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# Axial heterogeneity of organic cation transport along the rabbit renal proximal tubule: studies with brush-border membrane vesicles

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**Brush-border membrane vesicles prepared from rabbit kidney outer cortex (rich in S1 and S2) and outer medulla (rich in S3) were used to evaluate the axial heterogeneity of tetraethylammonium transport in the proximal tubule. The vesicle preparations had similar $K_m$ values but the $V_{max}$ values differed, suggesting that axial heterogeneity of tetraethylammonium secretion may be due to differences in transport across the brush-border membrane.**

Organic cations are extensively secreted by the proximal tubule of mammalian kidney [1]. In a study on isolated perfused proximal tubule segments of rabbit kidney [2] we have shown that the secretory (trans-epithelial) transport rate of TEA is different in the various sections of the proximal tubule. Maximal secretory rates were found in the S1 segment (according to the terminology of Woodhall et al. [3]) (early proximal convoluted tubule), intermediate rates in the S2 segment (initial pars recta) and the lowest secretory rates in the S3 segment (terminal pars recta). Per mm of tubule length, the S1 segment secreted almost twice as much TEA as the S3 segment. McKinney [4,5] observed the same axial heterogeneity in the transport of another organic cation, procainamide, by isolated proximal tubules of the rabbit. These results raised the question of which cell structure is responsible for the alteration in transport rate since secretion of TEA implies transport across the two successive membranes of the tubule epithelial cells; the basolateral and the brush-border membranes.

Studies on isolated non-perfused tubule segments (a preparation in which only the basolateral membrane is exposed to the surrounding medium) revealed that the basolateral membrane of the three segment types (S1, S2, S3) accumulated TEA to the same extent [2]. From these data we proposed the hypothesis that the luminal brush-border membrane might be the rate-limiting step during tubular secretion of TEA. According to this hypothesis, the transport rate of TEA should be larger in brush-border membranes from the S1 than in that of the S3, and the transport rate in the S2 tubules should be intermediate. Assuming a difference in transport rate in brush-border membrane, this difference could be due to a different density of one carrier type along the proximal tubule, or to different carrier types which transport TEA.

The present study was undertaken to try to validate our working hypothesis. For this, we ap-

Abbreviation: TEA, tetraethylammonium.

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plied the method used by Turner and Moran [6] to evaluate axial heterogeneity of the $Na^+$/D-glucose transport in the proximal tubule. The method has also been successfully used by other groups [7]. Accordingly, we prepared brush border membrane vesicles from rabbit outer renal cortex (which is rich in S1 and S2 cell types) and from the outer medulla (rich in the S3 cell type). The outer cortex was obtained by cutting the kidneys into transveral slices (1–2 mm thick) and by trimming away with a scalpel the outermost portion of the cortex (1–1.5 mm, 1.5–2.5 g per rabbit). The outer medulla was dissected after complete removal of the remnant of the cortex as a 1 mm stripe (0.5–0.9 g per rabbit) just underneath the corticomedullary junction. Since the material obtained from one animal was not sufficient for this study, this material was frozen in liquid nitrogen, pooled with the material from other rabbits (4–6 per preparation) and kept at −80 °C until use. In preliminary experiments, freezing the starting tissue did not change the results concerning the comparison between external cortex and external medulla transport capability.

The dissected material coming from the same animals was thawed and brush-border membrane vesicles were prepared the same day by a $Mg^{2+}$ precipitation/differential centrifugation method as described elsewhere [8]. For comparison, membrane vesicles were also prepared from frozen total cortex (pool of three rabbits per preparation).

The final membranes were suspended in 100 mM mannitol, 100 mM KCl and 20 mM Mes-Tris at pH 6.0 and stored in liquid nitrogen until use. The purity of the preparations was checked as described [9] by measuring the enrichment factor of the brush-border enzyme leucine-aminopeptidase over the initial homogenate: this factor was $10.7 \pm 1.2$ (mean ± SE, $n = 4$), $8.25 \pm 1.03$ ($n = 7$) and $12.45 \pm 0.99$ ($n = 8$) in total cortex, outer cortex and outer medulla, respectively. Absence of cross-contamination with basolateral membranes was checked by measuring the enrichment factors of the $(Na^+ + K^+)$-ATPase which were $1.8 \pm 0.3$ ($n = 4$), $0.96 \pm 0.07$ ($n = 6$) and $2.05 \pm 0.32$ ($n = 6$) for total cortex, outer cortex and outer medulla, respectively. The total intravesicular volume determined by glucose equilibrium was identical between the two brush-border membrane vesicle populations ($2.49 \pm 0.23$ and $2.67 \pm 0.70$ µl/mg protein (mean ± SE, $n = 3$) prepared from external cortex and external medulla, respectively).

In a previous study we have shown that TEA is exchanged with protons in brush-border membrane vesicles isolated from the whole cortex of the rabbit [8,9]. In the current work, the transport of [$^{14}$C]TEA (specific activity 3.7 mCi/mmol, New England Nuclear, Boston, MA, U.S.A.) was studied by imposing a pH gradient (intravesicular pH = 6, medium pH = 7.4) across the membranes. The three types of membrane vesicles were incubated for 15 s (initial linear uptake) at 25 °C in medium containing different concentrations of radiolabelled TEA (0.02–2 mM) and the transport was measured as described [8]. As an estimation of the passive TEA flux, the same experiments were repeated at 4 °C. The results are shown in Fig. 1. At 25 °C, all three preparations showed curvilinear uptake of TEA suggesting a carrier-mediated, saturable mechanism. Kinetic constants were de-

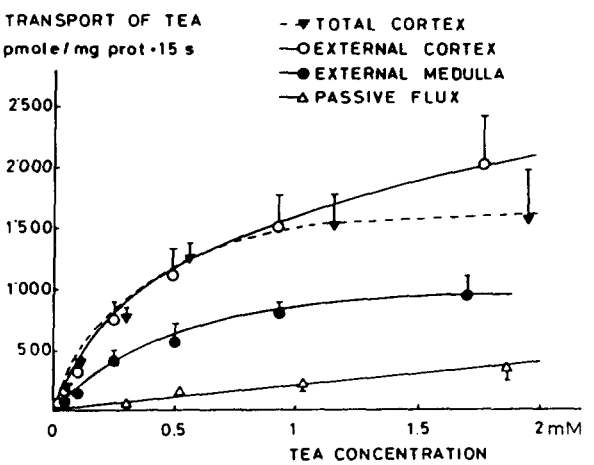


Fig. 1. Concentration dependence of TEA uptake by luminal membrane vesicles. The 15 s uptake of TEA was measured in the presence of a pH gradient ($pH_i = 6$, $pH_o = 7.4$) at 25 °C (▼, ○, ●) or at 4 °C to estimate the passive flux. Since the flux at 4 °C was identical for all three experimental conditions (data not shown), a mean of all three conditions is presented (△). All vesicle preparations were suspended in 100 mM mannitol, 100 mM KCl, 20 mM Mes-Tris (pH 6.0). The incubation medium contained 100 mM mannitol, 100 mM KCl, 20 mM Hepes-Tris (pH 7.4) and TEA at final concentrations ranging from 0.01 to 2 mM. 10 µl vesicles were mixed with 50 µl of incubation medium. Data represent the mean ± S.E. of four separate experiments run in triplicate.

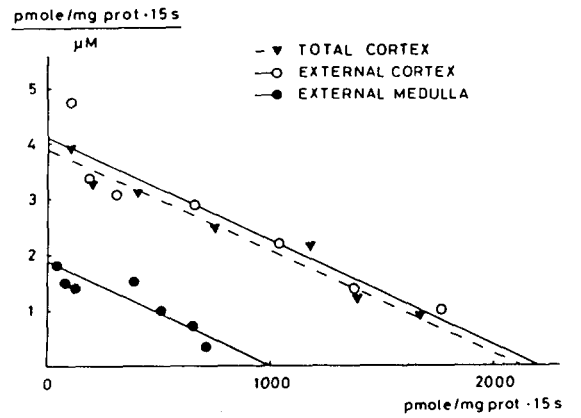


Fig. 2. Eadie-Hofstee plot of concentration dependence of TEA uptake by luminal membrane vesicles. The uptake observed at 4°C (passive flux) was substracted from total flux observed at 25°C (shown in Fig. 1). The kinetic constants calculated from these data were; $K_m$ 549, 533 and 582 μM and $V_{max}$ 2135, 2198 and 1036 pmol/mg protein per 15 s in total cortex (▼), outer cortex (○) and outer medulla (●), respectively. For further details see legend to Fig. 1.

termined using an Eadie-Hofstee plot (Fig. 2). In this plot, the straight line observed with membranes from total cortex suggests the presence of one kinetically resolvable carrier type for TEA transport in these brush-border membranes. This is confirmed additionally by observing the slopes of the data from outer cortex and outer medulla. The three slopes are parallel implying a common affinity for TEA among the three preparations (a $K_m$ of 549 (confidence limits for $P < 0.05$: 454–667) μM, 533 (confidence limits for $P < 0.05$: 400–833) μM and 582 (confidence limits for $P < 0.05$: 417–1000) μM, for total cortex, outer cortex and outer medulla, respectively). The apparent $V_{max}$ of total cortex and outer cortex were 2.13 (confidence limits for $P < 0.05$: 1.91–2.45) nmol/mg protein per 15 s and 2.2 (confidence limits for $P < 0.05$: 1.8–2.97) nmol/mg protein per 15 s, respectively, and differed considerably from that measured in outer medulla 1.04 (confidence limits for $P < 0.05$: 0.82–1.52) nmol/mg protein per 15 s.

These results imply that the membrane vesicles prepared from outer cortex or total cortex transport more TEA per mg protein than membrane vesicles prepared from outer medulla. The similar values of glucose equilibration space per mg protein observed between outer cortex and outer medulla brush-border membrane vesicles implied further that these two vesicle preparations have similar total volumes available for transport. If the ratio of (transport-capable) membrane area to volume is assumed to be the same between these preparations, then the results suggest that higher rates of TEA transport may be observed in the cells of the outer cortex versus outer medulla. Since similar $K_m$ values for TEA are observed in the three preparations, this (speculative) difference in transport could be due to a decrease in the density of a single type of carrier along the length of the proximal tubule.

These findings show that difference of TEA transport in isolated brush-border membrane vesicles parallels the observed differences in secretory capacity of TEA along the proximal tubule [2]. This suggests that TEA transport across the brush-border membrane might be responsible for differences in the secretory capacity.

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