PRELIMINARY NOTES

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Transport of amino acids by isolated rabbit renal tubules

Studies utilizing kidney cortex slices *in vitro* have contributed significantly to our understanding of renal amino acid transport. Amino acids are accumulated by this tissue preparation against concentration gradients by saturable processes dependent on oxidative metabolism and on the sodium concentration of the incubation medium[1,2]. However, interpretation of kinetic studies with kidney slices is complicated because all the tubule cells are not contiguous with the incubation medium and because the outermost tissue layers may act as a physical barrier between the medium and the inner core of tissue. This physical distortion is probably of little consequence in equilibrium studies, but it may be very significant in estimating initial rates of uptake, or in analyzing the direction of movement of substrate between incubation medium and intracellular space.

In 1962, BURG AND ORLOFF[3] prepared suspensions of isolated renal tubule segments by digestion of minced rabbit renal cortex with collagenase. This tissue preparation concentrated *p*-aminohippurate, consumed oxygen and maintained normal concentration gradients for sodium and potassium for a period of several h. HUANG AND LIN[4], MURTHY AND FOULKES[5], and KLEINZELLER, KOLINSKA AND BENES[6] subsequently used suspensions of isolated tubules to study *p*-aminohippurate uptake, *p*-aminohippurate efflux, and galactose accumulation, respectively. The present preliminary report demonstrates the usefulness of renal tubule suspensions in the study of amino acid transport.

Renal tubules were prepared from 4–5-lb male, white New Zealand rabbits according to the methods of BURG AND ORLOFF[3]. Minced kidney cortex was digested with collagenase (0.375 % solution) dissolved in modified Krebs–Ringer bicarbonate saline (pH 7.4) with or without added acetate. 1 ml of fetal calf serum was added to 20 ml of the final tubule suspension before incubation. The final incubation mixture contained 40–80 mg of tubules per ml of suspension. The suspension was placed in incubation tubes identical to those described by BURG AND ORLOFF[3], which permitted continuous agitation and oxygenation by bubbling 95 % oxygen and 5 % CO_2 through the suspension. A silicone antifoaming agent was sprayed on the surface of each tube prior to incubation. After a 3-min period of temperature equilibration, substrates were added to the tubes. Incubation times varied from 2 to 90 min. The experiments were terminated by pipetting duplicate 2-ml samples into tared tubes, cooling rapidly to 0°, and centrifuging at 48200 × *g* for 8 min in a refrigerated centrifuge (Servall RC2B). After withdrawing the incubation medium, the cell pellet was washed twice with ice cold buffer and the sides of the tube and surface of the pellet were dried by suction. 2 ml of distilled water was added to the cell pellet, and the tubules were lysed by boiling for 2 min followed by 2 passages through a No. 27 needle. Aliquots of the

tissue supernatant and incubation medium (deproteinized by boiling) were prepared for counting in a liquid scintillation spectrometer as described previously[7]. Extracellular space estimations using [^{14}C]inulin ranged from 19 to 21 % of the wet tissue weight. Total tissue water ranged from 75 to 78 % of the total wet weight. Values of 20 % and 76.5 %, respectively were used in the calculations. Uniformly ^{14}C-labelled glycine, 1-^{14}C-labelled α-aminoisobutyric acid and uniformly ^{14}C-labelled diaminobutyric acid were obtained from New England Nuclear Corporation. The collagenase was obtained from Nutritional Biochemicals Corporation. The fetal calf serum was purchased from Microbiological Associates.

TABLE I

UPTAKE OF LABELLED AMINO ACIDS BY ISOLATED RABBIT RENAL TUBULES

Renal tubules were incubated in modified bicarbonate buffer (pH 7.4) at 37° for the intervals shown above. See text for details.

*Substrate**	*Distribution ratio***	
	5 min	*45 min*
Diaminobutyric acid (0.05 mM)	3.1 ± 0.1 (3)	6.5 (2)
Glycine (0.05 mM)	3.1 ± 0.6 (18)	8.1 ± 1.3 (9)
α-Aminoisobutyric acid (0.02 mM)	1.3 ± 0.2 (13)	1.7 ± 0.2 (3)

* Initial medium concentration for each amino acid shown in parentheses.

** Defined as the ratio of counts/min per ml of intracellular fluid to counts/min per ml in the medium. Values represent mean ± 1 S.D. Figures in parentheses denote number of observations.

The three amino acids studied were concentrated against a chemical gradient by the tubule suspension as shown in Table I. The distribution ratios achieved with glycine and with diaminobutyric acid were significantly greater than those noted for α-aminoisobutyric acid. The data in Fig. 1 illustrate representative, comparative studies of α-aminoisobutyric acid and glycine uptake by kidney cortex slices and

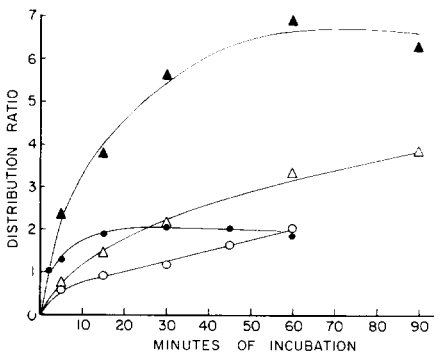

Fig. 1. Comparison of uptake of glycine and α-aminoisobutyric acid by rabbit kidney cortex slices and isolated renal tubule segments. Slices or tubules were incubated in modified bicarbonate buffer (pH 7.4) at 37°. After 150 min of incubation (not shown), the distribution ratio for glycine in the slices equalled that in the tubules. Each datum shown represents the mean of duplicate observations in a single representative study. Glycine uptake in tubules (▲—▲) and in cortex slices (△—△); α-aminoisobutyric acid uptake in tubules (●—●) and in cortex slices (○—○).

tubule suspensions from the same animal. Cortex slices were prepared from one kidney, and isolated tubules from the other. The initial rate of α-aminoisobutyric acid and glycine uptake was significantly greater in the tubule suspensions than in the slices. Furthermore, although the equilibrium distribution ratios were the same in tubules and slices, the approach to equilibrium was much slower in the slices.

Glycine uptake was studied under several experimental conditions (Table II). Uptake was markedly inhibited by 2,4-dinitrophenol, cyanide, ouabain and deletion of sodium from the incubation medium. Substitution of either choline or Tris for sodium resulted in essentially identical experimental findings. Additional experiments, to be reported subsequently, indicate that glycine uptake by the tubule suspension is saturable and obeys Michaelis–Menten kinetics.

TABLE II

INFLUENCE OF SODIUM ION AND METABOLIC INHIBITORS ON GLYCINE UPTAKE BY ISOLATED RENAL TUBULES

*Experimental conditions**	*Distribution ratio***
Control	2.6
2,4-Dinitrophenol (10^{-4} M)	1.0
Sodium cyanide (10^{-2} M)	0.5
Ouabain ($5 \cdot 10^{-4}$ M)	0.6
Sodium-free buffer	0.4

* Tubules were preincubated with inhibitors or in absence of sodium for 5 min at 37° prior to 5-min uptake experiments with glycine (0.05 mM). Sodium-free buffer was prepared by equimolar substitution with choline or Tris.

** Distribution ratios represent mean of duplicate observations in a single representative experiment.

These studies demonstrate that short segments of renal tubules prepared by collagenase digestion transport neutral and basic amino acids actively. The kinetic studies suggest that this tissue preparation has significant advantages over cortex slices in the study of amino acid influx and efflux.

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