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THE EFFECT OF VARIOUS AMINOGLYCOSIDE ANTIBIOTICS ON GLYCOGEN PHOSPHORYLASE ACTIVITY IN LIVER AND KIDNEY MEDULLA OF RABBIT

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Glycogen phosphorylase activity in both liver and kidney medulla of rabbit was stimulated in the presence of caffeine by various aminoglycoside antibiotics in the following rank order: gentamicin > neomycin > amikacin = kanamycin \geq tobramycin, while streptomycin did not affect the enzyme activity. In contrast, in the presence of AMP, the stimulatory action of antibiotics was not observed. Since in the gentamicin-treated rabbits stimulation of glycogen phosphorylase activity by about 30% in both liver and kidney medulla was accompanied by a decrease of liver glycogen content by about 60% it is likely that a decline in liver glycogen level following antibiotic treatment is due to an increased glycogen phosphorylase activity.

Aminoglycoside antibiotics are widely used for the treatment of infections caused by gram negative bacteria. They are known to produce severe nephrotoxicity [1, 2] resulting, among others, in alterations of carbohydrate metabolism [3, 4]. It has been reported [5] that in hepatocytes isolated from rats which received gentamicin (80 mg/kg) glycogen stores were promptly depleted in two hours after injection of antibiotic. Thus, studies were undertaken to establish both the effect of various aminoglycoside antibiotics on glycogen phosphorylase activity in liver and kidney medulla of rabbit as well as liver glycogen content and activity of this enzyme in the gentamicin-treated animals.

MATERIALS AND METHODS

Termond strain male rabbits (1.5-2 kg) were used throughout. Animals were treated with gentamicin by subcutaneous injection (80 mg of antibiotic/kg per day for 10 days [6]). Rabbit liver and kidney medulla postmitochondrial fractions were prepared by the method of Maddaiah & Madsen [7]. Glycogen

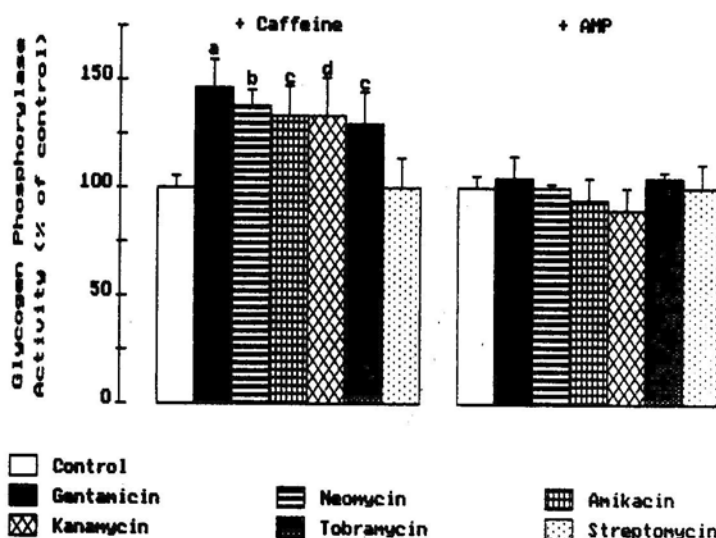


Fig. 1. The effect of various aminoglycoside antibiotics on glycogen phosphorylase activity in rabbit kidney medulla. The enzyme activity was measured in the presence of either 0.5 mM caffeine or 3 mM AMP in the incubation medium. a, $P < 0.005$; b, $P < 0.025$; c, $P < 0.01$; d, $P < 0.05$ versus control with no antibiotic

phosphorylase activity was determined by measuring the amount of orthophosphate generated in the reaction between glucose 1-phosphate and glycogen catalysed by the enzyme [8]. The incubation mixture (pH 6.8) contained 100 mM NaF, 0.5 mM caffeine or 3 mM AMP, 20 mM glucose 1-phosphate and postmitochondrial fraction (3-5 mg protein/ml). Antibiotics were added at 5 mM concentrations. During the incubation 100 μ l samples were withdrawn at regular intervals and placed in Eppendorf's tubes containing 10 μ l of 35% perchloric acid to stop the enzymatic reaction. Acid extracts obtained after deproteinization of samples were centrifuged and the supernatants were used for determination of phosphate by the method of Fiske & SubbaRow [9]. Liver glycogen content was estimated according to Pfleiderer [10]. Protein was measured by the method of Lowry using bovine serum albumin as a standard [11].

RESULTS AND DISCUSSION

There are no data in the literature on the effect of various aminoglycosides on glycogen phosphorylase activity. As shown in Fig. 1, in the presence of 0.5 mM caffeine in the incubation medium aminoglycoside antibiotics stimulated glycogen phosphorylase activity in the following rank order: gentamicin > neomycin > amikacin = kanamycin \geq tobramycin, while streptomycin did not alter the enzyme activity under these conditions. In the absence of caffeine

stimulation of the enzyme activity by the former antibiotics was smaller than that in the presence of the inhibitor (not shown). However, when the incubation mixture contained 3 mM AMP, the stimulatory action of antibiotics was not observed. Similar results were also obtained with respect to the effect of aminoglycosides on glycogen phosphorylase activity in rabbit liver (not shown). Since caffeine and AMP are an inhibitor and an allosteric activator of glycogen phosphorylase b, respectively [8], it seems likely that the stimulatory effect of aminoglycoside antibiotics on the enzyme activity results from interaction of antibiotics with phosphorylase a. The rank order of drugs based on the magnitude of stimulation of glycogen phosphorylase activity in rabbit, correlates well with their ability to produce nephrotoxicity [1, 12] and is similar to that observed for stimulation of cAMP- and Ca^{2+} -independent protein kinases of rat liver and ventral prostate [13].

Table 1

Liver glycogen content and glycogen phosphorylase activity in liver and kidney medulla of gentamicin-treated rabbits

All values are the average of 4 experiments \pm S.D. The enzyme activity was measured in the presence of either 0.5 mM caffeine or 3 mM AMP in incubation mixture

Animals	Tissue	Glycogen content (mg of glucose/mg of tissue)	Phosphorylase activity (nmol P/min per mg prot.)	
			+ caffeine	+ AMP
Control	liver	67 \pm 27	53 \pm 10	80 \pm 20
	kidney medulla	—	55 \pm 10	70 \pm 10
Gentamicin — treated	liver	28 \pm 3*	68 \pm 4**	81 \pm 15
	kidney medulla	—	70 \pm 5*	78 \pm 15

* P < 0.05; ** P < 0.01 versus the corresponding values for untreated animals.

As shown in Table 1, in rabbits treated with gentamicin (80 mg/kg per day for 10 days), an about 30% increase in glycogen phosphorylase activity in the presence of 0.5 mM caffeine was accompanied by an about 60% decline of liver glycogen content. The latter observation is in agreement with the data of Patel *et. al.* [5] who have reported a decreased glycogen level in hepatocytes obtained from gentamicin-treated rats. Since the stimulation of the enzyme was observed in the presence of 0.5 mM caffeine in the incubation medium, it seems likely that gentamicin elevates the activity and/or the content of phosphorylase a form. Lack of the stimulatory effect of AMP on the enzyme activity in the gentamicin-treated animals in comparison with that in untreated ones is consistent with this suggestion.

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