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Changes in Renal Brush-Border Sodium-Dependent Transport Systems in Gentamicin-Treated Rats

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To elucidate the mechanism of gentamicin induced renal dysfunction, renal functions and activities of various proximal tubular transport systems were studied in gentamicin-treated rats (Fisher 344). Gentamicin nephrotoxicity was induced by injecting gentamicin sulfate subcutaneously at a dose of 100 mg/kg · day for 7 days. The gentamicin injection resulted in a marked polyuria, hyposthenuria, proteinuria, glycosuria, aminoaciduria, phosphaturia, natriuresis, and kaliuresis, characteristics of aminoglycoside nephropathy. Such renal functional changes occurred in the face of reduced GFR, thus tubular transport functions appeared to be impaired. The polyuria and hyposthenuria were partly associated with a mild osmotic diuresis, but mostly attributed to a reduction in free water reabsorption. In renal cortical brush-border membrane vesicles isolated from gentamicin-treated rats, the Na^+ gradient dependent transport of glucose, alanine, phosphate and succinate was significantly attenuated with no changes in Na^+-independent transport and the membrane permeability to Na^+. These results indicate that gentamicin treatment induces a defect in free water reabsorption in the distal nephron and impairs various Na^+-cotransport systems in the proximal tubular brush-border membranes, leading to polyuria, hyposthenuria, and increased urinary excretion of Na^+ and other solutes.

Key Words: Gentamicin, Renal function, Renal brush-border membrane, Glucose, Amino acid, Phosphate, Succinate

INTRODUCTION

Use of aminoglycoside antibiotics in the treatment of Gram-negative bacterial infections has been complicated by nephrotoxic potential (Kaloyanides & Pastoriza-Munoz, 1980; Humes & O'Connor, 1988). The most prominent feature of aminoglycoside nephrotoxicity is proximal tubular dysfunction. It has been documented in humans and in experimental animals that gentamicin treatment results in glycosuria, aminoaciduria, phosphaturia, proteinuria (with β_2-microglobulinuria), and transport defects consistent with Fanconi-like syndrome (Ginsberg et al, 1976; Luft et al, 1978; Schentag et al, 1978; Cronin et al, 1980; Humes & O'Connor, 1988; Levi & Cronin, 1990). The mechanisms with which gentamicin induces these changes have not been entirely elucidated. Recent study by Levi and Cronin (1990) suggested that alterations in transport mechanisms in renal tubular brush-border membranes are involved. They observed in the renal cortical brush-border membrane vesicles (BBMV) isolated from gentamicin treated rats that the activities of Na^+ gradient-dependent phosphate transport (Na^+-Pi cotransport) and pH-gradient dependent Na^+ transport (Na^+-H^+ antiport) are significantly impaired. The present study provides evidences that similar changes occur in proximal tubular Na^+-dependent transport systems for glucose, amino acid and dicarboxylate as well.

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METHODS

Animals

Male Fisher 344 rats of 150~200 g were used. Gentamicin nephrotoxicity was induced by daily s.c. injections of gentamicin sulfate at a dose of 100 mg/kg · day for 7 days. The control animals were injected with saline.

Urine and blood analysis

Animals were kept in metabolic cages and were denied food and water for 24 hr. Urine was collected under mineral oil and was analyzed for Na^+/K^+ (Radiometer Flame Photometer FLM3), osmolality (Advanced Osmometer 3D2), creatinine (Wako Technical Bulletin No. 271-10509, Japan), glucose (Wako Technical Bulletin No. 270-66509), amino acids (LKB alpha plus amino acid analyzer), inorganic phosphate (Fiske & SubbaRow, 1925), protein (Bradford, 1976) and urea (Sigma Technical Bulletin No. 640). In some animals, blood samples were collected from the tail or heart and analyzed for creatinine and glucose.

Preparations of plasma membrane vesicles from renal cortex

Renal brush-border and basolateral membrane vesicles (BBMV and BLMV) were prepared from renal cortex by Percoll gradient centrifugation (Kinsella et al, 1979; Scalera et al, 1981) and magnesium precipitation (Booth & Kenny, 1974), as described in detail in a previous paper (Lee et al, 1990). The composition of vesicle buffer was 100 mM mannitol, 100 mM KCl, and 10 mM Hepes/Tris, pH 7.4. Purity of the BBMV and BLMV fractions was evaluated by analyzing activities of alkaline phosphatase (Wako Technical Bulletin No. 270-04609), a maker enzyme of the brush-border membrane (BBM), and Na^+-K^+-ATPase (Jφrgensen & Skou, 1971), a marker enzyme of the basolateral membrane (BLM). In the control group, the average specific activity of alkaline phosphatase in two separate batches of BBMV fraction (32.5 K-A unit/mg protein · hr) was 8.4-fold greater than that in the homogenate (3.9) and the Na^+-K^+-ATPase activity in the BLMV fraction (78.7 mol/mg protein · hr) was 8.3-fold greater than the homogenate value (9.5). However, the BBMV fraction was enriched in Na^+-K^+-ATPase (13.0 mol/mg protein · hr) 1.4-fold and the BLMV fraction was enriched in alkaline phosphatase (9.1 K-A unit/mg protein · hr) 2.4-fold, indicating that each fraction was slightly contaminated with opposite side membranes. Similar results were observed in the gentamicin group vesicles (alkaline phosphatase: 1.2, 11.2, and 2.5 K-A units/mg protein · hr in the homogenate, BBMV and BLMV, respectively; Na^+-K^+- ATPase: 9.3, 14.0 and 70.0 mol/mg protein · hr in the homogenate, BBMV and BLMV, respectively), although the alkaline phosphatase activity in each fraction was somewhat lower than the corresponding value in the control group, as observed by others (Knauss et al, 1983; Levi & Cronin, 1990).

Determination of substrate transport in membrane vesicles

Uptake of substrate by membrane vesicles was determined by a rapid filtration method (Hopfer et al, 1973). A 60 μl aliquot of vesicles (6~8 mg protein/ml) was incubated in 9 volumes of medium containing 100 mM NaCl or KCl, 100 mM mannitol, 10 mM Hepes/Tris (pH 7.4), and a substrate (D-[^{14}C]-glucose, L-[^{14}C]-alanine, [^{14}C]-succinate, $^{32}PO_4$, or ^{22}Na) at 25°C. At appropriate intervals, a 100 μl aliquot was removed and quickly filtered through Millipore filter (type HA, pore size 0.45 μm), which was soaked overnight in distilled water prior to use. The filter was washed with 5 ml of ice-cold stop solution (incubation medium without substrate). The radioactive materials in the filter was dissolved into 6 ml of Lumagel (Lumac, AC Landggaf, the Netherlands) and the radioactivity was counted on a liquid scintillation counter (Packard Tricarb 4530 C). Nonspecific binding of the radioactive material to the membrane was determined by incubating vesicles in distilled water containing 0.1% deoxycholate and radioactive substrate. The value of nonspecific binding was subtracted from the experimental value, and the vesicular uptake was expressed as pmol/mg protein for a given time.

All the radioactive compounds used were purchased from New England Nuclear (Boston, MA).

Statistical analysis

All results were presented as the mean ± SE and statistical evaluation of data was done using the Student's t-test (unpaired comparison).

RESULTS

Renal functions

Table 1 summarizes the effect of gentamicin treatment on urine flow (V), urine osmolality (U_{osm}), and urinary excretions of various solutes. Subcutaneous injections of gentamicin at a dose of 100 mg/kg · day for 7 days resulted in a marked increase in V and a decrease in U_{osm}. The average V in gentamicin-treated animals · day) was 3.5-fold greater ($P<0.001$) and the U_{osm} (852 ± 103 mosm/kg H_2O) was 62% lower ($P<0.001$) than the respective value in control (saline-treated) animals (V, 13.84 ± 0.63; U_{osm}, 2239 ± 106). The excretion of total osmotic substances (U_{osm} · V) was, however, not significantly different between the two groups (31.2 ± 2.5 and 41.5 ± 6.5 mosm/kg · day in control and gentamicin group, respectively), thus the gentamicin-induced polyuria and hyposthenuria appeared to be attributed mainly to an increase in water excretion.

The urinary excretion of creatinine (U_{cr} · V) was not significantly changed, indicating that the rate of creatinine production in the body was not altered by gentamicin. However, the creatinine clearance determined at the end of 7 day treatment appeared to be markedly reduced ($P<0.001$) in the gentamicin group (1.85 ± 0.65 l/kg · day) as compared with the controls (4.36 ± 0.37). This indicated that GFR was significantly reduced by gentamicin.

Despite this reduction in GFR, the urinary excretion of sodium (U_{Na} · V), potassium (U_K · V), phosphate (U_{Pi} · V) and glucose (U_g · V) was markedly increased in gentamicin-treated animals. The average increase was 3.4-fold in sodium (2.86 ± 0.18 mEq/kg · day in gentamicin group vs. 0.85 ± 0.15 in controls, $p<0.001$), 1.8-fold in potassium (5.01 ± 1.11 vs. 2.83 ± 0.32 mEq/kg · day, $p<0.10$), 1.9-fold in phosphate (46.59 ± 6.61 vs. 24.09 ± 1.89 mEq/kg · day, $p<0.01$), and 85-fold in glucose (209.2 ± 43.0 vs. 2.47 ± 0.28 mg/kg · day, $p<0.001$). The urinary excretion of urea (U_u · V) was, however, not significantly changed.

Table 2 shows that renal excretion of amino acids was also increased in gentamicin-treated animals. Although the degree of increase varied with amino acid, the gentamicin treatment increased excretion of all 16 amino acids examined, indicating that the renal transport systems for neutral, acidic and basic amino acids were universally impaired.

Transport of glucose, alanine, phosphate, and succinate in renal brush-border membrane vesicles

The foregoing results indicated that the gentamicin treatment enhanced the urinary excretions of glucose, amino acids and phosphate. Since these substances are normally reabsorbed in the proximal tubule by Na^+-coupled transport mechanisms, we next exam-

Table 1. Effect of gentamicin treatment on renal functions in rats

	Control (n=7)		Gentamicin (n=7)	
	Day 0	Day 7	Day 0	Day 7
V (ml/kg · day)	9.51 ± 0.71	13.84 ± 0.63	9.36 ± 0.67	47.89 ± 3.44*
U_{osm} (mosm/kg H_2O)	2733 ± 115	2239 ± 106	2822 ± 102	852 ± 103*
U_{cr} · V (mg/kg · day)	25.28 ± 1.39	27.90 ± 0.84	23.18 ± 1.66	29.23 ± 2.64
U_{osm} · V (mosm/kg · day)	25.6 ± 1.7	31.2 ± 2.5	26.5 ± 2.4	41.5 ± 6.5
U_{Na} · V (mEq/kg · day)	0.50 ± 0.05	0.85 ± 0.15	0.58 ± 0.11	2.86 ± 0.18*
U_K · V (mEq/kg · day)	2.69 ± 0.26	2.83 ± 0.32	2.69 ± 0.31	5.01 ± 0.10
U_{Pi} · V (mg/kg · day)	23.51 ± 2.02	24.09 ± 1.89	26.49 ± 2.35	46.59 ± 6.61*
U_g · V (mg/kg · day)	1.97 ± 0.19	2.47 ± 0.28	2.39 ± 0.15	209.2 ± 43.0*
U_u · V (mmol/kg · day)	14.20 ± 1.05	17.16 ± 1.61	14.95 ± 1.40	13.37 ± 3.31
C_{cr} (l/kg · day)		4.36 ± 0.37		1.85 ± 0.65

Note. Gentamicin group animals were subcutaneously injected with gentamicin sulfate at a dose of 100 mg/kg · day for 7 days. Values represent means ± SE. *Significantly different from the respective control value ($p<0.05$).

Table 2. Effect of gentamicin treatment on renal excretion of amino acids in rats

Amino Acid	Excretion (μmole/kg · day)			
	Control (n=4)		Gentamicin (n=5)	
	Day 0	Day 7	Day 0	Day 7
Glycine	9.05±1.16	11.48±1.71	8.78±0.55	30.65±7.03*
Alanine	2.65±0.43	2.98±0.38	2.67±0.26	12.19±2.93*
Valine	0.52±0.31	0.00±0.00	0.55±0.40	7.33±2.19*
Leucine	2.02±0.34	2.05±0.15	2.50±0.22	17.46±3.97*
Threonine	2.52±0.40	2.71±0.60	2.48±0.06	19.61±4.13*
Serine	1.95±0.37	2.22±0.51	2.47±0.20	11.37±2.93*
Glutamic acid	5.49±0.63	5.37±0.23	5.31±0.23	52.85±13.84*
Tyrosine	3.73±0.77	3.61±0.15	5.05±0.94	9.87±2.11*
Phenylalanine	0.66±0.31	0.17±0.19	0.41±0.28	8.34±2.26*
Glutamine	2.53±0.84	3.44±1.27	3.54±0.33	18.20±6.65*
Lysine	3.38±0.42	4.24±0.31	4.33±0.23	39.21±6.33*
Arginine	0.83±0.15	1.34±0.59	1.04±0.48	8.80±2.13*
Cystine	2.48±0.35	2.45±0.11	2.82±0.21	3.56±0.46
Methionine	2.57±0.87	1.36±0.12	2.62±0.66	6.77±2.55*
Proline	1.11±0.14	1.31±0.09	1.04±0.10	5.90±1.30*
Histidine	1.27±0.15	1.34±0.20	1.53±0.14	8.55±2.43*

Note. Gentamicin group animals were subcutaneously injected with gentamicin sulfate at a dose of 100 mg/kg · day for 7 days. Values represent means±SE. *Significantly different from the respective control value ($p<0.05$).

ined if these systems were impaired by gentamicin treatment. At the end of 7 day drug treatment animals were sacrificed and renal plasma vesicles were isolated.

Fig. 1 shows time courses of D-glucose uptake by renal BBMVs of control (saline-treated) and gentamicin-treated rats. Vesicles containing 100 mM mannitol, 100 mM KCl, and 10 mM Hepes/Tris (pH 7.4) were incubated in a medium containing 100 mM NaCl, 100 mM mannitol, 10 mM Hepes/Tris (pH 7.4) and 50 μM D-glucose (with 3 μM D-[^{14}C]glucose), thus the imposed Na^+ gradient was 100 mM. In control vesicles, the inwardly directed Na^+ gradient markedly stimulated D-glucose uptake over that observed in the absence of Na^+ (K^+ containing medium), showing a characteristic "overshoot" of the Na^+-driven transport process. In vesicles of gentamicin-treated animals, however, the initial rate of D-glucose uptake was markedly attenuated and overshoot phenomenon was not apparent. The Na^+-independent component of D-glucose uptake was not different between the two groups.

In order to evaluate the effect of gentamicin treatment on the D-glucose facilitated transport in the basolateral membrane we examined the D-glucose uptake by BLMV. The results, however, indicated that there was no significant changes in the gentamicin group (Data not shown).

Fig. 2 illustrates time courses of L-alanine uptake by renal cortical BBMVs of control and gentamicin-treated rats. In the presence of a 100 mM inwardly-directed Na^+ gradient, the L-alanine uptake increased rapidly showing a definite overshoot both in the control and gentamicin groups. However, the rate of uptake and the degree of overshoot appeared to be significantly reduced in the gentamicin group. The Na^+-independent L-alanine uptake was not different between the two groups.

Fig. 3 compares time courses of phosphate uptake by renal cortical BBMVs of control and gentamicin treated rats. The Na^+-gradient driven uphill transport was drastically inhibited in the gentamicin group with no change in Na^+-independent transport.

Fig. 4 depicts the effect of gentamicin treatment on

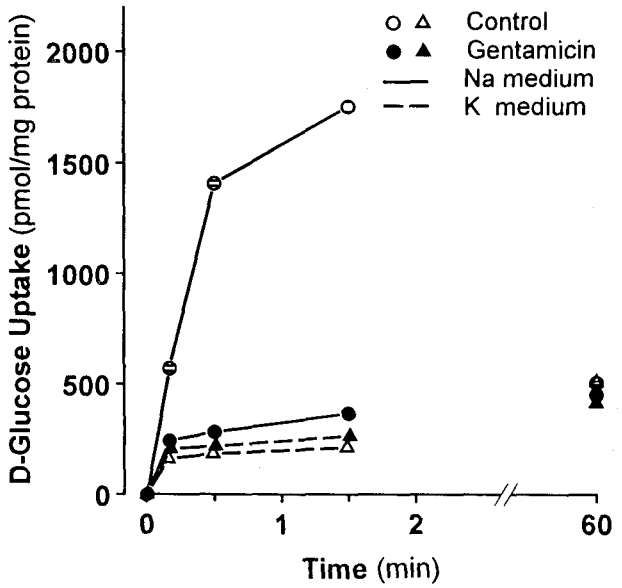

Fig. 1. Effect of gentamicin treatment on D-glucose transport system in renal cortical brush-border membrane. D-glucose uptakes by BBMVs of control and gentamicin-treated (100 mg/kg · day for 7 days) rats were measured at 25°C in the presence and absence of 100 mM inwardly-directed initial Na^+ gradient as a function of time. The medium contained 50 μM D-glucose, 3 μM D-[^{14}C]-glucose and 2 μM valinomycin. Data represent mean ± SE of 3 determinations from the same batch of vesicles derived from 10 rats in each group. For all data points the SE was less than the radius of symbol. All values of uptake for gentamicin group in Na^+-containing medium at times other than 60 min were significantly different ($p < 0.001$) from the corresponding control values.

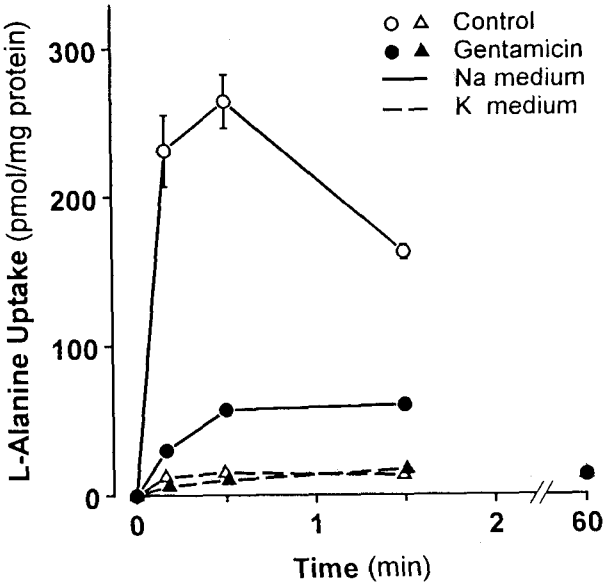

Fig. 2. Effect of gentamicin treatment on L-alanine transport system in renal cortical brush-border membrane. L-alanine uptakes by BBMVs of control and gentamicin-treated (100 mg/kg · day for 7 days) rats were measured at 25°C in the presence and absence of 100 mM inwardly-directed initial Na^+ gradient as a function of time. The medium contained 6 μM L-[^{14}C]-alanine and 4 μM valinomycin. Data represent mean ± SE of 3 determinations from the same batch of vesicles derived from 10 rats in each group. In the data point where no vertical bar is shown the SE is less than the radius of the symbol. All values of uptake for gentamicin group in Na^+-containing medium at times other than 60 min were significantly different ($p < 0.001$) from the corresponding control values.

succinate uptake by renal cortical BBMVs. Again, the Na^+-dependent transport component appeared to be markedly reduced in the gentamicin group.

The foregoing results indicate that the Na^+-dependent transports of glucose, alanine, phosphate and succinate were attenuated in the vesicles of gentamicin-treated animals. Since the cotransport of these substances with Na^+ depends on the electrochemical potential gradient for Na^+, it would decrease if the Na^+ gradient was more rapidly dissipated in the gentamicin group vesicles. To evaluate this possibility, ^{22}Na uptake by BBMVs was determined in the presence of 1 mM Na^+ and absence of substrate in the medium. The results indicated that gentamicin treatment had no apparent effect on the dissipation of the Na^+ gradient (Fig. 5).

DISCUSSION

Glucose transport

Under normal conditions, the filtered glucose is mostly reabsorbed in the proximal tubule. This process occurs in two steps. The filtered glucose first enters the tubular cell by Na^+-glucose cotransport mechanism at the BBM; it then leaves the cell at the BLM via the facilitated diffusion mechanism that is Na^+ independent (Ullrich, 1983; Burg, 1986).

In the present study, gentamicin treatment to rats resulted in a marked glycosuria (Table 1), as observed in other studies (Ginsburg et al, 1976; Luft et al, 1978; Cronin et al, 1980). This occurred with no significant increase in plasma glucose level, indi-

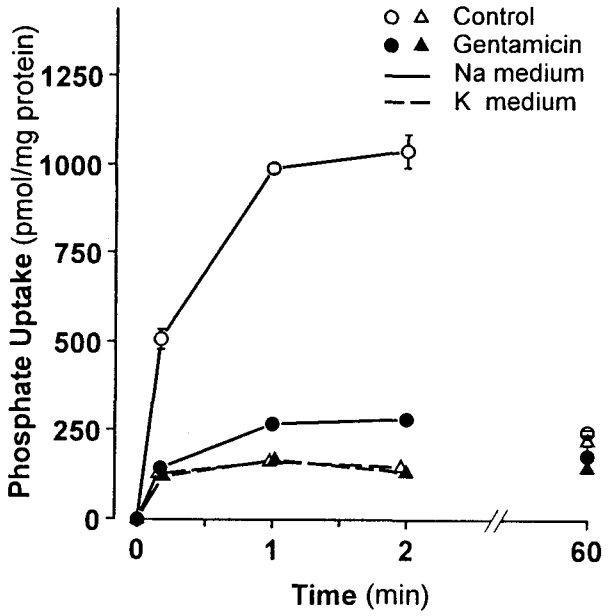

Fig. 3. Effect of gentamicin treatment on phosphate transport system in renal cortical brush-border membrane. Phosphate uptakes by BBMVs of control and gentamicin-treated (100 mg/kg · day for 7 days) rats were determined at 25°C in the presence and absence of 100 mM inwardly-directed initial Na^+ gradient as a function of time. The medium contained 100 μM $KH_2{}^{32}PO_4$. Data represent mean ± SE of 4 determinations from the same batch of vesicles derived from 10 rats in each group. All values of uptake for gentamicin group in Na^+-containing medium at times other than 60 min were significantly different ($p < 0.001$) from the corresponding control values.

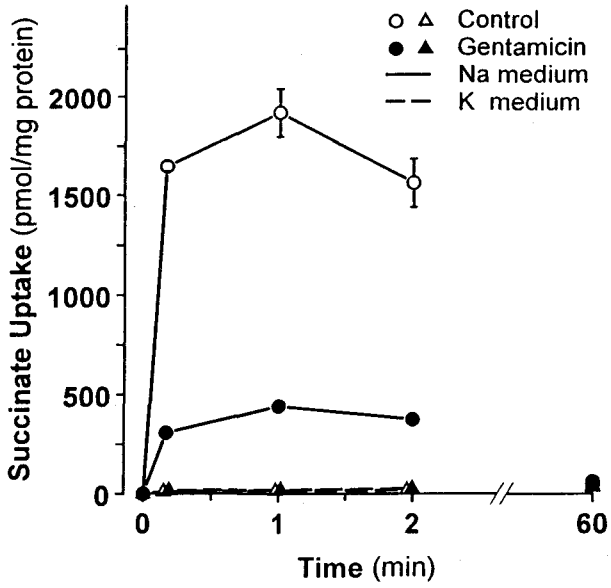

Fig. 4. Effect of gentamicin treatment on succinate transport system in renal cortical brush-border membrane. Succinate uptakes by BBMVs of control and gentamicin-treated (100 mg/kg · day for 7 days) rats were determined at 25°C in the presence and absence of 100 mM inwardly-directed initial Na^+ gradient as a function of time. The medium contained 10 μM [^{14}C]-succinate and 4 μM valinomycin. Data represent mean ± SE of 4 determinations from the same batch of vesicles derived from 10 rats in each group. All values of uptake for gentamicin group in Na^+-containing medium at times other than 60 min were significantly different ($p < 0.001$) from the corresponding control values.

cating that the glucose reabsorption in the renal tubule was attenuated. Vesicle experiments indicated that the first step of renal glucose reabsorption was impaired. In BBMVs isolated from gentamicin treated rats, the Na^+-dependent glucose uptake was markedly attenuated (Fig. 1), whereas the Na^+-independent glucose uptake (Fig. 1) and the permeability of the BBM to Na^+ (Fig. 5) were not significantly changed. It is, therefore, apparent that the Na^+-glucose cotransporter activity was specifically impaired.

The mechanism with which gentamicin affected the cotransporter is not clearly understood. According to Horio et al. (1986), a direct exposure of rabbit renal BBMV to gentamicin, *in vitro*, also inhibits the Na^+-coupled glucose transport, and the inhibition is due to a reduction in Vmax with no change in Km. It is, therefore, possible that during long-term exposure, gentamicin accumulated in the kidney directly interacts with the BBM, attenuating the cotransporter function. The gentamicin may directly alter the effective carrier number or turnover rate, or indirectly through alterations in membrane lipid composition. With respect to the latter possibility, it is known that 1) renal BBM has a high phosphatidylserine content and a high cholesterol-to-phospholipid ratio (Moritoris & Simon, 1985), and alterations in these lipid compositions affect Vmax of the Na^+-glucose cotransport without changing Km (Moritoris & Kinne, 1987), and 2) gentamicin inhibits phospholipase-C in the proximal tubular BBM (Schwertz et al, 1984) and induces changes in phospholipid composition with preferential increase in phosphatidylinositol, phosphatidylcholine,

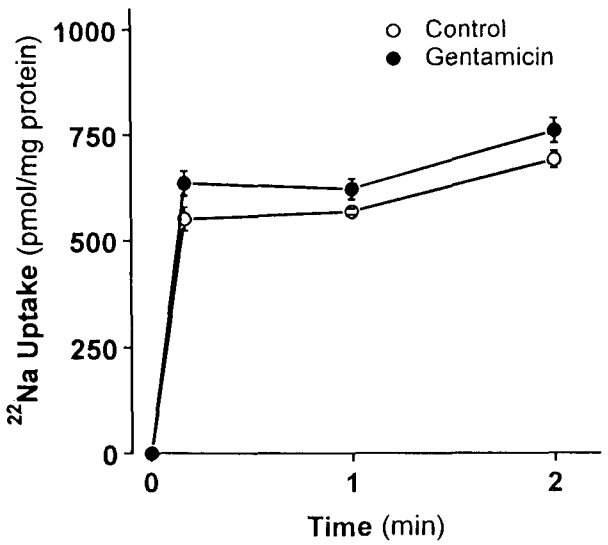

Fig. 5. Effect of gentamicin treatment on Na^+ permeability of renal cortical brush-border membrane. Na^+ uptakes by BBMVs of control and gentamicin-treated (100 mg/kg · day for 7 days) rats were determined at 25°C in the presence of 1 mM inwardly-directed initial Na^+ gradient and in the absence of substrate. The medium contained 1 μM ^{22}NaCl. Data represent mean ± SE of 3 determinations in each group.

and phosphatidylserine (Feldman et al, 1982; Knauss et al, 1983). Certainly, many more studies are required to precisely delineate the modification of Na^+-glucose cotransporter activity by gentamicin. Regardless of the mechanism, however, the present study clearly demonstrated that gentamicin treatment impairs the first step of the renal glucose reabsorption and this may be the most important etiologic mechanism of gentamicin-induced renal glycosuria.

Transport of alanine, phosphate and succinate

Under normal conditions, amino acids in the glomerular filtrate are mostly reabsorbed in the proximal tubule by their respective Na^+-coupled transport mechanisms in the BBM (Burg, 1986). In the present study, gentamicin treated rats showed increase in urinary excretion of various amino acids (Table 2) and their renal BBM showed a significantly impaired Na^+-alanine cotransport activity (Fig. 2). We, therefore, believe that one mechanism of the gentamicin-induced aminoaciduria is a defect in cotransport systems for amino acids in the renal tubular BBM. Again, the nature of interaction between gentamicin and the cotransporters is not understood.

Over 80% of inorganic phosphate filtered through glomeruli are normally reabsorbed in the renal tubule, almost all of this occurring in the proximal tubule (Greger et al, 1977; Ullrich et al, 1977; Bauman et al, 1985). Filtered phosphate initially moves from the lumen into the tubular cell via the Na^+-Pi cotransport mechanism in the BBM, then it diffuses across the BLM into the blood (Hammerman, 1986), the first process being the rate-limiting step (Gmaj & Murer, 1986; Biber, 1989). In the present study, gentamicin administrations to rats caused a significant increase in phosphate excretion (Table 1) and a decrease in Na^+-Pi cotransport activity in the BBM (Fig. 3). Similar results have been observed by others (Levi & Cronin, 1990) in gentamicin treated Sprague Dawley rats. Since, in the above study, the Vmax of Na^+-Pi cotransport and the Na^+-dependent phosphonoformic acid binding were reduced in the BBMV of gentamicin treated rats, the authors presumed that the number of Na^+-Pi cotransport units was diminished by gentamicin. However, the mechanism whereby gentamicin induces such changes has not been ascertained.

Krebs cycle intermediates filtered through glomeruli are reabsorbed in the proximal tubule (Glollman et al, 1963; Simpson, 1983). Studies involving renal membrane vesicles indicated that transport of dicarboxylates, such as succinate, in the renal BBM is mediated by the Na^+-dicarboxylate cotransporter (Wright et al, 1980; 1982). The results of the present study indicated that the activity of this cotransporter is inhibited by gentamicin. In the BBMV of gentamicin treated rats the Na^+-dependent succinate uptake appeared to be markedly attenuated (Fig. 4). We, therefore, speculate that a large amounts of Krebs cycle intermediates may be lost into urine in gentamicin treated animals.

In conclusion, the present study shows that gentamicin treatment in rats impairs various Na^+-dependent secondary active transport systems in the renal tubular brush-border membrane, which may lead to increased urinary excretions of Na^+ and other solutes. The subcellular mechanisms by which these changes are induced remain to be elucidated.

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REFERENCES

Bauman K, De Rouffignac C, Roinel N, Rumrich G, Ullrich KJ. Renal phosphate transport: inhomogeneity of local proximal transport rates and sodium dependence. *Pflugers Arch* 356: 287−298, 1985

Biber J. Cellular aspects of proximal tubular phosphate reabsorption. *Kidney Int* 36: 360−369, 1989

Booth AG, Kenny AJ. A rapid method for the preparation of microvilli from rabbit kidney. *Biochem J* 142: 575−581, 1974

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248−254, 1976

Burg MB. Renal handling of sodium, chloride, water, aminoacids, and glucose. In: Brenner BM, Rector FC ed, *The Kidney*. 3rd ed. Saunders, Philadelphia, Vol 1, p 145−175, 1986

Cronin RE, Bulger RE, Southern P, Henrich WC. Natural history of aminoglycoside nephrotoxicity in the dog. *J Lab Clin Med* 95: 463−474, 1980

Feldman S, Wang M, Kaloyanides GJ. Aminoglycosides induce a phospholipidosis in the renal cortex of the rat: an early manifestation of nephrotoxicity. *J Pharmacol Exp Ther* 220: 514−520, 1982

Fiske CH, SubbaRow YJ. The calorimetric determination of phosphorous. *J Biol Chem* 66: 375−400, 1925

Ginsburg DS, Quintanilla AP, Levin M. Renal glycosuria due to gentamicin in rabbits. *J Inf Dis* 413: 119−122, 1976

Glollman AP, Walker WG, Harrison HC, Harrison HE. Site of reabsorption of citrate and calcium in the renal tubule of the dog. *J Physiol* 205: 697−701, 1963

Gmaj P, Murer H. Cellular mechanisms of inorganic phosphate transport in kidney. *Physiol Rev* 66: 36−70, 1986

Greger R, Lang F, Marchand D, Knox FG. Site of renal phosphate reabsorption. Micropuncture and microinfusion study. *Pflugers Arch* 369: 111−118, 1977

Hammerman MR. Phosphate transport across the renal proximal tubular cell membranes. *Am J Physiol* 251: F385−F398, 1986

Hopfer U, Nelson K, Perrotto I, Isselbacher KJ. Glucose transport in isolated brush border membranes from rat small intestine. *J Biol Chem* 248: 25−32, 1973

Horio M, Fukuhara Y, Orita Y, Nakanishi T, Nakahama H, Moriyama T, Kamada T. Gentamicin inhibits Na^+-dependent D-glucose transport in rabbit kidney brush-border membrane vesicles. *Biochim Biophys Acta* 858: 153−160, 1986

Humes HD, O'Connor RP. Aminoglycoside nephrotoxicity. In: Schrier RW,. Gottschalk CW ed, *Diseases of the Kidney*. 4th ed. Little, Brown and Company, Boston/Toronto, Vol II, p 1229−1273, 1988

Jørgensen PL, Skou JC. Purification and characterization of Na-K-ATPase in preparations from the outer medulla of rabbit kidney. *Biochim Biophys Acta* 233: 366−388, 1971

Kaloyanides GJ, Pastoriza-Munoz E. Aminoglycoside nephrotoxicity. *Kidney Int* 18: 571−582, 1980

Kinsella JL, Holohan PD, Pessah NI, Ross CR. Isolation of luminal and antiluminal membranes from dog kidney cortex. *Biochim Biophys Acta* 552: 468−477, 1979

Knauss TC, Weinberg JM, Humes HD. Alterations in renal cortical phospholipid content induced by gentamicin: time course, specificity and subcellular localization. *Am J Physiol* 244: F535−F546, 1983

Lee HY, Kim KR, Woo JS, Kim YK, Park YS. Transport of organic compounds in renal plasma membrane vesicle of cadmium intoxicated rats. *Kidney Int* 37: 727−735, 1990

Levi M, Cronin RE. Early selective effects of gentamicin on renal brush-border membrane Na-Pi cotransport and Na-H exchange. *Am J Physiol* 258: F1379−F1387, 1990

Luft FC, Bloch R, Bloan RS, Yum MN, Costello R, Maxwell DR. Comparative nephrotoxicity of aminoglycoside antibiotics in rats. *J Infect Dis* 134: 541−548, 1978.

Molitoris BA, Simon FR. Renal cortical brush-border and basolateral membranes: cholesterol and phospholipid composition and relative turnover. *J Membr Biol* 83: 207−215, 1985

Molitoris BA, Kinne R. Ischemia induces surface membrane dysfunction. Mechanism of altered Na^+-dependent transport. *J Clin Invest* 80: 647−654, 1987

Scalera V, Huang YK, Hildermann B, Murer H. Simple isolation method for basal-lateral plasma membranes from rat kidney cortex. *Membr Biochem* 4: 49−61, 1981

Schentag JJ, Sutein TA, Plant ME, Jusko WJ. Early detection of aminoglycoside nephrotoxicity with urinary beta-2 microgloblin. *J Med* 9: 201−210, 1978

Schwertz DW, Kreisberg JI, Veukatachalam MA. Effects of aminoglycosides on proximal tubule brush border membrane phosphatidylinositol-specific phospholipase C. *J Pharmacol Exp Ther* 231: 48−55, 1984

Simpson DP. Citrate excretion: a window on renal metabolism. *Am J Physiol* 244: F223–F234, 1983

Ullrich KJ. Renal transport of organic solutes. In: Giebisch G, Tosteson DC, Ussing HH ed, *Membrane Transport in Biology*. Springer-Verlag, Berlin, Heidelberg, New York, Vol IV A, p 413–448, 1983

Ullrich KJ, Rumrich G, Kloess S. Phosphate transport in the proximal convolution of the rat kidney. I. Tubular heterogeneity, effect of parathyroid hormone in acute and chronic parathyroidectomized animals and effect of phosphate diet. *Pflugers Arch* 372: 269–274, 1977

Wright SH, Kippeu I, Klinenberg JR, Wright EM. Specificity of the transport system for tricarboxylic acid cycle intermediates in renal brush borders. *J Membr Biol* 57: 73–82, 1980

Wright SH, Kippeu I, Wright EM. Stoichiometry of Na^+-succinate cotransport in renal brush border membranes. *J Biol Chem* 257: 1773–1778, 1982