SIMILARITY OF L-PROLINE TRANSPORT SYSTEMS IN KIDNEY OF THE RAT IN-VITRO, AND OF MAN IN-VIVO

F. Mohyuddin and C. R. Scriver

deBelle Laboratory for Biochemical Genetics,
The McGill University-Montreal Children's Hospital Research Institute,
2300 Tupper Street,
Montreal 25, Canada.

Received August 5, 1968

Interpretation of a phenomenon in one species, by extrapolation from presumably relevant data obtained in another, has often been considered inadmissible. Yet, much of the interpretation of certain inherited disorders of amino acid transport in human kidney has depended on information gained from in-vitro experiments with rat kidney cortex slices (Scriver, 1967; Rosenberg, 1967). We have now obtained evidence that quantitative and qualitative characteristics of proline transport by rat kidney in-vitro, correlate closely with the corresponding characteristics documented in the living subject.

Renal tubular absorption of L-proline in man is achieved by more than one mode of uptake (Scriver and Wilson, 1967; Scriver, 1968). Proline shares one transport system with hydroxyproline and glycine. The capacity of this system is large, being about ten times the usual endogenous filtered

load, and uptake on it can be inhibited competitively by the co-substrates. Another system is used for absorption of proline at its usual endogenous concentration; this system has a small capacity and high affinity for its substrate, and it is shared only with hydroxyproline. The two proline transport systems are under separate genetic control.

The characteristics of L-proline uptake by rat kidney cortex slices were evaluated with the techniques described in detail elsewhere (Scriver and Mohyuddin, 1968). In the present series of experiments, the $^{14}CO_2$ evolved from labelled proline was trapped on filter paper soaked with KOH in the centre well of a Warburg flask during the incubation of the slice in Tris-electrolyte buffer (140 mOsm/L). L-proline-^{14}C (ul) (New England Nuclear, specific activity 180 mC/mM), the radiochemical purity of which was verified, was used to trace L-proline uptake and metabolism. The isotope in the medium and that which was liberated from the slice (Scriver and Mohyuddin, 1968) was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 4322, operating at 50 percent efficiency.

Progress curves of uptake into slices of 0.05 mM, 2.0 mM, and 12 mM L-proline, were similar in that more than 90 percent of the steady state was achieved in 30 minutes, at which time the slices had achieved "proline" uptake ratios of 3.6, 2.8, and 2.0 respectively, from the three initial medium concentrations.

When the progress of $^{14}CO_2$ evolution during uptake of L-proline-^{14}C (ul) was also measured, we found that considerably more of the substrate was metabolized at low initial extracellular concentrations than at high concentrations (Table I). One-dimensional chromatography, in butanol, acetic

TABLE I

Distribution of L-proline -^{14}C (ul) after 5 and 30 minutes' incubation of rat kidney cortex slices in Tris-electrolyte buffer (Scriver and Mohyuddin, 1968). $^{14}CO_2$ evolved from the slice was measured by collection on KOH-soaked filter paper in the centre well of the Warburg flask. ^{14}C-metabolites and L-proline left in the slice were estimated by chromatography of the supernatant solution obtained by boiling slice at end of incubation; the chromatogram was then run through a strip-scanner and the total isotope cpm determined at the various positions.

L-proline conc. in medium (mM)	5 minute incubation: percent of total ^{14}C cpm as L-proline	Metabolites	CO_2	30 minute incubation: percent of total ^{14}C cpm as L-proline	Metabolites	CO_2
0.1	59	29	12	34.5	40.5	25
0.8	63.5	27.5	9	37	38	25
2.0	66	30	4	37	40	23
12.0	81	15	4	58	34	8
120.00	96.5	2.5	1	92	6	2

acid, water (12:3:5), of the supernatant fluid obtained after the slice was boiled at the end of incubation, followed by scanning of the isotope distribution on the chromatogram by means of a gas flow strip-scanner, revealed that three ninhydrin-reactive positions on the chromatogram were radioactive; these positions were compatible with glutamic acid, ornithine and proline.

The effect of metabolism of the substrate in the slice on concentration-dependent uptake of L-proline under steady-

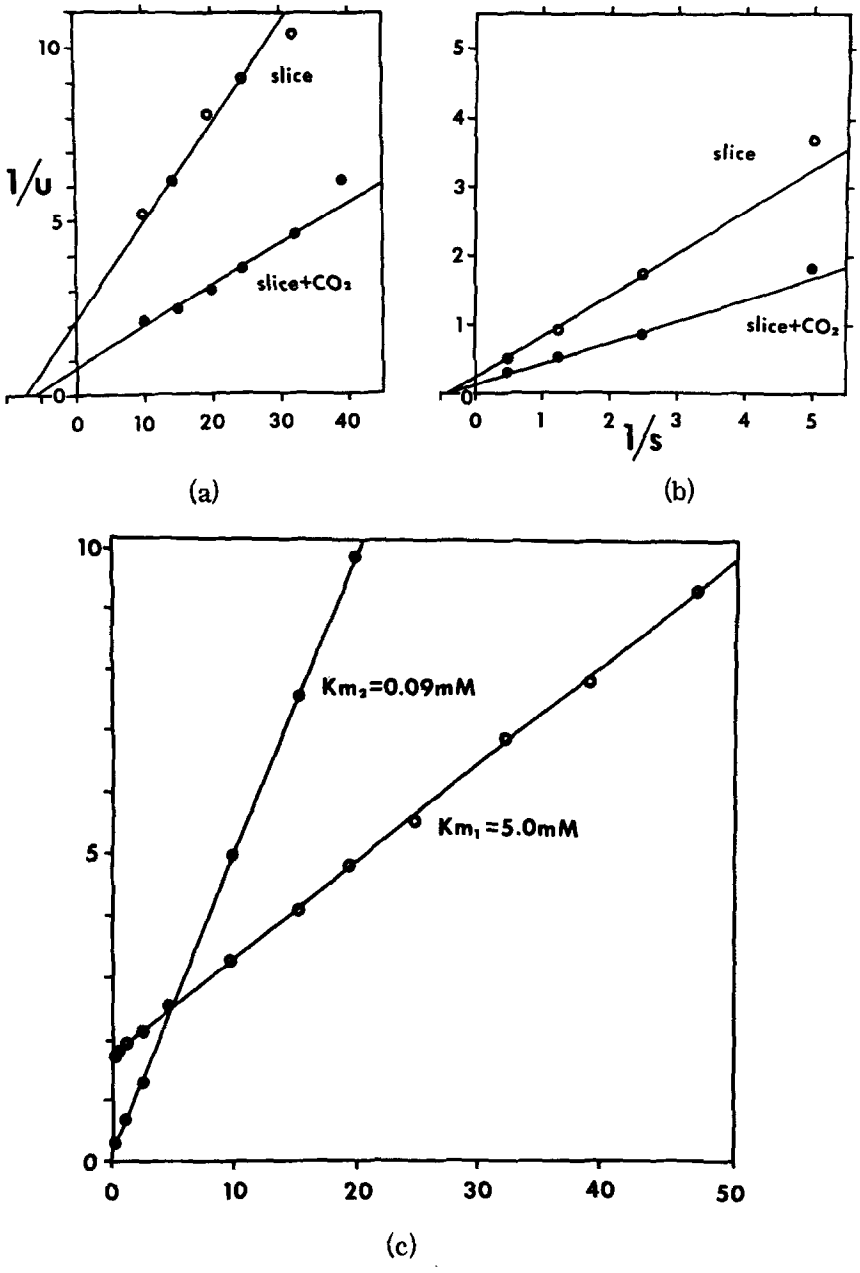

Figure 1: Uptake of L-proline by rat kidney cortex slices after 30 minute incubation at 37°C in Tris-electrolyte buffer pH 7.4; data presented as Lineweaver-Burk transformation of Michaelis equation. a) Uptake at low substrate concentration (≤0.2 mM); open circles show net uptake into slice alone;

solid circles indicate uptake when transfer of label into $^{14}CO_2$ is also accounted for. b) Uptake at substrate concentrations greater than 0.2 mM; symbols same as in part a). c) Plot obtained after numerical analysis, using data in figures 1a and 1b, assuming simultaneous uptake of proline on two systems; open circles indicate distribution of uptake at high concentrations; closed circles represent uptake at low concentrations. At 0.2 mM, uptake is equally divided on "low Km" and "high Km" systems.

---

state conditions was evaluated. Uptake under these conditions was corrected for diffusion (Akedo and Christensen, 1962); the corrected value was then used to obtain the conventional transformations of the Michaelis equation. Double reciprocal plots were drawn from uptake data, which accounted for or excluded the label appearing in CO_2 when the initial concentration of L-proline in the medium varied from 0.026 - 2.0 mM (Figure 1a, 1b). The apparent Km value varied according to the concentration range of substrate employed; at concentrations up to 0.2 mM, the value was about 0.15 mM (Figure 1a), while at concentrations from 0.2 - 12 mM, it was about 2.5 mM (Figure 1b). Metabolism did not significantly alter the first approximation of the Km value (Figures 1a and 1b). Moreover, the Km value at the higher substrate concentrations is practically identical to that determined under quite different conditions in earlier studies of L-proline transport by rat kidney cortex slices (Wilson and Scriver, 1967).

If more than one mode of uptake accounts for L-proline accumulation by rat kidney cortex slices, then at any one concentration, uptake will occur on each system simultaneously,

and the Michaelis constants, as determined by the usual method, will be in error. Therefore, calculation of the actual uptakes and the Km values was undertaken by iterative numerical analysis (Scriver and Mohyuddin, 1968); the data obtained at 15 different substrate concentrations were used in the analysis. The recalculated Km and V.max values for L-proline uptake at external concentrations below 0.2 mM were 0.09 mM and 0.57 μmoles/30 min/ml ICF respectively; at the higher concentration range, the corresponding values were 5.0 and 10.05 respectively (Figure 1c). Thus, at low concentrations (<0.2 mM), proline is accumulated predominantly by the "low Km" system, while the reverse is true for uptake at high concentrations (Figure 1c).

Qualitative characteristics of the two modes of L-proline transport in kidney were investigated by exposure of slices during incubation to chemical inhibitors and other amino acids. Incubation in the presence of ouabain (5×10^{-4}M), cyanide (10^{-2}M), iodoacetamide (10^{-2}M), and choline replacement of sodium (144 mM choline chloride), did not show significant differences between the inhibition of uptake at 0.1 mM and at 3.0 mM L-proline. However, when α-aminoisobutyric acid, hydroxy-L-proline and L-alanine, were added in varying concentrations to the medium, each had distinctly different effects on L-proline transport at high and low concentrations. At concentrations above 0.2 mM, there was strong competitive inhibition of proline uptake by all three amino acids. Earlier work (Wilson and Scriver, 1967) also showed that the imino acids and glycine interact competitively at such concentrations. At substrate concentrations from 0.02 - 0.2 mM, AIB and L-alanine did not inhibit uptake, even when present at

TABLE II

Characteristics of two modes for L-proline uptake by kidney in man and rat.

Feature	Human kidney (in-vivo) [a]	Rat kidney cortex slice [b]
A. Capacity ratio of two systems:		
"High" V.max / "Low" V.max	15	18
B. Km value (mM)		
"Low" system	0.1*	0.09
"High" system	> 1.0*	5.0
C. Specificity		
"Low" system	shared with hydroxy-L-proline	shared with hydroxy-L-proline
"High" system	shared with hydroxy-L-proline and glycine	shared with hydroxy-L-proline and glycine.

a) Described in detail elsewhere (Scriver, 1968).

b) Described in present communication, and previously (Wilson and Scriver, 1967).

* indirect estimates only.

up to 40 times the concentration of the substrate. Hydroxy-L-proline, on the other hand, still inhibited proline uptake competitively at these low concentrations.

L-proline transport by rat kidney in-vitro is thus

characterized by at least two modes which have different kinetics of uptake and different relationships to other amino acids (Table II). Rosenberg's group (Hillman et al, 1968), using an isolated tubule preparation, have also identified more than one mode for proline uptake in kidney. The point of greatest interest, however, is that proline uptake by rat kidney has a striking resemblance to its uptake by the human kidney in-vivo (Table II). This suggests that one could extrapolate from in-vitro data to the relevant situation in man. An obvious example where this would be useful is in the controversial area of dibasic amino acid transport (Schwartzman et al, 1967), which has been studied so intensively in the rat in an attempt to understand human cystinuria (Rosenberg, 1967).

ACKNOWLEDGEMENTS

Mr. David Slaughter kindly performed the numerical analysis at the McGill Computing Centre. This research was supported by grant AM-05117 from the National Institutes of Health, and grant MT-1085 from the Medical Research Council of Canada.

REFERENCES

Akedo, H. and Christensen, H.N.: J. Biol. Chem. 237, 118 (1962).

Hillman, R.E., Albrecht, I. and Rosenberg, L.E.: P. 55, Abst., In Proc. Soc. Ped. Res., Atlantic City (1968).

Rosenberg, L.E.: In Nyhan, W.L., ed., Amino Acid Metabolism and Genetic Variation, New York, McGraw-Hill (1967) p. 341.

Schwartzman, L., Blair, A. and Segal, S.: Biochim. Biophys. Acta 135, 120 (1967).

Scriver, C.R.: In Nyhan, W.L., ed., Amino Acid Metabolism and Genetic Variation, New York, McGraw-Hill (1967) p. 327.

Scriver, C.R.: J. Clin. Invest. 47, 823 (1968).

Scriver, C.R. and Mohyuddin, F.: J. Biol. Chem. 243, 3207 (1968).

Scriver, C.R. and Wilson, O.H.: Science 155, 1428 (1967).

Wilson, O.H. and Scriver, C.R.: Amer. J. Physiol. 213, 185 (1967).