MINIREVIEW

Basolateral Amino Acid Transport in the Kidney (43111)

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The polar nature of epithelial cells in general has long been recognized. Thus, in the early work of Koefoed-Johnsen and Ussing (1) transepithelial sodium transport was attributed to passive apical uptake followed by active extrusion across the basolateral cell membranes. Subsequent work with electrophysiologic techniques, purified membrane vesicles, and other approaches has fully confirmed this basic model. Much of the information on epithelial asymmetry derives from studies on the renal proximal tubule, as repeatedly reviewed (2).

Proximal tubular reabsorption and secretion, responsible for transporting large amounts of many solutes, are processes mediated by various asymmetric carrier systems. For instance, *p*-aminohippurate (PAH) is actively accumulated at the basolateral cell membranes, with a facilitated diffusion step responsible for its further transfer from epithelial cell into the lumen (3). Fractional reabsorption of amino acids is determined by the rate of active uptake at the apical cell membranes (4). Much less attention has been paid to the possible role of basolateral amino acid transport in reabsorption and in cellular nutrition. The present review will focus on the nature and the physiologic significance of basolateral amino acid transport in the proximal renal tubule.

Amino Acid Accumulation in Slices

Following the work of Cross and Taggart (5) on PAH accumulation in slices of the renal cortex, this convenient preparation became popular for studying the renal handling of sugars and amino acids. However, as pointed out by Foulkes (6), the length of the diffusion pathway through partially (or completely) collapsed tubular lumina makes it likely that a major fraction of accumulation in slices results from basolateral uptake, even though the possibility cannot be excluded that a solute could reach the apical membranes by leakage between cells, i.e., along paracellular pathways. It follows in any case that a large if indeterminate fraction of amino acids accumulated by slices during incubation *in vitro* presumably enters cells across their basolateral membranes.

Amino acid uptake by slices exhibits all of the characteristics of active transport processes, as described in a comprehensive review by Segal and Thier (7). Of particular relevance for the present discussion is the clear demonstration that accumulation results from action of carrier systems specific for different classes of amino acids such as dibasic, dicarboxylic, and neutral compounds. The likelihood, as detailed above, that basolateral membranes are to a large extent responsible for amino acid accumulation by slices then leads to the conclusion that specific amino acid carrier systems must be localized on these membranes. In general, as also revealed by *in vivo* studies (see below), the same substrate specificities are seen in basolateral transport of amino acids as in their brush border uptake (8).

Basolateral Amino Acid Uptake *In Vivo*

More direct evidence for basolateral amino acid transport was obtained *in vivo* by application of a double indicator dilution technique (9). In these studies, a bolus containing the test substance in combination with an extracellular and glomerular marker like creatinine was injected into a renal artery in an anesthetized dog; the nonfiltered fraction was recovered from the renal vein before appearance of filtered and reabsorbed solute. In the case of glucose and several other sugars, early venous recovery consistently fell below that of creatinine; apparently, the sugar moves out of the postglomerular extracellular spaces. The process proved structure-specific and sensitive to inhibition by

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phlorizin, and was ascribed to basolateral carrier systems mediating sugar uptake into tubular epithelial cells.

Similar results were subsequently obtained in studies on uptake of nonfiltered amino acids in the rabbit kidney (6). Figure 1, for instance, illustrates the renal artery to vein transit pattern of aspartate. The early venous deficit of the amino acid corresponded to almost 50% of the nonfiltered load (A). The uptake was depressed by another dicarboxylic acid (glutamate, B), but not by a basic amino acid (lysine, C). Finally, the uptake was found to be sensitive to heavy metals, as shown in D by the complete disappearance of the venous deficit in an animal poisoned by a previous injection of 1 μmol of uranyl nitrate/kg body wt. In this and other similar studies, uptake of acidic, neutral, and basic amino acids could thus be clearly separated from each other. The results all point to inhibitor-sensitive and structure-specific carrier systems mediating the renal uptake of amino acids from blood.

More recent studies showed that uptake as described here is a diffusion- or barrier-limited process (10). This is illustrated in Figure 2 by the results of an experiment with the nonmetabolizable amino acid analogue cycloleucine. Under control conditions, with a bolus transit time across the kidneys of 16 sec, as reflected by the mean transit time of inulin, the early venous deficit was small (though significant). In contrast, if the bolus was trapped in the kidney for 40 sec, following transient interruption of circulation by inflation of an aortic balloon from 4 to 44 sec after injection, the early venous deficit approximately doubled. Renal solute transport ability was not appreciably affected by the transient anoxia.

These findings, however, cannot prove that the early venous deficit arises from transfer of the amino acid across the basolateral membrane of tubular epithelium. This assumption had implicitly been made by Silverman *et al.* (9) in their study of renal handling of sugars and by Foulkes (6) in work with amino acids. Justification for the assumption was recently sought on the basis of the following argument. If the large amount of cycloleucine taken up during occlusion (Fig. 2) accumulates in the same cellular pool as does amino acid reabsorbed from the tubular lumen, then its rate of extrusion from this pool should be the same as for reabsorbed amino acid taken up across the brush border.

The time required for nonfiltered cycloleucine taken up during occlusion to return to renal vein plasma (extrusion delay) was measured as recently described (10). In short, an aortic bolus containing cycloleucine and inulin was transiently trapped in a nonfiltering (stop-flow) kidney. After reestablishment of circulation, the time required for cycloleucine, which had been taken up during occlusion, to return to renal

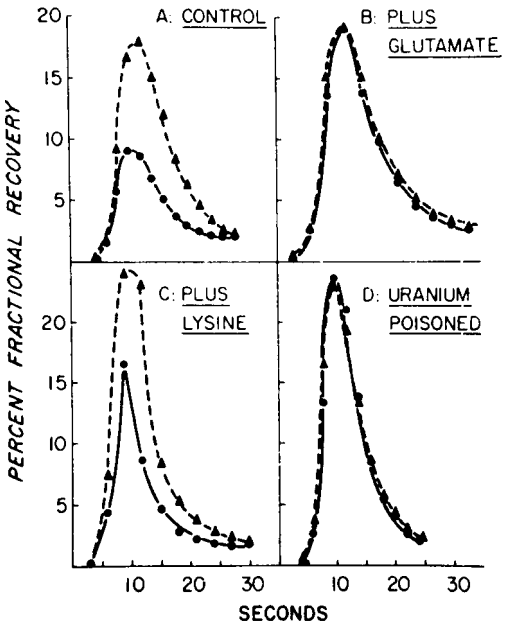

Figure 1. Renal artery to vein transit patterns of aspartate (reproduced from ref. 6, with permission). Renal venous recoveries of ^{14}C (●) and ^{3}H (▲) are shown following arterial bolus injection of [^{14}C] aspartate and [^{3}H]-inulin.

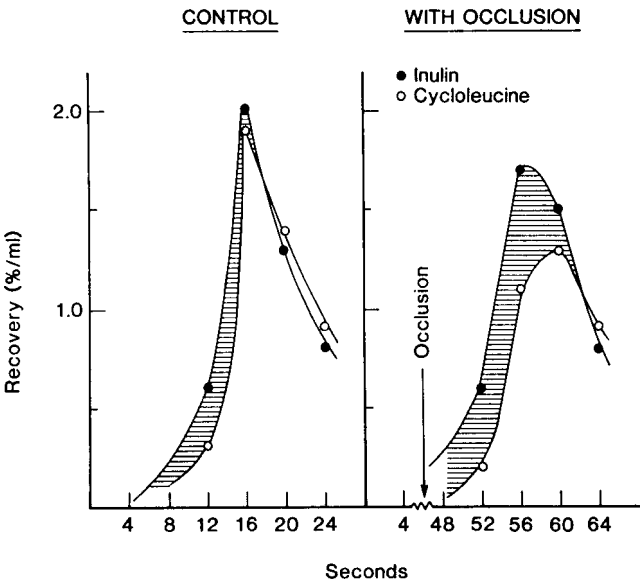

Figure 2. Barrier limitation of basolateral cycloleucine uptake (reproduced from ref. 10, with permission). The peak transit time of the bolus from artery to vein of 16 sec in controls was prolonged by 40-sec transient arterial occlusion. Renal venous recoveries are shown following arterial bolus injection of inulin and cycloleucine.

venous blood was determined. The mean value of the extrusion delay, corrected for the mean transit time of extracellular marker (inulin), found in six studies was 45 ± 6 sec.

The mean transepithelial transit time for cycloleucine reabsorbed from the lumen across the brush border had been found to approximate 40 sec (11), a value no

different from the extrusion delay of cycloleucine entering the tissue from the peritubular side. The logic of Occam's razor forbids the assumption of two separate cycloleucine pools in the renal cortex, each possessing the same kinetic properties. We are left with the conclusion, therefore, that the early venous deficit of the amino acid results from uptake into the same cellular pool as the one through which reabsorbed amino acid must cross on its way from lumen to peritubular interstitium. In other words, the deficit reflects basolateral uptake of the amino acid.

This conclusion is further supported by the observation that during aortic occlusion, a small fraction of cycloleucine accumulated in a nonfiltering kidney can be recovered ahead of inulin from the urine following release of both aortic and ureteral obstruction. One such study is illustrated in Figure 3 (10) and shows a small secretory peak of cycloleucine preceding the appearance of freshly filtered inulin in urine. The peak of net secretion is of course small because fractional reabsorption of cycloleucine from the lumen in the rabbit kidney normally exceeds 99%. Amino acid back-leak into the tubular lumen has been previously considered by several investigators (12).

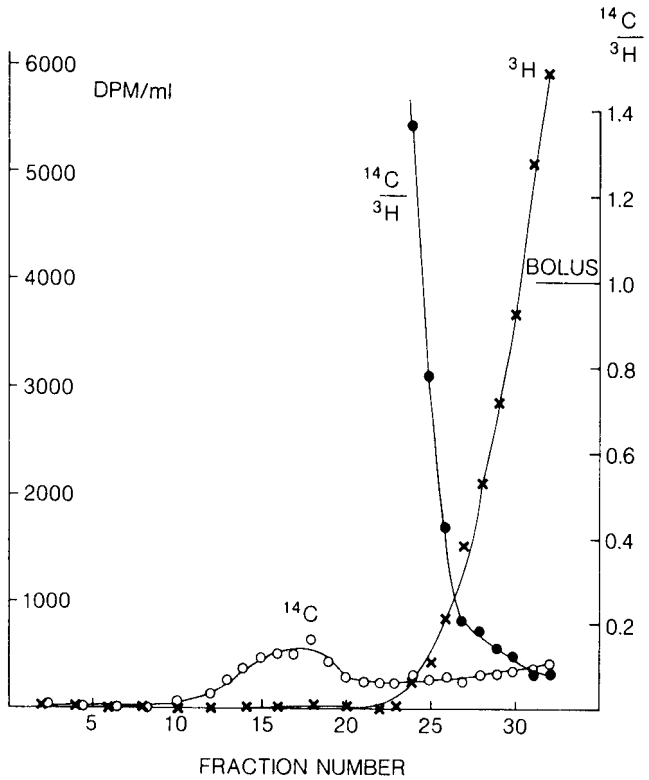

Figure 3. Transtubular movement of cycloleucine during occlusion (reproduced from ref. 10, with permission). A bolus containing [^{14}C] cycloleucine and [^{3}H]inulin was trapped for 40 sec by aortic occlusion in a kidney whose ureter had been clamped for 10 min (stop-flow). Six seconds after reestablishing circulation, the ureteral occlusion was removed and 0.1-ml urine fractions were collected.

Nature of Basolateral Amino Acid Uptake

Besides the fact that basolateral amino acid uptake is relatively structure specific, evidence from several sources indicates that it results from active transport. The most direct support for this conclusion comes from the work of Schafer and Barfuss (12) on the handling of glycine and α-aminoisobutyrate by isolated perfused tubules of the rabbit kidney. Analysis of tissue and surrounding medium showed that movement of the amino acid into the tissue occurred against the concentration gradient. In the rat kidney *in situ*, Samarzija and Fromter (13) observed that pertibular application of glutamate and aspartate leads to depolarization of the tubule cell; apparently, a sodium-dependent uptake mechanism transfers the acids across the basolateral membranes.

Additional direct support for active basolateral amino acid uptake was recently obtained with cycloleucine in the rabbit *in vivo* (14). In these studies labeled cycloleucine was continuously infused into rabbits undergoing mannitol diuresis. When steady-state plasma levels of cycloleucine had been approached, filteration was abolished by bilateral ureteral occlusion. Under those conditions, the intracellular pool of the amino acid should be extruded with a half-time of 40 sec (see above), so that after 10-min occlusion the distribution of cycloleucine between cells and plasma will reflect the steady-state flux balance across the basolateral membrane. Actually, identical results were obtained with free-flow kidneys, showing that the tissue level of the amino acid reflects primarily the flux balance across basolateral membranes. The distribution was expressed in terms of the concentration ratio of cell to plasma and is shown in Table I. The control value of 1.5 significantly exceeded the value of 1.0 which would have been expected for a concentration ratio determined entirely by passive fluxes. This conclusion is validated by the further observation that in the presence of certain inhibitors, the ratio fell to near unity.

The inhibitors shown had been administered for the simultaneous study of PAH accumulation, used as positive control in the analysis of steady-state basolateral accumulation of an actively transported solute.

Table I. Cycloleucine Accumulation in Cortex of Nonfiltering Kidney

	n	Ratio[a]
Control	12	1.5 ± 0.4[b]
+ Probenecid[c]	8	0.9 ± 0.2
+ PAH[c]	7	0.8 ± 0.1

[a] R = (mmol/ml cell H_2O)/(mmol/ml arterial plasma); mean ± SD for number of kidneys.
[b] Significantly higher than unity ($P < 0.001$).
[c] Probenecid (0.25–0.5 mmol) or PAH (1.5 mmol) was injected intravenously or intraarterially 4 min before termination of experiment.

Probenecid is the classical competitive inhibitor for the organic anion transport system responsible for PAH accumulation, and predictably both substances tested reduced ratio for PAH to near unity. In contrast, no action of probenecid and PAH on cycloleucine accumulation had been anticipated. These observations suggested the possibility that cycloleucine might share a common pathway with PAH across the basolateral membrane. On this view, the absence of significant amino acid secretion, so unlike that of PAH, could be readily explained by the existence on the brush border of high affinity reabsorptive systems for amino acids but not PAH; net secretion of amino acids is therefore expected to be quite small at best. The conclusion that the PAH transport system may be involved in cycloleucine uptake recalls an earlier finding that succinate, another inhibitor of PAH transport, and excess PAH itself depress basolateral uptake of acidic amino acids (2, 11). This is illustrated in Figure 4 which shows that injection of PAH reduces the early venous deficit of glutamate but not of lysine. The full involvement of the organic anion transport system in basolateral amino acid transport remains to be elucidated.

Role of Basolateral Transport in Amino Acid Reabsorption

The demonstration that active basolateral transport carries amino acids into tubular cells implies that the flux in the opposite direction, i.e., amino acid extrusion, represents movement down the activity gradient; this raises the question of the role, if any, of basolateral cell membranes in amino acid reabsorption.

Amino acid reabsorption can be described in terms of the two following processes in series (11): a reabsorptive flux J carries the amino acid across the brush border and into a cellular pool S (Step 1); extrusion from S across the basolateral membrane (Step 2) at low concentrations is a first-order process with rate constant k. At steady state, $S = J/k$ or $J \times TET$, where TET stands for the transepithelial transit time. Note that this scheme has all reabsorption passing through S, thus not permitting any paracellular leakage; basolateral uptake and luminal secretion are neglected because they are much smaller than the reabsorptive flux. To normalize values of S and J for differences in plasma concentrations, they are divided by plasma and expressed in ml and ml/min, respectively. The scheme is validated by the good agreement illustrated in Table II (11) between calculated and observed steady-state accumulations of three amino acids in renal cortex.

The following argument leads to the conclusion that it is primarily the rate of extrusion of reabsorbed solute which determines TET for reabsorbed sugars or amino acids. By definition, the 40-sec extrusion delay of cycloleucine taken up by tubule cells during occlusion (see above) must directly reflect its slow transfer across basolateral membranes. The fact that TET for reabsorbed cycloleucine approximately equals this basolateral extrusion delay indicates that TET is primarily determined by Step 2 described above. Such a delaying role of basolateral membranes is seen especially clearly in a comparison of the absorption of glucose and of α-methylglucoside (15). Because of its ready transport across basolateral membranes (9), glucose reabsorption should be characterized by a short TET and correspondingly low S. In contrast, there are no basolateral carriers for the glucoside (6, 9), so that its reabsorption should be characterized by a prolonged TET and large value of S. These predictions were fully confirmed, as shown in Table III. Note that for a similar reabsorptive flux, J, TET and S are around 8-fold larger for the glucoside than for glucose.

The series model similarly predicts that if extrusion results from a mediated process, then inhibitors of this process should increase S and prolong TET. This prediction was confirmed, as shown in Table IV (15), for the presumably competitive serine inhibition of leucine reabsorption and for the effect of mercury on that of

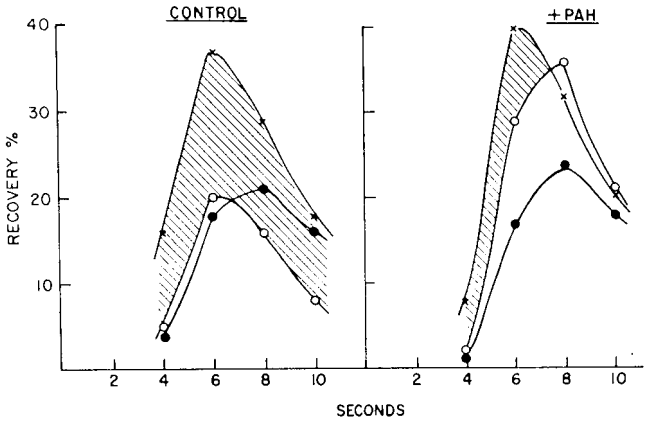

Figure 4. Basolateral interaction of PAH and glutamate (reproduced from ref. 2, with permission). Artery to vein transit curves are shown for inulin (x), ^{3}H (●), and ^{14}C (○) following bolus injection of inulin, [^{3}H] lysine, and [^{14}C]glutamate. PAH (1 mmol/kg) was administered intravenously 30 sec before the bolus.

Table II. Steady-State Cortical Accumulation of Amino Acids[a] (Modified from Ref. 11)

Amino acid	n	S (ml)		
		Observed	Calculated	Observed/ Calculated
Leucine	7	3.8	3.1	1.1 ± 0.3
Cycloleucine	5	3.5	3.3	1.1 ± 0.1
Aspartate	6	2.7	3.0	1.0 ± 0.2

[a] S represents the size of the cellular isotope pool normalized by the plasma concentration. Mean values are shown for observed and calculated sizes of S. The ratio of S (observed)/S(calculated) is expressed as the mean of paired values (±SD) for number (n) of kidneys.

Table III. Comparison of Reabsorption of Glucose and α-Methylglucoside[a] (Modified from Ref. 15)

	J (ml/min)	S (ml)	TET (sec)
Glucose	5.0 ± 0.8	1.6 ± 0.5	15 ± 5
α-Methylglucoside	4.8 ± 1.0	12 ± 3	>120

[a] Results represent mean ± SD. Fractional reabsorption of both compounds equalled 99% of filtered load, so that their reabsorptive flux (J) in ml/min approximated the glomerular filtration rate. TET represents the observed transepithelial transit time.

Table IV. Effects of Inhibitors of Basolateral Extrusion on Cellular Pool of Reabsorbed Amino Acid[a] (Modified from Ref. 15)

	n	J (ml/min)	S (ml)	TET (sec)
Action of serine on leucine accumulation				
Control	7	5.2 ± 1.1	3.8 ± 1.3	44
+ Serine	8	4.8 ± 0.8	6.7 ± 1.7	84
Action of Hg on cycloleucine accumulation				
Control	5	5.0 ± 0.4	3.5 ± 0.2	44
Mercury-treated	5	1.1 ± 0.3	10.5 ± 3.7	590

[a] TET is the calculated value of the transepithelial transit time; J and S, both normalized for plasma concentration, represent mean values for number (*n*) of kidneys ± SD.

cycloleucine. Note, for instance, that even though mercury reduced the reabsorptive flux of cycloleucine to 22% of control, it increased S by a factor of 3.

The fact that mercury inhibits reabsorption, i.e., decreases J, while at the same time increasing the size of S, shows that this and other metals (16) can affect amino acid reabsorption at both brush border and basolateral cell membranes. Fractional reabsorption from the lumen is determined primarily by the active transport process at the brush border (4), but the length of TET depends primarily on function at the basolateral membranes.

The toxicologic implication of depressed basolateral amino acid transport, i.e., of prolongation of TET for amino acid reabsorption, remains unclear. In the rabbit, inhibition of basolateral aspartate transport by *p*-chloromercuribenzoate did not alter fractional reabsorption of filtered aspartate (6), showing that at least at low amino acid concentrations, basolateral transport mechanisms are not essential for reabsorption. It is worth emphasizing that the preferential inhibition of basolateral transport does not necessarily imply that the carrier systems involved are intrinsically more sensitive to the mercurial than those on the brush border membrane. Such a conclusion would be justified only if under the conditions of these experiments, concentrations of inhibitor, substrate, and carriers had been comparable at both membranes; no information is available on this point.

Nature of Basolateral Cadmium Inhibition

Reference was made above to the inhibition of basolateral aspartate transport by mercurials. Similar results have been observed with different metals and various amino acids. Cadmium, for instance, inhibits basolateral uptake of aspartate and glutamate in rabbits (17). Movement of amino acids in the opposite direction, i.e., their extrusion from cell into peritubular interstitium, is also sensitive to metal action, as revealed by the intracellular accumulation of reabsorbed amino acids in poisoned kidneys (15). Surprisingly, however, the steady-state accumulation ratio R (see above) of cycloleucine across basolateral cell membranes was found to be the same in control and in cadmium-exposed rabbits (14), so that active uptake of cycloleucine and its passive extrusion must have been equally affected by cadmium. An explanation suggested for this finding is that the metal here does not exert a cytotoxic effect but acts indirectly on cycloleucine fluxes, perhaps by altering physical variables such as blood flow distribution or cell volume. This view is strengthened by the additional observation that in the nonfiltering kidney the R value for PAH, another solute actively taken up across the basolateral membrane, also remains unaltered following cadmium administration (14).

Nutritive Function of Basolateral Amino Acid Transport

The cells of at least the early portions of the proximal tubule presumably can obtain all of their required amino acids from the glomerular filtrate; this is no longer the case further along the tubule, where the high fractional reabsorption of filtered amino acids may have removed them essentially completely from the lumen. Accordingly, and in common with all other cells, these cells must rely on their ability to extract essential nutrients from blood or interstitial fluid. Not surprisingly, therefore, and in the absence of the apical amino acid transport systems characteristic of proximal tubule cells, slices, for instance, of renal papilla (18) and cerebral cortex (19), as do those of renal cortex, possess the ability to concentrate amino acids. This fact not only raises additional questions about the relevance of amino acid accumulation in renal cortical slices to the process of amino acid reabsorption from the lumen, but also supports the conclusion that active amino acid uptake across basolateral membranes reflects normal cellular function and helps assure an adequate supply of these essential nutrients (8). To what extent such uptake may result in the case of certain amino acids from their affinity for the organic anion transport system remains uncertain.

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