1. Time courses of ATP-dependent fluorescence quenching (intravesicular acidification) and ATP-dependent TEA uptake by renal cortical endosomes derived from normal (control) rats.

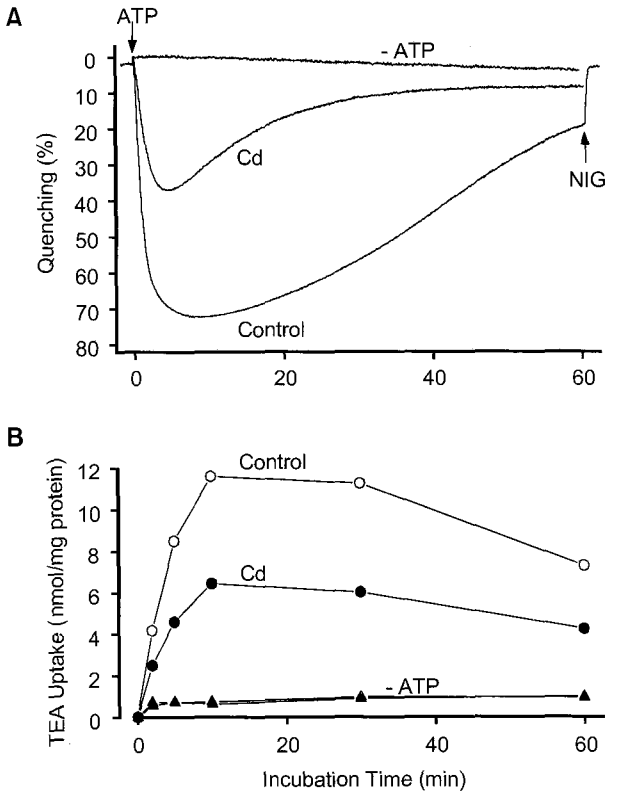

Fig. 2. Comparison of ATP-dependent fluorescence change (intravesicular acidification) and ATP-dependent TEA uptake between endosomes from control and Cd-intoxicated rats. The ATP concentration was 2 mM.

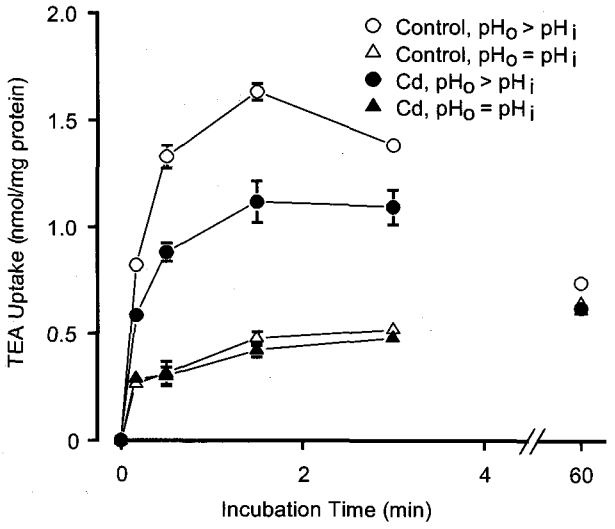

Fig. 3. Time courses of TEA uptake by renal cortical endosomes from control and Cd-intoxicated rats in the presence and absence of an imposed pH gradient. The initial pH gradient between the outside and inside media (pHo/pHi) was 7.4/5.4. Data represent the mean±SE of 3 determinations.

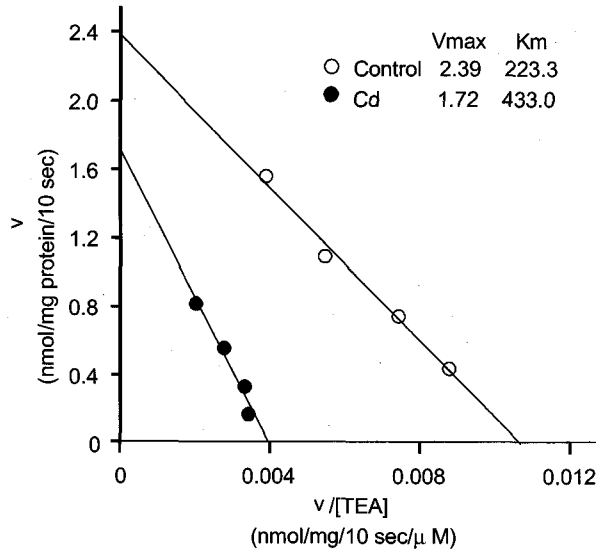

Fig. 4. Kinetics of pH gradient-dependent TEA uptake by renal cortical endosomes of control and Cd-intoxicated rats. The initial (10 sec) rate of pH-gradient dependent TEA uptake (v) was plotted against v/[TEA] (Hofstee plot) in each group. Covariance analysis indicates that the two regression lines are significantly ($p<0.05$) different in the y-intercept (Vmax) and the slope (1/Km). Data represent the mean±SE of 5 determinations.

dient (pHi = pHo = 7.4). In the control group, the uptake in the presence of H^+ gradient showed a definite overshoot (i.e., a transient accumulation over the equilibrium level) during the early period, a characteristic of H^+-gradient driven transport system. In the Cd group, the TEA uptake in the absence of H^+ gradient was not changed, but it was significantly reduced in the presence of H^+ gradient, as

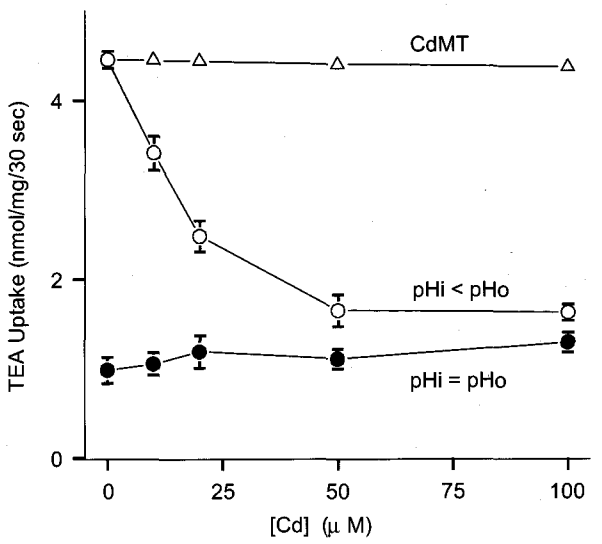

Fig. 5. TEA uptakes by renal cortical endosomes directly exposed to free Cd or Cd bound to metallothionein (CdMt) *in vitro*. 30-sec rates of TEA uptake in the presence (pHo/pHi=7.4/5.4) and absence (pHo/pHi=7.4/7/4) of an imposed pH gradient was plotted against the $CdCl_2$ or CdMt concentration in the medium. Data represent the mean±SE of 3 determinations.

compared with the control. These indicate that the H^+/TEA antiport system itself was impaired by Cd intoxication.

In order to explore the mechanism of this impairment, we next carried out kinetic analyses of H^+/TEA antiport in the control and Cd group endosomes. The initial rates (v) of H^+ gradient-dependent TEA uptake (the difference between the uptakes in the presence and absence of H^+ gradient) were analyzed as a function of the substrate (TEA) concentration. Hofstee plots of the data (Fig. 4) indicated that the Vmax (y-intercept of the regression line) of the H^+-driven TEA uptake was significantly lower, while the Km (the slope of the regression line) was significantly higher in endosomes from Cd-intoxicated animals than in those from the control animals. These results suggest that the capacity of the endosomal membrane to transport TEA by H^+/TEA antiport mechanism was reduced by Cd intoxication.

TEA uptake by endosomes directly exposed to free Cd in vitro

Fig. 5 depicts the effect of free Cd directly applied to the renal cortical endosomes on the initial rate of TEA uptake. Endosomes from normal animals were preincubated with various concentrations (0, 10, 20, 50, and 100 μM) of $CdCl_2$ for 10 min, then tested for TEA uptake. The TEA uptake in the absence of pH gradient was not affected, but that in the presence of pH gradient was progressively inhibited as the $CdCl_2$ concentration increased from 0 to 100 μM. Within the same concentration range, Cd bound to metallothionein (CdMt) showed no apparent effect on the pH-gradient dependent TEA uptake. These data indicate that the interaction of free Cd with endosomal membrane led to an inhibition of intrinsic H^+/TEA antiporter activity.

DISCUSSION

Cationic drugs and foreign compounds of different types and structures are secreted into urine via transcellular transport in the renal proximal tubule. Organic cations, such as TEA, are first accumulated into the tubular epithelial cell across the basolateral membrane (BLM) and then extruded across the brush-border membrane (BBM) into the tubular lumen. The transport across the BLM is brought about by a facilitative, but passive, electrogenic carrier-mediated system that is driven by the inside negative membrane potential (Kinsella et al, 1979; Takano et al, 1984; Smith et al, 1988), and the transport across the BBM by an electroneutral exchange of cellular OC^+ with luminal H^+ via H^+/OC^+ antiporter (Takano et al, 1984; Sokol et al, 1985; Dantzler et al, 1989; Ott et al, 1991). During renal OC^+ excretion by some species, intracellular concentrations greatly exceed the value predicted by the potential-driven mechanism thought to mediate their basolateral uptake. It was, therefore, suggested that the cytoplasmic free OC^+ concentration might be much lower than the total tissue concentration due to a sequestration within intracellular compartments such as endosomes (Pritchard et al, 1994). The endosomal accumulation of OC^+ is mediated by H^+/OC^+ antiport system, and is driven by a pH gradient maintained by the proton pump (H^+-ATPase) activity in the endosomal membrane (Pritchard et al, 1994).

Our previous studies on Cd-intoxicated animals have shown that the potential-sensitive OC^+ transport system in the BLM is not affected but the H^+/OC^+ antiport system in the BBM is seriously impaired by Cd intoxication (Lee et al, 1990; Kim et al, 1998). The data reported here show that the endosomal OC^+ accumulation is also significantly inhibited by Cd intoxication. In renal cortical endosomes derived from Cd-intoxicated rats, the ATP-dependent TEA uptake was markedly attenuated as compared with that in control endosomes (Fig. 2B). The attenuation was attributed in part to a defect in endosomal acidification (Fig. 2A) and in part to an inhibition of H^+/TEA antiport (Fig. 3). The latter effect was associated with a reduction in Vmax and an increase in Km (Fig. 4), which suggested that the density of H^+/TEA antiporter in the endosomal membrane was reduced and the biochemical characteristics of the transporter was changed by Cd. The mechanisms with which Cd induces these changes are not clearly understood at present. The reduction in Vmax may be due to an inhibition of *de novo* synthesis of H^+/OC^+ antiporter or to an inhibition of recycling of the proteins via vesicle trafficking. In any event, reduction of functional H^+-ATPase and H^+/OC^+ antiporter units would reduce the capacity of endosomal membrane for acidification and H^+/TEA antiport.

This, however, may not be the sole mechanism for the reduction of endosomal acidification and TEA uptake. During chronic exposure, the Cd ingested into the body is bound to metallothionein (Mt), a heavy metal binding low molecular protein (Foulkes, 1982; Elinder & Nordberg, 1986). The CdMt complex is transported to the kidney via blood circulation, filtered through glomeruli, and reabsorbed into the proximal tubule cell by receptor-mediated endocytosis (Fowler & Nordberg, 1978; Squibb et al, 1979). The CdMt entered the cell is degraded in the lysosome, releasing free Cd in the cytoplasm (Fowler & Nordberg, 1978; Squibb & Fowler, 1984; Min et al, 1986; Dorian et al, 1992). In addition to the inhibition of specific mRNA expression, the Cd released from this degradation may directly interact with various enzymes and membrane transporters, such as H^+-ATPase and H^+/OC^+ antiporter in the endosome. The experiments on the *in vitro* Cd effects strongly favor this possibility. Direct exposure to renal endosomes to free Cd ($CdCl_2$) at $10\sim100\,\mu M$ resulted in a dose-dependent inhibition of H^+/TEA antiport (Fig. 5). Similar effects of free Cd on the endosomal H^+-ATPase activity have also been observed in our previous study (Kim et al, 2000). The concentrations of Cd used in these studies were within the range of unbound (free) Cd observed in renal cortical tissues of Cd-intoxicated animals ($\sim13\,\mu g/g$, $\sim115\,\mu M$) at the time of onset of renal dysfunction (Nomiyama & Nomiyama, 1986). It is, therefore, likely that a direct inhibition of endosomal H^+/OC^+ antiporter and H^+-ATPase activities by free Cd also occurs *in vivo* in Cd-intoxicated animals. The exposure to CdMt had no effect on the H^+/TEA antiport (Fig. 5), as well as H^+-ATPase activity (Kim et al, 2000), indicating that only free Cd interacts with the endosomal membrane.

Pritchard et al (1994) proposed two possible roles of renal endosomal H^+/OC^+ antiport. First, the transport could serve simply to reduce the concentration of free OC^+ within the tubular cells, which would reduce the energy barrier against which basolateral transport must take place and decrease the potential for toxicity of OC^+ during secretory transport. The second possibility is direct participation of endosomal uptake in transcellular movement of OC^+ during secretion, i.e., trafficking of endosomes loaded with OC^+ to the luminal membrane, where their contents are released and then return to the cell interior. Both are speculative and have not been firmly established. But, if existed, the second mechanism would effectively facilitate OC^+ secretion, as the transmembrane pH gradient, the driving force of H^+/OC^+ antiport, is much greater in the endosome than in the BBM (Pritchard et al, 1994). Such membrane recycling would also account for the presence of H^+/OC^+ antiporter in the BBM. In this context, it is important to point out that the capacity of H^+/OC^+ antiport in renal cortical BBM is markedly reduced in Cd-intoxicated animals (Kim et al, 1998). This reduction may be associated with an interaction of Cd with endosome, as well as a direct interaction with BBM.

In conclusion, the results of the present study suggest that, in long-term Cd-exposed animals, free Cd ions liberated from lysosomal degradation of CdMt in renal proximal tubule cells may impair the endosomal accumulation of OC^+ by directly inhibiting the H^+/OC^+ antiporter activity and indirectly by reducing the intravesicular acidity, the driving force of H^+/OC^+ exchange. Such effects of Cd, together with those on the luminal H^+/OC^+ antiporter, would lead to an inhibition of renal OC^+ secretion in intact animals.

ACKNOWLEDGEMENT

This work was supported in part by a grant from Kosin Medical College.

REFERENCES

Adams RG, Harrison JF, Scott P. The development of cadmium-induced proteinuria, impaired renal function, and osteomalacia in alkaline battery workers. *Quart J Med* 38: 425–443, 1969

Ahn DW, Park YS. Transport of inorganic phosphate in renal

cortical brush-border membrane vesicles of cadmium-intoxicated rats. *Toxicol Appl Pharmacol* 133: 239−243, 1995
Axelsson B, Piscator M. Renal damage after prolonged exposure to cadmium. An experimental study. *Arch Environ Health* 12: 360−373, 1966
Bernard A, Buchet H, Roels H, Masson P, Lauwerys R. Renal excretion of proteins and enzymes in workers exposed to cadmium. *Eur J Clin Invest* 9: 11−12, 1979
Bernard A, Lauwerys R, Gengoux P. Characterization of the proteinuria induced by prolonged oral administration of cadmium in female rats. *Toxicology* 20: 345−347, 1981
Bluementhal SS, Ren L, Lewanad DL, Kreizoski SK, Petering DH. Cadmium decreases SGLT1 messenger RNA in mouse kidney cells. *Toxicol Appl Pharmacol* 149: 49−54, 1998
Choi JS, Kim KR, Ahn DW, Park YS. Cadmium inhibits albumin endocytosis in OK renal epithelial cells. *Toxicol Appl Pharmacol* 161: 146−152, 1999
Dantzler WH, Brokl OH, Wright SH. Brush-border TEA transport in intact proximal tubules and isolated membrane vesicles. *Am J Physiol* 256: F 290−F297, 1989
Dorian C, Gattone VH II, Klaasen CD. Accumulation and degradation of the protein moiety of cadmium-metallothionein (CdMT) in mouse kidney. *Toxicol Appl Pharmacol* 117: 242−248, 1992
Elinder CG, Nordberg M. Metallothionein. In: Friberg L, Elinder CG, Kjellstrom T, Nordberg GF ed, *Cadmium and Health: A Toxicological and Epidemiological Appraisal.* Vol 1. CRC Press, Boca Raton, FL, p 65−79, 1986
Flick DF, Krayabill HF, Dimitroff JM. Toxic effect of cadmium. A review. *Environ Res* 4: 71−85, 1971
Foulkes EC. Role of metallothionein in transport of heavy metals. In: Foukes EC ed, *Biological Roles of Metallothionein.* Elsvier /North Holland, New York, p 131−140, 1982
Fowler BA, Nordberg GF. The renal toxicity of cadmium metallothionein: Morphometric and X-ray microanalytical studies. *Toxicol Appl Pharmacol* 46: 609−623, 1978
Friberg L. Health hazards in the manufacturer of alkaline accumulators with special reference to chronic cadmium poisoning. *Acta Med Scand* 138: supple 240: 1−124, 1950
Gieske TH, Foulkes EC. Acute effects of cadmium on proximal tubular function in rabbits. *Toxicol Appl Pharmacol* 27: 292−299, 1974
Goyer RA, Tsuchiya K, Leonard DL, Kahyo H. Aminoaciduria in Japanese workers in the lead and cadmium industries. *Am J Clin Pathol* 57: 635−642, 1972
Herak-Kramberger CM, Brown D, Sabolic I. Cadmium inhibits vacuolar H^+-ATPase and endocytosis in rat kidney cortex. *Kidney Int* 53: 1713−1726, 1998
Herak-Kramberger CM, Spindler B, Biber J, Murer H, Sabolic I. Renal type II Na/Pi-cotransporter is strongly impaired whereas the Na/sulphate-cotransporter and aquaporin 1 are unchanged in cadmium-treeated rats. *Pflugers Arch Eur J Physiol* 432: 336−344, 1996
Hopfer U, Nelson K, Perrotto I, Iseelbacher KJ. Glucose transport in isolated brush border membranes from rat small intestine. *J Biol Chem* 248: 25−32, 1973
Iwao S, Tsuchiya K, Sakurai H. Serum and urinary beta-2-microglobulin among cadmium-exposed workers. *J Occup Med* 22: 399−402, 1980
Kazantzis G, Flynn FV, Spowage JS, Trott DG. Renal tubular malfunction and pulmonary emphysema in cadmium pigment workers. *Quart J Med* 32: 165−192, 1963
Kim KR, Kim GC, Choi JS, Ahn DW, Park YS. Renal transport systems for organic anions and cations in cadmium-exposed rats. *Toxicol Appl Pharmacol* 149: 144−149, 1998
Kim KR, Lee HY, Kim CK, Park YS. Alteration of renal amino acid transport system in cadmium-intoxicated rats. *Toxicol Appl Pharmacol* 106: 102−111, 1990
Kim KR, Park YS. Phlorizin binding to renal outer cortical brush-border membranes of cadmium-injected rabbits. *Toxicol Appl Pharmacol* 133: 244−248, 1995
Kim MS. Kim KR. Ahn DW. Park YS. Cadmium inhibition of renal endosomal acidification. *Kor J Physiol Pharmacol* 4: 63−72, 2000
Kim YK, Choi JK, Kim JS, Park YS. Changes in renal function in cadmium-intoxicated rats. *Pharmacol & Toxicol* 63: 342−350, 1988
Kinsella JL, Holohan PD, Pessah NI, Ross CR. Transport of organic ions in renal cortical luminal and antiluminal membranes. *J Pharmcol Exp Ther* 209: 443−459, 1979
Lee HY, Kim KR, Park YS. Transport kinetics of glucose and alanine in renal brush-border membrane vesicles of cadmium-intoxicated rabbits. *Pharmacol & Toxicol* 69: 390−395, 1991
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
Min KS, Kobayashi K, Onosaka S, Ohta N, Okada Y, Tanaka K. Tissue distribution of cadmium and nephropathy after administration of cadmium in several forms. *Toxicol Appl Pharmacol* 86: 262−270, 1986
Nomiyama K, Nomiyama H. Critical concentration of 'unbound' cadmium in the rabbit renal cortex. *Experientia* 42: 149, 1986
Nomiyama K, Nomiyama H, Yotoriyama M, Matsui K. Sodium dodecyl sulfate acrylamide gel electrophoretic studies of low-molecular-weight proteinuria, an early sign of cadmium health effects in rabbits. *Ind Health* 20: 11−18, 1982
Nomiyama K, Sato C, Yamamoto A. Early signs of cadmium intoxication in rabbits. *Toxicol Appl Pharmacol* 24: 625−635, 1973
Nomiyama K, Sugata Y, Yamamoto A, Nomiyama H. Effects of dietary cadmium on rabbits. I. Early signs of cadmium intoxication. *Toxicol Appl Pharmacol* 31: 4−12, 1975
Nordberg GF, Piscator M. Influence of long-term cadmium exposure of urinary excretion of protein and cadmium in mice. *Environ Physiol Biochem* 2: 37−49, 1972
Ott RJ, Hui AC, Yuan G, Giacomini KM. Organic cation transport in human renal brush-border membrane vesicles. *Am J Physiol* 261: F443−F451, 1991
Piscator M. Proteinuria in chronic cadmium poisoning. III. Electrophoretic and immunoelectorphoretic studies on urinary proteins from cadmium workers, with special reference to the excretion of low molecular weight proteins. *Arch Environ Health* 12: 335−344, 1966
Pritchard J, Sykes DB, Walden R, Miller DS. ATP-dependent transport of tetraethylammonium by endosomes isolated from rat renal cortex. *Am J Physiol* 266: F966−F976, 1994
Sabolic I, Burkhardt G. Characteristics of the proton pump in rat renal cortical endocytotic vesicles. *Am J Physiol* 250: F817−F826, 1986
Sabolic I, Burkhardt G. ATP-driven proton transport in vesicles from the kidney cortex. *Methods in Enzymol* 191: 505−520, 1990
Sabolic I, Haase W, Burkhardt G. ATP-dependent H^+ pump in membrane vesicles from rat kidney cortex. *Am J Physiol* 248: F835−F844, 1985
Schroeder HA. Cadmium as a factor in hypertension. *J Chronic Dis* 18: 647−656, 1965
Smith PM, Pritchard JB, Miller DS. Membrane potential drives organic cation transport into teleost renal proximal tubules. *Am J Physiol* 255: R492−R499, 1988
Sokol PP, Holohan PD, Ross CR. Electroneutral transport of organic cations in canine renal brush border membrane vesicles (BBMV). *J Pharmacol Exper Ther* 233: 694−699, 1985
Squibb KS, Fowler BA. Intracellular metabolism and effects of circulating cadmium-metallothionein in the kidney. *Environ Health Perspect* 54: 31−35, 1984
Squibb KS, Ridlington JW, Carmichael NG, Fowler BA. Early cellular effect of circulating cadmium-thionein in kidney proximal tubules. *Environment Health Perspect* 28: 287−296, 1979
Takano I, Inui KI, Okano T, Saito H, Hori R. Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. *Biochim Biophys Acta* 773: 113−124, 1984
Tsuchiya K. Production and use of cadmium in Japan. In: Tsuchiya K ed, *Cadmium Studies in Japan: A Review.* Kodensa, Tokyo and Elsveier/North Holland, Amsterdam, New York, Oxford, p 7−9, 1978