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Inhibition of gentamicin uptake into rat renal cortex by aminoglycoside antibiotics *in vitro*

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Nephrotoxicity has been established as a major complication of antibacterial therapy with aminoglycoside antibiotics. The degree of this injury is proportional to the concentration of aminoglycosides in the kidney. The site of accumulation is the renal cortex, as has been demonstrated in experimental animals [1–3] and in man [4–6]. It is believed that the extent of the uptake of aminoglycosides in renal tissue correlates positively with the number of free amino groups on the drug molecule [7–9]. Accumulation of aminoglycosides has been studied in the renal cortical slice model. The uptake of gentamicin in this system is at least in part an active process [10–12] and recently specific competition between the uptake of gentamicin and other aminoglycosides was suggested [12–14].

The aim of this work was to define the type of inhibition of gentamicin uptake into slices of rat renal cortex by other aminoglycosides and to assess the quantitative parameters of this inhibition.

Materials and methods

Adult Wistar rats (males) weighing 220–260 g were used. Animals were exsanguinated and both kidneys were quickly removed and placed in ice-cold phosphate buffer, pH 7.4. The capsule was stripped off and thin cortical slices were cut free and stored in ice-cold medium [15] for no longer than 10 min. Slices weighing 70–80 mg were incubated in 4.5 ml of phosphate buffer under 100% O_2 at 37° in a shaking incubator (100 cycles/min).

The time course of the uptake was studied by incubating the slices in medium which contained 4 μg gentamicin/ml and [^{3}H]gentamicin (Radiochemical Centre, Amersham, U.K.), 160 Bq/ml, for 2.5, 3, 5, 7, 10, 12, 14, 15, 18, 20, 25 and 35 min.

To determine the kinetics of gentamicin uptake the duration of the incubation was held constant at 7 min and the incubation medium contained gentamicin in the following concentrations: 4, 8, 12, 20, 40 and 100 μg/ml with constant addition of the gentamicin label. In further experiments neomycin, streptomycin (Sigma Chemical Co., St. Louis, MO), kanamycin (a gift from Bristol-Myers Int. Corp., NY) or netilmicin (a gift from Essex Chemie AG, Lucerne, Switzerland) was added to the incubation medium at a concentration of 40 μg/ml. The slices were removed after the incubation had finished, rinsed with 96% ethanol, blotted, weighed and digested with Soluene 350 (Packard Instrument Co. Inc., Downers Grove). The digests were decolorized by H_2O_2 and mixed with Dimilume scintillation solution (Packard). The ^{3}H radioactivity was determined in a liquid scintillation spectrometer (Packard 300 CD)with quench correction by the external standard method. The ^{3}H radioactivity of an 1 ml aliquot of incubation medium was determined after mixing it with 10 ml of InstaGel (Packard).

The uptake of gentamicin in renal cortex was expressed as the ratio of radioactivity in 1 g tissue to that in 1 ml of medium (*S/M* ratio). The tissue concentration of [^{3}H]gentamicin after 7 min of incubation was calculated by multiplying the *S/M* ratio by the medium concentration of gentamicin. Statistical evaluation of the results included analysis of variance, linear regression and Student's *t*-test. The results are given as means ± S.E.M. of 4–6 experiments.

Results and discussion

The gentamicin *S/M* ratio, shown in Fig. 1, increased linearly with time up to approximately 15 min. Thereafter no further increase of the ratio was observed; rather there appeared to be a tendency to a slow decrease. The values

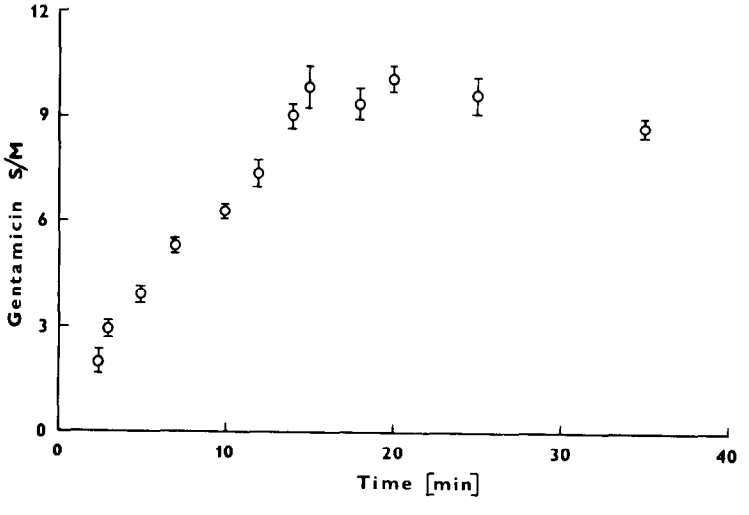

Fig. 1. Time dependence of the gentamicin slice-to medium ratio. Means ± S.E.M.

Table 1. Slice-to-medium ratio of gentamicin alone and in the presence of other aminoglycosides (40 μg/ml). Means ± S.E.M.

Aminoglycoside	Concentration of gentamicin in medium (μg/ml)					
	4	8	12	20	40	100
Gentamicin alone	5.29 ± 0.15	5.05 ± 0.22	4.86 ± 0.13	4.40 ± 0.14	4.04 ± 0.09	2.64 ± 0.06
Gentamicin and netilmicin	5.22 ± 0.14	5.02 ± 0.23*	4.77 ± 0.11	4.86 ± 0.15†	3.42 ± 0.07*	2.35 ± 0.06
Gentamicin and streptomycin	4.87 ± 0.14†	4.60 ± 0.18	4.46 ± 0.10†	4.58 ± 0.17	3.69 ± 0.13	2.37 ± 0.06
Gentamicin and kanamycin	4.12 ± 0.08*	3.99 ± 0.09*	4.23 ± 0.24†	3.60 ± 0.08*	3.12 ± 0.07*	2.04 ± 0.07*
Gentamicin and neomycin	3.21 ± 0.07*	2.98 ± 0.09*	3.03 ± 0.05*	2.82 ± 0.06*	2.80 ± 0.07*	2.12 ± 0.04*

* Significantly different to gentamicin alone at $P < 0.01$.
† Significantly different to gentamicin alone at $P < 0.05$.

Table 2. Binding parameters of gentamicin to rat renal cortical slices

Aminoglycoside	B_{max} (μg/g per 7 min)	K_m (μg/ml)
Gentamicin alone	477.47	86.67
Gentamicin and netilmicin	456.19	83.44
Gentamicin and streptomycin	437.81	83.75
Gentamicin and kanamycin	498.35	116.41
Gentamicin and neomycin	495.85	152.51

of the *S/M* ratio obtained were significantly higher than those obtained in comparable experiments [10, 11, 13]. However, the time that elapsed between sacrifice of animals and the start of incubation in our experiments was no longer than 10 min, while other authors reported a delay of 30 min or more. In our experiments total binding of gentamicin is reported, which also may be reflected in greater *S/M* ratios. However, other authors [10, 16] reported, in order to differentiate between specific and non-specific binding of gentamicin, data corrected for uptake in a N_2 atmosphere or after additional administration of excess cold gentamicin to previously applied [^{3}H]gentamicin. It may be seen from Fig. 1 that the *S/M* ratio at the 7 min time interval was approximately in the middle of the linear part of the *S/M* ratio time dependence. It may be assumed that this linear increase represents the initial rate of gentamicin uptake into slices. Therefore, the incubation time of 7 min was chosen for inhibition experiments. The *S/M* ratios obtained after 7 min incubation with gentamicin alone and with gentamicin in the presence of other aminoglycoside antibiotics in the medium are shown in Table 1. A tendency to saturation of the gentamicin uptake into the slices is obvious. Lower *S/M* ratios of gentamicin were observed in the presence of other aminoglycoside antibiotics in the incubation medium than in their absence. This indicates an inhibition of the gentamicin renal cortical uptake by other aminoglycoside antibiotics. To determine the type of inhibition, Lineweaver–Burk plots of gentamicin uptake into slices in the presence of other aminoglycosides were constructed (Fig. 2). Straight lines were fitted to experimental points, and the corresponding values of binding capacity, B_{max} and K_m are shown in Table 2. As there were no significant differences between values of B_{max} for binding of gentamicin in the absence or presence of other aminoglycoside antibiotics (inset in Fig. 2), a competitive type of inhibition is suggested. It follows from the comparison of the K_m values that neomycin and to a lesser degree kanamycin successfully competed with gentamicin for binding to cortical slices, whereas netilmicin and streptomycin did not exhibit this effect. This correlates well with the number of free amino groups on the respective molecule: neomycin 5, kanamycin 4, netilmicin 3, and streptomycin 2 guanido groups. The nephrotoxic potential of the aminoglycoside antibiotics in rats *in vivo* decreases

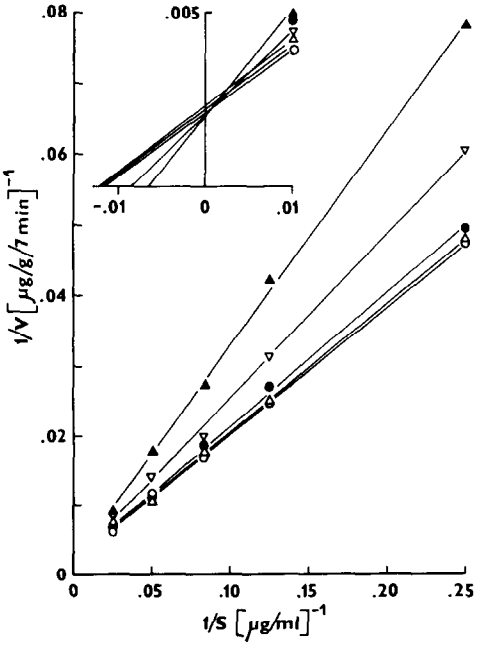

Fig. 2. Binding of gentamicin alone (○) to renal slices and in the presence of streptomycin (△), netilmicin (●), kanamycin (▽) and neomycin (▲).

in the following order: gentamicin, kanamycin, amikacin, sisomicin, tobramycin, netilmicin and streptomycin [17]. Neomycin, the most toxic aminoglycoside, is used in local therapy only. The ranking of aminoglycosides with respect to the decrease of their ability to compete with gentamicin for binding sites on renal cortex *in vitro* in our experiments is only partly in accordance with the order of their nephrotoxic potential *in vivo*.

In conclusion it may be said that the uptake of gentamicin into rat renal cortex *in vitro* is saturable and characterized by $K_m = 86.67\ \mu g/ml$ and $B_{max} = 477.47\ \mu g/g$ per 7 min. This uptake could be competitively inhibited by neomycin and kanamycin which are aminoglycoside antibiotics with more free amino groups on their molecule than gentamicin (3). This is in accord with the proposed charge interaction between the cationic polybasic aminoglycosides and the anionic tissue components of the kidney [16].

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Inhibition of acetylcholinesterase and cholinesterase by ellipticine derivatives

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Ellipticine and its derivatives are indole alkaloids with antitumoral properties [1, 2]. The structure of these compounds is depicted in Fig. 1. The cytotoxicity of ellipticines may be related to their affinity for deoxyribonucleic acid to which they combine by intercalation. Elliptinium acetate (**IIb**), a quaternary ammonium derivative, has induced remissions in patients with breast cancer and other malignancies [3, 4]. Some of the side effects observed, such as dyspnea and muscle cramps, suggested an interaction between elliptinium and the cholinergic system [5]. Previous studies on the structure–activity relationship have shown that many reversible inhibitors of acetylcholinesterase (EC 3.1.1.7, AcChE) and butyryl cholinesterase (EC 3.1.1.8, BuChE) contain one or two quaternary ammonium groups [6, 7], or eventually a protonated tertiary amine. The present study reports the interaction of ellipticine derivatives with the catalytic activity of acetylcholinesterase from rat brain and of human sera pseudocholinesterase.

Materials and methods

The substrates (i.e. acetylthiocholine ATC and butyrylthiocholine BTC), the chromogen dithionitrobenzoic acid (DTNB) and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, MO). 2*N*-methyl-9-hydroxyellipticinium* and BD 40 M were obtained from Institut Pasteur Production (Paris, France), and other derivatives were gifts of Dr. Auclair (L.A. 147 C.N.R.S.). Edrophonium chloride was obtained from Hoffmann-La Roche & Co., A.G. (Basel, Switzerland). All other reagents were of analytical grade.

Enzymatic preparations. The two enzymatic preparations used in this study were acetylcholinesterase extracted from the cerebral cortex of male Wistar rats and cholinesterase of human serum.

The rat cortices were rapidly removed after decapitation and placed in a cold phosphate buffer, pH 7.4, containing 1% of non-ionic surfactant (Triton X-100). Cortices were then homogenized by Polytron action, and 8 ml of buffer was added to give a final volume of 10 ml. After centrifugation (15 min, 40,000 *g*, 4°), the supernatant was removed and used immediately or frozen at −20°. Enzymatic solutions were always used within 4 days after preparation. Human serum cholinesterase (BuChE) was obtained by centrifugation of whole blood collected on heparin anticoagulant, and diluted to 50% in phosphate buffer.

Cholinesterase assay. The determination of enzyme

* Elliptinium acetate (DCI) : Celiptium.®