Cellular Localization of Amino Acid Carriers in Renal Tubules[1] (36291)

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It is well known that, in the normal animal, a major fraction of filtered amino acids is reabsorbed from tubular fluid by structure-specific transport mechanisms in the proximal tubule. In the case of glucose reabsorption active transport occurs across the luminal cell membrane (LM) of the rabbit tubule (1). Further evidence is required to localize the amino acid carriers at that site. This need arises especially out of the recent demonstration that the peritubular membrane (PTM) may also play part in active amino acid transport (2, 3). Subsequent experiments showed that heavy metals, whose administration causes aminoaciduria, exert their effect on LM (4). The present report provides more direct evidence for the localization at LM of the system responsible for reabsorption of dicarboxylic amino acids.

Methods. The preparation employed for these studies resembles that previously used (5) to adapt the double indicator-dilution technique of Silverman *et al.* (6) to the rabbit. In the present experiments catheters were inserted into the left renal artery and vein, and the ureters of male white New Zealand rabbits (av wt, 2 kg). The kidney was perfused with aortic blood at a constant rate controlled by a finger pump; perfusion pressure was monitored with a Harvard Apparatus Co. pressure transducer. In order to minimize recirculation of tracers total venous outflow was collected for 60 sec after the arterial injections of 0.15 ml of saline containing 1 μCi of ^{14}C-aspartate and 15 μCi of ^{3}H-methoxyinulin. Venous collections were replaced with equal volumes of 6% dextran in saline. Analytical and radiological techniques, as well as the labeled compounds used were all previously described (2). Mean transit times ($\bar{t}$) were computed as usual by integration of concentrations as function of time, up to the point at which fractional recovery fell below 2% of the cumulative total. Inulin recovery was set at 100%.

Results. Artery-to-urine transit times of ^{14}C (injected as aspartate) and of ^{3}H-labeled inulin are compared in Fig. 1 before and after injection of glutamate. In the amount administered, glutamate strongly depressed reabsorption of filtered ^{14}C; no significant effect of glutamate was seen, however, on the relative mean transit times of ^{14}C and of ^{3}H. The ratio of $\bar{t}_{asp}/\bar{t}_{in}$ (in control studies on 7 animals) averaged 1.02 $\pm$ 0.03 (SD; one additional experiment gave a value of 1.37 and was excluded), and 0.95 $\pm$ 0.04 after glutamate injection in 6 animals.

Discussion. The volume of distribution V of a solute between points of injection and collection is defined operationally as the product of flow and mean transit time. After arterial injection, tracer requires only seconds to reach the glomerulus, as contrasted with minutes elapsing between filtration and collection. The mean transit time of inulin therefore represents passage time from glomerulus to ureter. Calculation of the urinary volume of distribution of inulin (V_{in}) is rendered uncertain by the fact that flow of tubular fluid exceeds that of urine. V_{in} computed as $\bar{t}_{in} \times$ urine flow must therefore be a minimum value. Nevertheless, the observation that $\bar{t}_{asp} = \bar{t}_{in}$ implies that $V_{asp} = V_{in}$. It follows that aspartate, which has moved into cells from either tubular fluid or from interstitial spaces, does not enter the urine during the collection period.

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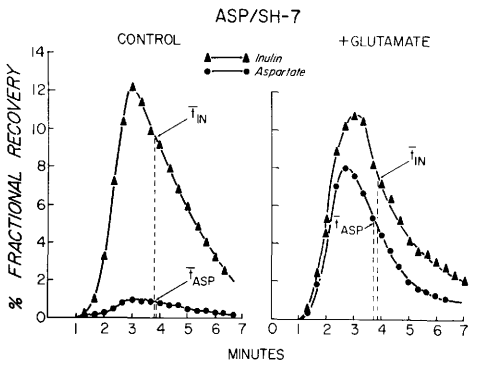

FIG. 1. Effect of glutamate on artery-to-urine transit of aspartate: Rabbit Asp/Sh-7, 2.3 kg. Continuous intravenous infusion: 1.5 ml/min 10% mannitol in 0.9% NaCl, containing 1 μmole of PAH, 10 μmoles of creatinine/ml. Blood flow in renal vein, 13.1 ml/min; perfusion pressure, 70/50 mm Hg; final weight of undrained kidney, 12.1 g. (Period I) Urine flow, 0.6 ml/min; clearances of creatinine, 1.7; PAH, 9.1 ml/min, E_{PAH}, 89%; arterial hematocrit, 25%; renal venous plasma amino acid, 4 mM; reabsorption of filtered ^{14}C, 91%. (Period II) 15 min after beginning of intraarterial infusion of 0.5 M Na_2 glutamate at 0.15 ml/min: renal venous plasma amino acid, 14 mM; urine flow, 0.7 ml/min; clearances of creatinine, 1.7; PAH, 8.2 ml/min; E_{PAH}, 78%; hematocrit, 23%; reabsorption of filtered ^{14}C, 39%.

Administration of glutamate reduced aspartate reabsorption on the average from above 90% to below 40%. If this block to aspartate transport occurred at a point beyond LM, within cells or even at PTM, aspartate would accumulate at such sites and diffuse back into the lumen. In turn such backdiffusion would add a portion of the intracellular compartment to V_{asp} and would therefore prolong $\bar{t}_{asp}$ beyond $\bar{t}_{in}$. If we assume that 20% of the kidney (by volume) consists of proximal tubular cells the intracellular space potentially involved in amino acid reabsorption in the study shown in Fig. 1 amounts to about 2 ml. Addition of this volume to V_{in} would have almost doubled the minimum value of 2.3 ml calculated for V_{in}. As a result $\bar{t}_{asp}$ should have significantly exceeded $\bar{t}_{in}$ in the presence of glutamate. The fact that this did not occur justifies the conclusion that the block in aspartate transport must have occurred at or near LM.

In a previous report dealing with the renal reabsorption of α-aminoisobutyrate (AIB), it had been similarly concluded that heavy metals inhibit the reabsorptive process at the level of the luminal membrane (4). One might argue that such a conclusion need not imply that the carrier itself is situated at LM; conceivably metals could decrease passive permeability of the membrane to amino acids and thus deny access of substrate to the carrier system located at a point beyond LM. Available evidence (7) does not support this interpretation. It further seems unlikely that the interaction between aspartate and glutamate should represent effects on passive permeability. The glutamate inhibition of aspartate movement described here presumably results from competition of the two dicarboxylic acids for a common carrier. The earlier results with AIB, and the present observations on aspartate reabsorption are thus most simply explained in terms of transport systems at the luminal border of tubule cells.

Summary. Determination of the mean transit time of aspartate from glomerulus to ureter, in presence or absence of a competitive inhibitor of aspartate reabsorption (glutamate), provides evidence that the carrier system responsible for aspartate reabsorption is localized on the luminal cell membrane.

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