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Biochemical heterogeneity of arginine metabolism along kidney proximal tubules

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Biochemical heterogeneity of arginine metabolism along kidney proximal tubules. By using an *in vitro* single tubule micromethod of high specificity, in four different species of mammals it has been observed that (a) arginine synthesis from citrulline (arginine synthase activity, E.C. 6.3.4.5 and E.C. 4.3.2.1) is restricted to the early portions of proximal convoluted tubules, whereas (b) urea production from arginine (arginase activity, E.C. 3.5.3.1.) is present mainly in the cortical (CPST) and even more in the outer medullary (OSPST) portions of straight proximal tubules. The data suggest that (a) in early PCT cells, the citrulline reabsorbed from glomerular filtrate is converted into arginine, which in turn crosses peritubular cell membranes together with reabsorbed arginine, and (b) the urea formed in CPST and OSPST cells might passively diffuse into the luminal fluid entering Henle's loops. Such urea secretion might contribute to sustain the process of urea recycling in kidney medulla and thereby participate in the mechanism of urine concentration.

It is well established from enzyme activity measurements that kidney tissue from mammals contains both the arginine synthase enzyme complex (EC 6.3.4.5 and EC 4.3.2.1) and arginase (E.C. 3.5.3.1), that is, the enzyme systems that in the liver form arginine from citrulline plus aspartate, and urea plus ornithine from arginine, respectively [1–7].

To establish which cell types contain these two enzyme activities in the kidney, we developed a highly sensitive and specific *in vitro* micromethod that permits measurement, in single microdissected pieces of kidney tubules, of the $^{14}CO_2$ formed either from [^{14}C-guanido]arginine in the presence of urease in the incubate (arginase activity [8]), or from [^{14}C-ureido]citrulline in the presence of arginase and urease in the incubate (arginine synthase activity [9]). The labeled substrates were used at physiological concentrations (about 0.2 mM for arginine and 0.1 mM for citrulline).

Results and discussion

The data obtained in the rat [8, 9], mouse [10, 11], rabbit [10, 11], and in a desert rodent species, *Meriones shawi* [12] revealed qualitatively similar patterns of distribution in all species, which, however, were quite different for the two types of enzyme.

Arginine synthesis from citrulline was restricted to proximal tubules, with an activity decreasing markedly from the convoluted portion (PCT) to the cortical (CPST) and even more the outer medullary (OSPST) portions of straight tubules. In contrast, urea production from arginine increased from PCT to CPST and even more to OSPST in all species. In addition, arginase was also present, though to a lesser extent, in collecting tubules of some species, in particular the rat [8], and in medullary thick ascending limbs of the rabbit [10].

The heterogenity of distribution along proximal tubules for these two enzymes have been recently analyzed in great detail in the kidney of *Meriones shawi* [12]. Figure 1 illustrates the average data obtained in successive PCT portions from the same nephrons. The two activities are clearly dissociated from each other, as arginine synthesis decreased sharply along this segment, whereas urea production exhibits the opposite pattern.

Regarding arginine synthesis, the results shown on Figure 1 suggest, as discussed in [12], that the citrulline reabsorbed from glomerular filtrate across luminal cell borders may represent the actual substrate for this enzyme under physiological *in vivo* conditions, since, (a) the curve of activity decreases along early PCT portions in a way similar to amino acid concentration in tubular fluid, and (b) the enzyme complex is almost absent in late PCT portions and in straight tubules, where amino acid reabsorption is nearly completed. Figure 2 depicts what the overall mechanism of arginine synthesis from citrulline could be in early PCT cells. Note that this mechanism does not require any transport process for citrulline accross basolateral cell membranes.

Regarding urea production from arginine, the situation is quite different. Arginase activity increases in late PCT portions and CPST (Fig. 1) to reach a maximum in OSPST, that is, in nephron portions where arginine is absent from luminal fluid under physiological conditions. One has to postulate, therefore, that arginine enters the cell compartment in those segments by facilitated diffusion across the peritubular cell border, as depicted on Figure 3. A passive process would be sufficient in view of the inside negative cell membrane potential. Moreover, arginase activity would reduce arginine concentration in the cell, whereas the urea formed would tend to accumulate. In late proximal segments, the permeability to urea is known to be low, and urea concentration in tubular fluid is higher than in blood plasma under normal conditions. It is not known, however, which of the two cell borders acts as the limiting permeability barrier to urea diffusion in those segments. If, as postulated on Figure 3, apical membranes were much more permeable to urea than basolateral membranes, the larger part of the urea formed from arginine in the cell compartment would eventually be added to the luminal fluid (Fig. 3).

The physiological role of urea formation from arginine in late proximal segments is difficult to assess, in particular because the

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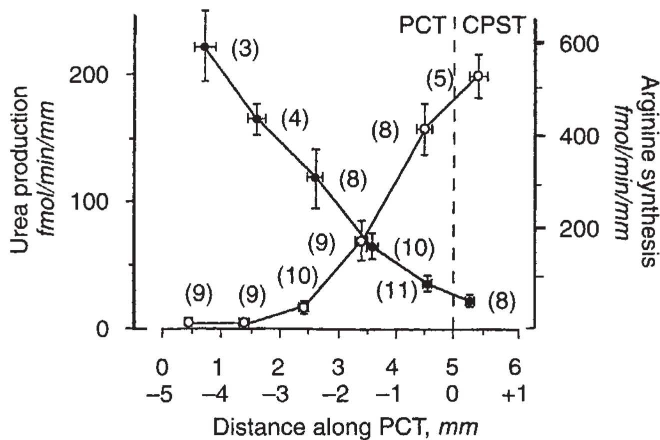

Fig. 1. *Metabolism of arginine in proximal convoluted tubules of Meriones shawi.* Microdissected PCT measuring 4 to 6 mm in length were segmented in successive portions of about 1 mm each, either starting from the attached glomerulus (for urea production measurements, fmol · min^{-1} · mm^{-1}; ○) or from the transition between PCT and CPST (for arginine synthesis measurements, fmol · min^{-1} · mm^{-1}; ●). In parentheses are the numbers of samples. Modified with permission from [12].

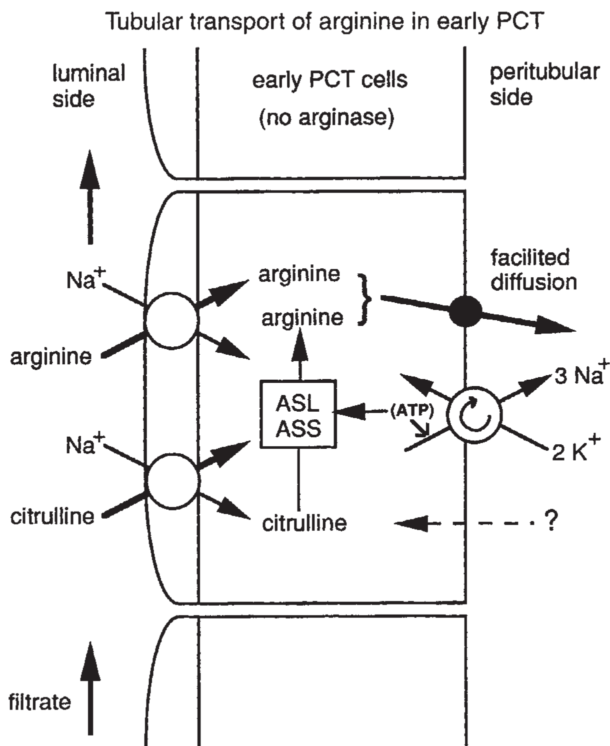

Fig. 2. *It is assumed that, in the cell compartment, the arginine produced from reabsorbed citrulline mixes to the pool of reabsorbed arginine.* Abbreviations are: ASS, arginosuccinate synthase, EC 6.3.4.5; ASL, arginosuccinate lyase, EC 4.3.2.1. These two enzymes form the arginine synthase complex.

rate of this metabolic process is quantitatively low as compared to the rate of urea flow from glomerular origin. It amounts to about 1 versus 50 pmoles per minute per nephron, that is, to only 2% according to a rough calculation [12]. Consequently, the urea

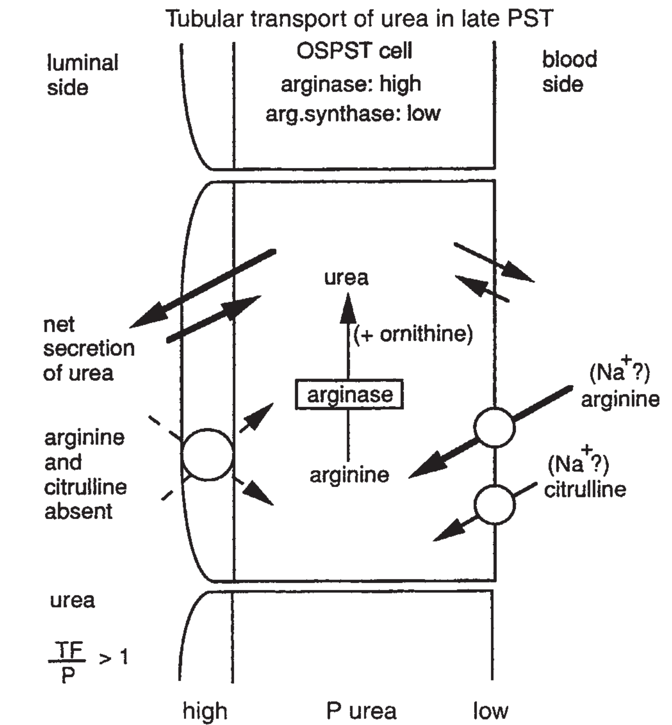

Fig. 3. *The size of arrows in this schematic diagram indicates the rate of the respective flux.* Refer to text for more details.

formed in the kidney can hardly result in a significant increase of urea excretion in final urine. Nevertheless, a process of urea secretion in pars recta, whatever its mechanism might be [13, 14], could contribute to sustain the medullary recycling of urea in kidney medulla, and thereby participate in the mechanism of urine concentration by a counter current. The following observations support this assumption. (1) The main site of urea production (OSPST) is located in the outer medulla, namely, at the entrance to the loop of Henle. (2) When measured under similar experimental conditions, urea production by OSPST was more than threefold greater in species with a high ability to concentrate urine (250 fmol · mm^{-1} · min^{-1} in rat [1], mouse [4], and *Meriones shawi* [5]) than in species with a low concentrating ability (guinea pig or rabbit, 75 fmol · mm^{-1} · min^{-1} [4]). (3) In *Meriones shawi*, in which these aspects could be analyzed, both OSPST and the adjacent thin descending limb (DTL) produced more urea in juxtamedullary nephrons than in superficial nephrons [12]: 320 versus 190 fmol · mm^{-1} · min^{-1} for OSPST, and 40 versus 9 fmol · mm^{-1} · min^{-1} for DTL, respectively.

In conclusion, it should be recalled that arginase activity results in equimolar productions of urea and ornithine. Ornithine, in turn, is the precursor of the polyamine pathway via the action of ornithine decarboxylase. The above-mentioned observations, however, point to a role of urea rather than ornithine in urine concentration, even though a physiological function of ornithine cannot be excluded *a priori*.

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