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SPECIFICITY AND METAL SENSITIVITY OF RENAL AMINO ACID TRANSPORT

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SUMMARY

1. This study describes some properties of amino acid transport systems at the peritubular cell membrane in rabbit kidneys, and compares them with characteristics of amino acid uptake at the luminal membrane.

2. Mutual inhibition studies led to the conclusion that at least four separate systems are involved in transport of L-amino acids at the peritubular membrane. These four systems, defined on the basis of their substrate specificities mediate uptake, respectively, of (a) dicarboxylic amino acids, (b) phenylalanine, (c) alanine, serine, cysteine, glycine, and (d) basic amino acids.

3. In their structural specificity peritubular systems resemble those responsible for amino acid reabsorption at the luminal cell membrane. In contrast to luminal transport of dicarboxylic compounds, however, their peritubular uptake is inhibited by low concentrations of Hg; movement of other amino acids is not affected. Similarly, a peritubular action of Cd was observed, specific for aspartic and glutamic acids.

INTRODUCTION

Renal reabsorption of filtered amino acids is well known to be mediated by several structure-specific transport systems[1], localized on the cell membrane facing the tubular lumen (luminal membrane)[2]. It is their inhibition which leads to the aminoaciduria of heavy-metal poisoning. Amino acid transport across the membrane separating tubular cells from the peritubular interstitium (peritubular membrane) has also been reported[3,4]. The present paper further describes properties of these peritubular processes. It will be shown that at least four carrier systems must be invoked, differing in their substrate specificities and their sensitivity to inhibition by compounds of Cd and of Hg.

METHODS

As in previous reports[5] rabbits (New Zealand white males, body weight 2–3 kg) were prepared for these studies by creation of an aortic pocket at the level of the left renal artery. The aorta below the renal artery, as well as the right kidney and other vessels were tied off. Renal venous effluent was collected through an

indwelling catheter into a venous reservoir from which it could be returned to the jugular vein. Renal blood flow in this preparation ranged from 20 to 30 ml/min. In experiments involving injection of Cd and Hg, compounds which may alter renal resistance, total renal blood flow was kept constant by cannulation of the left renal artery and insertion of a finger pump between aorta and kidney[2]; renal blood flow in these animals averaged 10–12 ml/min. Infusions, isotope injections and analytical techniques have all been previously described[2,3]. In competition experiments the carrier acid was added to a final concentration of 1 M to the 0.15-ml tracer bolus containing ^{14}C-labeled amino acid (2 μCi) and [^{3}H]methoxy-inulin (30 μCi). Unless otherwise indicated all amino acids were of the L configuration.

Uptake of a test substance at the peritubular cell membrane was quantitated by measurement of the area between its recovery curve and that for inulin. Inulin here served as reference substance and its recovery was equated to 100%. Control activity, *i.e.* the area between the recovery curves before injection of inhibitors was then set at 100, and inhibition of uptake could thereupon be expressed in terms of the diminution of that area.

RESULTS

Interaction between amino acids at peritubular membrane

The fact that transport of aspartate across peritubular membrane is inhibited by excess glutamate but is insensitive to lysine (in the same concentration as glutamate) has previously provided evidence for structural specificity of aspartate transport[3]. Results shown in Figs 1–3 confirm and extend these earlier findings. In Fig. 1a the large area between the recovery curve for inulin and that for alanine

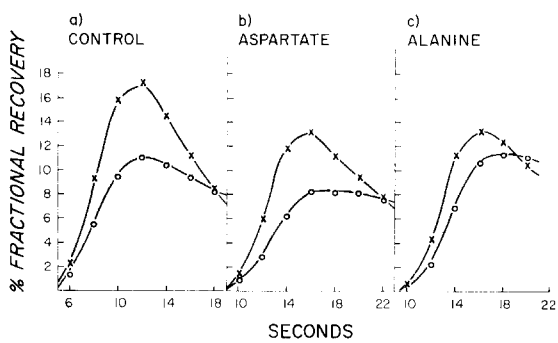

Fig. 1. Peritubular uptake of alanine. Recovery curves are shown for inulin (×—×) and alanine (○--○).

reflects the extensive uptake of alanine across, or at least binding of alanine to the peritubular cell membrane. Similar uptake was observed with a variety of other amino acids including glycine, leucine, methionine, D- and L-tryptophan and serine. Reaction with α-aminoisobutyrate is small[6]. Fig. 1b shows that the uptake of alanine is little altered by the inclusion of 1 M aspartate in the injection of tracer alanine. By contrast, in Fig. 1c the area between the curves was reduced to 55% of that in the aspartate experiment (Fig. 1b) following the addition of 1 M unlabeled

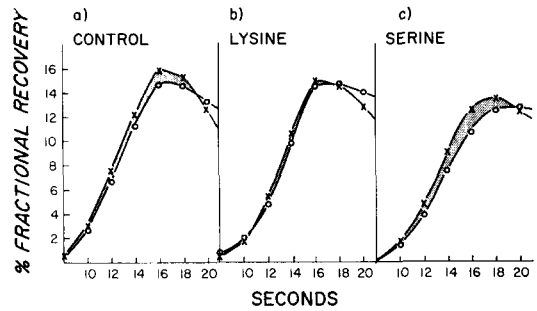

Fig. 2. Peritubular uptake of arginine. Recovery curves are shown for inulin (×—×) and arginine (○—○).

alanine. Similarly, Fig. 2 shows that lysine essentially abolishes disappearance of arginine, whereas equimolar amounts of serine remain without effect. The relatively low uptake of arginine (and lysine) compared to that of *e.g.* alanine or aspartate was consistently observed and is further detailed in Tables II and III. Unlike alanine and arginine, phenylalanine does not appear to cross-react with other amino acids tested (see Fig. 3).

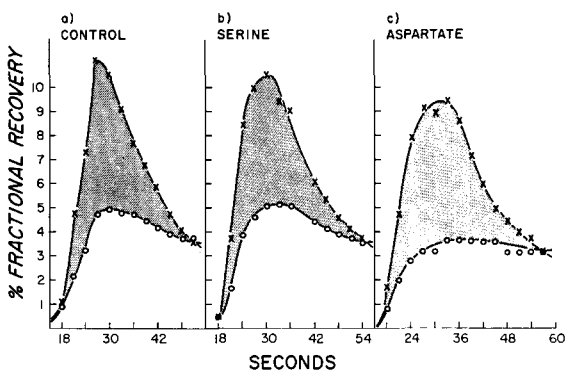

Fig. 3. Peritubular uptake of phenylalanine. Recovery curves are shown for inulin (×—×) and phenylalanine (○—○).

Figs 1–3 illustrate representative experiments on interaction between various L-amino acids at the peritubular membrane. A summary of all experiments performed to characterize this interaction is presented in Table I. No interaction between dicarboxylic acids and alanine or serine was noted in five studies. Similarly four experiments each failed to show any interaction between dicarboxylic compounds and basic amino acids and phenylalanine, respectively. Serine and alanine did not affect reaction of basic amino acids with the peritubular membrane in two experiments, and four studies failed to reveal any interaction between phenylalanine and serine or alanine. In three further studies serine uptake was little altered by D-alanine at concentrations at which L-alanine caused very strong inhibition.

Effects of heavy metals

We have previously reported that a variety of compounds of Hg including

TABLE I

INTERACTION BETWEEN AMINO ACIDS AT THE PERITUBULAR MEMBRANE

Carrier amino acid was added to a final concentration of 1 M to tracer bolus; number of experiments shown in parentheses.

Tracer	*Uptake*	
	Depressed by	*Not depressed by*
Alanine	Serine (1)	Aspartate (3)
	Alanine (1)	Lysine (1)
Phenylalanine		Alanine (3)
		Serine (1)
		Aspartate (3)
		Glutamate (1)
Aspartate	Glutamate (4)	Lysine (4)
		Serine (1)
Arginine	Lysine (2)	Serine (1)
Serine	Glycine (2)	Aspartate (1)
	Cysteine (2)	D-Alanine (3)
	Alanine (2)	

TABLE II

EFFECT OF *p*-CHLOROMERCURIBENZOATE ON PERITUBULAR AMINO ACID TRANSPORT

Percent inhibition refers to diminution of area between recovery curves of inulin and tracer amino acid as in Fig. 4; *p*-chlomercuribenzoate was administered intraarterially 20 min before second tracer injection. Dose/kg 50 μmoles.

Expt. No.	*Amino acid*	E_{PAH}	*Loss of* ^{14}C		
		%	%	Δ	*Inhibition* (%)
Acidic compounds					
1	Glutamate	89→	21.4→ 0	−21.4	100
2	Glutamate	92→84	36.7→13.8	−22.9	62
3	Glutamate	84→79	33.9→16.3	−17.6	52
4	Aspartate	91→89	61.6→44.2	−17.4	28
5	Aspartate	88→81	54.5→26.8	−27.7	51
6	Aspartate	91→47	23.5→ 0	−23.5	100
7	Aspartate	87→35	14.2→ 3.9	−10.3	73
8	Aspartate	87→83	40.3→22.7	−17.6	56
9	Aspartate	36→33	7.6→ 1.6	− 6.0	79
			32.6	−18.2	
Neutral compounds					
1	Methionine	97→76	18.8→17.3	− 1.5	8
2	Methionine	91→48	25.6→19.6	− 6.0	23
3	Phenylalanine	96→44	41.8→46.4	+ 4.6	0
4	Phenylalanine	93→84	21.9→26.8	+ 4.9	0
5	Alanine	86→81	10.9→14.1	+ 3.2	0
			23.8	+ 1.0	
Basic compounds					
1	Arginine	81→78	16.9→19.9	+ 3.0	0
2	Arginine	85→21	13.0→ 9.8	− 3.2	25
3	Arginine	87→30	9.5→10.5	+ 1.0	0
4	Arginine	91→33	7.7→ 8.6	+ 0.9	0
			11.8	+ 0.4	

HgCl, neohydrin, *p*-chloromercuribenzoate and methylmercuric chloride all depress uptake of dicarboxylic acids at the peritubular membrane[6]. These findings are now extended to other classes of amino acids, using *p*-chloromercuribenzoate as test compound. Results of these studies are collected in Table II. It will be seen, in agreement with earlier findings, that at the dose level utilized *p*-chloromercuribenzoate consistently inhibited transport of aspartate or glutamate. Under identical conditions, however, little effect on transport of any other amino acid tested could be observed. Note, as pointed out above, the much smaller disappearance of basic compounds than of the other classes of amino acids at the peritubular membrane.

Experiments similar to those described in Table II were further carried out with Cd. This metal was injected intraarterially, in the presence of excess mercaptoethanol, as described elsewhere[7,8]. No uptake of Cd by the kidney, nor any effects on amino acid transport could be observed in the absence of the thiol compound (unpublished experiments). As illustrated in Fig. 4, injection of Cd–mercaptoethanol

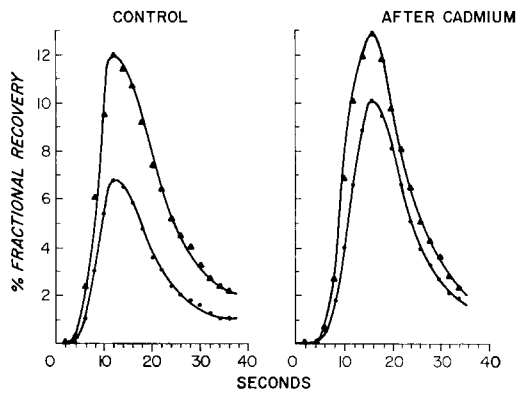

Fig. 4. Effect of Cd on peritubular uptake of glutamic acid. Cd was administered as detailed under Table III. Recovery curves are shown for inulin (▲—▲) and glutamate (●—●).

significantly inhibited uptake of glutamate at peritubular membrane. Table III summarizes other similar studies with Cd and shows by comparison with Table II, that in general the sensitivity of systems responsible for uptake of different classes of amino acids is the same to Cd as to Hg.

The specific inhibition of dicarboxylic acid transport can also be observed in animals which, like those described elsewhere in a report dealing with effects of Cd on renal function[7], had received an injection of Cd *plus* mercaptoethanol 3 days before the experiment. As illustrated in Fig. 5 for one of seven such studies, transport of glutamate was minimal under conditions where phenylalanine uptake at peritubular membrane remained significant. In control animals, the two compounds are transported at similar rates (see Tables II and III).

DISCUSSION

The present paper confirms and extends the previous observation that the peritubular membrane of renal tubular epithelium in the rabbit kidney reacts with a variety of amino acids. On the basis of mutual competition studies and of

TABLE III

EFFECT OF Cd ON PERITUBULAR AMINO ACID TRANSPORT

As for Table II; Cd was administered intraarterially over 1 min immediately after control period, and 20 min before the second study. Dose/kg 2.5 μmoles, *plus* 200 μmoles mercaptoethanol.

Exp. No.	*Amino Acid*	E_{PAH}	*Loss of* ^{14}C		
		%	%	Δ	*Inhibition* (%)
Acidic compounds					
1	Glutamate	93→84	10.8→ 3.8	− 7.0	65
2	Glutamate	91→89	48.1→24.6	−23.5	49
3	Glutamate	88→88	38.6→29.2	− 9.4	24
4	Glutamate	87→90	16.8→ 5.3	−11.5	68
5	Aspartate	96→92	33.1→19.6	−11.5	41
6	Aspartate	61→12	24.3→14.9	− 9.4	39
			28.6		
Neutral compounds					
1	Phenylalanine	—	38.1→38.7	+ 0.6	0
2	Phenylalanine	71→62	26.6→19.6	− 7.0	26
3	Phenylalanine	93→75	27.8→29.6	+ 1.8	0
4	Phenylalanine	53→40	41.9→40.0	− 1.9	5
5	Alanine	32→10	33.9→30.8	− 3.1	9
6	Serine	85→65	28.6→28.0	− 0.6	2
7	Serine	>95→95	36.5→32.9	− 3.6	10
			33.3	− 2.0	
Basic compounds					
1	Arginine	89→89	16.7→13.8	− 2.9	17
2	Arginine	88→88	9.3→ 5.9	− 3.4	37
3	Arginine	96→86	7.1→ 6.2	− 0.9	13
4	Lysine	84→85	0.9→ 9.9	+ 9.0	0
5	Lysine	95→91	5.4→ 6.5	+ 1.1	0
			7.9	+ 0.6	

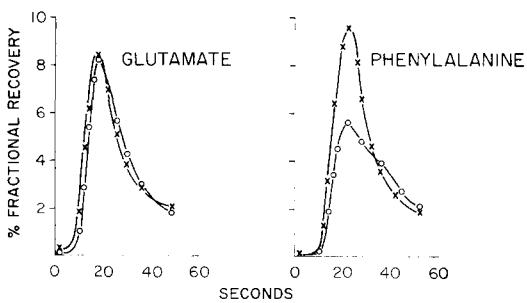

Fig. 5. Specificity of Cd effect on peritubular amino acid transport. In this study, the animal had received 10 μmoles $CdCl_2$ and 350 μmoles mercaptoethanol/kg by intravenous injection 3 days prior to the experiment (see ref. 7). Recovery curves are shown for inulin (×—×) and amino acid (○—○).

inhibition experiments with heavy metals the observed mediation of amino acid transport requires the postulation of at least four distinct carrier systems: The

first of these reacts only with dicarboxylic acids and is the only one found to be sensitive to inhibition by heavy metals under present conditions; a second system shows high affinity for glycine, serine, alanine and cysteine; the third system appears specific for phenylalanine; lastly a fourth system slowly transports basic amino acids.

This classification of amino acid transport systems into four entities is based, admittedly, on analysis of only a limited number of compounds. It is entirely likely that a more extensive investigation might uncover additional structure requirements for substrates of the different systems. Interestingly, however, the pattern of substrate specificity of the peritubular transport systems resembles at least in part that observed at the luminal membrane[1]. This similarity extends to the ability of the peritubular membrane to distinguish between optical isomers. In their sensitivity to heavy metals, by contrast, the peritubular systems differ from those at the luminal membrane. Previous reports described the finding that in amounts which inhibit aspartate transport at the peritubular membrane, *p*-chloromercuribenzoate remains without effect on aspartate reabsorption[3-6]. We have further observed that unlike the relatively specific effect of Cd on the peritubular membrane in animals 3 days after poisoning, reabsorption of all amino acids at the luminal membrane is strongly depressed[7]. The peritubular mechanisms can thus be distinguished from those on the luminal side of tubular epithelium. As pointed out before, peritubular transport accounts for intracellular solute accumulation *in vitro* but plays no role in the process of amino acid reabsorption[3]. The dissociation between cellular accumulation of amino acids and their transepithelial transport has been confirmed in the rat[8]. The physiological significance of these peritubular processes remains unclear.

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