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SHORT COMMUNICATIONS

[^{3}H]Gentamicin uptake in brush border and basolateral membrane vesicles from rat kidney cortex

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Aminoglycoside antibiotics are accumulated by the mammalian kidney where they induce nephrotoxicity in man [1] and experimental animals [2, 3] Evidence for renal uptake of aminoglycosides via tubular reabsorption and peritubular (vascular) extraction has been reported [4–7], however, the relative contribution of brush border (tubular) and basolateral (vascular) uptake in the renal accumulation process has not been determined

Renal accumulation of the aminoglycoside gentamicin has been studied by renal techniques including *in vivo* clearance [8], cortical slices [9], autoradiography [10, 11], microperfusion, micropuncture and incubation of nephron segments [5, 12], isolated brush border membranes [13] and the isolated perfused kidney [4] While the movement of gentamicin into renal tissue via reabsorption from the glomerular filtrate by the brush border surface is evidenced by these various techniques, the ability of the basolateral surface of the proximal tubule to extract gentamicin is also clearly demonstrated

The use of isolated membrane vesicles to study renal transport mechanisms offers the advantage of being able to measure separately the processes occurring on the two tubular cell surfaces Thus, quantitative, kinetic comparisons can be made Although brush border uptake of [^{3}H]gentamicin has been measured in rats [13] and rabbits [14], preliminary comparisons between brush border and basolateral membrane affinity and capacity for this aminoglycoside have been reported only recently [15] Purified renal membrane vesicles were utilized in the present study to examine the uptake of [^{3}H]gentamicin in these anatomically and functionally distinct surfaces of the kidney in order to ascertain by direct measurement any qualitative or quantitative differences in their interaction with this aminoglycoside

Materials and methods

Male Sprague–Dawley rats (200–250 g) were killed by cervical dislocation, and the kidneys were immediately removed and placed in ice-cold saline Cortical tissue was separated from the medulla, weighed, and homogenized in 10 vol (w/v) of 0 25 M sucrose using a Teflon–glass homogenizer In selected experiments, female rats were utilized (150–200 g) and kidney tissue was obtained and prepared as above

Brush border and basolateral membrane vesicles were prepared by a modification of the method of Kinsella and co-workers [16, 17] The modification consisted of collecting basolateral membranes on a discontinuous sucrose gradient composed of 8 and 38 7% sucrose Brush border and basolateral membranes were separated by precipitation of basolateral membranes in a buffer containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 1 mM $MnCl_2$ in 100 mM mannitol, 25 mM *N*-2-hydroxyethylpiperazine-*N*^{1}-2-ethanesulfonic acid (HEPES), pH 7 0 with Tris Purity of the two membranes relative to the starting homogenate was determined by measuring the enhancement of marker enzymes associated with the membranes Membranes were suspended in a buffer consisting of 300 mM mannitol and 10 mM HEPES, pH 7 0 with Tris

Uptake assays were performed at 25° in the above buffer unless specified differently in the text The assays were performed by incubating membrane vesicles with radiolabeled gentamicin for various time periods The reaction was initiated by the addition of membrane protein (100 μg) and was stopped by the addition of 40-fold excess (2 ml) of cold buffer Following the addition of cold buffer, the membranes were immediately collected on 0 45 micron Millipore filters under vacuum, and uptake was quantitated by counting radioactivity by liquid scintillation spectroscopy

Protein was determined by the method of Lowry *et al* [18], using bovine serum albumin as the standard. Na^+, K^+-ATPase was measured by a linked-enzyme assay in which oxidation of NADH was monitored continuously at 340 nm in the presence and absence of 1 mM ouabain [19] Alkaline phosphatase was measured at 420 nm by the *p*-nitrophenyl phosphate method [20], and succinic dehydrogenase was monitored at 600 nm by a method described by Earl and Korner [21]

Gentamicin sulfate was obtained from the Schering Corp (Kenilworth, NJ) [^{3}H]Gentamicin was obtained from Amersham Radiochemicals (Des Plaines, IL) (86 mCi/mmole)

Data are presented with standard errors Statistical significance was determined by Dunn's multiple comparison method [22] Kinetic plots were prepared by linear regression analyses

Results and discussion

Brush border and basolateral renal membrane vesicles were isolated and purified as indicated by enhancement of marker enzyme activities relative to the starting material Alkaline phosphatase, the brush border membrane marker, and Na^+, K^+-ATPase, the basolateral membrane marker, showed greater than 10-fold enrichment in the respective membrane fractions Cross-contamination of the membrane fractions was not evident since neither membrane showed enhancement relative to the starting material of the enzyme marker associated with the opposing membrane Both membranes showed a lack of enrichment of the mitochondrial enzyme marker, succinic dehydrogenase, indicating that the membrane fractions were free of mitochondrial contamination

The time courses of [^{3}H]gentamicin uptake in brush border and basolateral membrane vesicles are shown in Fig. 1 As evidenced by these data, uptake in both membranes was quite rapid and saturable It was also noted that basolateral uptake of [^{3}H]gentamicin was significantly higher than brush border uptake To further examine this difference, the uptake of [^{3}H]gentamicin was measured as a function of concentration Figure 2 displays the saturability of both concentration curves and confirms the quantitative differences in their uptake The data were further examined by Scatchard analysis as shown in Fig 3 A single population of binding sites was observed in both membranes However, the basolateral membrane exhibited a 2-fold greater capacity (N_{max}) for [^{3}H]gentamicin uptake than the brush border membrane Brush border kinetic

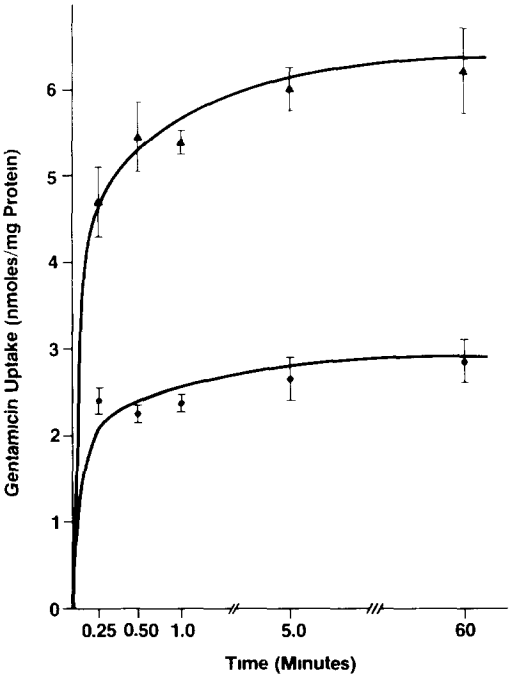

Fig 1 Time course of [^{3}H]gentamicin uptake in rat brush border (●) and basolateral (▲) membranes The uptake of 0 4 mM [^{3}H]gentamicin was measured by a rapid filtration assay following incubation with membrane vesicles for the time period indicated The data represent mean values of three experiments ± standard errors

parameters are in relatively good agreement with those previously reported in the rat by Sastrasinh *et al* (K_a = 25 μM, N_{max} = 2 1 nmoles/mg protein) [13] Basolateral binding parameters were similar to values previously observed [15] and confirm the quantitative difference between brush border and basolateral binding of gentamicin Similar data have been reported for [^{3}H]netilmicin binding to rat renal brush border and basolateral membrane vesicles [23]

To distinguish whether the uptake of [^{3}H]gentamicin observed in these experiments represented binding to or transport across the membrane vesicles the effects of media osmolarity and temperature on [^{3}H]gentamicin uptake were examined (Table 1) It is clear from these experiments that the uptake observed in this study largely represents binding as evidenced by the relative lack of sensitivity to alterations in intravesicular space and temperature A slight effect of osmolarity was observed in basolateral membranes which was statistically significant Uptake was only slightly affected by temperature in both membranes although the changes were statistically significant

Based upon biochemical parameters of renal function it has been observed that female rats are less sensitive to nephrotoxicity, due to aminoglycoside antibiotics than male rats [24] Since renal cortical levels of gentamicin were reported to be lower in females than in males [24] the comparative binding of gentamicin to renal membranes was investigated Scatchard analyses (Fig 4) revealed basolateral binding parameters in females very similar to those of males Female brush border membranes on the other hand, exhibited a lower affinity (K_d) of binding than males It is possible that the decreased affinity for brush border uptake in females compared to males may explain the lower renal accumulation observed *in vivo* which in turn may relate to less pathogenic injury

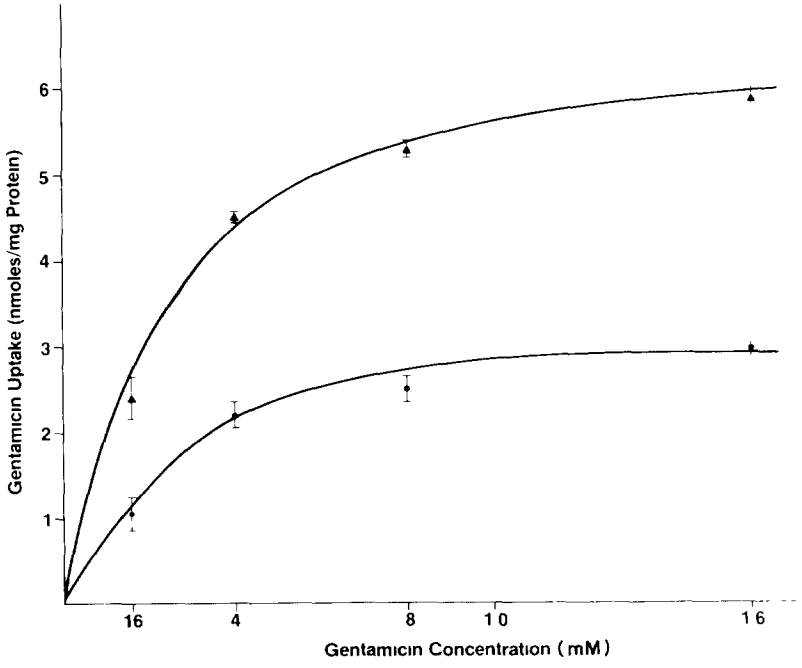

Fig 2 [^{3}H]Gentamicin uptake in rat brush border (●) and basolateral (▲) membrane vesicles as a function of concentration Equilibrium uptake was measured after a 15-min incubation of membrane vesicles with various concentrations of gentamicin Data displayed represent specific uptake Non-specific uptake was determined by the addition of 10-fold excess cold gentamicin to the uptake assay (specific uptake = total uptake − non-specific uptake) The data are mean values of three experiments ± standard errors

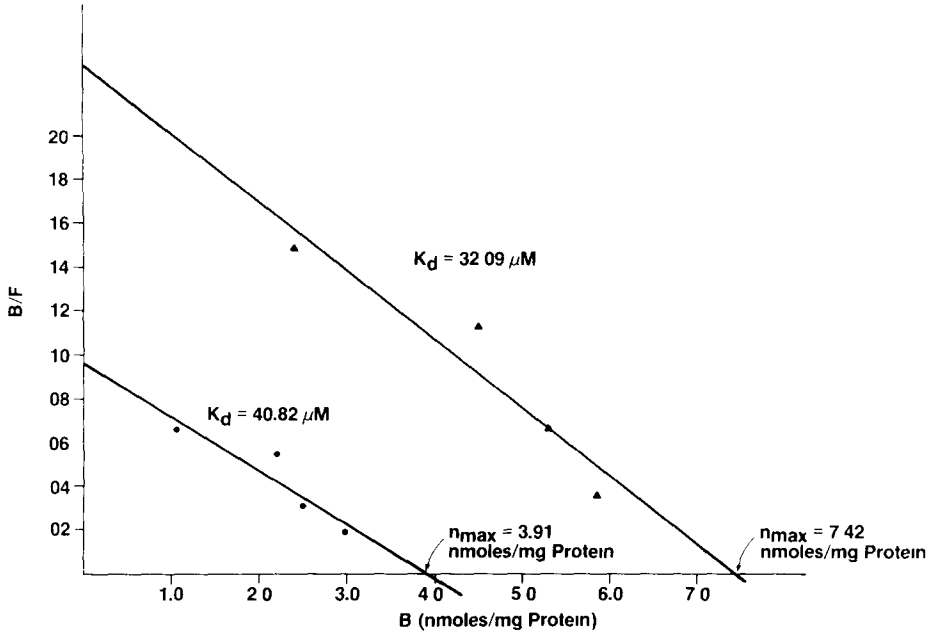

Fig 3 Scatchard plots of specific [^{3}H]gentamicin uptake in rat brush border (●) and basolateral (▲) membranes Equilibrium uptake was measured after a 15-min incubation of membrane vesicles with various concentrations of gentamicin B/F = bound/free

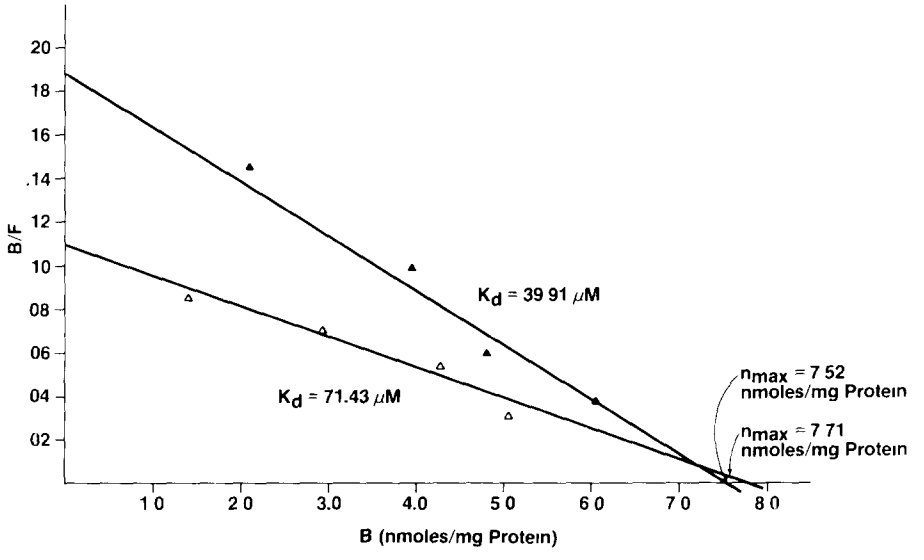

Fig 4 Scatchard plots of specific [^{3}H]gentamicin uptake in female brush border (△) and basolateral (▲) membranes Equilibrium uptake was measured after a 15-min incubation of membrane vesicles with various concentrations of gentamicin B/F = bound/free

These data provide evidence at the cellular membrane level that renal accumulation of gentamicin from the basolateral as well as the brush border surface of the kidney may be a significant process Furthermore, since concentrations of gentamicin in the glomerular filtrate and peritubular vasculature can be considered approximately equal (assuming little or no protein binding) [25], our data indicate that more basolateral than brush border binding of gentamicin may occur *in vivo* In fact, higher levels of gentamicin were found bound to basolateral compared to brush border membranes following *in vivo* injection of [^{3}H]gentamicin [17] However, the quantitative relationship between the degree of binding observed in these studies and the extent of renal transport or internalization via these membrane surfaces is unknown Collier *et al* [4] quantitated differences in the renal accumulation of gentamicin via luminal and vascular exposure utilizing the isolated perfused kidney in filtering and nonfiltering modes

Table 1 Effects of osmolarity and temperature on gentamicin uptake in rat renal brush border and basolateral membrane vesicles

	Gentamicin uptake* (nmoles/mg protein)	
	Membrane fraction	
	Brush border	Basolateral
Osmolarity†		
50	2 28 ± 0 15	6 21 ± 0 09
100	2 58 ± 0 09	5 96 ± 0 07
200	2 56 ± 0 12	5 61 ± 0 39
400	2 29 ± 0 02	5 53 ± 0 13
800	2 37 ± 0 09	5 54 ± 0 08
Δ Uptake‡	−0 09	+0 67§
Temperature		
4°	2 13 ± 0 044	5 12 ± 0 167
25°	2 44 ± 0.152	5 22 ± 0 338
37°	2 91 ± 0 321	6 09 ± 0 262
Δ Uptake‖	+0 78§	+0 97§

* Fifteen-second uptakes as described in text
† Osmolarity = mM sucrose
‡ Δ Uptake = uptake at 50 mM sucrose − uptake at 800 mM sucrose
§ $P < 0\ 05$, Dunn's test for multiple comparisons
‖ Δ Uptake = uptake at 37° − uptake at 4°

They found that renal accumulation of the drug in the filtering kidney was four times that of a non-filtering kidney Based upon these limited data, brush border transport may contribute more on a quantitative basis to renal accumulation than basolateral uptake Qualitatively, however, basolateral membrane binding may be more important in the pathogenic process since functional changes in basolateral membranes have been reported following gentamicin exposure [17]

In summary, this study provides a comparative analysis of renal brush border and basolateral membrane uptake of [^{3}H]gentamicin which demonstrates a significantly greater capacity of the basolateral membrane to bind gentamicin These data support the hypothesis that the basolateral as well as the brush border surface of the proximal tubular cell may contribute significantly to the renal accumulation of aminoglycosides

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