Biochimica et Biophysica Acta 939 (1988) 52–56
Elsevier

BBA 73900

Contraluminal uptake of serine in the proximal nephron

Akira Shimomura [a], Frank A. Carone [a] and Darryl R. Peterson [b]

[a] *Department of Pathology, Northwestern University Medical School, Chicago, IL and* [b] *Department of Physiology and Biophysics, University of Health Sciences / The Chicago Medical School, North Chicago, IL (U.S.A.)*

(Received 30 November 1987)

Key words: Serine incorporation; Proximal tubule; Amino acid transport; (Rabbit kidney)

Rabbit proximal nephron segments were microperfused in vitro to determine whether active contraluminal uptake of serine occurs in the renal proximal tubule during bath-to-lumen transport (influx) of the L- and D-isomers in the convoluted (pars convoluta) and straight (pars recta) segments. It is known that several amino acids are actively reabsorbed in the proximal nephron by a mechanism involving co-transport with sodium at the luminal membrane. There is some evidence that certain amino acids may also be accumulated across the contraluminal membrane by an energy-dependent mechanism, indicating that net reabsorption is the result of two oppositely directed active transport processes. During in vitro microperfusion of rabbit proximal nephron segments in this study, inward movement of L- and D-serine occurred in a bath-to-cell direction against a concentration gradient in the range 305–2735:1, indicating active uptake at the contraluminal membrane. The concentration gradients were maintained during influx of both isomers of serine in the proximal tubule. L-Serine accumulation by tubular cells was similar in the pars convoluta and recta, and significantly greater than that of D-serine, which was the same in both regions of the proximal tubule. The data support the conclusion that renal handling of serine involves active contraluminal uptake of the L- and D-isomers in both regions of the proximal tubule, and suggest that contraluminal events play an important role in renal handling of amino acids.

Introduction

Several studies have shown that amino acids are reabsorbed in the proximal tubule of the mammalian nephron [1–6]. The process involves carrier-mediated active transport at the luminal membrane [4,6]. Inward movement across the apical plasmalemma is Na^+ dependent for most amino acids, occurring via a co-transport mechanism [6–8]. Outward passage across the contraluminal cell membrane is accomplished by passive means along a concentration gradient [6]. Several reabsorptive systems have been described for tubular transport of the various classes of amino acids [8]. A number of studies, mostly indirect, have suggested that contraluminal uptake of amino acids may occur as well [9–17], implying that net reabsorption is the result of active processes moving in opposite directions. Such a process is pertinent to overall handling of amino acids, and may be related to their secretion under pathological conditions [18–21]. The present study was designed to determine directly whether active contraluminal accumulation of serine occurs in the proximal nephron.

Correspondence: D.R. Peterson, Department of Physiology and Biophysics, University of Health Sciences/The Chicago Medical School, 3333 Green Bay Road, North Chicago, IL 60064, U.S.A.

Materials and Methods

Cellular accumulation of radiolabeled serine was observed during bath-to-lumen flux (influx) in the rabbit proximal nephron, to determine whether active contraluminal uptake of serine may occur. Proximal straight kidney tubular segments from female New Zealand white rabbits were microperfused in vitro at 37° C (pH 7.4) by the method of Burg et al. [22]. The perfusate was composed of Krebs-Ringer bicarbonate (115 mM NaCl/5.0 mM KCl/10 mM CH_3COONa/1.2 mM $MgSO_4$/1.2 mM NaH_2PO_4/1.0 mM $CaCl_2$/25 mM $NaHCO_3$). The bathing medium measured approx. 1 ml in volume and consisted of Krebs-Ringer bicarbonate/5.5 mM glucose/bovine serum albumin (6 g/100 ml). Influx was quantified by placing L- or D-[^{3}H]serine ($2 \cdot 10^{-6}$ M; 0.6 μCi/ml and 1.0 μCi/ml, respectively) in the bathing medium of microperfusing nephron segments and measuring the radiolabel appearing in the collection fluid for approx. 1 h. The collection pipet was sealed with Sylgard 184 (Dow Corning Co.) to prevent leakage of bathing medium into the collected fluid. Influx was calculated using the following equation [23]:

$$J_{b\to l} = \frac{C_c}{L \cdot X_b} \cdot \frac{dV_c}{dt} \qquad (1)$$

dV_c/dt is the collection rate (nl/min); C_c is the cpm of ^{3}H in the collection fluid (cpm/nl); L is the length of the tubule in mm; X_b is the cpm of ^{3}H per mol of serine (cpm/mol) in the bath. Measurements were made following a 10 min equilibration time in both proximal convoluted and straight tubular segments, and influx was determined during 60 min of perfusion to substantiate that steady-state conditions had been reached. The collection rate was determined by observing the filling time of a pre-calibrated inner, volumetric collection pipet [24]. Radioactive samples were counted for the ^{3}H label in 15 ml Instagel (Packard). Influx was independent of perfusion rate when measured over 1 h at rates ranging from 3.5 to 40 nl/min.

After 70 min of perfusion, accumulated serine was measured in each tubule segment by quickly washing it in Krebs-Ringer bicarbonate and dissolving the tissue in 100 μl of 3% trichloroacetic acid. Washing time was always less than 1 min. Accumulation of radiolabeled serine was expressed in terms of cell water. This value was estimated by measuring the cell volume from photographs of the perfusing tubular segment and converting to the corresponding volume of water according to the following formula [1]

$$V = 0.7(r_o^2 - r_i^2)\pi L \qquad (2)$$

V is the volume of cell water; r_o and r_i are the outer and inner radii of the perfusing nephron segment; L is the tubular length, which averaged about 1 mm. 0.7 is a correction factor for intracellular water [25]. Such a method has been shown to closely approximate the actual value for cell water, determined by direct techniques [1,26]. [^{14}C]Inulin was always included in the bathing medium during measurement of influx, and cellular accumulation of [^{3}H]serine was corrected for the inulin space. Preliminary studies with non-perfused proximal straight nephron segments revealed that radiolabeled L-serine in the bath ($2 \cdot 10^{-6}$ M) equilibrates with the intracellular fluid within 40 min (Fig. 1). Since the tubule lumena were collapsed, it may be assumed that uptake occurred primarily across the contraluminal membrane. A slightly modified bath was used composed of: 125 mM NaCl/5 mM KCl/1.2 mM $MgSO_4$/1.2 mM KH_2PO_4/1.0 mM $CaCl_2$/25 mM Tris-HCl (pH 7.4)/5.5 mM glucose/bovine serum albumin (6 g/100 ml). Previous studies

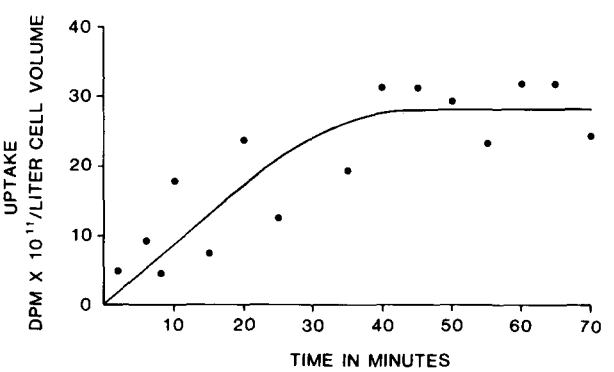

Fig. 1. Uptake of L-[^{3}H]serine ($2 \cdot 10^{-6}$ M) by non-perfused proximal straight nephron segments plotted as a function of time during 70 min of incubation. Equilibrium conditions were achieved within 40 min.

with glycine suggest that relatively little [^{3}H]serine would be metabolized by the tubule under the conditions of the experiments in this investigation [1,27,28]. To test this directly, 17 non-perfused proximal straight nephron segments were incubated with L-[^{3}H]serine ($2 \cdot 10^{-6}$ M) in the above medium for 70 min, after which the segments were washed in buffer and extracted in boiling water. Using thin-layer chromatography (cellulose) and a pyridine/water solvent (10 : 3, v/v), the extracted radiolabeled material was found to contain 0% glycine, 94% serine and 6% other.

To determine whether sodium affects contraluminal uptake of serine in the proximal tubule, proximal straight nephron segments were incubated with L-[^{3}H]serine ($2 \cdot 10^{-6}$ M) for 70 min at 37° C in the presence of a normal, sodium-containing medium (i.e., the same as that used for the equilibration study, containing 125 mM NaCl) and in a sodium-deficient medium (i.e., the same as above, but less than 6 mM NaCl). Uptake was expressed as dpm per liter of cell volume, by the methods described earlier.

Finally, to test whether non-specific binding contributes significantly to serine uptake, proximal straight nephron segments were momentarily exposed to L-[^{3}H]serine ($2 \cdot 10^{-6}$ M) in a cold (0–4° C) bathing medium and immediately washed, as described above. The bath was identical to that used to establish equilibration time. No binding was observed.

Influx and cellular concentration of serine were compared between groups of nephron segments by the group *t*-test.

Results

Bath-to-lumen flux (influx) of L- and D-serine was measured in proximal straight nephron segments, during approx. 1 h of transport. The concentration of [^{3}H]serine was $2.0 \cdot 10^{-6}$ M in the bathing medium, and influx was measured by counting the label appearing in the collection fluid. The results are compiled in Table I and show that under the experimental conditions used the rate of influx of radiolabeled serine is the same in both segments of the proximal tubule, except for a statistically lower rate for L-serine in the proximal pars recta.

TABLE I

INFLUX OF L- AND D-SERINE

Values are mean ± S.E. with the number of tubules shown in parentheses (10^{-15} mol · min^{-1} · mm^{-1} tubule segment). PCT, pars convoluta tubule; PST, pars recta tubule.

	PCT	PST
L-Serine	5.6 ± 0.5 (8)	3.4 ± 0.3 (9) [a]
D-Serine	6.2 ± 1.2 (8)	6.4 ± 0.7 (7)

[a] Significantly smaller than other values, at $P < 0.01$.

Table II describes the cellular concentrations of serine during influx. Since serine conceivably could enter tubular cells across the luminal membrane following its influx into the luminal compartment, cellular accumulation was measured during microperfusion with radiolabeled serine in the luminal fluid only (efflux), as a control. L- and D-serine were perfused at an initial concentration of $2 \cdot 10^{-6}$ M (3.0 and 5.0 μCi/ml, respectively) under the same experimental conditions as those used for the influx studies. Thus, the average luminal concentration of serine during efflux (lumen-to-bath flux) was always greater than that achieved during influx. The results are reported in Table II. Sequestration due solely to uptake at the contraluminal membrane under these conditions is the difference between both values and is shown in Table III along with the ratio of cell-to-bath concentrations of serine. The data indicate that accumulation of L-serine was similar in the pars convoluta and recta, and significantly greater than

TABLE II

CELLULAR CONCENTRATION OF L- AND D-SERINE

Values mean ± S.E. with the number of tubules in parentheses. (10^{-3} mol · l^{-1} cell water. PCT and PST, as in Table I.

	PCT	PST
During influx		
L-Serine	5.70 ± 0.80 (6) [a]	4.90 ± 1.30 (9) [a]
D-Serine	0.90 ± 0.01 (8)	1.08 ± 0.02 (7)
During efflux		
L-Serine	0.23 ± 0.05 (6)	0.59 ± 0.12 (10)
D-Serine	0.29 ± 0.05 (6)	0.33 ± 0.08 (6)

[a] Significantly greater than D-serine, at $P < 0.01$.

TABLE III

CELLULAR CONCENTRATION OF L- AND D-SERINE DUE TO CONTRALUMINAL UPTAKE

Values are mean ± S.E. with the number of tubules in parentheses (10^{-3} mol·l^{-1} cell water). C_c, concentration in the cell; C_b, concentration in the bath. PCT and PST, as in Table I.

	PCT		PST	
	concn.	C_b/C_c	concn.	C_b/C_c
L-Serine	5.47 ± 0.80 (6) [a]	2735	4.31 ± 1.30 (9) [a]	2155
D-Serine	0.61 ± 0.01 (8)	305	0.75 ± 0.02 (7)	375

[a] Significantly greater than D-serine, at $P < 0.01$.

that of D-serine, which was the same for both regions of the proximal tubule. The net cellular concentration of serine during influx (Table III) increased to 305–2735-times that in the bathing medium ($2 \cdot 10^{-6}$ M).

To examine the effects of sodium on contraluminal uptake of serine by the proximal tubule, L-[^{3}H]serine ($2 \cdot 10^{-6}$ M) was incubated for 70 min at 37°C with non-perfused proximal straight nephron segments in normal or sodium-deficient media, and uptake was determined. Removing most of the sodium from the incubation medium reduced serine uptake by 85% (40 ± 2.8 dpm · 10^{11}/l cell volume compared to 6 ± 1.0 dpm in the absence of Na^+). This difference was significant at the $P < 0.001$ level of confidence.

Discussion

Active uptake of serine occurs across the contraluminal membrane of the proximal tubule. During in vitro microperfusion of proximal convoluted and straight segments of rabbit nephrons, both L- and D-serine entered tubular cells from the bathing medium against a cell-to-bath concentration gradient in the range 305–2735 : 1. Since these values were corrected for possible luminal uptake of serine which might occur during influx, the data show that an energy-dependent mechanism must exist for its uptake across the contraluminal membrane of proximal tubular cells. Early indirect studies implied that peritubular uptake of amino acids may occur in the kidney [9,12–15], but a more definitive approach was necessary. More recently, Barfuss et al. [10] showed active accumulation of glycine from the bathing medium of microperfused rabbit proximal straight, but not convoluted nephron segments. The present study demonstrates that both the pars convoluta and recta possess the ability to sequester actively serine across the contraluminal membrane, and that the tubular segments function similarly to accumulate the respective L- and D-isomers. This was made apparent by reducing the serine concentration to a subphysiological level in the bathing media, which was done to prevent any toxic effects of the D-isomer [29–31]. The observation that L-serine is accumulated to a greater extent than D-serine in both proximal tubular segments is consistent with Silbernagl's proposal [4] that contraluminal uptake of amino acids serves a nutritional function. If this is the case, one might expect selective sequestration of the naturally occurring L-form of amino acids.

The mechanism for active contraluminal uptake of serine in the proximal tubule appears to be dependent upon sodium. Incubation of non-perfused proximal straight nephron segments in normal and sodium-deficient media revealed that the presence of sodium greatly enhanced the cellular uptake of L-serine. Since the lumena of these tubules were collapsed, it is reasonable to assume that little serine may reach the luminal plasmalemma, and thus uptake is primarily a measure of transport across the contraluminal membrane. This is consistent with the observations of Barfuss et al. [10] that sodium stimulates contraluminal uptake of glycine in proximal nephron, and is supported further by the studies of Schwab and Hammerman [32] showing sodium stimulation of glycine transport in basolateral membrane vesicles from the dog kidney. The data contradict the finding of Kragh-Hansen and Sheikh [33,34] that serine uptake by renal cortical basolateral membranes from the rabbit is sodium independent. The negative findings of the latter membrane vesicle study may reflect variability associated with a mixed population of tubular and non-tubular membranes derived from the renal cortex.

Influx of L- and D-serine was observed to accompany their active contraluminal uptake in both segments of the proximal tubule. Whether transepithelial transport occurs by a transcellular or paracellular route in this case cannot be

determined by the present experimental design. Nevertheless, the high intracellular concentrations of serine that were achieved make it likely that some of the amino acid was secreted across the luminal membrane, and that the transcellular pathway made a significant contribution to its movement from bath-to-lumen. Influx may have been underestimated somewhat by back-flow of serine from lumen-to-bath following its transport into the luminal compartment, but the data clearly demonstrate that influx occurred as the cells were maintaining a concentration gradient for both isomers of serine.

This study has shown that both L- and D-serine may be actively transported inwardly across the contraluminal membrane of proximal convoluted and straight segments of the kidney tubule. Such a process is accompanied by influx of serine in the proximal nephron, which becomes a significant factor in characterizing renal handling of this, and presumably other amino acids. These findings emphasize the importance of characterizing contraluminal as well as luminal events in defining the reabsorption and excretion of amino acids.

Acknowledgements

This work was supported in part by USPHS Grants No. AM14610, AM33984, and a grant from the Chicago Heart Association.

References

1 Barfuss, D.W. and J.A. Schafer (1979) Am. J. Physiol. 236, F149–F162.
2 Segal, S. and S.O. Their (1973) in Handbook of Physiology (Orloff, J. and Berliner, R.W., eds.), Vol. 8, Renal Physiology, p. 653, American Physiological Society, Washington D.C.
3 Schafer, J.A. and D.W. Barfuss (1980) Am. J. Physiol. 238, F335–F346.
4 Silbernagl, S., Foulkes, J.A. and P. Deetjen (1975) Rev. Physiol. Biochem. Pharmacol. 74, 105–167.
5 Silbernagl, S. and Völkl, H. (1977) Pflugers Arch. 367, 221–227.
6 Silbernagl, S. (1985) in The Kidney: Physiology and Pathophysiology (Seldin, D.W. and Giebisch, G., eds.), p. 1677, Raven Press, New York.
7 Ullrich, K.J., Rumrich, G. and S. Kloss (1974) Pflugers Arch. 351, 49–60.
8 Ullrich, K.J. (1979) Annu. Rev. Physiol. 41, 181–195.
9 Ausiello, D.A., Segal, S. and Their, S.O. (1972) Am. J. Physiol. 222, 1473–1478.
10 Barfuss, D.W., Mays, J.M. and J.A. Schafer (1980) Am. J. Physiol. 238, F324–F333.
11 Burckhardt, G., Kinne, R., Stange, G. and Murer, H. (1980) Biochim. Biophys. Acta 599, 191–201.
12 Foulkes, E.C. (1971) Biochim. Biophys. Acta 241, 815–822.
13 Foulkes, E.C. (1972) Fed. Proc. 31, 298.
14 Foulkes, E.C. and Gieske, T. (1973) Biochim. Biophys. Acta 318, 439–445.
15 Greth, W.E., Their, S.O. and Segal, S. (1973) J. Clin. Invest. 52, 454–462.
16 Sacktor, B. (1977) Curr. Top. Bioenerg. 6, 39–81.
17 Samarzija, I. and Fromter, E. (1982) Pflugers Arch. 393, 215–221.
18 Crawhall, J.C., Scowen, E.F., Thompson, C.J. and Watts, R.W. (1967) J. Clin. Invest. 46, 1162–1171.
19 Frimpter, G.W., Horwith, M., Furth, E., Fellows, R.E. and Thompson, D.D. (1962) J. Clin. Invest. 42, 281–288.
20 Melancon, S.B., Dallaire, L., Lemieux, B., Robitaille, P. and Potier, M. (1977) J. Pediatr. 91, 422–427.
21 Teijema, H.L., Van Gelderen, H.H., Giesberts, M.A.H., Serena, M.L. and De Angulo, L. (1974) Metabolism 23, 115–123.
22 Burg, M.B., Grantham, J.J., Abramow, M. and Orloff, J. (1966) Am. J. Physiol. 210, 1293–1298.
23 Schafer, J.A., Troutman, S.L. and Andreoli, T.E. (1974) J. Gen. Physiol. 64, 582–607.
24 Chonko, A.M., Irish, J.M. and Welling, D.J. (1978) in Methods in Pharmacology (Martinez-Maldonado, M., ed), p. 221, Plenum Press, New York.
25 Tune, B.M. and Burg, M.B. (1974) Am. J. Physiol. 221, 580–585.
26 Burg, M.B. and Weller, P.F. (1969) Am. J. Physiol. 217, 1053–1056.
27 Hillman, R.E., Albrecht, I. and Rosenberg, L.E. (1968) J. Biol. Chem. 243, 5566–5571.
28 Rosenberg, L.E., Berman, M. and Segal, S. (1963) Biochim. Biophys. Acta 71, 664–675.
29 Carone, F.A. and Ganote, C.E. (1975) Archiv. Pathol. 99, 658–662.
30 Ganote, C.E., Peterson, D.R. and Carone, F.A. (1974) Am. J. Pathol. 77, 269–282.
31 Peterson, D.R. and Carone, F.A. (1979) Anat. Rec. 193, 383–387.
32 Schwab, S.J. and Hammerman, M.R. (1985) Am. J. Physiol. 249, F338–F345.
33 Kragh-Hansen, U. and Sheikh, M.I. (1984) J. Physiol. 354, 55–67.
34 Kragh-Hansen, U. and Sheikh, M.I. (1986) Renal Physiol. 9, 52.