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In Vitro Response of Hepatic Lysosomes to Endogenous and Exogenous Cationic Compounds (41881)

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Abstract. An investigation of the effect of four cationic compounds on rat liver lysosomes was carried out. Lysosomes from homogenized rat liver were isolated by differential centrifugation at 0–5°C in 0.3 *M* sucrose. These lysosomes were then incubated for 1 hr at 37°C in 0.25 *M* glycine solution containing widely varied concentrations of the test agent. The lysosomes were resedimented and the *N*-acetyl-β-glucosaminidase (NAG) activity was measured in the supernatant and in the remaining pellet after disruption. Spermine, ferric ion, mepacrine, and gentamicin all produced dose-dependent effects on these lysosomes. Low concentrations of these compounds inhibited the release of NAG into the supernatant while high concentrations enhanced the release of NAG. This effect of these cationic compounds on the lysosomal membrane may be a mechanism by which they produce cellular toxicity with the organ or tissue selectivity being related to the distribution of the cation.

Since the discovery of the lysosome by de Duve and co-workers (1), the functional integrity of the membrane of this organelle has been accepted as essential to its definition. This is primarily due to the ability of enzymes contained within lysosomes to digest nearly all cellular constituents. The lysosomal membrane is known to be composed, to a large degree, of lipids (3, 4). Consistent with this, other investigators have demonstrated lysosomal membrane stability to be inversely related to lipolytic activity (5) and, but less sensitive, to the actions of proteases (6).

We have recently published data showing effects of cations on renal cortical lysosomes and suggested that cationic compounds which reach the proximal renal tubular lysosomes can cause renal injury by decreasing lysosomal phospholipase activity (7, 8). In this study, we examined the response of lysosomes from hepatic tissue to endogenous and exogenous cationic compounds to determine if lysosomes from other types of cells might respond in a manner similar to lysosomes from kidney cells.

Materials and Method. Spermine and gentamicin sulfate were purchased from Sigma Chemical Company, St. Louis, Missouri. Mepacrine hydrochloride was purchased from ICN Pharmaceuticals Inc., Plainview, New York.

Male Fisher 344 rats weighing between 200 and 250 g were anesthetized with subcutaneous sodium pentobarbital. Rats were perfused transcardially with 10 ml of cooled 0.3 *M* sucrose containing 1 m*M* EDTA at pH 7.0 (hypertonic sucrose). Livers were removed and placed in a rinsing bath of hypertonic sucrose at 0–5°C. The entire homogenization and isolation procedure was carried out at 0.5°C. After rinsing, the livers were homogenized in hypertonic sucrose with 5 strokes of a manual all glass Dounce homogenizer and 5 strokes of a Potter–Elvejehm homogenizer with Teflon pestle rotating at 1500 rpm. The homogenate was then centrifuged twice in a Sorvall RC-2B centrifuge with an SS-34 rotor at 750*g* for 10 min. The sediment was discarded. The supernatants were recentrifuged at 10,000*g* for 10 min. The supernatant and other cellular debris overlying the light brown crude lysosomal–mitochondrial pellet (lysosomal pellet) was discarded. The lysosomal pellet was then resuspended in 3 ml of hypertonic sucrose and resedimented as a wash procedure and the supernatant was discarded.

A pH 7.0 solution of 0.25 *M* glycine (isotonic glycine) was prepared and compounds to be tested were dissolved at 10 times a final concentration in isotonic glycine. The lysosomal pellet was also resuspended in isotonic glycine. Two-tenths milliliter of drug/glycine solution was added to 1.8 ml of lysosomal suspension (incubation mixture) in separate centrifuge tubes, gently vortexed, and incu-

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bated for 1 hr at 37°C in a gently shaking water bath. Two-tenths milliliter of isotonic glycine containing no drug was added to 1.8 ml of lysosomal suspension and incubated as a control. Immediately after incubation, the incubation mixtures were cooled, resedimented, and the supernatants were removed. The remaining pellets were disrupted in 2 ml of 0.1% Triton X-100 containing 100 m*M* KCl.

Supernatants and disrupted pellets were diluted appropriately and assayed for the activity of the lysosomal enzyme *N*-acetyl-β-glucosaminidase (EC 3.2.1.30) (NAG) by our previously published method (7). The percentage NAG released was calculated as the ratio of the NAG activity in the supernatant to the total NAG activity in the supernatant and the pellet multiplied by 100.

Results and Discussion. Hepatic lysosomes incubated in the presence of ferric ion or spermine in isotonic glycine demonstrated the responses shown in Fig. 1. Low concentrations of these compounds inhibited NAG release while high concentrations enhanced NAG release. A similar response was observed when mepacrine or gentamicin was added to the incubation mixture (Fig. 2).

These effects on liver lysosomes are similar to those produced by aminoglycosides (7) and other cations (8) on kidney lysosomes. Other investigators have shown mepacrine (9), the polyamino compounds gentamicin (10, 11) and spermine (12), as well as ferric ions and other metal ions (13), to be phospholipase inhibitors. These compounds appear to have little in common except for the mechanism by which they are thought to inhibit phospholipase enzymes. These cations appear to bind to the negative charge of the phospholipid substrate thereby inhibiting phospholipase enzyme–substrate interaction (9, 10, 12, 13). This is a mechanism of phospholipase inhibition that has been described for many cationic compounds (9, 14).

We have used ferric ion and spermine as examples of endogenous compounds and gentamicin and mepacrine as examples of exogenous compounds. These compounds are not degraded by lysosomal enzymes so they can remain intact in the lysosome. This is in contrast to albumin and some other proteins which can inhibit lysosomal phospholipases (5) but are degraded by lysosomal enzymes, a process which disinhibits the phospholipase. Amino acid hydrolysis products of proteins permeate the lysosomal membrane poorly, (15, 16) and have been suggested to diffuse out of the lysosomes through pores in the membrane which are dependent on phospho-

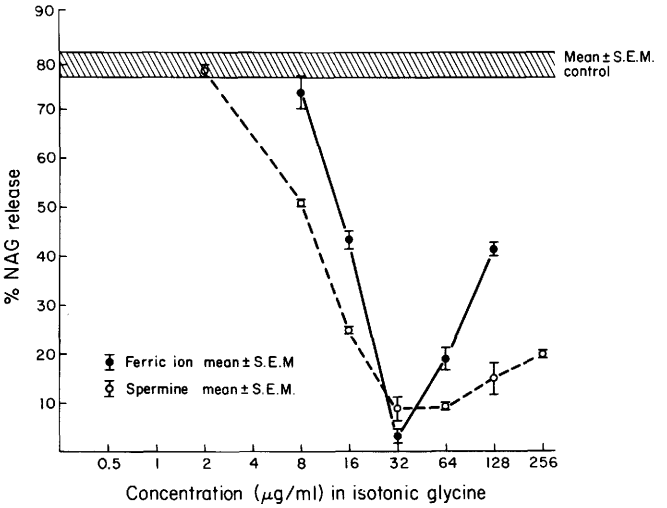

FIG. 1. The percentage NAG release from hepatic lysosomes as a function of the concentration of ferric ion or spermine in isotonic glycine. Values are means ± SEM of four replicate determinations at each drug concentration except for 128 μg/ml ferric ion and 2, 8, 16, and 64 μg/ml spermine which were done in duplicate.

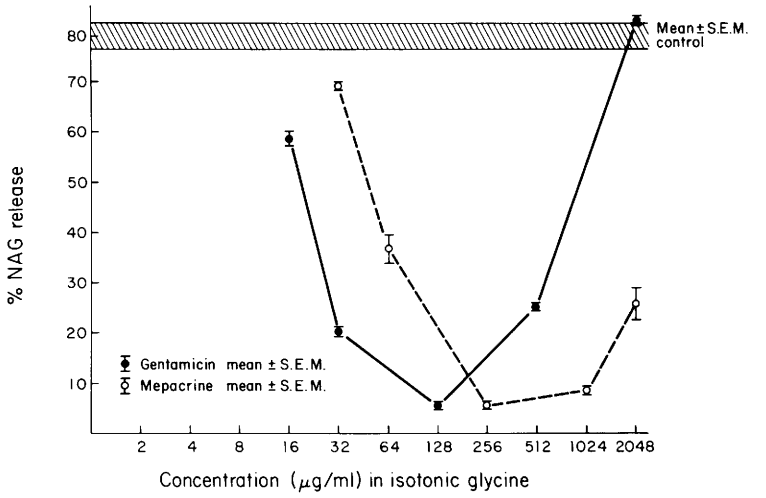

FIG. 2. The percentage NAG release from lysosomes as a function of the concentration of gentamicin or mepacrine in isotonic glycine. Values are means ± SEM of four replicate determinations at each drug concentration except for 8 and 6 μg/ml gentamicin and 8, 32, and 256 μg/ml mepacrine which were done in duplicate.

lipase activity as described by Lucy (4). Support for this mechanism of efflux is the failure to demonstrate carrier-mediated processes for transport of amino acids out of lysosomes (15). If phospholipase activity is inhibited, then these osmotically active hydrolysis products will remain inside the lysosome drawing water into the organelle. This should cause it to swell and even eventually rupture. Stimulation of endogenous proteolysis has been shown to transiently increase the swelling, fragility, and osmotic sensitivity (17, 18) of lysosomes as well as decrease their density (18). In addition to this, the compounds we examined, spermine (19), gentamicin (20), mepacrine (21), and iron (22) have been associated with lysosomal swelling and lipid accumulation in animals. Lysosomes which have accumulated gentamicin (23) and iron (24) have also been associated with increased lysosomal fragility as well as lysosomal enzyme accumulation. This accumulation of enzymes may be related to the inhibition of secretion caused by stabilization of lysosomes (4, 8).

The four compounds tested have little structural similarity. Yet these compounds appear to produce similar effects on lysosomes from renal (7, 8) and hepatic tissue. The selective toxicity of aminoglycosides (20), polyamines (19), iron (24), and the cationic amphiphillic class of drugs (of which mepacrine is a prototype) to specific types of cells may be related to differences in mechanisms by which these compounds distribute to or are accumulated by different cell types. Iron may reach high concentrations in lysosomes through sequestration of heme-containing proteins in overload states (25). Gentamicin seems to accumulate through pinocytosis by proximal renal tubular cells (26) but probably less so by other cells in which it would have to compete with serum proteins for pinocytotic uptake (27). Mepacrine, as well as other cationic amphiliphillic drugs, appears to accumulate by simple diffusion and ion trapping of the weak bases at the low pH of lysosomes (9, 21). Accumulation of spermine by lysosomes has not been shown. However, in situations where spermine is pathologically increased in extracellular fluid, several pathways might be utilized. Spermine may bind to sites for pinocytosis as in renal cells (28). Since it also binds covalently to some serum proteins (29) it may be pinocytosed as a part of this structure and released inside the lysosome with hydrolysis of the protein (29).

In summary, the cationic compounds, ferric ion, spermine, mepacrine, and gentamicin, produce a biphasic effect on hepatic lysosomes, *in vitro*, just as cationic compounds affect kidney lysosomes *in vitro* (7, 8). Low concentrations of these compounds stabilize lysosomes

while high concentrations disrupt them. This lysosomal effect may be a mechanism by which cationic compounds injure cells. The organ or tissue selectivity by the particular chemical would then be related to the distribution of the chemical to the lysosomes of the specific cells involved.

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