THE pH-DEPENDENT INFLUENCE OF AMINOGLYCOSIDE ANTIBIOTICS ON IODOHIPPURATE ACCUMULATION IN RABBIT RENAL CORTICAL SLICES

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The effects of aminoglycoside antibiotics on ^{125}I-hippurate (OIH) accumulation in rabbit renal cortical slices were assessed *in vitro* using incubation media with pH-values ranging from 6.4 to 8.4 and containing streptomycin, kanamycin, amikacin, gentamicin and tobramycin in concentrations ranging from 100 to 2,000 μg base/ml. The aminoglycoside-induced inhibition of OIH accumulation was clearly pH-dependent and most pronounced at alkaline pH-values. At pH 6.4 and 7.4 the aminoglycosides had either no or only moderate effects on OIH accumulation, while all drugs produced a distinct depression in accumulation at pH 7.9 and 8.4. The microbiologically inert *N*-acetyl gentamicin had no influence on accumulation. The influence of aminoglycosides on OIH accumulation is probably related to the pKa-values of these drugs and implies the presence of free amino groups.

In a previous study it was demonstrated that aminoglycoside antibiotics *in vitro* depressed the accumulation of ^{125}I-hippurate (OIH) in rabbit renal cortical slices[1]. However, the measured effects were partially explained by aminoglycoside-induced pH-changes in the incubation media. Therefore, in the present investigation the influence of aminoglycosides on OIH accumulation has been studied under well-defined pH conditions.

Materials and Methods

Measurement of ^{125}I-hippurate accumulation

The technique employed is a modification of the method reported by CROSS and TAGGART[2] and has been described in details previously[1]. Rabbit renal cortical slices were incubated in Erlenmeyer vessels for 60 minutes at 25°C with 100% oxygen in the gas space, while being shaken at 100 cycles/minute. Each vessel contained 90~110 mg slices, 10 ml CROSS-TAGGART medium[2] and 20 μCi *o*-^{125}I-hippurate/liter, corresponding to approximately 3 μmol/liter. The slices and aliquots of the incubation media were counted in a gamma scintillation counter, and the OIH accumulation expressed as the slice to medium ratio, *i.e.* the ratio of the counts in 1 g tissue to those in 1 ml incubation medium.

Aminoglycoside studies

Prior to incubation the following aminoglycosides were added to the incubation media in concentrations ranging from 100 to 2,000 μg base/ml: Streptomycin sulfate (714 μg base/mg), kanamycin sulfate (777 μg base/mg), amikacin base (895 μg base/mg), gentamicin sulfate (571 μg base/mg), tobramycin base (925 μg base/mg) and microbiologically inert *N*-acetyl gentamicin[3] (690 μg calculated base/mg) as a control substance. After addition of aminoglycosides pH was adjusted to values ranging from 6.4 to 8.4 with 5 N HCl or 5 N NaOH and using a pH-meter. Incubations were then performed as described above. Each experiment included 4 control vessels with identical pH and without aminoglycoside. The OIH accumulation in the aminoglycoside-containing vessels was expressed in per cent of the accumulation in the control vessels.

STUDENT's *t*-tests for paired and unpaired values were employed in statistical evaluation of the results.

Results

The effects of aminoglycosides on OIH accumulation are summarized in Tables 1~3 and Figs. 1~3. The results represent the averages of 4~8 experiments. According to the accumulation profiles (dose response curves) the aminoglycosides could be divided in three groups. I. Streptomycin. II. Kanamycin and amikacin. III. Gentamicin and tobramycin.

Streptomycin

Accumulation was significantly depressed by concentrations $\geq$500 μg/ml at pH 6.4 (Table 1 and Fig. 1), but unaffected even by high concentrations at pH 6.9 and 7.4, whereas a profound drop was observed at pH 7.9 and 8.4.

Table 1. Effects of streptomycin and *N*-acetyl gentamicin on ^{125}I-hippurate accumulation in rabbit renal cortical slices at different pH-values in the incubation media.
Results are expressed in per cent of the accumulation in aminoglycoside-free controls (mean±S.D.).

		^{125}I-Hippurate accumulation				
		pH 6.4	pH 6.9	pH 7.4	pH 7.9	pH 8.4
Streptomycin base (μg/ml)	100	86.9± 6.2	104.4± 4.4	95.4± 5.6	65.8± 2.3*	72.2±24.6*
	500	79.4± 8.6*	101.9± 3.7	92.6± 6.8	45.9± 8.6*	50.4±15.8*
	1,000	85.1±11.7*	96.7± 2.5	86.2± 8.3	18.4± 1.4*	12.0± 9.8*
	2,000	72.2±11.5*	97.1± 2.0	93.1± 8.0	13.8± 8.5*	9.1± 7.1*
N-Acetyl gentamicin base (μg/ml)	100	104.4±11.0	111.4± 7.8	103.9± 6.7	97.9± 5.8	98.7±16.5
	500	100.0± 9.6	99.0±11.4	90.6± 8.5	97.3± 7.4	110.3±13.2
	1,000	88.2± 6.3	94.9± 9.2	98.8±11.6	101.9±14.3	92.6±14.3
	2,000	72.9± 6.0*	84.4±16.7	98.1± 5.8	86.0± 8.9	104.3±19.0

* $P<0.05$

Fig. 1. Effects of streptomycin and *N*-acetyl gentamicin on ^{125}I-hippurate accumulation in rabbit renal cortical slices.
Accumulation is expressed in per cent of values in aminoglycoside-free controls.

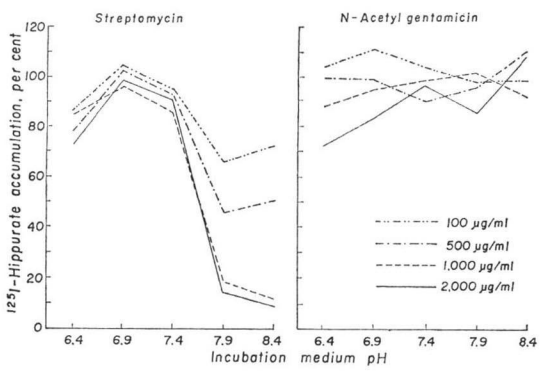


Fig. 2. Effects of kanamycin and amikacin on ^{125}I-hippurate accumulation in rabbit renal cortical slices.
Accumulation is expressed in per cent of values in aminoglycoside-free controls.

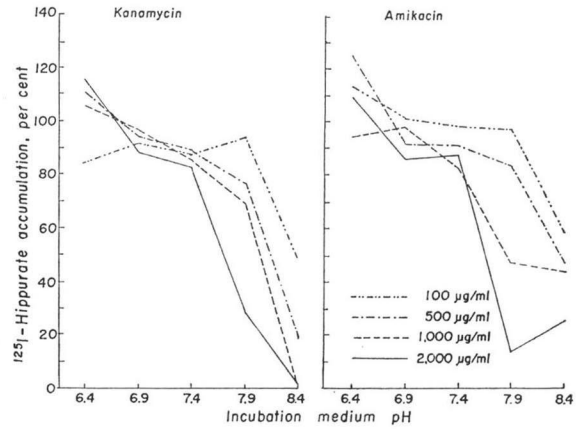


Table 2. Effects of kanamycin and amikacin on ^{125}I-hippurate accumulation in rabbit renal cortical slices at different pH-values in the incubation media.

Results are expressed in per cent of the accumulation in aminoglycoside-free controls (mean±S.D.).

		^{125}I-Hippurate accumulation				
		pH 6.4	pH 6.9	pH 7.4	pH 7.9	pH 8.4
Kanamycin base (μg/ml)	100	84.0± 8.2	92.1±10.6	88.3± 8.2	94.0± 9.0	48.9±13.2*
	500	113.6± 6.0	94.2± 3.2	88.6±21.6	76.3± 7.8*	18.4±15.0*
	1,000	105.3±43.2	95.8±13.6	85.4±10.2	69.5±13.2*	0.6± 0.8*
	2,000	115.1±25.6	88.2±14.6	82.6± 4.8	28.3±13.2*	0.0*
Amikacin base (μg/ml)	100	113.6±17.6	101.0±14.0	98.5± 3.6	97.4±15.8	58.4±11.9*
	500	125.3±23.2	91.5±15.6	90.1± 7.0	84.2±23.4	46.7±10.0*
	1,000	94.8±11.8	102.3± 6.0	81.9± 5.0	47.5±21.2*	44.4± 7.0*
	2,000	108.6±20.4	85.2± 5.2	87.4±13.7	14.4± 9.2*	26.1±26.0*

* $P<0.05$

Table 3. Effects of gentamicin and tobramycin on ^{125}I-hippurate accumulation in rabbit renal cortical slices at different pH-values in the incubation media.

Results are expressed in per cent of the accumulation in aminoglycoside-free controls (mean±S.D.).

		^{125}I-Hippurate accumulation				
		pH 6.4	pH 6.9	pH 7.4	pH 7.9	pH 8.4
Gentamicin base (μg/ml)	100	88.5± 9.2	69.3± 5.5*	115.1±12.0	102.3±20.3	68.3± 7.8*
	500	78.3±10.4	71.5± 7.0*	113.5±10.7	84.2± 4.2	22.6± 6.6*
	1,000	64.1±13.9*	67.6± 3.5*	102.3±13.4	64.1±11.0*	4.9± 2.3*
	2,000	60.7±10.0*	64.2± 8.3*	119.3± 3.5	44.9± 1.8*	1.3± 1.7*
Tobramycin base (μg/ml)	100	94.3± 7.8	81.7±10.9*	90.7± 4.0	77.7± 3.8*	55.6± 7.2*
	500	93.3± 6.9	81.7±12.6*	103.7± 6.4	33.0±16.8*	6.0± 6.6*
	1,000	88.0± 8.6	83.7± 6.2*	98.0± 1.7	26.2± 9.2*	2.5± 3.0*
	2,000	75.3±30.7	73.0± 7.5*	94.0± 0.7	5.0± 1.7*	0.0*

* $P<0.05$

Kanamycin and Amikacin

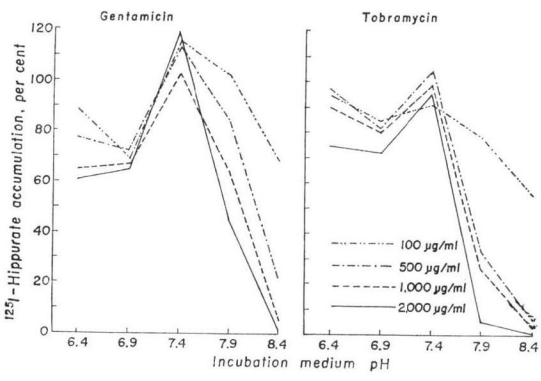
Had no significant influence on accumulation at pH 6.4, 6.9 and 7.4 (Table 2 and Fig. 2), but produced a distinct decrease at pH 7.9 and 8.4. There were no significant differences between the accumulation profiles of the two drugs, except at pH 8.4 where kanamycin depressed accumulation more than amikacin in concentrations $\geq$ 500 μg/ml.

Gentamicin and Tobramycin

At pH 6.4 gentamicin produced a significant decrease in accumulation in concentrations $\geq$ 1,000 μg/ml, while the influence of tobramycin was insignificant (Table 3 and Fig. 3). At pH 6.9 both drugs produced a significant decrease in

Fig. 3. Effects of gentamicin and tobramycin on ^{125}I-hippurate accumulation in rabbit renal cortical slices.

Accumulation is expressed in per cent of values in aminoglycoside-free controls.



accumulation already from 100 μg/ml, which was relatively independent of the aminoglycoside concentration. Accumulation was unaffected at pH 7.4, and distinctly reduced at pH 7.9 and 8.4. Gentamicin had significantly higher accumulation profiles than tobramycin at pH 7.9 and 8.4.

N-Acetyl gentamicin

Produced a significant decrease in accumulation only in the highest concentration at pH 6.4, while accumulation was unaffected at all other pH-values (Table 1 and Fig. 1). *N*-Acetyl gentamicin had significantly higher accumulation profiles than gentamicin at pH 6.4, 6.9, 7.9 and 8.4, but not at pH 7.4.

Common features for the microbiologically active aminoglycosides were the absent effects on accumulation at pH 7.4, and the pronounced decrease observed at more alkaline pH-values. Accordingly there were no significant differences between the accumulation profiles of the various aminoglycosides at pH 7.4. When arranged according to their influence on OIH accumulation at pH 7.9 the order became: Gentamicin < kanamycin = amikacin < streptomycin = tobramycin. At pH 8.4 the order was: Amikacin < streptomycin < gentamicin < tobramycin = kanamycin.

Discussion

The accumulation of OIH in renal cortical slices is distinctly affected by the presence of aminoglycosides. Other authors have also demonstrated an *in vitro* inhibitory effect of gentamicin on *p*-aminohippurate accumulation in rat cortical slices[4,5], but have not mentioned that this partially could be explained through aminoglycoside-induced pH-changes in the incubation media.

The accumulation profiles varied with the type of aminoglycoside employed in the experiment and were identical for drugs with related chemical structure. Differences in accumulation profiles were most pronounced in the acid area, where streptomycin, gentamicin and tobramycin depressed accumulation slightly, while this was unaffected by kanamycin and amikacin. All drugs had no influence at pH 7.4 and produced a distinct decrease in the alkaline area. Kanamycin and amikacin displayed "simple" accumulation profiles, showing a gradual decrease in accumulation contemporary with an increase in pH, whereas streptomycin, gentamicin and tobramycin had "complex" accumulation profiles in the acid area. These features impede a simple interpretation of the results as regards the last-mentioned three drugs and suggest that different mechanisms are responsible for the effects on accumulation in the acid and alkaline area, respectively.

The mechanism of the aminoglycoside-induced inhibition of OIH accumulation is unclarified, but the effect is probably exerted on a toxic-metabolic intracellular level. These drugs are accumulated in high concentrations in proximal tubular cells and compromise among other things mitochondrial function[6].

According to the theory of nonionic diffusion uncharged molecules penetrate more easily through biological membranes than charged molecules[7]. An increase in pH of the incubation medium reduces the charges of the basic aminoglycoside molecules and facilitates entrance into tubular cells. The low intracellular pH favours retention by protonation and contribute to the high intracellular concentration[8]. The measured decrease in OIH accumulation at alkaline pH-values is therefore probably partially explained by enhanced intracellular penetration due to diminished ionization. As a parallel it is known that the antimicrobial effect of aminoglycosides is pH-dependent and greatest at alkaline pH-values[9], possibly due to better penetration into bacteria. The pKa-values of the individual amino groups determine the total charge of the aminoglycoside molecule at any given pH. A high pKa should therefore be related to poor intracellular penetration and to a smaller effect on OIH accumulation. However, there was no clear connection between the average pKa-value of the individual aminoglycoside and its influence on accumulation, indicating that other differences in chemical structure are of importance for the effect on tubular cells.

N-Acetyl gentamicin in which the amino groups have been blocked by acetylation is microbiologically inert and non-toxic[3] and had no influence on OIH accumulation. Consequently the presence of free

amino groups is essential for the depressive effect on accumulation.

All the studied aminoglycoside antibiotics possess nephrotoxic properties. Streptomycin, on a weight basis, is the least toxic, kanamycin and amikacin occupy an intermediate position, while gentamicin and tobramycin display the greatest degree of nephrotoxicity[10,11,12]. There existed no correlation between the *in vivo* recognized renal toxicity of these drugs and the measured *in vitro* influence on OIH accumulation at neutral and alkaline pH-values. However, at pH 6.9 gentamicin and tobramycin had significantly greater effects on accumulation than the other aminoglycosides and as a parallel it has been reported that gentamicin nephrotoxicity is potentiated by metabolic acidosis in rats[13]. However, systematic studies of the influence of changes in acid-base balance on aminoglycoside nephrotoxicity have not been performed.

Alkalisation of the urine is recommended in aminoglycoside treatment of urinary infections, in order to achieve maximum antimicrobial effect[10,14]. However, the presented results suggest that alkalosis potentiate the nephrotoxicity of these drugs. Furthermore monosaccharides of the D-glucarate species protect against aminoglycoside-induced renal damage, possibly through a decrease in urinary pH[15]. Therefore the indication for urinary alkalisation should be carefully reconsidered.

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