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Proline transport into isolated rat glomeruli

Previous publications from this laboratory have described transport of amino acids by mammalian kidney *in vivo* and *in vitro*. The latter studies[1-3] employed the incubated rat-kidney-cortex slice technique, a procedure which exposes tubules, glomeruli and other cellular components in the slice to the incubation medium. Several investigators have developed new methods to obtain kidney of more specific composition. LOWENSTEIN *et al.*[4] were thus able to study amino acid transport in the rat renal papilla where anaerobic metabolism predominates, while several other groups[5-8] have studied membrane transport in rabbit renal tubules. We have adapted the method of FONG AND DRUMMOND[9] for isolating rat glomeruli to study the specific contribution of glomeruli to amino acid uptake by kidney. We can thus determine whether glomerular cells, which are adapted to function quite differently from tubular cells, exhibit any important differences in their characteristics of membrane transport and whether there is differentiation of membrane transport functions in different types of cells in the same organ.

The kidneys of adult female Long–Evans hooded rats were perfused to remove the blood; glomeruli were then obtained by mincing and sieving the tissue[9]. Cold oxygenated Tris buffer (pH 7.4, 300 mosM) was substituted for the Krebs–Ringer phosphate buffer, previously recommended for the washing and suspension of glomeruli. The preparations of glomeruli were over 90 % pure, the yield being about 8 mg wet tissue per rat. This amount of glomeruli was incubated in 2 ml of Tris buffer containing 2.8 mM glucose; the ^{14}C-labeled solute and unlabeled carrier were added to the initial medium in 25-ml erlenmeyer flasks and incubation was performed at 37° in a Dubnoff incubator oscillating at 300 strokes/min to keep the glomeruli in suspension.

Glomeruli were recovered from the incubation medium (1 ml) by capture on weighed millipore filters (average pore size, 0.45 μ) suspended over a flask under gentle vacuum. The thin layer of tissue on the filter was then washed twice with chilled buffer. This part of the method is an adaptation of the filter technique used by BRITTEN AND MCCLURE[10] to study membrane transport and metabolism in microorganisms. The filters containing the glomeruli were then removed and dried for 18 h at 90°. After reweighing to determine the dry weight of tissue, the filters were glued to aluminum planchets and the radioactivity was measured in a Nuclear-Chicago gas-flow counter. Corrections were made for the adsorption of buffer salts and of isotope on the filter and for interference with counting by the filter itself.

Uniformly labeled sucrose (specific activity, 5 mC/mmole), and uniformly ^{14}C-labeled L-proline (specific activity, 210 mC/mmole), were obtained from New England Nuclear Corp.; their radiochemical purity was confirmed by partition chromatography, electrophoresis and scanning, as described earlier[1,2]. Phlorizin (J. T. Baker Chemical Co.) was used with sucrose to determine extracellular space, as described by ROSENBERG *et al.*[11]. It was necessary to employ sucrose because excessive adsorption of inulin by the filters prevented an accurate estimation of extracellular water with this compound.

Glomeruli were scraped from filters, weighed on tared pans of aluminum foil,

dried and reweighed. Total tissue water was 84 ± 4 % of the total wet weight. Incubation in the presence of sucrose and phlorizin resulted in a constant distribution of sucrose after 20 min; the sucrose (or extracellular) space was 17.6 ± 0.8 % of the wet tissue weight. Intracellular water was therefore 66.4 % of the wet weight; this value may include water sequestered in Bowman's space.

Progress curves for net accumulation of L-proline at 0.05 mM in the initial medium, reveal that equilibration of uptake is achieved quite slowly (Fig. 1).

Physical and chemical treatment of glomeruli by methods which will disrupt membranes and cellular integrity or will inhibit energy metabolism and SH-dependent or Na^+-dependent reactions, inhibit net accumulation of 0.10 mM L-proline. Proline uptake is also inhibited when the incubation medium contains 1.0 mM hydroxy-L-proline or 1.0 mM α-aminoisobutyric acid; 1.0 mM L-leucine does not have this effect (Table I).

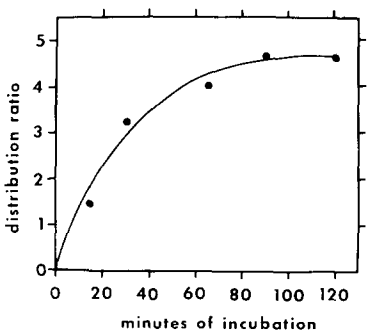

Fig. 1. Progress curve for uptake by rat glomeruli of uniformly ^{14}C-labeled L-proline (●—●). Distribution ratio refers to intracellular ^{14}C:extracellular ^{14}C. After addition of ^{12}C-carrier, substrate was at 0.05 mM in the initial medium. At 120 min, 27 % of the intracellular proline label is still free proline.

TABLE I

EFFECT OF INHIBITORS ON L-PROLINE UPTAKE BY RAT GLOMERULI

Glomeruli were prepared and incubated with 0.10 mM L-proline for 120 min, as described. All experiments were performed in triplicate in parallel with triplicate controls prepared from the same batch of glomeruli.

Condition	*Percent of distribution ratio in control**	*p (F test)***
Boiling (10 min)	0	<0.001
Freezing and thawing (3 times)	16	<0.001
Sodium cyanide (0.1 mM)	39	<0.05
2,4-Dinitrophenol (0.1 mM)	49	<0.001
Iodoacetamide (10 mM)	14	<0.001
Ouabain (0.5 mM)	41	<0.001
Choline chloride (144 mequiv/l)	24	<0.001
Hydroxy-L-proline (1 mM)	55	<0.02
α-Aminoisobutyric acid (1 mM)	73	<0.01
L-Leucine (1 mM)	65	Not significant

* Distribution ratio in control glomeruli was 4.2 for radioactivity, and 1.13 for L-proline, assuming that 27 % of the intracellular label was still proline.

** See ref. 12.

Accumulation of L-proline by glomeruli is saturable and uptake obeys Michaelis–Menten kinetics. The first approximation for the apparent K_m is 0.1 mM when the initial substrate concentration varies between 0.04 and 0.4 mM. This value is equivalent to that obtained in cortex slices under similar conditions of incubation[3].

FONG AND DRUMMOND[9] showed that isolated glomeruli oxidize [^{14}C]glucose to CO_2. We examined the metabolism of labeled L-proline after incubation of rat glomeruli for 120 min. The distribution of label into soluble metabolites and the hot-ethanol-insoluble fraction was determined by methods described previously[1,3]. Isotope from uniformly ^{14}C-labeled L-proline was distributed as follows: proline, 27 %; glutamic acid, 27 %; ornithine, 6 %; CO_2, 9 %; "insoluble", 31 %.

Rat glomeruli thus exhibit mediated uptake of L-proline. This uptake shares many features which characterize uptake by the intact slice[1–3] and tubule[8]. Metabolism of proline by the intact cortex slice has been shown to influence the observed rate of proline migration across the membrane[3]; on the other hand, the apparent kinetics of proline binding to the transport site and competitive interactions at the site are not influenced by the metabolic fate of the substrate after its uptake[3]. Thus, we assume that the vigorous metabolism of L-proline by glomeruli does not determine the binding characteristics of proline uptake which we have described.

More extensive studies are being carried out to determine whether the mechanisms which control membrane transport of imino acids and α-aminoisobutyric acid[1–3] are expressed in glomeruli, in the manner observed in a more traditional "transport" structure, such as the renal tubule.

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