L-Arginine Transport in Rat Proximal Tubules
Microperfusion Studies on Reabsorption Kinetics*

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Summary. The reabsorption of L-arginine in the proximal tubule of the rat kidney was measured in microperfusion experiments. The following results were obtained:

1. The fractional reabsorption of L-arginine decreases with increasing L-arginine concentration.
2. At a given concentration the reabsorption rate increases with decreasing tubular volume flow.
3. \( V_{\text{max}} \) and \( K_m \) of this saturable transport system were estimated; at a constant tubular volume flow of 20 nl/min the mean values are:

\[
V_{\text{max}} = 7.1 \times 10^{-10} \text{ [mol \cdot cm}^{-2} \cdot \text{sec}^{-1}] \\
K_m = 1.2 \times 10^{-3} \text{ [mol \cdot l}^{-1}] 
\]

It is concluded, that L-arginine is removed from tubular fluid by a saturable system. Simple diffusion of L-arginine is not detectable.

Key words: Microperfusion — Renal Tubule — L-Arginine Reabsorption — Kinetics.

Microinjection experiments [1] and stop flow studies [6] indicate that L-arginine reabsorption in the mammalian kidney mainly takes place in the proximal tubule. From clearance experiments [10,16,7] it was concluded that L-arginine has a tubular transport maximum and with in vitro techniques it was demonstrated that L-arginine is concentrated against a chemical gradient by a saturable transport system in proximal tubular cells [11].

In order to obtain more direct information about the renal handling of L-arginine in vivo, we studied proximal tubular transport of L-arginine in the rat directly with a microperfusion technique.

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Methods

Surgical Procedure. Male rats (220 - 280 g B.W., Wistar strain) were anesthetized by intraperitoneal injection of 110 - 120 mg/kg B.W. of Inactin (Promonta, Hamburg). The left kidney of the animals were prepared for microperfusion in the usual way. Details have been reported recently [13].

Microperfusion. Using the technique of Sonnenberg and Deetjen [14], sections of proximal tubules were perfused with equilibrium solutions which contained 115 mEq/l Na⁺, 5 mEq/l K⁺, and 4 mEq/l Ca²⁺. These fluids were buffered by TES¹ [8] to a pH value of 7.4. In some experiments we used also phosphate buffer for comparison with TES. The osmolarity of the perfusion fluids was adjusted to 300 mosm/l by addition of mannitol. The perfusion solutions contained L-arginine at initial concentrations of 0.36 mmol/l, 2.0 mmol/l, 10.0 mmol/l or 22.0 mmol/l. The perfusion rates were 11 nl/min, 20 nl/min, or 35 nl/min respectively. At the end of each perfusion experiment the tubules were filled with Latex and, after maceration of the kidney with HCl, the length of the perfused section was measured. For further modifications of the original microperfusion technique which are in use now in our laboratory we refer to a recent description [4].

Materials. L-arginine-¹⁴C (U) monohydrochloride (10 mCi/mmol), L-arginine (guanido-¹⁴C) monohydrochloride (50 mCi/mmol), and inulin-³H (300 mCi/mmol), all obtained from Radiochemical Centre, Amersham, England, were used as tracers. TES-buffer were obtained from Serva, Heidelberg, W. Germany.

Radioactive Measurement. The radioactivity was counted in a Packard Tricarb Liquid Scintillation Spectrometer. 16 ml of a mixture of 800 ml toluene, 200 ml ethanol absol. p. a., 7 g PPO and 300 mg POPOP were used as scintillation fluid. More details of the procedure of the radioactive measurement were reported recently [13].

Calculations. Commonly, saturable transports are described in analogy to Michaelis-Menten kinetics by the maximum transport rate Vmax and the substrate concentration Kₘ at 1/2 Vmax. Assuming that the proximal tubule is a cylindric tube we calculated the reabsorption rates in the following way: Using equilibrium perfusion fluids the volume flow ˙V is constant along the proximal tubule. The contact time is calculated from the perfusion distance by the volume flow ˙V and by the tubular radius r which depends on ˙V (see [3]). We estimated the reabsorption rate V(t) at the concentration C(t) of each sample by the formula (I):

\[ V(t) = \frac{dC}{dt} \cdot \frac{r}{2}, \]  

where r = tubular radius and \( \frac{dC}{dt} \) is calculated from Eq. (II):

\[ \ln C_t = a \cdot t + \ln C_0 \]  

(II)

\( t = \) contact time, \( C_t = \) concentration at t, \( C_0 = \) concentration at \( t = 0, a = \) slope

Inserting (II) in (I) results in:

\[ V(t) = \frac{r}{2} \cdot C_0 \cdot a \cdot e^{a \cdot t}; \]  

(III)

Using ˙V(t) [Eq. (III)] and S = C₁ of each point of Fig. 1 we plotted 1/˙V vs. 1/S, S/˙V vs. S, and ˙V vs. ˙V/S and estimated the regression lines. Vmax and Kₘ were derived from each plot.

All regression lines are calculated using the method of least squares. The regression coefficients in Figs. 1 and 3 were compared for significance by the formula given by Sachs [12].

¹ Tris-(hydroxymethyl)-methyl-2-aminoethan-sulfonic acid.