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CHANGES IN ORGANIC ACID TRANSPORT SYSTEM IN CADMIUM INTOXICATED RAT KIDNEYS

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= Abstract =

Kinetics of p-aminohippurate (PAH) transport was studied in rats treated with $CdCl_2$ at a subcutaneous injection dose of 2mgCd/kg·day. The Cd-treatment for 4~16 days resulted in a marked reduction of the maximum rate of active PAH influx (Vmax) without any change in substrate affinity of the transport system (Km). The passive influx and the efflux of PAH across the basolateral membrane and the rate of renal tissue oxygen consumption were not apparently attenuated in Cd-treated animals.

These results indicate that the mechanism of impaired renal PAH excretion in Cd-treated animals is a loss of effective organic acid carriers in the basolateral membrane of proximal tubules.

INTRODUCTION

Exposure to inorganic cadmium (Cd) produces proximal tubular nephropathy in laboratory animals[1,2,15] and impaired renal functions in humans[4,9,18]. One of the characteristic functional changes documented in Cd-exposed animals is a reduction of p-aminohippurate (PAH) excretion. Nomiyama et al.[23,24] observed in rabbits that the PAH clearance reduced markedly after acute administrations of $CdCl_2$ (2~12 mg Cd per animal, i.a.) and the Tm_{PAH} decreased gradually during chronic treatment with $CdCl_2$ (0.5~1.5 mg Cd/kg·day s.c.). Although these observations indicate that the renal tubular transport system for the organic acids is impaired by Cd, the underlying mechanism is not clearly elucidated.

We therefore investigated in the present study the kinetic behavior of the organic acid transport system in Cd-treated rat kidneys, using PAH as a model substance.

MATERIALS AND METHODS

Sprague-Dawley male rats (250~300g) were maintained for upto 40 days under standard laboratory conditions with *ad libitum* access to food and water, unless otherwise mandated by experimental protocol.

After 4 days of baseline period the experimental group recieved a daily dose of 2 mg Cd/kg (body wt.) $CdCl_2$ solution and the control group recieved

the same volume of saline over 16 days (treatment period), following which animals were maintained as in the baseline period for 20 days (recovery period). At appropriate intervals rats were killed, and the kidneys were perfused with saline. Kidneys were then immediately removed, decapsulated, and placed in an ice-cold incubation medium (composition in mM:NaCl, 95; Na-acetate, 5; KCl, 10; $CaCl_2$, 1.5; Tris-HCl, 40, pH 7.6 at 25℃). Renal cortical slices approximately 0.5 mm thick were cut using a Stadie-Rigger tissue slicer and placed in the above medium.

In the influx studies, approximately 100 mg slices were transfered to an incubation vessel containing 9 ml of incubation medium saturated with oxygen and equilibrated at 25℃. After 15 min of preincubation, 1 ml of appropriate PAH stock solution was added and incubation was carried out for a 15 min period. To estimate passive uptake, one series of vessels contained 1 mM iodoacetic acid and was gassed with nitrogen. Active uptake into the cell was computed by subtracting the uptake value of the metabolically inhibited slices from that of the uninhibited slices. Upon completion of incubation, slices were removed from the medium, blotted on filter paper and weighted. The tissue was then placed in distilled water overnight to leach out PAH from the tissue. PAH concentration was determined on both the leaching and final incubation media by the method of Smith et al.[35].

In the efflux studies, slices were first loaded with PAH by incubating them in a medium containing 1 mM PAH for 60 min. Slices were removed, quickly rinsed, blotted and transferred to vessels containing 10 ml PAH-free medium. The medium included 1 mM iodoacetic acid and was gassed with nitrogen in order to eliminate reuptakes of PAH effluxed into the medium. At 5 min intervals, aliquots of the medium were removed and replaced by fresh PAH-free medium. At the end of a 20 min period the slices were removed, rinsed, blotted, and placed in distilled water to leach out PAH. PAH concentrations in the leaching solution and medium samples were determined as described above. The total PAH effluxed into the medium and the PAH remaining in the tissue were combined to determine the initial amount of PAH in the slices. The percentage of PAH remaining in the tissue at the end of each efflux period was plotted as a function of efflux time using a semi-logarithmic plot The slope of this line represents the efflux rate constant.

In some experiments, the rate of oxygen consumption of slices was determined using a polarographic oxygen monitor system (Yellow Springs Instrument, Model 53) Approximately 20 mg slices were placed in a reaction chamber containing 2.5 ml of incubation medium saturated with air at 37℃

After 1 min of equilibration, changes in Po_2 in the medium was measured with a Clark-type oxygen electrode (Yellow Springs Instrument, Model 5331) and recorded on a potentiometric recorder (Kipp & Zonen, Model BD40). From the initial slope of Po_2 vs. time curve the rate of oxygen consumption (Qo_2, μmoles o_2/min·100g wet tissue) was calculated.

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

RESULTS

Previous studies using rat renal slices[19] have indicated that the initial velocity of PAH uptake can be obtained anytime between 0 to 30 min period. Thus, in the present study we measured PAH accumlation during 15 min incubation for kinetic analysis of PAH influx.

Fig. 1 depicts the effect of Cd-treatment on PAH influx. Renal cortical slices of rats treated with

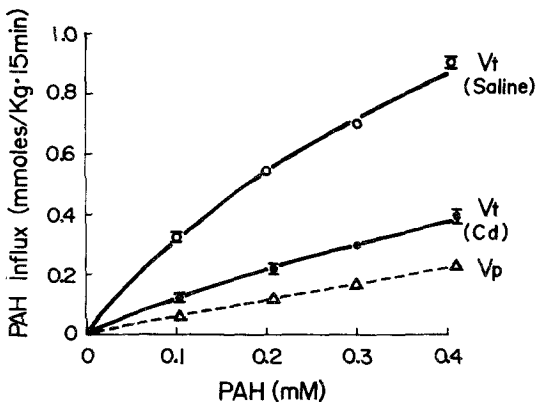

Fig. 1. Influx of PAH into rat renal cortical slices as a function of final medium concent ration of PAH. Vt and Vp represent the total and passive influx of PAH. Data represent the mean± SE of 3 rats treated with saline or with $CdCl_2$ (2 mg/kg·*day) for 16 days.*

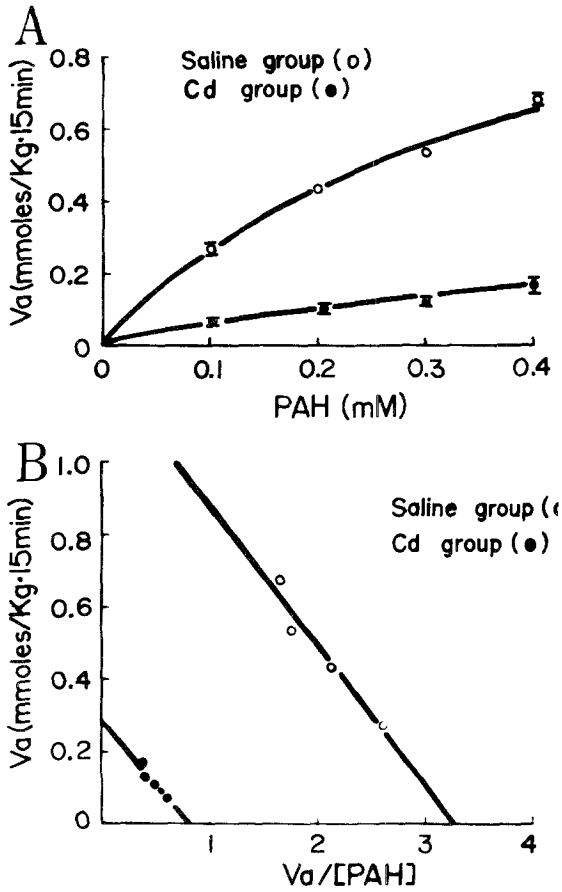

Fig. 2. Hofstee plot of active PAH influx (Va) in saline- and Cd-treated rat renal slices. In this plot, intercept of a line with y-axis represents Vmax and the slope represents -Km.

saline (control) or with Cd (2 mg/kg·day) for 16 days were incubated in media containing various concentrations of PAH. Experiments were carried out with or without addition of iodoacetate (1 mM) and nitrogen. In both control and Cd-treated animals, the total influx (i.e., active+passive influx) increased curvilinearly as the medium concentration of PAH increased, although the value at a given PAH concentration was much higher in the former than in the latter. On the other hand, the passive influx, measured in metabolically poisoned slices, increased linearly with the medium concentration of PAH, and showed an identical slope in the two groups.

Fig 2A illustrates the active influx (Va), computed by subtracting the passive influx from the total influx in each group. Hofstee plot of the data (Fig 2B) shows that the relationship between Va and Va/[PAH] was linear in both control and Cd-treated animals This indicates that in either cases the active influx follows a simple Michaelis-Menten Kinetics, i.e.,

$Va = Vmax \cdot [PAH]/(Km + [PAH])$, where Vmax is the maximal influx (i.e., capacity of influx), Km is the [PAH] for Vmax/2 Thus, the total influx (Vt) can be expressed as:

$Vt = Vmax \cdot [PAH]/(Km + [PAH] + D \cdot [PAH])$,

where D is the coefficient for passive influx (slope of the dashed line in Fig 1). As described above, D was not changed by Cd-treatment. Thus, any change in PAH influx in Cd-treated animals must

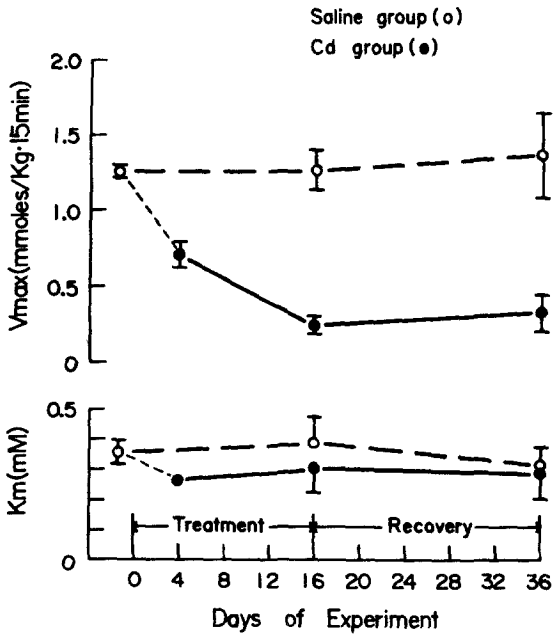

Fig. 3. Changes in Vmax and Km for PAH influx into renal cortical slices during and after Cd-treatment. Each point and vertical bar represents the mean±SE of 3 rats.

be due to alternation in Vmax and/or Km.

Fig. 3 summarizes values of Vmax and Km obtained in the control and Cd-treated groups at various intervals during the course of experiment. As is seen, the Vmax in the control group did not change throughout the experimental period, whereas that in the Cd-group declined gradually to about 20% of the baseline value at the end of 16 days of Cd-treatment and remained unchanged during the following 20 days of recovery period. However, the value of Km appeared to be not significantly altered in the Cd-group as well as control group during the entire experimental period.

To determine whether the reduction of PAH uptake in Cd-treated animals was a consequence of a depressed tissue respiration, oxygen consumption of renal slices was determined at the end of treatment. The results, however, indicated that there was no apparent difference in the tissue oxygen consumption between the control (146±9 μmoles O_2/min·100g) and Cd-treated (123±9) rats (Table 1).

Table 1. Oxygen Consumption of Renal Cortical Slices.

Group	Qo_2 (μmoles O_2/100gm·min)	p
Saline	146.3±9.4	> 0.05
Cadmium	122.5±8.7	

Values represent mean±SE of 9 determinations in rats treated with saline or $CdCl_2$ (2mg Cd/Kg·day) for 16 days.

In an another series of experiments the effect of Cd-treatment on the PAH efflux from renal cortical slices were investigated. Slices, obtained from rats treated with saline or with Cd for 16 days, were

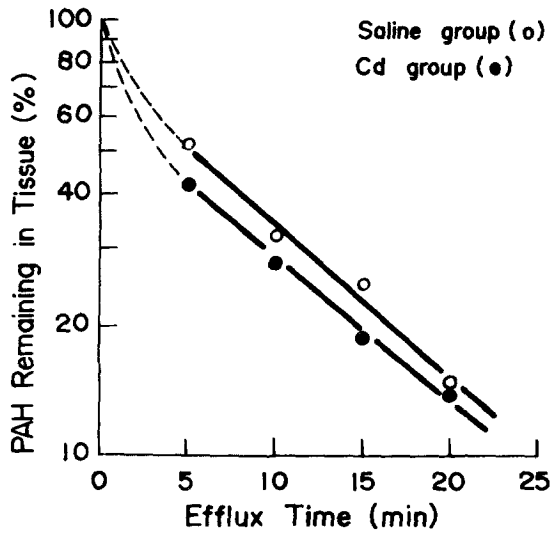

Fig. 4. Efflux of PAH from preloaded kidney slices from rats treated with saline or with Cd (2 mg/kg·day) for 16 days. The efflux was determined in the presence of 1 mM iodoacetate and N_2 bubbling. Data represent the mean±SE of 4 rats in each group.

loaded with PAH by incubating them aerobically in a medium containing 0.1 mM PAH for 60 min and the efflux of PAH into a PAH free medium was determined anaerobically in the presence of 1 mM iodoacetate. As shown in Fig. 4, PAH efflux was not altered by Cd-treatment. The average rate constant of efflux (K) in Cd-treated rats (0.080 ± 0.001 min^{-1}) was of the same magnitude as that in control animals (0.073 ± 0.003 min^{-1}).

DISCUSSION

During chronic exposure, Cd is gradually accumulated in the kidney cortex[17,23]. This accumulation takes place mainly in proximal tubules, because the Cd in plasma is transported bound to metallothionein, a protein ligand for Cd, which is readily filtered through glomeruli and reabsorbed into the proximal tubular cells by endocytosis[6,10,17,26,37]. After entering lysosomes, the Cd-metallothionein complex is catalyzed, liberating free Cd, which in excess amount induces nephrotoxicities[11,17,25]. The mechanisms with which the free Cd alters tubular functions have not been elucidated.

Previous studies[16,23,24] indicated that the ability of renal PAH excretion is reduced in Cd-treated animals. In the present study, we have attempted to identify the mechanism of this reduction. The site of PAH secretion in the mammalian kidney is localized in the proximal tubule[3,7,31,38]. The PAH anion first enters the tubular cell against steep electrical and chemical gradient across the basolateral membrane and then passively diffuses into the lumen across the luminal membrane[39]. Several studies using isolated renal cortical slices[13,27,28,30], proximal tubular fragments[14,33] and perfused proximal tubular segments[34] have provided strong evidence that the basolateral membrane step is a saturation process involving carriers (i.e., the organic acid transport system).

In the present study, the rate of PAH accumulation in the renal cortical slices was significantly reduced in Cd-treated rats (see Fig. 1). Since in the slice preparation tubular lumens are collapsed[8], such a result may indicate that the basolateral membrane step of PAH transport was impaired in Cd-treated animals. In the kinetic analysis, the Km for active PAH influx was similar in the control and Cd-exposed rats (see Figs. 2 and 3), indicating that substrate affinity of the carrier was not changed by Cd-treatment. However, the maximum rate of PAH influx (Vmax) appeared to be significantly attenuated after Cd-treatment (see Figs. 2 and 3) The Vmax is a function of the capacity of the carrier system (i.e., the number of transport site) and the proportion of the adsorbed molecules which dissociate in the forward direction per unit time[21]. Since the Km was not changed, it is unlikely that carrier-substrate dissociation was retarded in Cd-treated rats. The more likely reason is a decrease in carriers.

The number of carriers per unit mass of tissue will be determined by the total area of basolateral membranes and the density of carrier in the membrane. Some histological observations indicated that the area of basolateral membrane is declined in the kidney of Cd-treated animals. For instance, an electronmicroscopic study of Scott et al.[32] revealed general loss of basal infoldings in proximal tubules of the Cd-treated rat. If a similar change occurred in the present study, it would attribute to the reduction of Vmax. However, the efflux data suggest that loss of basolateral membrane, if anything, was not significant The rate constant of PAH efflux in metabolically poisoned slices, which may be a function of the basolateral membrane area, was of the same magnitude in the control and the Cd-treated rats (see Fig. 4). In this connection, it is important to point out that a similar change in

PAH transport kinetics as observed in the present study has also been seen in a study of normal kidney slices incubated in a Cd-containing medium. In the latter study the Vmax of PAH influx was significantly attenuated without Km change by the Cd (1 mM) in the incubation medium (unpublished data by authors). Since it seems unlikely that the geometric area of basolateral membrane is quickly changed by Cd, we speculate that the major effect of Cd in both acute and chronic exposures is not on the area but on the carrier density in basolateral membranes of the proximal tubule It may be that Cd inactivates or mobilizes certain fraction of carriers through its binding to the membrane.

Another reason which may account for the reduction of Vmax is a change in the Na-K-ATPase system. The Na-K-ATPase activity of renal cortical microsomes was significantly inhibited in Cd-exposed animals in the present study (data are not shown). Several studies in isolated renal cortical slices have suggested a functional link between PAH transport and Na-K-ATPase activity. The incubation of slices in low Na^+ or ouabain-containing media (conditions which restrains Na-K-ATPase activity) results in a decrease in the Vmax of PAH influx without change in the Km[13,19,36]. The ouabain inhibition of PAH uptake could be reversed by increasing medium K^+ levels[5,29]. There is a positive correlation between PAH uptake and Na-K-ATPase activity at various concentrations of Na^+, K^+ or ouabain[20,36] The precise role of the enzyme in supporting transport of PAH has not identified. It has been postulated that the Na-K-ATPase activity directly energizes the transport system[12] or indirectly stimulates the transport by providing a Na-gradient[29]. Regardless of the mechanism, the effect of Cd inhibition of Na-K-ATPase on active PAH transport must be mediated through a change in effective carrier density in the basolateral membrane.

Crucial to our argument concerning the effects of the Cd-treatment on active PAH transport is also effects played by Cd on tissue metabolism. Electronmicroscopies of the kidney in Cd-exposed animals[22] have disclosed marked swellings of mitochondria in the proximal tubular epithelium. Whether a similar change occurred in Cd-treated rats in the present study is not known, However, an equal rate of renal tissue oxygen consumption in the control and Cd-treated rats (see results) suggest strongly that the Cd-treatment did not impair the energy-producing catabolism linked to active transport of PAH

The recovery of PAH transport capacity in Cd-exposed animals was insignificant during 20 days after exposure ceased (see Fig. 3). Other functional changes, such as polyuria, proteinuria and osmotic diuresis, were significantly reversed during this period (unpublished data by authors). Why the damage in PAH transport system is not readily reversible is not certain. In any event, this fact emphasizes that the PAH clearance and the Tm_{PAH} can not be used as measures of the renal plasma flow and the functional tissue mass if an animal has been exposed to a toxic level of Cd.

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