Renal transport of cystine by isolated renal tubules and brush-border membrane vesicles

J. W. Foreman, P. D. McNamara and S. Segal

INTRODUCTION

Human cystinuria, an inherited disease characterized by increased excretion of cystine and the dibasic amino acids, lysine, ornithine, and arginine, has focused attention on the nature of the renal tubule reabsorption of these amino acids. In 1951, Dent and Rose proposed that cystine and the dibasic amino acids were handled by a common system in the kidney that was defective in human cystinuria. This hypothesis was strengthened by the fact that lysine infusion in both man and dog increased the excretion of cystine and the other dibasic amino acids. A common system for the accumulation of dibasic amino acids in renal tubule cells has been demonstrated using cortical slices from both human and rat kidney. Further support for a common system came from microperfusion studies in rat proximal tubules, demonstrating arginine inhibition of cystine uptake from the tubule lumen.

On the other hand, evidence for cystine uptake separate from the...
dibasic amino acids has been demonstrated. Cystine uptake by renal cortical slices from both humans\textsuperscript{5} and rats\textsuperscript{6} was not inhibited by the dibasic amino acids. Further, dibasic amino-acid uptake by cortical slices from cystinuric patients was defective, but cystine uptake was not\textsuperscript{5}. Additional support for the separate nature of renal transport processes came from descriptions of patients with cystinuria without dibasic amino-aciduria\textsuperscript{9} and patients with hyperdibasic aminoaciduria without cystinuria\textsuperscript{9,10}. In canine cystinuria, some dogs have only an increase in cystine excretion without significant dibasic amino acid excretion\textsuperscript{11}.

Because of these ambiguities, we examined the nature of cystine and dibasic amino-acid transport using the isolated renal tubule and isolated brush-border membrane vesicles from the rat. The isolated tubule offered advantages over the cortical slice in that concerns about substrate penetration, tissue thickness and oxygenation, which could obscure the interaction of cystine and dibasic amino acids, were minimized. The isolated brush-border membrane vesicle preparation allowed the examination of the interaction of cystine and dibasic amino acids at the membrane locus of transport, without complications of cellular metabolism.

**ISOLATED RENAL TUBULES**

Isolated renal tubules were prepared using a modification\textsuperscript{12,13} of the method described by Burg and Orloff\textsuperscript{14}. Renal cortex from adult male Sprague–Dawley rats was finely minced and then digested in 0.375% collagenase for 45 min. The tubules were then washed free of collagenase and filtered through surgical gauze, such that only cortical tubular fragments remained, as shown in Figure 13.1. The uptake studies were performed in Krebs–Ringer bicarbonate buffer containing 5% fetal calf serum and 10 mmol/l sodium acetate in special flasks, which allowed continuous bubbling of \( \text{O}_2: \text{CO}_2 \) (95:5) through the incubation mixture. The results are expressed as a distribution ratio of radioactivity [ratio of cpm/ml of intracellular fluid to the cpm/ml of the medium].

Figure 13.2 demonstrates the progressive uptake of 0.025 mmol/l \[^{35}\text{S}\text{]}\text{cystine} until a steady state, where influx of the tracer equalled efflux, was reached after 60 min of incubation with a distribution ratio of 38.58 ± 0.76. With 0.5 mmol/l \[^{35}\text{S}\text{]}\text{cystine} as the substrate, the distribution ratio of radioactivity at each time point was lower than that observed with 0.025 mmol/l cystine, suggesting that the uptake of cystine by isolated renal tubules was saturable and carrier-mediated.