# AMINOGLYCOSIDE-INDUCED MODIFICATIONS OF MEMBRANE PHOSPHOINOSITIDE METABOLISM. A COMPARATIVE STUDY

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Several lines of evidence indicate that the first steps in the pathogenesis of aminoglycoside nephrotoxicity involve the accumulation of the antibiotic in the proximal tubule where it interacts with the plasma membrane of epithelial cells[1,2]. In brush border membranes, acidic phospholipids and most particularly phosphoinositides, phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PI-P) and phosphatidylinositol-4,5-bisphosphate (PI-P$_2$), have been recently shown to be an integral component of the aminoglycoside binding site[3]. In addition, the aminoglycosides have been shown to modify the metabolism of phosphoinositides in the kidney cortex[4~7]. In a biomembrane model such as the ghost membrane isolated from rat erythrocyte, results from this laboratory[8] indicated that aminoglycosides impaired the phosphoinositide interconversion since these drugs induced a decrease and an increase in the $^{32}$P-labelling of PI-P$_2$ and PI-P, respectively likely by inhibiting the PI-P kinase; the existence of a possible relationship between the toxicity of aminoglycosides and their capacity to impair the phosphoinositide metabolism was also suggested. This finding stimulated us to further study the interaction between aminoglycosides and phosphoinositide metabolism within the membrane. For this purpose, using isolated red cell ghosts, experiments were designed to compare the effects of five aminoglycosides in clinical use, namely amikacin, dibekacin, gentamicin, netilmicin and tobramycin on the $^{32}$P-labelling of the polyphosphoinositides PI-P and PI-P$_2$.

## Materials and Methods

Fifteen to eighteen week-old male rats of the Wistar strain were supplied by Iffa Credo (France). [$\gamma$-$^{32}$P]ATP (2,000 ~ 3,000 Ci/mmol) was purchased from Amersham (UK). Amikacin, dibekacin and netilmicin were generously provided by Bristol, Bristol/Bellon and Unilabo, respectively. Gentamicin and tobramycin were from Sigma. Blood sampling and preparation of isolated ghost membranes were performed as already detailed[9]. For $^{32}$P incorporation into membrane phospholipids, 0.3 mg membrane protein were preincubated for 10 minutes at 37°C in 50 mM Tris-HCl buffer pH 7.5 in the presence (or absence for controls) of the antibiotic. Then 5 mM MgCl$_2$ and 2 mM ATPNa$_2$ (containing 5 ~ 10 $\mu$Ci [$\gamma$-$^{32}$P]ATP) final concentrations, were added in a total volume of 0.5 ml. The reaction was stopped 15 minutes later and samples were processed for phospholipid extraction, separation and $^{32}$P-phosphoinositide analysis as described previously[9]. In order to compare the effects of antibiotics on the $^{32}$P incorporation into phosphoinositides, the following experimental protocol was used. In a single experiment, the five aminoglycosides namely, amikacin, dibekacin, gentamicin, netilmicin and tobramycin were studied on the same membrane preparation obtained from one rat. Each aminoglycoside was studied in duplicate at two concentrations ($6 \times 10^{-5}$ and $6 \times 10^{-4}$ M). Control samples (processed in the absence of drug) were in quadruplicate. The $^{32}$P radioactivities associated with PI-P$_2$ and PI-P in each drug-treated sample were expressed as the percentage of these radioactivities in controls. Seven experiments were performed, each one using a different membrane preparation in order to take into account variations between animals.

## Results and Discussion

Using isolated ghosts as a biomembrane model, a previous study has shown that concentration of $6 \times 10^{-5}$ M neomycin was required to induce an increase in [$^{32}$P]PI-P as well as a concomitant decrease in [$^{32}$P]PI-P$_2$. We therefore chose aminoglycoside concentrations of $6 \times 10^{-5}$ and $6 \times 10^{-4}$ M for the present study. When used at $6 \times 10^{-5}$ M, all the drugs tested did not significantly affect the $^{32}$P-labelling of PI-P$_2$ (Fig. 1). At such a concentration, whereas amikacin did not modify significantly the $^{32}$P-labelling of PI-P this latter was significantly enhanced by the other aminoglycosides studied (Fig. 1). When used at

Fig. 1. Effect of aminoglycosides on the $^{32}$P-labelling of PI-P$_2$ (upper) and of PI-P (lower).

Values are expressed as the percentage of those obtained in controls (*i.e.* when the drugs were omitted) which were $1.67\pm0.19$ and $0.60\pm0.08$ $^{32}$P nmol/15 minutes/mg protein for [$^{32}$P]PI-P$_2$ and [$^{32}$P]PI-P, respectively (mean$\pm$SEM, n$=$7).

○ Gentamicin, ● dibekacin, △ netilmicin, ▲ amikacin, □ tobramycin.
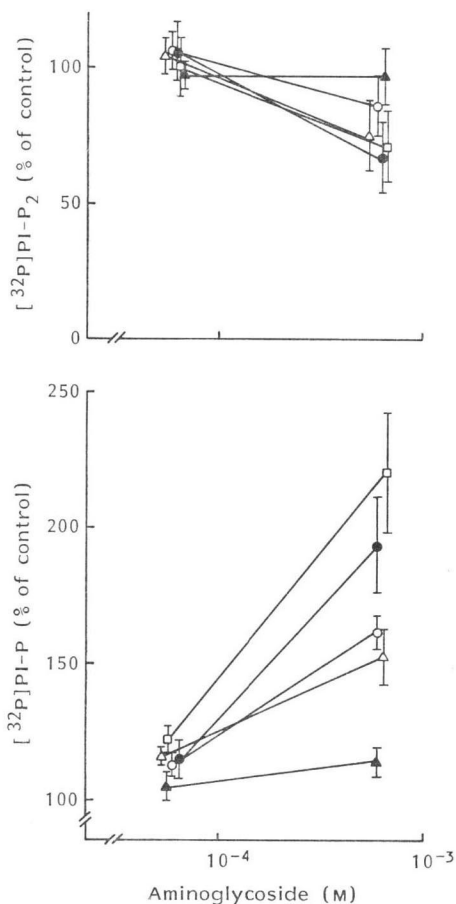


$6\times10^{-4}$ M, Fig. 1 also shows that all aminoglycosides tested, except amikacin, induced a significant decrease in [$^{32}$P]PI-P$_2$ and a significant increase in [$^{32}$P]PI-P. It is noteworthy that the total radioactivity incorporated into both PI-P$_2$ and PI-P in aminoglycoside-treated samples (and irrespective of the drug used) did not differ significantly from that incorporated into controls ($2.16 \sim 2.51$ *versus* 2.27 $^{32}$P nmol/15 minutes/mg protein). This agrees with what has been reported with neomycin-treated samples[5] and suggests that aminoglycosides share a similar mechanism of action

for impairing the phosphoinositide interconversion.

Although the potency with which a single aminoglycoside affected the $^{32}$P incorporation into PI-P and PI-P$_2$, varied with the animal used for membrane preparation, the data obtained in the course of our experiments revealed that the order of potency of the drugs studied was very similar from one experiment to another. This prompted us to use the paired Student's t test for comparing the five aminoglycosides studied. When the antibiotics were used at $6\times10^{-5}$ M, no significant difference could be detected between them, probably because at this concentration the drug-induced modifications were too small (Fig. 1). When the drugs were used at $6\times10^{-4}$ M the statistical analysis revealed that: if one considers their ability to decrease [$^{32}$P]PI-P$_2$, (i) amikacin was less effective than dibekacin ($P<0.05$), netilmicin ($P<0.02$), gentamicin and tobramycin ($P<0.001$) and (ii) gentamicin was less effective than dibekacin ($P<0.05$) and than tobramycin ($P<0.01$); if one considers their ability to increase [$^{32}$P]PI-P, (i) amikacin was less effective than the other four antibiotics ($P<0.01$ vs. dibekacin, tobramycin or netilmicin and $P<0.001$ vs. gentamicin), (ii) netilmicin, gentamicin and dibekacin were less effective than tobramycin ($P<0.01$) and (iii) netilmicin was less effective than dibekacin ($P<0.01$). Not included in this comparative investigation, a separate experiment showed that neomycin was more potent than tobramycin. Indeed when compared to control values, [$^{32}$P]PI-P$_2$ and [$^{32}$P]PI-P were 51 and 222%, respectively in the presence of $6\times10^{-4}$ M tobramycin whereas these values were 28 and 323%, respectively in the presence of $3\times10^{-4}$ M neomycin.

Aminoglycoside concentrations in the millimolar range could not be used appropriately in the system studied since too high drug concentrations have been proved to decrease both [$^{32}$P]PI-P$_2$ and [$^{32}$P]PI-P (results not shown). Although the use of only two drug concentrations prevents calculation of ED$_{50}$ values for the compounds tested, the experimental design of the present investigation nevertheless allows the comparative study of the effect of aminoglycosides on the phosphoinositide interconversion. The results clearly indicate that tobramycin is 2 times as potent as amikacin. On the other hand, the results do not permit a clear distinction between dibekacin, genta-

micin and netilmicin which were approximately
$1.4 \sim 1.7$ times as potent as amikacin. Thus the
order of ability to impair the $^{32}$P-labelling of poly-
phosphoinositides is: tobramycin > gentamicin $\simeq$
dibekacin $\simeq$ netilmicin > amikacin; neomycin ap-
pears to be more potent than tobramycin. In ad-
dition, streptomycin has been described as similar
to amikacin[8]. The above ranking order resem-
bles the order of aminoglycosides when ranked
according to their cationic charge (streptomy-
cin = 3; amikacin = 4; tobramycin = netilmicin =
dibekacin = gentamicin = 5; neomycin = 6). This
suggests that a charge interaction between the
basic drugs and the acidic membrane phospholi-
pids could account, at least in part, for the ef-
fects observed here. Such an interaction has
been described to be involved in the binding of
aminoglycosides to brush border membranes[3].
However, our observation that tobramycin was
significantly more potent than dibekacin, genta-
micin and netilmicin suggests also that other
determinants such as the molecular structure may
confer to an aminoglycoside its potency to impair
the phosphoinositide metabolism.

The nephrotoxic potential of aminoglycosides
appears also to correlate with their cationicity
(for review see ref 10). This potential appears
however to be determined by both the extent to
which the drug accumulates within tubules and
the intrinsic toxic effect of the drug on the tubular
cell itself[11]. In the kidney cortex, the lesser ac-
cumulation of tobramycin compared to gentami-
cin may explain therefore the lower degree of ne-
phrotoxicity of the former compound[10,11] al-
though its capacity to alter phosphoinositide
metabolism was greater. The $Ca^{2+}$ binding to
membranes and to lipid monolayers has also been
demonstrated to be altered by aminoglycosi-
des[12~14]. Although it still remains to establish
that this can be related with the drug-induced
alteration of phosphoinositide metabolism, it is
noteworthy that polyphosphoinositides have
been described as intimately involved in the pro-
cesses of transmembrane $Ca^{2+}$ flux (ref 15 and
references therein). These lipids also participate
actively in the structure and function of mem-
branes[16]. It is therefore conceivable that amino-
glycoside-induced modifications of phosphoino-
sitide metabolism would lead to alterations of
membrane permeability and/or membrane trans-
port capacity that can play critical roles in the
pathogenesis of cellular injury. In this respect

the recent identification of polyphosphoinositides
and of PI-kinase activity in lysosomes[17] may be
relevant since aminoglycosides accumulate in
these organelles where they cause phospholipido-
sis and myeloid body formation before cell ne-
crosis.

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