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EFFECT OF GENTAMICIN ON THE SUBCELLULAR DISTRIBUTION OF RENAL β-*N*-ACETYLGLUCOSAMINIDASE ACTIVITY

HERMAN MEISNER

Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, U.S.A.

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Abstract—The effect of gentamicin on lysosomal enzyme activity in renal cortex of Sprague–Dawley rats was examined. In rats given 60 mg gentamicin/kg subcutaneously for 8 days, renal β-*N*-acetylglucosaminidase activity increased by 57 per cent while cathepsin B_1 and dipeptidylpeptidase I activities did not change. The specific activity of glucosaminidase in partially purified lysosomes, however, was only 10 per cent higher. The subcellular distribution of glucosaminidase activity in gentamicin-treated rats shifted towards the less dense mitochondrial and 10,000 *g* supernatant fractions, which showed a 55 and 80 per cent increase, respectively, of total activity. It is concluded that gentamicin caused an increased activity, and reduced latency, of lysosomal glucosaminidase.

The place of gentamicin, tobramycin, and other aminoglycosides in the treatment of severe gram negative infections is firmly established, but so also is the occurrence of nephrotoxicity [1–4]. The high frequency in humans, which is between 2 and 10 per cent [5, 6], is related to the fact that this group of polybasic drugs is excreted almost entirely by the kidney [7]. A certain proportion of the filtered drug is reabsorbed by the proximal tubules, which accumulate the drug up to ten times above that found in other organs [1, 8–10]. At the subcellular level, lysosomes within the proximal tubular cells have been found to contain much higher concentrations of gentamicin than other organelles [8, 10].

The effects of gentamicin and other aminoglycosides on enzyme activities in urine have been well studied [1, 11]. It was found, for example, that β-*N*-acetylglucosaminidase, β-galactosidase, and α-fucosidase activities are greatly elevated in rats fed doses of gentamicin of 30–60 $mg \cdot kg^{-1} \cdot day^{-1}$. Recently, Morin *et al.* [12] have shown that gentamicin administration to rats at 50 $mg \cdot kg^{-1} \cdot day^{-1}$ for 8 days decreases renal cortex sphingomyelinase, γ-glutamyltranspeptidase, and cathepsin B activity. Because urinary β-*N*-acetylglucosaminidase activity is very sensitive to aminoglycosides, and the hydrolytic activity is high, we have chosen to investigate the effects of gentamicin on this enzyme in different subcellular fractions or renal cortex.

METHODS

Groups of Sprague–Dawley male rats, six per experimental condition, weighing between 150 and 200 g, were given daily subcutaneous injections of gentamicin. Rats were fed Purina rat chow and housed in temperature and light controlled rooms. Urine was collected for 24 hr, under conditions designed to minimize contamination, as described by Parry and Brosnan [13]. Animals were decapitated, the kidneys were placed in ice-cold saline, and the cortices were removed. The tissue was minced, and a 20% (w/v) homogenate prepared with a loose fitting Potter–Elvejhem homogenizer in 0.45 M sucrose, 1 mM Na^+-EDTA, pH 7.4 [14, 15]. The homogenate was centrifuged at 800 rpm for 5 min in a Sorvall RC2B at 4° to yield a pellet (N_1) and supernatant fraction. The latter was again centrifuged at 800 rpm for 5 min, the pellet saved (N_2), and the supernatant fraction centrifuged for 2 min at 10,000 rpm to give a supernatant (S) and pellet. The tan colored top portion containing mitochondria and brush border (M) was carefully washed off with a pipet, leaving the crude lysosomal (L) fraction.

β-*N*-Acetylglucosaminidase activity was assayed colorimetrically according to Patel *et al.* [16], with 2.5 mM PNP-*N*-acetyl-β-glucosaminide, 60 mM citrate phosphate, pH 4.2. Cathepsin B_1 was measured fluorimetrically with 2 mM α-*N*-benzoyl-DL-arg-β-naphthylamide according to McDonald and Ellis [17]. Dipeptidylpeptidase I was determined with 2 mM gly-arg-β-naphthylamide [18]. In both cases, the fluorimeter was calibrated with β-naphthylamine, taking the necessary precautions with this carcinogenic substance. Cytochrome oxidase was measured by the decrease in A_{550} of the alpha band of ferrocytochrome *c* [19]. Protein was measured by a modification of the Lowry method that allows linearity up to 0.4–0.5 O.D. at 650 nm [20].

Gentamicin was a gift from the Schering Corp., Bloomfield, NJ.

RESULTS AND DISCUSSION

In preliminary dose–response experiments (not shown), we found that maximum urinary activity of glucosaminidase or leucine aminopeptidase and tissue activity of glucosaminidase were reached after a dose of 60 mg gentamicin/kg over a period of 5–6

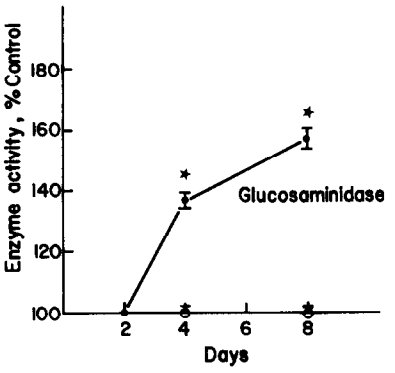

Fig. 1. Effect of gentamicin on β-*N*-acetylglucosaminidase, dipeptidylpeptidase I, and cathepsin B_1 activity in rat renal cortex. Rats were given 60 mg gentamicin·kg^{-1}·day^{-1}, and enzyme activities were determined in cortex homogenates as described in Methods. Initial activities were 4.33 ± 0.16 μmoles·mg^{-1}·hr^{-1} (filled circles, β-*N*-acetylglucosaminidase); 4.62 ± 0.60 nmoles·mg^{-1}·hr^{-1} (crosses, dipeptidylpeptidase I); and 37.2 ± 3.6 nmoles·mg^{-1}·hr^{-1} (open circles, cathepsin B_1). Bars = S.E.M. five to six rats. Key: (*) difference from control value is statistically significant at $P < 0.01$.

days. Consequently, gentamicin was given at a concentration of 60 mg/kg or greater, to achieve maximum enzyme stimulation.

The time course of the effect of gentamicin on lysosomal enzyme activity in whole homogenates from renal cortex is shown in Fig. 1. In rats fed 60 mg gentamicin·kg^{-1}·day^{-1}, tissue glucosaminidase activity was enhanced by 37 per cent at 4 days and by 57 per cent after 8 days, while no change was observed in cathepsin B_1 or dipeptidylpeptidase I activity. Although not shown, the glucosaminidase isoenzymes A and B, which are represented in the rat in a 9:1 ratio, were increased proportionally (H. Meisner, unpublished observation). The lack of an effect of gentamicin on cathepsin B_1 activity differs from the observations of Morin *et al.* [12], who state that the activity of this enzyme is reduced in rats after 6 days at 50 mg gentamicin·kg^{-1}·day^{-1}. The extent of the decrease in enzyme activity was not given, however.

Table 1. Effect of gentamicin on tissue and urine levels of β-*N*-acetylglucosaminidase*

Condition	Renal cortex (μmoles/hr)	Urine (μmoles/hr)
Control (6)	1384 ± 21	5.26 ± 0.28
Gentamicin (6)	2000 ± 32	12.2 ± 0.66
% Increase	44	132
	$P < 0.01$	$P < 0.01$

* Rats were given gentamicin, 80 mg·kg^{-1}·day^{-1} for 4 days. Urine was collected over a 24-hr period, from day 3 to day 4. On day 4, the animals were killed, and glucosaminidase activity was measured. The number of rats is given in parentheses. Results are expressed as the mean ± S.E.M. for each experiment. Statistical significance was evaluated by Student's *t*-test.

A comparison of the effect of gentamicin on renal and urinary levels of glucosaminidase from rats given 80 mg·kg^{-1}·day^{-1} for 4 days is shown in Table 1. In 24 hr urine samples taken between days 3 and 4, total glucosaminidase activity increased 132 per cent, while the tissue level rose only 44 per cent, from 1384 to 2000 μmoles/hr. Despite the greater percentage increase in urine, only 0.5 per cent of the total enzyme activity was found in the urine compared to tissue, in both groups of rats. Thus, a considerably greater increase in total glucosaminidase protein took place intracellularly in experimental rats.

The effect of gentamicin, 100 mg·kg^{-1}·day^{-1} for 5 days, on intracellular distribution of glucosaminidase activity in kidney cortex is shown in Fig. 2 in the form of a deDuve plot [21], in which the specific activity is plotted against the percent of total protein recovered within each fraction. Tissue glucosaminidase activity in the whole homogenate increased by 44 per cent, from 7.4 ± 0.4 to 10.7 ± 0.5 μmoles·mg^{-1}·hr^{-1} ($P < 0.001$). The specific activity in isolated lysosomes, however, rose only by 9.9 per cent from 16.6 ± 0.6 to 18.2 ± 0.2 μmoles·mg^{-1}·hr^{-1} ($P < 0.01$). The discrepancy was not due to incomplete recovery of enzyme activity, which was approximately 100% but, rather, could be accounted for when the subcellular distribution of glucosaminidase was examined. The mitochondrial and supernatant fractions from experimental rats showed a significant increase of 55 and 80 per cent, respectively, in specific activity. Total glucosaminidase activity (specific activity × protein) increased by 89 per cent in the 10,000 *g* supernatant fraction. Thus, the distribution of lysosomal glucosaminidase activity had shifted towards the less dense fractions, a result that has also been found by others [12, 22]. In Fig. 3, cytochrome oxidase activity was measured as an index of the purity of different subcellular fractions. Aside from a slight decrease of activity in whole homogenates of gentamicin-treated rats from 11.1 ± 0.2 to 9.6 ± 0.2 μmoles·mg^{-1}·hr^{-1}, the most noteworthy result was that the lysosomal fraction in kidney cortex from drug-treated rats was less contaminated with cytochrome oxidase than in control kidneys. This indicates that the increase of glucosaminidase activity in purified lysosomes, which was smaller than in whole homogenates, was not simply due to a more impure fraction. On the contrary, the lysosomal fraction from drug-treated rat kidneys was more pure than control lysosomes.

In this study, it has been demonstrated that not only did glucosaminidase activity increase in renal cortex after treatment with gentamicin, but the enzyme activity pattern shifted towards a subcellular fraction having a lower density. Two interpretations of the latter result are possible. First, the increase in enzyme activity occurred predominantly in the smaller, lower density lysosomes. Arguing against this are the experiments of Silverblatt and Kuehn [10], who showed that gentamicin accumulates intracellularly in rat cortex in larger, and presumably heavier, lysosomes. Second, lysosomal enzyme latency decreased in gentamicin-treated animals, resulting in an increase in glucosaminidase activity in the cytosol. In support of this, Morin *et al.* [12, 23]

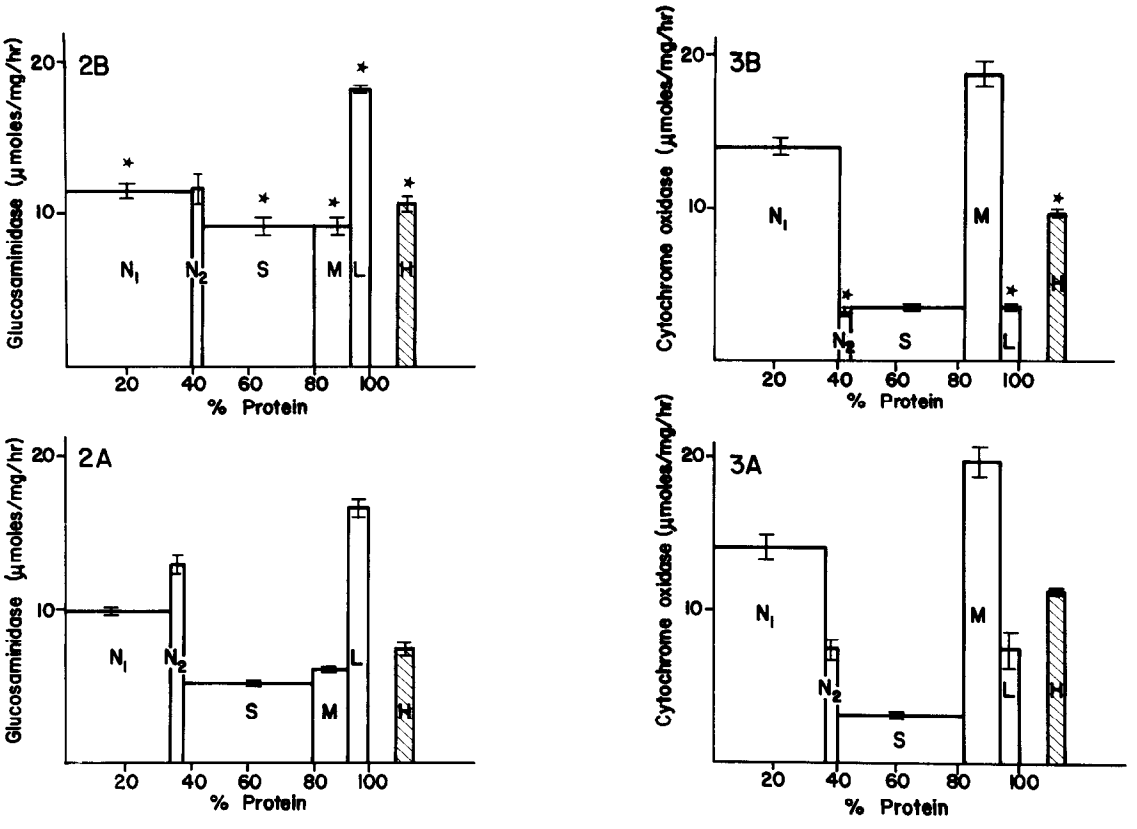

Fig. 2 and 3. Subcellular distribution of β-*N*-acetylglucosaminidase (Fig. 2) and cytochrome oxidase (Fig. 3) activity in kidney cortex of control (Figs. 2A and 3A) and gentamicin-treated (Figs. 2B and 3B) rats. Groups of six rats were given 100 mg gentamicin·kg^{-1}·day^{-1} for 5 days, and enzyme activity was measured as described in Methods. Abbreviations: N_1, first nuclear pellet; N_2, second nuclear pellet; S, 10,000 *g* supernatant; M, crude mitochondria; L, crude lysosomes; and H, total homogenate. Bars = S.E.M. of six rats. Key: (*) difference from control activity is statistically significant at $P < 0.01$.

have shown that aminoglycosides, added *in vitro* and *in vivo*, cause a dose- and time-dependent increase of glucosaminidase activity in the extra-lysosomal supernatant fraction. In addition, many studies have shown that diverse compounds change lysosomal enzyme release, an action correlated with cyclic AMP level [24, 25]. Measurement of the subcellular distribution of dipeptidylpeptidase I and cathepsin B_1 activity, and cyclic AMP levels, is being carried out currently, to determine the relationship between release of lysosomal hydrolases and cellular toxicity.

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