Kor J Physiol (1996) 30(2), 279~288

Effect of Cadmium on Organic Acid Transport System in Renal Basolateral Membrane

Ghi Chan Kim, Kyoung Ryong Kim, Jee Yeun Kim and Yang Saeng Park

Department of Physiology, Kosin Medical College

=ABSTRACT=

Chronic exposure to cadmium impairs various renal tubular functions, including organic acid (anion) secretion. To investigate the mechanism of cadmium-induced alterations in the organic anion transport system, kinetics of p-aminohippurate (PAH) uptake was studied in renal cortical basolateral membrane vesicles (BLMV) isolated from cadmium-intoxicated rats (adult male Sprague-Dawley). Cadmium intoxication was induced by subcutaneous injections of $CdCl_2$ (2 mg Cd/kg per day) for 3 weeks. The renal plasma membrane vesicles were prepared by Percoll gradient centrifugation. The vesicular uptake of ^{14}C-PAH was determined by rapid filtration technique using Millipore filter. Cadmium intoxication resulted in a marked attenuation of Na^+-dependent, α-ketoglutarate (αKG)-driven PAH uptake with no changes in Na^+ and αKG-independent transport component. Kinetic analysis indicated that Vmax, but not Km, of the Na^+-dependent, αKG-driven component was reduced. A similar reduction of Na^+-dependent, αKG-driven PAH uptake was observed in normal membrane vesicles directly exposed to inorganic cadmium *in vitro*, and this was accompanied by an inhibition of both Na^+-dependent αKG uptake and αKG-PAH exchange activity. These results indicate that during chronic exposure to cadmium, free cadmium ions liberated in the proximal tubular cytoplasm directly interact with the basolateral membrane and impair the active transport capacity for organic anions, most likely due to an inhibition of both Na^+-dicarboxylate cotransporter and dicarboxylate-organic anion antiporter activities.

Key Words: Organic acid, Kidney, Basolateral membrane vesicle, Cadmium

INTRODUCTION

The renal secretion of organic anion is a principal mechanism involved in the elimination of foreign substances and metabolites from the body. Several studies in the past have indicated that the function of this transport system is changed by cadmium exposure. For instance, Vander (1963) found in dogs injected (i.v.) with a cadmium-cysteine complex a significant reduction of the maximum tubular ability to secrete p-aminohippurate (Tm_{PAH}) within 0.5~2 hr of an administration. Gieske and Foulkes (1974) observed a marked suppression of PAH clearance occurring in rabbits 3 days after a single i.v. injection of a $CdCl_2$ (2~12 mg Cd-350 μmole mercaptoethanol/kg). Nomiyama (1973, 1978) also found in rabbits that PAH clearance fell after an acute intra-arterial administration of $CdCl_2$ (2~12 mg Cd per animal) and the Tm_{PAH} decreased gradually during chronic treatment with $CdCl_2$ (0.5~15 mg Cd/kg · day, s.c. injections). In a kinetic

study of PAH uptake in renal cortical slices of rats exposed to $CdCl_2$ (2 mg Cd/kg · day s.c. injections for 3~16 days), we have observed a significant reduction in the maximum rate of active influx (Vmax), with no change in Km (Kim et al, 1988). While these observations suggest that the capacity of renal tubules to secrete organic acids is attenuated by cadmium, the underlying mechanism(s) remains unidentified.

The present study was therefore undertaken to directly evaluate the activity of organic anion transport system at the membrane level using proximal tubular basolateral membrane vesicles (BLMV) isolated from cadmium-intoxicated rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats of 200~300 g were used. Cadmium intoxication was induced by subcutaneous injections of $CdCl_2$ (2 mg Cd/kg · day) for 3 weeks. At 1-week intervals animals were kept in metabolic cages and collected urine for 24 hours. If animals showed a significant glycosuria, they were considered to be cadmium intoxicated and were sacrificed for vesicle study.

Preparation of plasma membrane vesicles from kidney cortex

Renal plasma membrane vesicles were isolated by a procedure similar to those described by Kinsella et al. (1979) and Scalera et al. (1981). Kidneys were perfused through the abdominal aorta with an ice-cold solution containing 150 mM NaCl. The cortex was cut off, minced, and placed in 250 mM sucrose-20 mM Hepes/Tris, pH 7.4 (sucrose buffer). The cortical tissues from 5~6 animals were pooled and were homogenized with 25 strokes in a motor-driven glass homogenizer with a tight-fitting teflon pestle (clearance 0.15 mm) at 1,500 rpm. The tissue homogenate was centrifuged at 1,075 g for 10 min in a Sorvall refrigerated centrifuge (Sorvall RC-5B, SS-34 Rotor). The supernatant was saved, and the pellet was suspended again in half the original volume of sucrose buffer and homogenized with 10 strokes at 1,500 rpm. The homogenate was centrifuged as above for 10 min at 1,075 g. The supernatant was decanted and combined with the previous supernatant (Fraction 1). Fraction 1 was centrifuged at 14,460 g for 15 min, the resulting supernatant and the soft light portion of the pellet were taken, and pooled (Fraction 2). Fraction 2 was then centrifuged at 47,800 g for 30 min, the supernatant and the lower dark pellet were discarded, and the upper fluffy layer of the pellet was suspended in sucrose buffer to a total volume of 26.5 ml (Fraction 3). This constituted the microsomal fraction. Plasma membranes in this fraction were purified further by centrifugation on a gradient of Percoll (a suspension of colloidal silica particles which generates a self orienting density gradient during centrifugation). 3.5 ml stock solution of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) was added to the fraction 3 (final Percoll concentration 11.7 %); the solution was mixed by inversion and centrifuged at 47,800 g for 40 min. The spontaneously formed Percoll gradient was fractionated from the top by careful pipetting and was collected in 1 ml fractions. Initially, each fraction was assayed for Na^+-K^+-ATPase (a marker enzyme of basolateral membrane) and alkaline phosphatase (a marker enzyme of brush border membrane) by the method of Jorgensen and Skou (1971) and Wako Technical Bulletin No. 270-04609, respectively. Routinely thereafter, fractions were pooled according to the distribution of marker enzymes to obtain aliquots enriched in either basolateral membrane vesicles (BLMV) or brush-border membrane vesicles (BBMV). Typically, the first 4 ml were discarded; 5~15 ml were pooled as BLMV and 16~30 ml were pooled as crude BBMV. Each pooled fraction was mixed with an equal volume of sucrose buffer, and the Percoll was removed by centrifuging at 100,000 g for 1 hr in an ultracentrifuge (Sorvall OTD-75, TFT 70.38 Rotor). The Percoll forms a glassy pellet, and

membranes form a fluffy layer on top of the Percoll. In the case of BLMV, they were resuspended in a vesicle buffer (100 mM mannitol, 100 mM KCl, and 20 mM Hepes/Tris, pH 7.4) by passing the membrane pellet several times through a 25 gauge needle. The protein concentration of the vesicle fraction was adjusted to be 6~8 mg/ml. The vesicle preparations were stored at −70°C until used. The specific activities of the marker enzymes in each preparation were as follows: In the control group, the Na^+-K^+-ATPase activity in the BLMV was 15.7 μmol Pi/mg protein · hr, approximately 10-fold that in the cortical homogenate and the BBMV preparations were enriched in alkaline phosphatase, with a specific activity of 21.3 K-A units/mg protein · hr, 7.6-fold greater than that of the homogenate value. However, Na^+-K^+-ATPase in the BBMV fraction was 1.9-fold enriched and alkaline phosphatase activity in the BLMV fraction was 3.2-fold enriched, indicating that each fraction was slightly contaminated with opposite side membranes. Similar results were obtained in the cadmium group, although the alkaline phosphatase activity in each fraction was significantly lower than that of the corresponding value in the control group.

Sidedness of vesicles was determined for BLMV preparations by a methodology similar to those employed by Kinsella et al (1979) and Windus et al. (1984). The sidedness was inferred from the asymmetry of the Na^+-K^+-ATPase such that ouabain acts on the enzyme from the exterior of intact vesicle whereas ATP acts from the interior. It was assumed that ATP and ouabain are not permeable to sealed vesicles. BLMV preparations, suspended in 25 mM imidazole (pH 7.4) and 2 mM EDTA, were disrupted with 0.6 mg/ml deoxycholate at 25°C for 30 min. Total ATPase and ouabain (1 mM)-sensitive ATPase activities were measured before and after disruption. The fraction of membranes for which orientation was not assignable (i.e., open vesicles) was determined by measuring the quotient of ouabain-sensitive ATPase before disruption and the quotient of ouabain-sensitive ATPase after disruption. The fraction of inside out vesicles (IOV) was assessed by subtracting the fraction not assignable from the quotient of total ATPase before disruption to total ATPase after disruption. The fraction of right-side out vesicles (ROV) was determined by calculation 1 - fraction not assignable - fractions everted. Such determinations revealed that the fractions of ROV, IOV, and open vesicles were 0.23, 0.07, and 0.70 in the control group and 0.25, 0.10, and 0.65 in the cadmium group; thus more than 70% of the sealed vesicles were oriented right-side out in both groups.

Determination of substrate transport in membrane vesicles

Transport of substrate in membrane vesicles was determined using a rapid filtration method (Hopfer et al, 1973). For the PAH uptake, an aliquot of membrane vesicles was incubated in 9 volumes of incubation medium (100 mM NaCl or KCl, 100 mM mannitol, 10 μM α-ketoglutarate (αKG), and 20 mM Hepes/Tris, pH 7.4) containing 50 μM ^{14}C-PAH at 25°C. At appropriate intervals, a 100 ml aliquot was removed and quickly filtered through Millipore filter (Type HA, pore size 0.45 μm), which was soaked overnight in distilled water prior to use. The filter was washed with 5 ml of ice-cold stop solution (incubation medium containing 1 mM probenecid). ^{14}C-PAH in the filter was dissolved into 6 ml of Lumagel (Lumac, AC Landggaf, the Netherlands) and the ^{14}C activity was counted on a liquid scintillation counter (Packard Tricarb 4530 C). Nonspecific binding of the radioactive material to the plasma membrane was determined by incubating vesicles in distilled water containing 0.1% deoxycholate and ^{14}C-PAH. The value of nonspecific binding was subtracted from the experimental value, and the vesicular uptake was expressed as pmoles per mg protein for a given time.

A similar procedure was used in the determination of ^{14}C-αKG uptake. The compositions of the intra- and extravesicular media are described in the figure legend.

All the radioactive compounds used in this study were purchased from New England Nuclear (Boston, MA, USA).

Statistical analysis

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

RESULTS

Transport of p-aminohippurate (PAH) in renal basolateral membrane vesicles (BLMV) from cadmium-intoxicated rats

The peritubular uptake of PAH is known to be a "tertiary active" process involving a Na^+-coupled influx of dicarboxylate and a dicarboxylate-PAH exchange (Shimada et al, 1987; Pritchard, 1988; Burckhard & Ullrich, 1989). Thus, in the present study we evaluated the effect of cadmium intoxication on this system.

Fig. 1 illustrates the time course of PAH uptake by renal cortical BLMV of normal rats. The imposition of an inwardly-directed Na^+ gradient in the presence of 10 μM αKG markedly stimulated PAH uptake over that observed in the absence of Na^+ ($K^+_i = K^+_o$), showing a definite "overshoot". The Na^+ gradient alone produced a very modest stimulation of PAH uptake, but in this case there was no overshoot phenomenon. As shown in Fig. 2, in the absence of Na^+, αKG did not stimulate the PAH uptake. Furthermore, the Na^+-dependent, α KG-stimulated PAH transport was completely inhibited by 1 mM probenecid. These results confirm earlier findings by others (Shimada et al, 1987; Pritchard, 1988) and support their notion that the

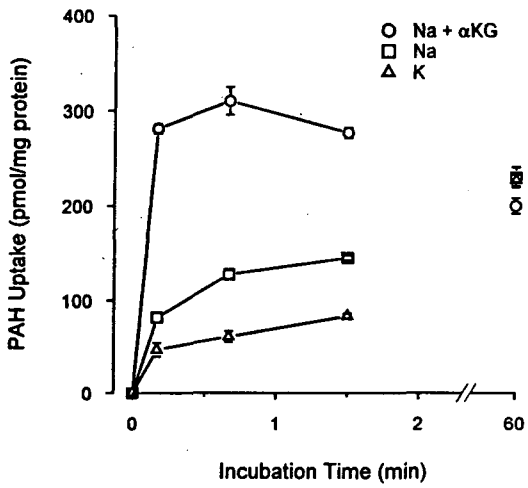

Fig. 1. Time course of PAH uptake by rat renal cortical basolateral membrane vesicles. Vesicles containing (in mM) 100 mannitol, 100 KCl, and 20 Hepes/Tris (pH 7.4) were incubated at 25°C in a buffer containing 100 mM NaCl and 10 μM α-ketoglutarate, or 100 mM NaCl, or 100 mM KCl in addition to 50 μM PAH (10 μM ^{14}C-PAH), 100 mM mannitol, and 20 mM Hepes/Tris (pH 7.4). Values are means ± SE of 3 determinations.

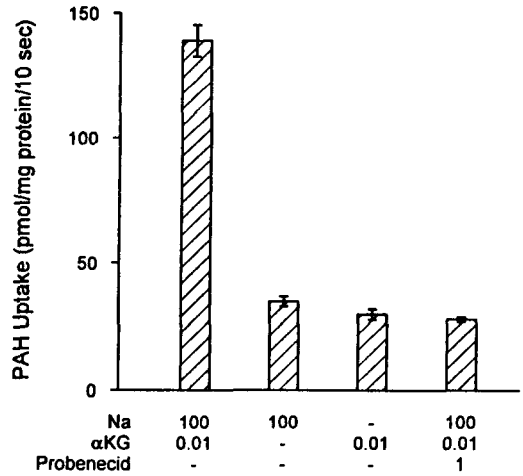

Fig. 2. Initial (10 sec) rates of PAH uptake by rat renal cortical basolateral membrane vesicles at various medium composition. Vesicles containing (in mM) 100 mannitol, 100 KCl, and 20 Hepes/Tris (pH 7.4) were incubated at 25°C in a buffer containing 100 mM NaCl and 10 μM α-ketoglutarate, or 100 mM NaCl, or 10 μM α-ketoglutarate, or 100 mM NaCl, 10 μM α -ketoglutarate and 1 mM probenecid in addition to 50 μM PAH (10 μM ^{14}C-PAH), 100 mM mannitol, and 20 mM Hepes/Tris (pH 7.4). Data represent mean ± SE of 3 determinations.

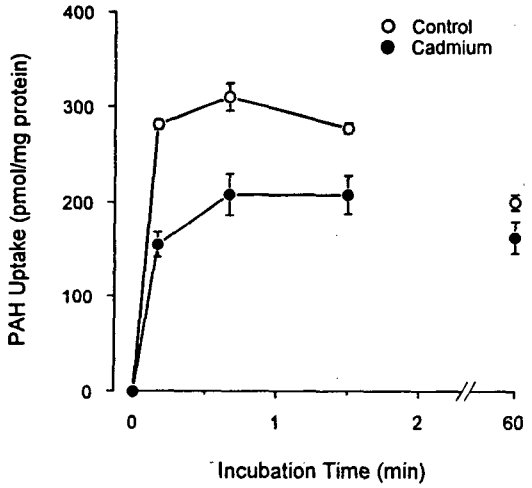

Fig. 3. Time courses of PAH uptake by renal cortical basolateral membrane vesicles of control and cadmium-intoxicated rats in Na^+ and α-ketoglutarate containing medium. Vesicles containing (in mM) 100 mannitol, 100 KCl, and 20 Hepes/Tris (pH 7.4) were incubated at 25°C in a buffer containing 100 mM NaCl and 10 μM α-ketoglutarate in addition to 50 μM PAH (10 μM ^{14}C-PAH), 100 mM mannitol, and 20 mM Hepes/Tris (pH 7.4). Values are means ± SE of 3 determinations.

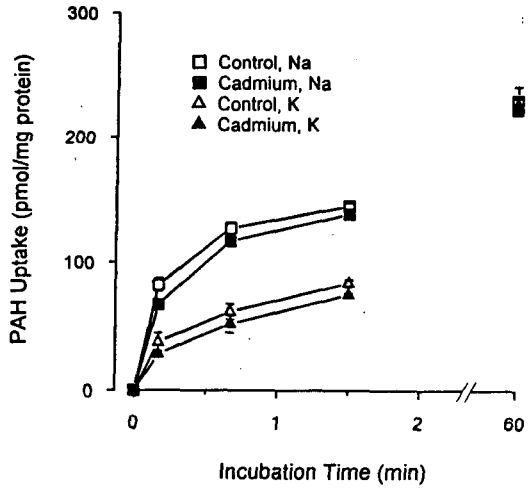

Fig. 4. Time courses of PAH uptake by renal cortical basolateral membrane vesicles of control and cadmium-intoxicated rats in Na^+- or K^+- containing media lacking αKG. Vesicles containing (in mM) 100 mannitol, 100 KCl, and 20 Hepes/Tris (pH 7.4) were incubated at 25°C in a buffer containing 100 mM NaCl or 100 mM KCl in addition to 50 μM PAH (10 μM ^{14}C-PAH), 100 mM mannitol, and 20 mM Hepes/Tris (pH 7.4). Values are means ± SE of 3 determinations.

basolateral membrane PAH transport is a tertiary active process.

Fig. 3 compares the time courses of PAH uptake by BLMVs of control and cadmium-intoxicated rats. The vesicles were incubated in a Na^+ and αKG containing medium. Clearly, the uptake by cadmium group vesicles was markedly suppressed as compared with that by normal rat vesicles. The patterns of PAH uptake with Na^+ alone or with no Na^+ ($K^+_i = K^+_o$) were not different between the control and cadmium group vesicles (Fig. 4). These results indicate that the Na^+-dependent, αKG-driven PAH transport system was specifically impaired by cadmium intoxication.

Fig. 5A illustrates kinetics of PAH uptake by renal cortical BLMVs. The 5-sec rates of PAH uptake were measured at PAH concentrations of 10 ~400 μM. Preliminary study indicated that the PAH uptake increased almost linearly up to 5 sec. In vesicles of both control and cadmium-intoxicated rats, the uptake in the presence of both Na^+ and αKG increased curvilinearly as the PAH concentration increased, providing an evidence for saturability, but the uptake in the absence of Na^+ (but in the presence of αKG) increased linearly with the substrate concentration. Cadmium intoxication markedly attenuated the saturable component, but it had no effect on the linear component. The Na^+-dependent, αKG-driven PAH uptake was estimated by subtracting the linear component from the saturable component. The Lineweaver-Burk plots of the data (Fig. 5B) indicated that the Vmax, not the Km, was decreased in the vesicles of cadmium-intoxicated animals.

Effect of cadmium on the PAH transport system in normal membrane vesicles

Although the foregoing results indicated that the

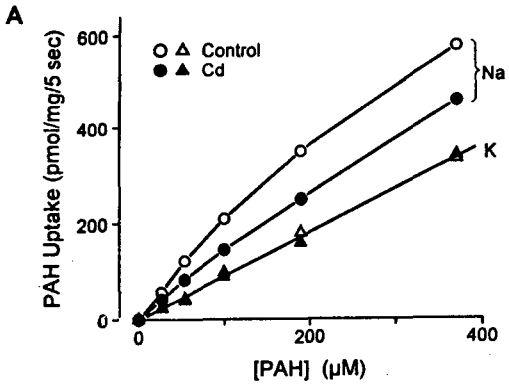

Fig. 5A. Initial (5 sec) rates of PAH uptake by renal cortical basolateral membrane vesicles of control and cadmium-intoxicated rats as a function of PAH concentration in the medium. The uptake was measured in the presence of Na^+ and α-ketoglutarate (10 μM) or K^+ and α-ketoglutarate. Other conditions of incubation are the same as Fig. 3. Data represent mean ± SE of 3 determinations.

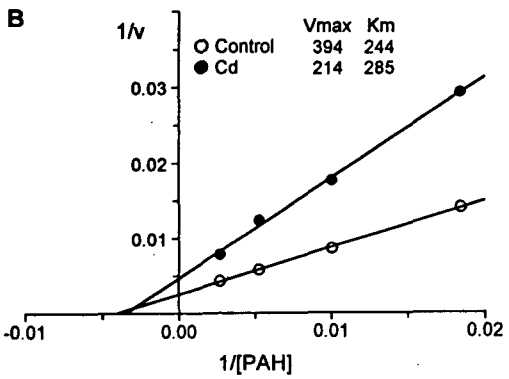

Fig. 5B. Lineweaver-Burk plot of the carrier-mediated PAH uptake. Data are based on Fig. 5A.

capacity for active PAH transport per unit basolateral membrane area was reduced the underlying mechanism was not forthcoming. It could be due to alterations in PAH-dicarboxylate antiporter activity or to changes in Na^+-dicarboxylate cotransporter which provides counter anions to exchange with PAH.

In order to clarify this point and to evaluate direct effect of cadmium on the membrane, we next in-

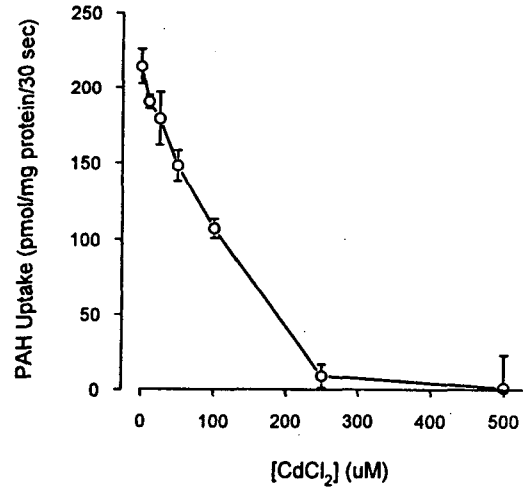

Fig. 6. Na^+ and α-ketoglutarate dependent PAH uptake by renal cortical basolateral membrane vesicles as a function of cadmium concentration in the preincubation medium. Renal BLMVs isolated from normal rats were preincubated in media containing various concentrations of $CdCl_2$ for 30 min at 37°C and then the rates of Na^+ and αKG dependent PAH uptake were determined for 30 sec at 25°C. Other conditions of incubation are the same as Fig. 3. Values are means ± SE of 4 determinations.

vestigated changes in Na^+-dicarboxylate cotransporter and PAH-dicarboxylate antiporter activities in renal BLMVs directly exposed to free cadmium *in vitro*. Renal BLMVs isolated from normal animals were first preincubated in $CdCl_2$ containing medium for 30 min and then tested for transport function.

The first series of experiments was to find out an appropriate concentration of $CdCl_2$ in the preincubation medium. The Na^+-dependent, αKG-driven PAH uptake was determined as a function of $CdCl_2$ concentration in the preincubation medium. As illustrated in Fig. 6, the uptake decreased gradually as the $CdCl_2$ concentration in the preincubation medium increased, showing the half maximal inhibition at about 100 μM. On the basis of these results, the cadmium concentration in the preincubation medium was fixed at 100 μM in all subsequent experiments.

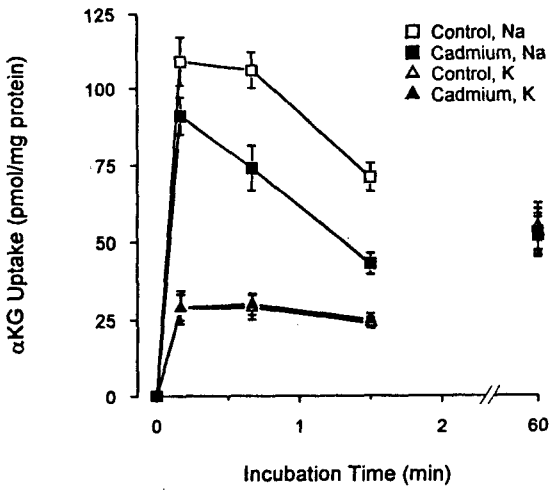

Fig. 7. Time courses of α-ketoglutarate uptake by control and cadmium treated renal basolateral membrane vesicles in the presence and absence of Na^+. Renal cortical BLMVs isolated from normal rats were preincubated in cadmium (100 μM)-containing media for 30 min at 37°C and then αKG uptake was determined during incubation in 100 mM Na^+- or K^+-containing medium at 25°C. The αKG concentration in the medium was 10 μM (with 2 μM ^{14}C-αKG). The mannitol and buffer concentrations in intra- and extra-vesicular media are the same as Fig. 3. Values are means ± SE of 6 determinations.

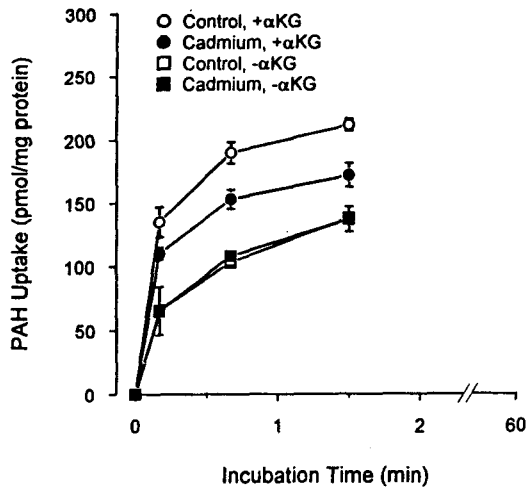

Fig. 8. Time courses of PAH uptake by control and cadmium treated renal basolateral membrane vesicles with and without α-ketoglutarate preloading. Renal cortical BLMVs isolated from normal rats were preincubated in cadmium (100 μM)- or cadmium (100 μM) and αKG (2 mM)-containing media for 30 min at 37°C and then PAH uptake was determined at 25°C. The composition of incubation medium was (in mM) 100 mannitol, 100 KCl, 20 Hepes/Tris (pH 7.4), and 50 μM PAH (10 μM ^{14}C-PAH). Values are means ± SE of 6 determinations.

Fig. 7 shows time courses of αKG uptake in control and cadmium (100 μM)-treated vesicles. In both cases the uptake in Na^+-containing medium revealed an overshoot phenomenon, indicating a Na^+-gradient driven uphill transport. However, the degree of overshoot appeared to be significantly reduced in cadmium-treated vesicles. The αKG uptake in the absence of Na^+ (K^+-containing medium) was not different between the control and cadmium-treated vesicles. These results indicated that the Na^+-dicarboxylate cotransport system in the basolateral membrane was impaired by cadmium treatment.

Fig. 8 summarizes the effect of cadmium treatment on the PAH-αKG exchange activity of the BLMV. PAH uptake was determined in the presence or absence of intravesicular αKG as a counter anion. The PAH uptake in the absence of intravesicular αKG was not different between the control and cadmium-treated vesicles. However, the uptake in the presence of intravesicular αKG appeared to be significantly retarded in cadmium-treated vesicles. These results indicate that the PAH-dicarboxylate exchange mechanism was impaired by cadmium.

DISCUSSION

The secretion of PAH is one of the most sensitively affected renal functions in cadmium-exposed animals (Vander, 1963; Gieske & Foulkes, 1974). Several *in vivo* (Vander, 1963; Nomiyama,

1973) and *in vitro* (kidney slice) (Kim et al, 1988; Park et al, 1988) studies have shown that the renal tubular capacity to transport PAH is reduced after cadmium exposures. The mechanism responsible for this alteration may be multiple.

The capacity of a transport will be determined by the total number of active carriers in the tissue which, in turn, is a function of the total membrane area and the density of carrier in the membrane. An electron microscopic study of Scott et al (1977) revealed a general loss of basal infoldings of proximal tubules in the cadmium-treated rat. Since the carrier systems for PAH secretion reside in the basolateral membrane (Tune et al, 1969; Shimomura et al, 1981), the loss of basal infoldings with a consequent reduction of the basolateral membrane area will limit the number of carriers mediating the PAH transfer.

The capacity of PAH transport in the renal tissue may also be affected by the activity of Na^+-K^+-ATPase. According to a current view (Shimada et al, 1987; Pritchard, 1988; Burckhard & Ullrich, 1989), PAH enters the proximal tubular cell at its peritubular face in exchange for dicarboxylate anion (PAH-Anion antiport), such as glutarate and αKG, which entered the cell by a Na^+-coupled transport (Na^+-dicarboxylate cotransport, "secondary active") at the basolateral membrane. Thus, the PAH entry is a "tertiary active" transport, indirectly coupled with the basolateral Na^+ gradient provided by the Na^+-K^+-ATPase pump ("primary active"). If this were the case, then the peritubular uptake of PAH would be critically dependent on the Na^+-K^+-ATPase activity. Indeed, several studies in isolated renal cortical slices have shown a positive correlation between PAH uptake and Na^+-K^+-ATPase activity at various concentrations of Na^+, K^+ or ouabain (Spencer et al, 1979; Maxild et al, 1981). Moreover, incubations of renal cortical slices in low Na^+ (Gerencser et al, 1973; Misanko et al, 1977) or ouabain (Spencer et al, 1979) containing media, which restrain the Na^+-K^+-ATPase activity, reduce Vmax of PAH uptake. In recent studies, we have observed that both the Na^+-K^+-ATPase activity and the Vmax of PAH uptake are attenuated in renal cortical tissues of cadmium-intoxicated animals (Kim et al, 1988) as well as in tissues directly exposed to cadmium *in vitro* (Park et al, 1988). Thus, the reduction of PAH transport capacity by cadmium in intact tissue could be a consequence of changes in Na^+-K^+-ATPase system as well as geometric changes in renal basolateral membrane.

The results of the present study indicate that an alteration of PAH transport system itself is also involved. In the renal cortical BLMV isolated from cadmium-intoxicated rats, the Na^+-dependent, αKG-driven PAH transport was markedly attenuated (Fig. 3), whereas the Na^+ and αKG-independent component of PAH transport was not significantly changed (Fig. 4). It is, therefore, apparent that the carrier-mediated transport mechanism for PAH was impaired by cadmium exposure. Kinetic analysis (Fig. 5) indicated that the suppression of carrier-mediated PAH transport was due to a reduction in Vmax (capacity) and not Km (substrate affinity). This suggest that the capacity for PAH transport per unit basolateral membrane area was reduced. Such changes in transport capacity could be resulted from a loss of PAH-dicarboxylate antiporter units or from changes in Na^+-dicarboxylate cotransporter which provides counter anions to exchange with PAH. The results of *in vitro* cadmium treatment studies suggested that both the Na^+-αKG cotransporter (Fig. 7) and αKG-PAH exchanger (Fig. 8) activities were impaired by cadmium. The precise mechanism with which cadmium induces these changes remain to be determined.

Finally, crucial to our argument concerning the effect of *in vivo* cadmium treatment on renal membrane function is the effect of cadmium on the membrane integrity. If the membrane was irreversibly damaged, one may not expect any active transport function. However, we have previously observed in rats treated with cadmium as in the present study that various renal functional changes gradually recovered after cessation of cadmium

exposure (Kim et al, 1988). We, therefore, believe that irreversible cell damages did not occur in the present model of cadmium nephrotoxicity.

In conclusion, the present study shows that cadmium intoxication in the rat impairs active transport mechanisms for organic anions in the proximal tubular basolateral membrane and this may be due to a direct alteration of the membrane by free cadmium ions. The precise mechanism by which the membrane is altered is yet to be determined.

ACKNOWLEDGMENT

This work was supported in part by a grant from Korea Science and Engineering Foundation (90-07-00-03). The authors greatly acknowledge Ms Jung Sook Kim for technical helps.

REFERENCES

Burckhard G & Ullrich KJ (1989) Organic anion transport across the contraluminal membrane - Dependence on sodium. *Kidney Int* **36,** 370-377

Gerencser GA, Park YS & Hong SK (1973) Sodium influence upon the transport kinetics of p-aminohippurate in rabbit kidney slices. *Proc Soc Exp Biol Med* **144,** 440-444

Gieske TH & Foulkes EC (1974) Acute effects of cadmium on proximal tubular functions in rabbits. *Toxicol Appl Pharmacol* **27,** 292-299

Hopfer U, Nelson K, Perrotto I & Isselbacher KJ (1973) Glucose transport in isolated brush border membranes from rat small intestine. *J Biol Chem* **248,** 25-32

Jorgensen PL & Skou JC (1971) Purification and characterization of Na^+-K^+-ATPase in preparations from outer medulla of rabbit kidney. *Biochim Biophys Acta* **233,** 366-388

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

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

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

Maxild J, Moller JV & Sheikh I (1981) Involvement of Na^+-K^+-ATPase in p-aminohippurate transport by rabbit kidney tissue. *J Physiol* **315,** 189-201

Misanko BS, Park YS & Solomon S (1977) Effect of hypophysectomy on p-aminohippurate transport kinetics in rat renal cortical slices. *J Endocr* **74,** 121-128

Nomiyama K (1973) Development mechanism and diagnosis of cadmium poisoning. *Kankyo Hoken Report 24.* Japanese Public Health Association, Tokyo, p11-15

Nomiyama K (1978) Experimental studies on animals, in vivo experiments. In: Tsuchiya K (ed) *Cadmium Studies in Japan.* Kodansha Ltd, Tokyo, p45-97

Park YD, Choi JK & Park YS (1988) Effect of cadmium on renal organic anion transport in vitro. *Korean J Physiol* **22,** 55-62

Pritchard JB (1988) Coupled transport of p-aminohippurate by rat kidney basolateral membrane vesicles. *Am J Physiol* **255,** F597-F604

Scalera V, Huang YK, Hildermann B & Murer H (1981) Simple isolation method for basolateral plasma membranes from rat kidney cortex. *Memb Biochem* **4,** 49-61

Scott R, Aughey E & Sinclair J (1977) Histological and ultrastructural changes in rat kidney following cadmium injection. *Urol Res* **5,** 15-20

Shimada H, Moewes B & Burkckhart G (1987) Indirect coupling to Na^+ of p-aminohippuric acid uptake into rat renal basolateral membrane vesicles. *Am J Physiol* **253,** F795-F801

Shimomura A, Chonko AM & Grantham JJ (1981) Basis for heterogeneity of para-aminohippurate secretion in rabbit proximal tubules. *Am J Physiol* **240,** F430-F436

Spencer AM, Sack J & Hong SK (1979) Relationship between PAH transport and Na^+-K^+-ATPase activity in the rabbit kidney. *Am J Physiol* **236,** F126-F130

Tune BM, Burg MB & Patlak CS (1969) Characteristics of p-aminohippurate transport of proximal renal tubules. *Am J Physiol* **217,** 1057-1063

Vander AJ (1963) Effects of zinc, cadmium, and mercury on renal transport systems. *Am J Physiol* **204,** 781-784

Windus DW, Cohn DE, Klahrs & Hammerman MR (1984) Glutamine transport in renal basolateral vesicles from dogs with metabolic acidosis. *Am J Physiol* **246,** F78-F86