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FURTHER STUDIES OF THE RESPONSE OF KIDNEY LYSOSOMES TO AMINOGLYCOSIDES AND OTHER CATIONS

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Abstract—Rat renal cortical lysosomes were isolated in 0.3 M sucrose containing 1 mM EDTA by differential centrifugation. Lysosomes were incubated in isotonic sucrose or isotonic glycine with various concentrations of endogenous and exogenous compounds at 37° for 1 hr. Lysosomes were resedimented, and the *N*-acetyl-β-glucosaminidase (NAG) activity was measured in the supernatant fraction and the disrupted pellet and the percentage of total NAG released was calculated. Gentamicin and its C_1 and C_2 components had similar potencies for inhibiting NAG release from lysosomes at low concentrations. The release of alpha-galactosidase and beta-galactosidase from lysosomes was also inhibited by streptomycin and gentamicin. Mepacrine at low concentrations stabilized lysosomes and at high concentrations disrupted lysosomes. This drug also enhanced the effect of low concentrations of gentamicin on lysosomes. Inositol hexaphosphoric acid was a potent antagonist of the effect of low concentrations of gentamicin and mepacrine on lysosomes. Rats were treated with gentamicin at doses of 40, 80 and 160 mg/kg for 1 and 3 days. NAG excretion in gentamicin-treated groups as compared to saline controls was unchanged at day 1. Only the 160 mg/kg treatment group showed a tendency toward elevated renal cortical NAG at day 1 ($P < 0.06$). All treatment groups had elevated renal cortical NAG at day 3, while the 160 mg/kg group also had elevated NAG excretion. Lysine, arginine, L-canavanine and polymyxin B all affected NAG release from lysosomes *in vitro*. Lysine enhanced the disruptive effect of high gentamicin concentrations on lysosomes. Ferric and ferrous ions, tested over widely varied concentrations, inhibited NAG release at low concentrations while enhancing NAG release at high concentrations. We therefore conclude that the nephrotoxicity of aminoglycoside and other endogenous and exogenous renally excreted cationic compounds may be produced by their effects on lysosomes in the proximal renal tubule.

Recent studies of the pathogenesis of acute renal tubular necrosis caused by aminoglycosides have focused on the inhibitory effects of these drugs on phospholipid metabolism and its probable significance [1–3]. We have postulated recently a mechanism by which renal proximal tubular injury can result from a decrease in lysosomal phospholipid metabolism caused by aminoglycosides concentrated in the lysosome [4]. We now report *in vivo* and *in vitro* studies completed in an effort to further investigate the action of aminoglycosides and other exogenous and endogenous compounds on the proximal renal tubular lysosomal system.

MATERIALS AND METHODS

Gentamicin C_1 and C_2 and sterile injectable gentamicin were donated by or purchased from the Schering Corp., Kenilworth, NJ. Mepacrine hydrochloride was purchased from ICN Pharmaceuticals, Inc., Plainview, NY. 4-Methylumbelliferyl derivatives of *N*-acetyl-β-glucosamide, alpha-galactoside and beta-galactoside, used as enzyme substrates, were purchased from the Sigma Chemical Co., St Louis, MO. Inositol hexaphosphoric acid (phytic acid), spermine and sulfates of streptomycin, gentamicin, L-canavanine, and polymyxin B were purchased from the Sigma Chemical Co.

Enzyme assays. *N*-Acetyl-β-glucosaminidase (EC 3.2.1.30) (NAG) was measured by our previously published method [4]. Alpha-galactosidase (EC 3.2.1.22) and beta-galactosidase (EC 3.2.1.23) were assayed by the fluorimetric measurement of the rate of release of 4-methylumbelliferone (MU) from the appropriate methylumbelliferylgalactoside. A 0.5 mM substrate concentration was used for the assay of alpha-galactosidase activity. For measurement of beta-galactosidase activity, a 0.25 mM concentration of the 4-methylumbelliferyl-β-galactoside was used.

A 50 μl aliquot of the diluted enzyme source was added to 950 μl of the substrate dissolved in 0.02 M sodium citrate buffer at pH 4.8. The reaction was incubated for 1 hr at 37° in a gently shaking water bath. The reaction was immediately quenched by the addition of 3 ml of 0.25 M sodium glycinate buffer (pH 10.7). The 4-methylumbelliferone formed was measured as the increase in fluorescence in an Aminco–Bowman spectrophotofluorometer (excitation 365 nm, emission 450 nm). The fluorescence of known concentrations of 4-methylumbelliferone was measured, and a linear calibration curve was constructed. Enzyme activity was expressed as nanomoles of MU liberated per hour per milliliter.

Lysosomal preparation. A lysosomal-mitochondrial pellet from the renal cortex of male Fisher 344 rats was isolated by differential centrifugation at 0–5°, incubated with and without drug in 0.25 M

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glycine at pH 7.0 (isotonic glycine) or 0.25 M sucrose at pH 7 (isotonic sucrose) at a temperature of 37° and resedimented after 1 hr according to our previously published method [4]. The supernatant fraction was removed and the resedimented pellets were disrupted in 0.1% Triton X-100. The percent enzyme released was measured as the enzyme activity present in the supernatant fraction divided by the total of the enzyme activity in the supernatant fraction and in the resedimented pellet after disruption.

In vivo *effects of gentamicin treatment.* Male Fisher 344 rats of equal weights were housed in standard metal cages and allowed water and standard lab chow *ad lib.* They were divided into four groups. Three groups received sterile injectable gentamicin at doses of 40, 80 or 160 mg/kg, respectively, in one subcutaneous injection at 8:00 a.m. daily. The fourth group received daily subcutaneous injections of sterile saline as concurrent controls.

Animals were taken from each group on day 1 (24 hr after one injection) and day 3 (24 hr after the third injection) for study. The rats were anesthetized with sodium pentobarbital. Under direct vision, urine was withdrawn from the bladder through a 21 gauge needle. Urine specimens were placed on ice for later NAG (same day) and creatinine (Cr) measurements. Rats were perfused transcardially with ice-cooled 1 mM EDTA solution in 0.3 M sucrose at pH 7.0 until the kidneys blanched, after which a kidney was removed. The renal cortical tissue was quickly separated, weighed and minced with sharp scissors. The tissue was homogenized at 0–5° in a 9:1 vol. of 100 mM KCl in 0.1% Triton X-100, using a Potter–Elvehjm homogenizer with a Teflon pestle rotating at 1500 rpm. Aliquots of this homogenate were diluted at least 1:300 in distilled water for same day NAG enzyme determination.

Urine studies. Creatinine in the urine was measured by an automated method (manufactured by Gilford Diagnostics, Cleveland, OH) of the alkaline picrate reaction [5]. Urinary NAG was expressed as NAG activity per mg urinary creatinine.

In vivo *effects of chronic gentamicin treatment.* Male Fisher 344 rats were divided into two groups having equal mean weights. They were housed in standard metal cages and allowed water and standard lab chow *ad lib.* One group was given sterile injectable gentamicin, 20 mg/kg subcutaneously, at 8.00 a.m. and 4.00 p.m. daily. The second group received an equal volume of saline as concurrent controls. All animals survived until time of sacrifice. After 28 days rats were killed. Kidneys were removed and renal cortices were homogenized by our previously published method [4]. An aliquot of the homogenate was diluted in 0.1% Triton X-100 and analyzed for NAG activity per gram renal cortex. Lysosomes were isolated from the remaining homogenate by differential centrifugation, resuspended, and incubated in isotonic sucrose. After resedimentation, NAG activity was measured in the pellet and in the supernatant fraction. The percentage, of the total enzyme activity, release into the supernatant fraction on incubation was determined.

RESULTS

Rat renal cortical lysosomes released NAG enzyme in response to incubation in an isotonic solution of glycine. We previously reported the ability of gentamicin to inhibit the release of NAG in a dose-related fashion at low *in vitro* concentrations of gentamicin [4]. Gentamicin, gentamicin C_1 and gentamicin C_2 did not differ significantly in their abilities to inhibit NAG release from lysosomes. The total enzyme activity was the same in each tube indicating no NAG enzyme inhibition by the drugs. Triton X-100 also had no effect on enzyme activity when tested.

Lysosomes from rat renal cortx also released alpha-galactosidase (Fig. 1) and beta-galactosidase (Fig. 2) on incubation in isotonic glycine. The aminoglycosides gentamicin and streptomycin were also able to inhibit the release of these enzymes from lysosomes in a dose-related fashion.

NAG release from renal cortical lysosomes incubated with 16 μg/ml of phytic acid in isotonic glycine did not differ from that released by incubation with glycine alone. The responses of lysosomes incubated in the presence of various concentrations of gentamicin alone in isotonic glycine, and in gentamicin with 16 μg/ml of phytic acid were tested. Percent response was calculated by the following formula:

$$\% \text{ Response} = \frac{\text{mean \% NAG release by control} - \text{\% NAG release by drug}}{\text{mean \% NAG release by control}} \times 100.$$

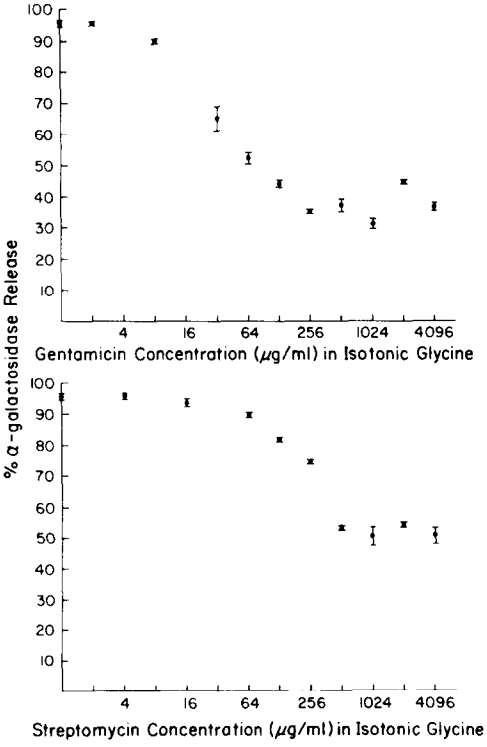

Fig. 1. Alpha-galactosidase release from lysosomes as a function of the concentration of streptomycin in isotonic glycine. $P < 0.01$ at nadir compared to control. Points are mean ± S.E.

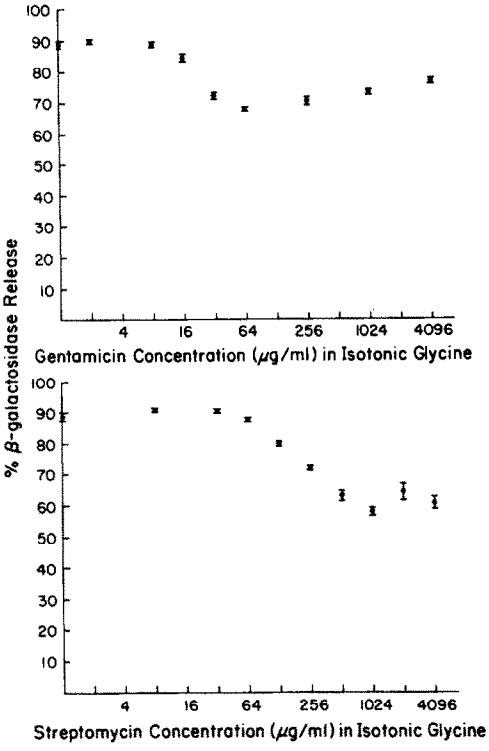

Fig. 2. Beta-galactosidase release from lysosomes as a function of the concentration of streptomycin and gentamycin in isotonic glycine. $P < 0.01$ at nadir compared to control.

Phytic acid inhibited the effect of gentamicin on lysotomes at all concentrations of gentamicin tested (Fig. 3).

To determine the role of lysosomal phospholipases in the observed NAG release pattern, a phospholipase inhibitor, mepacrine [6], was tested at low and high concentrations in a renal cortical lysosomal suspension. This compound exhibited a biphasic effect of inhibition of NAG release at low concentrations with enhanced NAG release at high concentrations (Fig. 4). A mixture of mepacrine produced a greater response than either of the drugs alone at the concentration in the mixture. Their effects appeared additive. Phytic acid at a concentration of 16 μg/ml also inhibited the effect of mepacrine at low concentrations in isotonic glycine (Fig. 5).

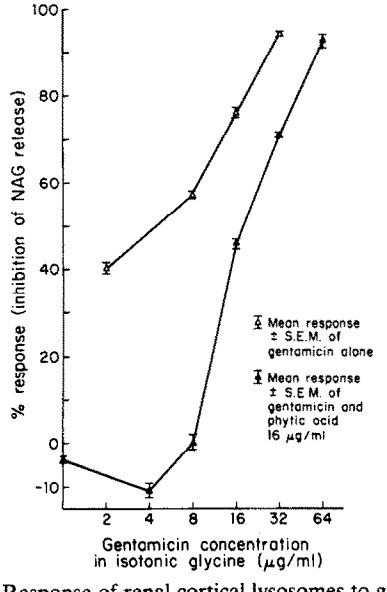

Fig. 3. Response of renal cortical lysosomes to gentamicin in the presence of a constant concentration of phytic acid in isotonic glycine. The response of lysosomes to phytic acid in glycine did not differ from the response of lysosomes in glycine alone.

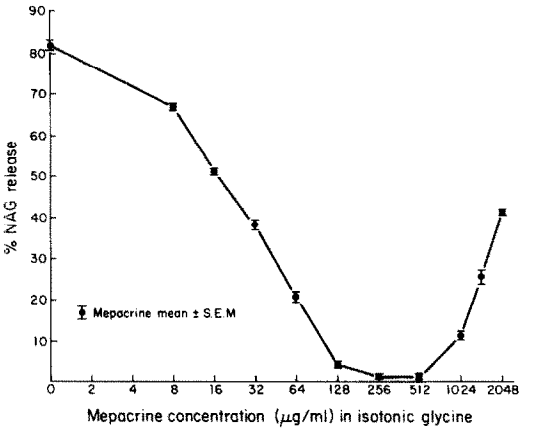

Fig. 4. NAG release from lysosomes in the presence of wide concentrations of mepracine in isotonic glycine.

Rats, when treated with gentamicin for 1 and 3 days, exhibited the responses shown in Fig. 6. After 1 day of gentamicin treatment the excretion of NAG into the urine did not differ between treatment groups and control. Only the group of rats treated with 160 mg/kg of gentamicin showed a tendency toward elevated NAG activity in the renal cortex as

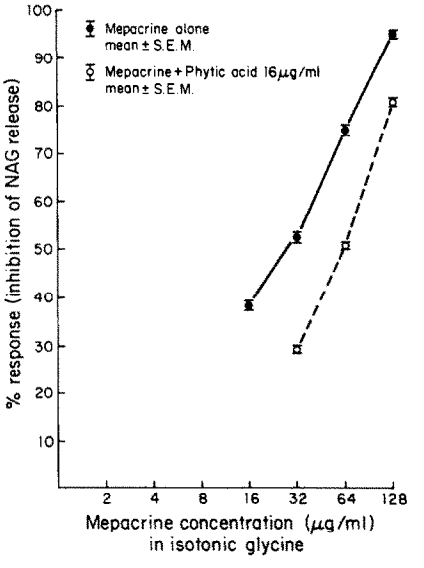

Fig. 5. Response of lysosomes to mepacrine alone or with phytic acid.

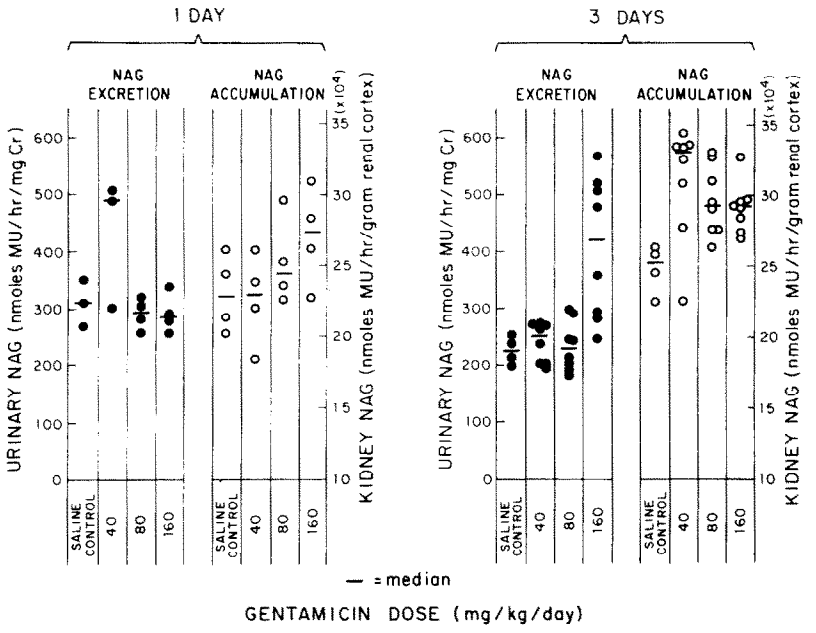

Fig. 6. Effect of gentamicin treatment on the levels of NAG enzyme in the renal cortex and the urine of rats over time.

compared to controls (P 0.06). After day 3 of gentamicin treatment, the NAG activity was elevated in the renal cortex of all three treatment groups compared to controls with P values of 0.004, 0.002 and 0.002 for the 40, 80 and 160 mg/kg groups respectively. Rats treated with 160 mg/kg of gentamicin had a lower NAG per gram renal cortex than the 40 mg/kg treatment group ($P < 0.05$). After 3 days of treatment with 160 mg/kg gentamicin, rats had elevated NAG excretion into the urine compared to controls, with a P value of <0.004.

When rats were treated with gentamicin for 28 days, the NAG activity in the renal cortex was 354.3 ± (S.E.M.) μM MU liberated per gram of tissue per hour. The NAG activity per gram renal cortex of control animals was 217.3 ± 11.6 ($P < 0.01$). Lysosomes isolated from the same gentamicin-treated animals released 19.6 ± 2.5% of NAG activity into the supernatant fraction on incubation in isotonic sucrose. Lysosomes from control animals released 46.5 ± 4.3% of NAG activity ($P < 0.001$).

Rat renal cortical lysosomes were incubated in isotonic glycine in the presence of the polyamine, spermine. Spermine inhibited NAG release from lysosomes in a dose-related fashion under these conditions (Fig. 7). This effect of spermine on lysosomes was inhibited by the addition of 16 μg/ml of phytic acid.

Rat renal cortical lysosomes were also incubated in isotonic sucrose and isotonic glycine. There was a biphasic response of lysosomes to the addition of gentamicin in both of these incubation media. Gentamicin inhibited NAG release at low concentrations and enhanced NAG release at high concentrations in these preparations (Fig. 8).

The basic amino acids lysine and arginine, L-canavanine (an analog of arginine), and polymyxin-B were tested for their effects on lysosomal NAG release *in vitro*. Lysine and arginine when incubated in isotonic glycine caused a dose-related inhibition of NAG release. These same two basic amino acids had a dose-related disruptive effect on lysosomes when incubated in isotonic sucrose (Fig. 9). L-Canavanine and polymyxin-B, a polycationic peptide antibiotic, inhibited release of NAG from lysosomes on incubation in isotonic sucrose.

Rat renal cortical lysosomes were incubated with high gentamicin concentrations in isotonic glycine alone or isotonic glycine containing 2048 μg/ml (0.014 mM) of lysine. Lysine enhanced the disruptive

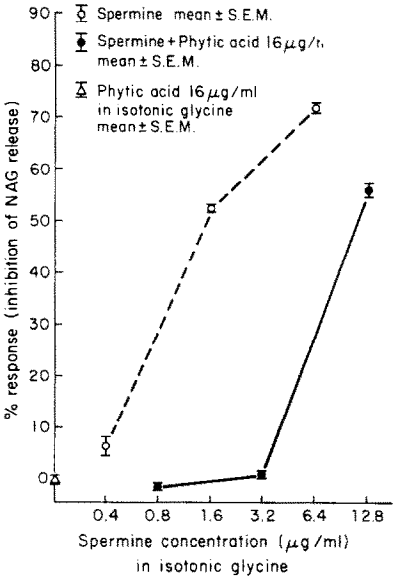

Fig. 7. Concentration–response curves for the spermine effect on lysosomes with and without phytic acid in isotonic glycine.

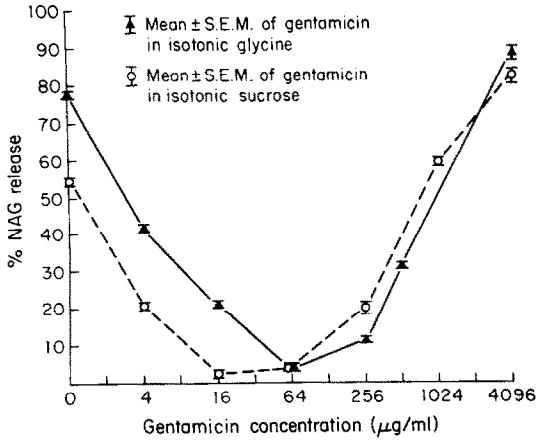

Fig. 8. NAG release from lysosomes in the presence of widely varied concentrations of gentamicin in isotonic glycine and in isotonic sucrose.

effect of high concentrations of gentamicin on lysosomes with P values of <0.01 at all three concentrations tested (Fig. 10). The addition of 50 mM glycine to the same high concentrations of gentamicin in isotonic glycine had no effect on the disruptive response to gentamicin.

Renal cortical lysosomes were also incubated in the presence of widely varied concentrations of ferric and ferrous ions in isotonic glycine. These cations also caused a biphasic effect on lysosomal NAG release (Fig. 11). Low concentrations of these ions inhibited NAG release while high concentrations enhanced NAG release.

DISCUSSION

We have observed previously that low concentrations of aminoglycosides inhibit NAG release *in vitro* while high concentrations enhanced NAG release. We have proposed that this might occur, *in vivo*, through aminoglycoside binding to lysosomal phospholipid membrane, causing a decrease in phospholipid metabolism. The resulting increase in hydrophobicity of the lysosomal membrane would inhibit the efflux of water-soluble products of ongoing macromolecular degradation. This could lead to osmotic disruption of lysosomes with the intracellular release of hydrolytic enzymes causing cell injury [4].

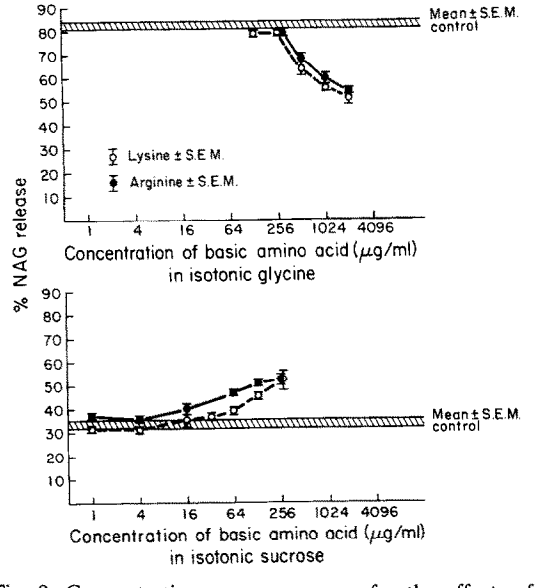

Fig. 9. Concentration–response curves for the effects of basic amino acids on lysosomes incubated in isotonic glycine and isotonic sucrose. At high concentrations of amino acids, NAG release was significantly different from controls ($P < 0.001$).

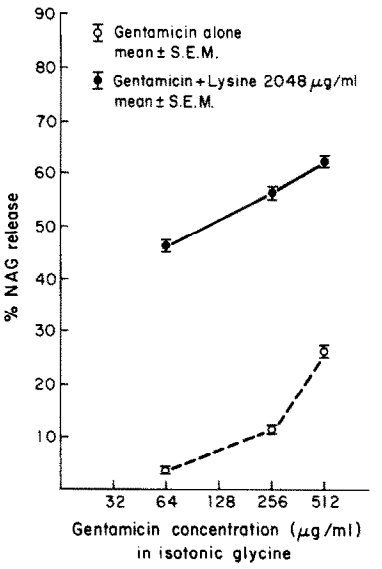

Fig. 10. Concentration–response curves for the lysosomal effect of high concentrations of gentamicin with and without 2048 μg/ml of lysine in isotonic glycine. At each gentamicin concentration, the two groups differed ($P < 0.01$).

Gentamicin inhibits kidney lysosomal phospholipases A and C [1]. This is consistent with the *in vitro* stabilization of the lysosomal membrane at low concentrations which we observed since Weglicki *et al.* [7] showed that lysosomal phospholipase activity is inversely related to the stability of the membrane. This stabilization effect appears to be relevant to the production of nephrotoxicity in rats [4]. The nephrotoxic potential of gentamicin has been found to be

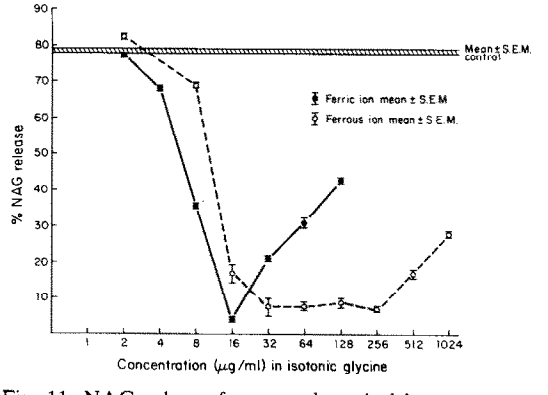

Fig. 11. NAG release from renal cortical lysosomes as a function of the concentrations of ferric and ferrous ions in isotonic glycine.

the same as its C_1 component [8]. Gentamicin, gentamicin C_1 and gentamicin C_2 did not differ in their abilities to stabilize renal cortical lysosomes.

We monitored the stability of renal cortical lysosomes using two additional lysosomal enzymes, alpha-galactosidase and beta-glactosidase. These enzymes, like NAG, are secreted from proximal tubular lysosomes and appear in the urine [9]. The lysosomal release of alpha-galactosidase and beta-galactosidase was inhibited in the presence of gentamicin and streptomycin. Gentamicin was more potent than streptomycin in inhibiting the release of each of these enzymes.

Phosphatidylinositol and its phosphate derivatives have been suggested to be brush border membrane receptors for the aminoglycosides [10]. Phosphatidylinositol is a substrate or phospholipase C which is inhibited by aminoglycosides [1]. This phospholipid also accumulates in the kidney cortex in response to gentamicin treatment [3]. Phytic acid, which resembles the aqueous acidic portion of phosphatidylinositol and its mono- and diphosphate derivatives, inhibited the stabilization of lysosomes by gentamicin. The polycationic aminoglycosides are thought to bind to the negative charge of the phospholipid thereby inhibiting phospholipase enzyme–substrate interaction [1, 11]. Phytic acid with its six phosphate groups may compete with membrane phospholipids to bind the gentamicin, allowing less membrane stabilizing activity. Spermine has been shown to be an inhibitor of non-lysosomal phospholipases A and C [12]. It appears to stabilize the lysosomal membrane by the same mechanism as gentamicin [4]. The effect of spermine on renal cortical lysosomes was also inhibited by phytic acid. The effects of spermine on isolated lysosomal phospholipases have not been examined.

The phospholipase inhibitor mepacrine has been shown previously to stabilize lysosomal membranes [13]. We observed a biphasic effect of mepacrine on renal cortical lysosomal NAG release similar to that observed for aminoglycosides [4]. Low concentrations of mepacrine inhibited NAG release while high concentrations enhanced NAG release. This cationic amphiphilic drug is also thought to inhibit phospholipases by its ability to bind to phospholipid substrate [11, 14]. This is consistent with our findings that mepacrine enhances the stabilization of lysosomes by gentamicin. Phytic acid is also able to inhibit stabilization of lysosomes by mepacrine. Other investigators have observed phospholipase inhibitors to produce a biphasic effect on lysosomes *in vitro* [13].

N-Acetyl-β-glucosaminidase is normally excreted into urine, probably by a process of exocytosis of lysosomes from the proximal tubule [9]. This process of secretion would be inhibited by the stabilization of the lysosomes through phospholipase inhibition [15]. We treated rats with gentamicin at three doses and compared NAG accumulation by the renal cortex and NAG excretion into the urine in the three groups. The results of this *in vivo* experiment suggest that NAG accumulation by the kidney cortex precedes the elevated NAG excretion into the urine caused by aminoglycoside nephrotoxicity. This is best illustrated by the 160 mg/kg treatment group. Only this group began to show evidence of NAG accumulation in the kidney cortex after one dose. After three doses, this treatment group exhibited both a lesser renal cortical NAG than the other treatment groups and an increase in NAG excretion. Although the 40 and 80 mg/kg treatment groups accumulated NAG after three doses, these animals did not have elevated NAG excretion. Other investigators have observed that doses of gentamicin of 30 mg/kg or greater, administered to rats, significantly increased the 24-hr urinary NAG excretion in 3–4 days [16–17]. The changes with time of the renal cortical and urinary NAG levels appear to parallel the biphasic effect of aminoglycosides on lysosomes *in vitro*, i.e. stabilization followed by disruption. Consistent with these findings Meisner [16] found that the kidneys of rats treated with gentamicin accumulated lysosomal enzymes and had less dense and more fragile lysosomes than controls. This decrease in density simultaneous with increasing fragility may be due to water accumulation caused by an increase in intralysosomal osmotic activity as we have postulated. The accumulation of lipid may also contribute to the decrease in density.

Our data also demonstrate that lysosomes isolated from rats which have accumulated NAG enzyme *in vivo* in response to chronic gentamicin treatment were more stable than control lysosomes *in vitro*. These data suggest that lysosomal stabilization by aminoglycosides occurs *in vivo*. This stabilization probably contributes to the accumulation of lysosomal enzymes by the renal cortex.

The aminoglycosides, spermine, and the basic amino acids at high concentrations have been shown by Just and coworkers to bind to brush border membrane binding sites involved in the pinocytotic uptake of proteins by the proximal tubule [18]. This group of investigators showed that the affinity of basic compounds for this membrane appears to increase with the number of cationic amino groups [18]. Just and Habermann [18] also demonstrated a disruptive effect of gentamicin and the base polypeptide aprotinin on renal cortical lysosomes, *in vitro*, at a high concentration of 200 μg/ml. The effect of such proteins on the lysosomal membrane would be short-lived since they would be degraded by the proteolytic enzymes of the lysosomes [18]. In contrast, aminoglycosides, polyamines and other polycationic compounds which are not degraded by lysosomes would continue to occupy these sites on the membrane.

The lysosomal membrane is known to be permeable to amino acids [19]. This is demonstrated *in vitro* by the failure of isotonic solutions of amino acids to provide osmotic protection of lysosomes leading to their disruption and leakage of enzymes [19]. Sucrose does not permeate the lysosomal membrane [19]. Isotonic sucrose provides a more stable osmotic environment with less leakage of enzymes [19]. Our data demonstrate that the addition of widely varied concentrations of gentamicin to lysosomes suspended in both isotonic glycine and isotonic sucrose produced a biphasic effect. Low concentrations of gentamicin stabilized lysosomes and high concentrations disrupted them.

We examined the effects of lysine and arginine on renal cortical lysosomes. These amino acids, like the

aminoglycosides, are not degradable by lysosomal enzymes. Arginine and lysine at high concentrations inhibited NAG release from lysosomes incubated in isotonic glycine. These amino acids had a disruptive effect on lysosomes in the initiallly more stable environment of incubation in isotonic sucrose. At low concentrations L-canavanine, a naturally occurring analog of arginine, and polymyxin-B, a nephrotoxic cationic polypeptide antibiotic, inhibited NAG release in isotonic sucrose.

The observed effect of basic amino acids on lysosomes *in vitro* may be related to the permeability of the particles in the incubation medium. The addition of basic amino acids to lysosomes in isotonic sucrose provides an increase in the number of more permeant particles. This may have contributed to the disruptive effect. When osmotic activity was maximized by incubation of lysosomes in glycine, the response to addition of basic amino acids was a concentration-dependent stabilization.

Lysine at high doses has been shown to produce renal toxicity in rats [20–22]. Mogensen *et al.* [23] reported evidence of renal toxicity in humans in response to arginine infusion. Lysine has been shown recently to increase the renal toxicity of a single dose of gentamicin [21]. This is consistent with our data suggesting a nephrotoxic effect of basic amino acids, and our data showing the ability of lysine to enhance the disruptive effect of gentamicin on lysosomes *in vitro*.

We also examined the effects of ferric and ferrous ions on lysosomes. Ferric ion as well as several other metal ions are lysosomal phospholipase inhibitors [24]. Ferric and ferrous ions may reach high concentrations in proximal tubular lysosomes as a consequence of tubular handling of myoglobin [25] and hemoglobin [26, 27]. Both myoglobin and hemoglobin are proximal tubular toxins probably by mechanisms other than, or in addition to, tubular obstruction [28]. Ferric ion was more potent than ferrous ion in causing stabilization followed by disruption of renal cortical lysosomes. Iron accumulation by hepatic lysosomes of humans has been shown to increase the fragility of these organelles and cause lysosomal enzyme accumulation [29, 30].

Several investigators have described the progression in some patients of mild renal insufficiency to frank renal failure even in cases where the initiating renal insult has been resolved [31–33]. We have outlined a mechanism by which aminoglycoside antibiotics might produce a nephrotoxic response. Furthermore our findings suggest that endogenous compounds can produce toxicity by the same mechanism or enhance the toxicity of administered compounds such as aminoglycosides. These compounds can be cleared by glomerular filtration, accumulate in renal failure, and, at elevated concentrations in glomerular filtrate, can be incorporated into the proximal tubular lysosomal system. These compounds also appear to be nephrotoxic at high doses. We suggest that, under conditions in which an increase in glomerular flow is produced through a diminished number of functioning nephrons [34], a positive feedback mechanism may exist to produce a further decline in renal function. Low molecular weight cationic peptides are normally filtered by the glomerulus [35] and bind to proximal tubular binding sites for pinocytosis [36]. Cationic peptides, containing basic amino acids, and other cationic compounds which accumulate in renal insufficiency [37] may combine to exert a toxic effect on the lysosomes of remaining nephrons. This is consistent with the observation of Hostetter *et al.* [34] of increased numbers of swollen lysosomes, some containing myelin whorls, in glomerulii and proximal tubules associated with partial renal ablation. These changes are similar to those produced histopathologically by aminoglycoside treatment [38] and may be caused by endogenous cations acting via a mechanism similar to aminoglycosides. Although these findings may be suggested to be a nonspecific response of sick cells, swollen lysosomes containing osmiophilic myelin whorls have been associated consistently with the lysosomal accumulation of cationic phospholipase inhibitors [11, 14].

Our *in vitro* data were obtained with a lysosomal–mitochondrial fraction from renal cortex. Although it seems unlikely, one cannot rule out the possibility of mitochondrial factors being involved in the response of lysosomes to the tested compounds. Since mitochondrial membranes also contain phospholipids, these organelles will probably compete with lysosomes for the binding of these cationic compounds *in vitro*.

We therefore suggest that renally excreted cationic compounds may produce nephrotoxicity by their effects on proximal tubular lyosomal phospholipid metabolism. Under conditions in which the number of functioning nephrons is reduced, cationic compounds which accumulated may contribute to a further decline in renal function by the postulated mechanism.

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