

Molecular mechanisms of drug-induced hearing loss

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Although the ototoxic actions of a variety of drugs have long been documented, the biochemical mechanisms underlying such toxicity largely remain to be established. For example, recent advances have provided us with information about the actions of salicylates (aspirin) and diuretics (furosemide) but we are not yet able to specify the mechanisms by which these drugs damage the cochlea. On the other hand, the considerable amount of biochemical and pharmacological data on the effects of aminoglycosides (streptomycin, neomycin, gentamicin and related compounds) has enabled us to formulate a rational hypothesis of their mechanism of action.

We have previously presented evidence for an involvement of polyphosphoinositides in the ototoxic actions of aminoglycosides. Recent electrophysiological and pharmacokinetic studies have shown in addition that aminoglycosides occupy at least two distinct compartments in the course of their actions. Further studies of drug uptake *in vitro* and of drug toxicity in cochlear perfusions suggested the involvement of an active (energy-requiring) aminoglycoside transport system. These and other data are compatible with the following multi-step model of aminoglycoside toxicity:

(1) The initial step in the reaction sequence is an electrostatic interaction of aminoglycosides with the plasma membrane. The resulting displacement of calcium accounts for acute effects but the action is reversible and antagonized by divalent cations.

(2) An energy-dependent uptake process is required for the expression of toxicity. It can be prevented by select metabolic blockers.

(3) A crucial step in subsequent intracellular drug actions is the binding of aminoglycosides to phosphatidylinositol bisphosphate inhibiting its hydrolysis and preventing its physiological function. Hydrolysis of phosphatidylinositol bisphosphate is believed to be the key reaction in a fundamental transducing mechanism initiated by a number of physiological stimuli and resulting in a signal cascade to mobilize intracellular calcium stores.

(4) Other reactions possibly regulated by polyphosphoinositides (such as the synthesis of prostaglandins and the polymerization of actin) are potentially affected by aminoglycoside action.

(5) Aminoglycosides may interfere with further intracellular reactions which are controlled by calcium or polyamines.

Introduction

The awareness that certain drugs in clinical use may cause temporary or permanent disorders of the auditory or vestibular systems goes back at least a century. At that time the toxic effects of quinine, the salicylates and oil of chenopodium had been described in the scientific literature (North, 1880; Schwabach, 1884). Since then the number of drugs potentially interfering with the function of the labyrinth has grown considerably. One of the intriguing aspects of this phenomenon is that ototoxic drugs fall into several unrelated therapeutic classes. In addition to the analgesics and antipyretics (salicylates, quinine) there are the antibacterial aminoglycosides (for example, neomycin, gentamicin) and polypeptide antibiotics (viamycin, vancomycin), some metallo compounds

(arsenicals, mercurials), 'loop' diuretics (ethacrynic acid, furosemide) and antineoplastic drugs (*cis*-dichlorodiammin-platinum). There is no apparent structural relationship between these drugs nor is there a predictable pattern of the pathology they may induce. Some act preferentially on the cochlea, some on the vestibular system, and within the cochlea they may act on the organ of Corti or the stria vascularis. The types of cellular damage they induce are also quite dissimilar as are the manifestations of toxicity such as tinnitus, temporary or permanent hearing loss (for a review of the pathology of drug-induced ototoxicity see Hawkins, 1976, and Wersäll, 1986).

Considering these multifaceted drug actions, it is not surprising that a unifying hypothesis of ototoxicity does not exist. Each drug has to be considered separately and generalizations cannot

be made. Furthermore, to date only few of these drugs have been investigated for the molecular mechanism of their toxicity, among them most notably the aminoglycoside antibiotics. In addition, some recent information on the pharmacology and biochemistry of diuretic- and salicylate-induced ototoxicity (Rybak et al., 1984; Escoubet et al., 1985) shows promise of leading to a rational understanding of their action.

The aminoglycosides, still widely used for their antibacterial efficacy, were discovered in the 1940s (see Waksman, 1953) and their toxicity was described shortly after the first clinical application for the treatment of tuberculosis. Drugs of this class induce both nephrotoxicity which is generally reversible, and ototoxicity which is generally irreversible. Some of the aminoglycosides preferentially damage the cochlea (for example, amikacin), some the vestibular apparatus (streptomycin), while others show only gradual, if any predilections (gentamicin, tobramycin). The ototoxicity of the aminoglycosides will be the focus of our discussion in this chapter.

Results and Discussion

Pharmacokinetics

The first systematic investigations into aminoglycoside ototoxicity were pharmacokinetic studies of the drug distribution in the perilymph of animals (Voldrich, 1965; Vrabec et al., 1965; Stupp, 1970). This approach – analysis of an extracellular fluid rather than the affected tissues – is only indirect and its justification may have been the technical feasibility at the time. It had, however, considerable impact as these first studies seemed to show an ‘accumulation’ of the aminoglycosides in perilymph which was postulated to be the primary cause of their toxicity and organ specificity.

Recent detailed and well-controlled pharmacokinetic investigations, however, demonstrated that an accumulation of gentamicin in the inner ear fluids over plasma levels did not occur (Tran Ba Huy et al., 1981, 1983, 1985). Furthermore, the concentrations of several aminoglycosides attained in inner ear fluids were shown to be unrelated to the resultant toxic effects (Ohtsuki et al., 1982; Ohtani et al., 1985). Conversely, direct application of different aminoglycosides in identical concentrations into the cochlea by perilymphatic per-

fusion produced a differential loss of the cochlear microphonic potential, indicating that toxicity was largely an intrinsic property of the drugs rather than related to their concentration in inner ear fluids (Lodhi et al., 1980; Anniko et al., 1982). In addition to studies of inner ear fluids the determination of neomycin levels (Desrochers and Schacht, 1982) and gentamicin pharmacokinetics (Tran Ba Huy et al., 1986) in inner ear tissues showed that the concentration the drug reached in these tissues was not higher than in organs that do not show pathologies such as liver or lung.

The combined evidence, then, seems to rule out a mechanism of toxicity and organ specificity based solely or primarily on drug concentrations reached in inner ear fluids or tissues. This does not preclude the existence of drug uptake or transport systems in the affected cells. It means, however, that the mechanism (or mechanisms) of toxicity must lie in the specific biochemical interactions of the drugs with their target cells.

Biochemical studies

A major problem that arises in the study of any drug-induced pathology is the need to differentiate between primary and secondary actions. For example, after the initial insult to a cell by a drug a wide variety of biochemical pathologies may be observed that result not from a direct interaction with the drug itself but from the metabolic response of the cell to the primary insult. Such pathologies may be part of the chain of events leading to cell death or, in contrast, may be cellular repair mechanisms. In either case, they do not reflect the specific mechanism of drug action. It is the specific site(s) that need to be elucidated for a rational hypothesis of drug action.

Such a rational hypothesis of aminoglycoside action has been lacking until now. Not because of a lack of demonstrable biochemical effects but paradoxically an abundance of them. Aminoglycosides have been reported to affect DNA, RNA and protein synthesis, enzymes of energy metabolism and transport, gangliosides, mucopolysaccharides and lipids – virtually all categories of cellular components. Yet these findings have not led to a coherent explanation of the toxic mechanism probably because most of them are linked to secondary effects.

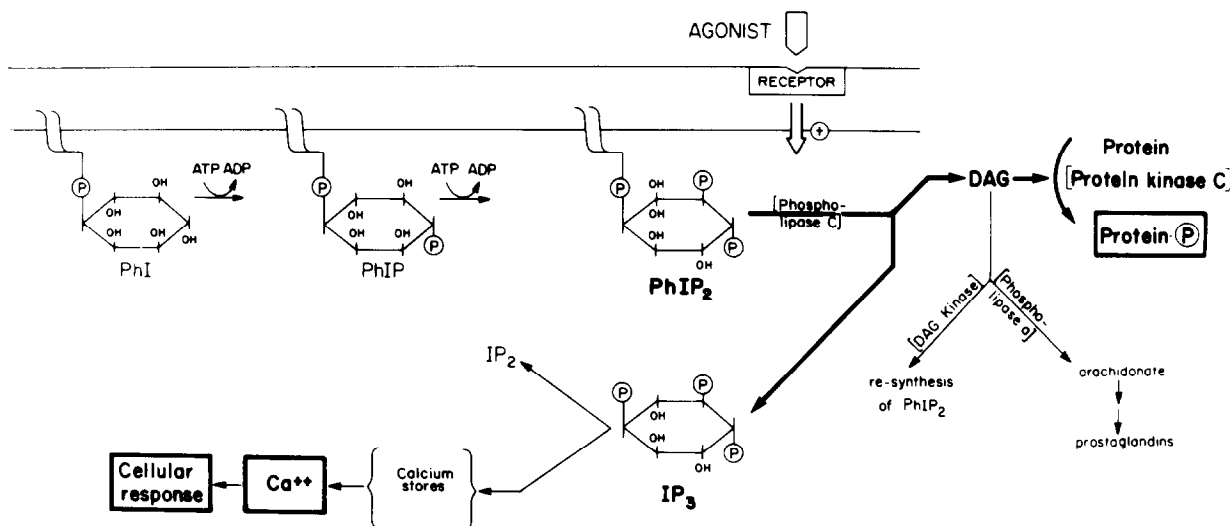


Fig. 1. The phosphoinositide cascade. Binding of the agonist to its receptor at the outer plasma membrane stimulates the phosphodiesteratic cleavage of phosphatidylinositol bisphosphate (PhIP₂) located in the inner plasma membrane. The resulting products, diglyceride (diacylglycerol, DAG) and inositol trisphosphate (IP₃) act as second messengers and elicit the physiological response of the cell via activation respectively, of a protein kinase and the release of Ca²⁺ from intracellular stores. The action of IP₃ is terminated by its hydrolysis to inositol bisphosphate (IP₂), and PhIP₂ is resynthesized from DAG via phosphatidic acid, CDP diglyceride, phosphatidylinositol (PhI) and phosphatidylinositol phosphate (PhIP). Synthesis of prostaglandins may also proceed from DAG. After Nishizuka (1984) and Berridge (1984).

We have previously presented evidence for an involvement of polyphosphoinositides in the toxic actions of aminoglycosides. Polyphosphoinositides are acidic phospholipids of the plasma membrane synthesized in two sequential phosphorylation reactions from phosphatidylinositol (Fig. 1). Phospholipids provide the structural framework of cell membranes, regulate membrane function and mediate the communication of the cell with the external environment.

All environmental stimuli are encoded at the cell membrane into molecular signals that traverse the membrane and elicit the appropriate physiologic response in the cell. Recent evidence suggests that polyphosphoinositides, which are located on the cytoplasmic site of the plasma membrane, constitute a fundamental transmembrane signaling system for neuromodulators and hormones which use calcium as an ultimate cellular messenger (Nishizuka, 1984; Berridge, 1984; Fig. 1). The initial step in this action is a stimulation of the phosphodiesteratic hydrolysis of membrane-bound phosphatidylinositol bisphosphate liberating diacylglycerol and inositol trisphosphate as second

messengers and initiating an intracellular signal cascade. In this cascade, diacylglycerol activates a specific protein kinase C, and inositol trisphosphate mobilizes intracellular calcium, two signals that trigger the cellular response. Sensory stimuli, e.g. visual excitation, may be processed by this mechanism (Brown et al., 1984).

Whether cochlear phosphoinositide metabolism is responsive in such a fashion remains to be established. We have investigated phospholipids in the mature and developing mammalian cochlea, in the otocyst in organ culture, in the fish and the frog lateral line and in the ear of the Noctuid moth (Stockhorst and Schacht, 1977; Kilian and Schacht, 1980; Anniko and Schacht, 1981; Tachibana et al., 1983; Schacht, 1984). All hair-cell systems contain polyphosphoinositides and the incorporation of radioactive [³²P]orthophosphate consistently shows a fast kinetics and isotopic equilibrium indicative of the high rate of turnover of these lipids. Sound induced an increase in ³²P labeling of phosphatidylinositol phosphate and phosphatidylinositol bisphosphate in the ear of the Noctuid moth (Kilian and Schacht, 1980). Furthermore, the

polyphosphoinositide cascade can be speculated to be involved in several aspects of auditory transduction, based on modulation of membrane properties and structural elements.

Extensive *in vivo* and *in vitro* studies from our laboratory have provided evidence that phosphatidylinositol bisphosphate may be a specific binding site for aminoglycosides (Schacht et al., 1977; Schacht, 1979). The configuration of the negative charges of phosphatidylinositol bisphosphate allows a specific three-point binding to the positively charged aminoglycoside. Hydrogen bonding may further stabilize the complex and other cationic groups of the antibiotic may 'anchor' the complex in the cell membrane at secondary sites. Due to this binding the metabolism of phosphatidylinositol bisphosphate is inhibited by aminoglycosides both *in vivo* (Orsulakova et al., 1978; Stockhorst and Schacht, 1977) and *in vitro* (Lodhi et al., 1979). In addition, specific changes in membrane structure and fluidity were induced by neomycin in lipid monolayers and liposomes which contained phosphatidylinositol bisphosphate (Lodhi et al., 1979; Wang et al., 1984a,b).

Following our initial reports on altered phosphoinositide metabolism in the neomycin-intoxicated ear and kidney (Schacht, 1974; Schibeci and Schacht, 1977) confirmation of aminoglycoside/phosphoinositide interactions has come from a number of laboratories studying cochlear and renal lipid metabolism and physicochemical models. Incorporation of ^{32}P into cochlear polyphosphoinositides was enhanced by sound and depressed by neomycin (Yanagisawa et al., 1982). Aminoglycoside-induced altered renal phospholipid metabolism, particularly that of phosphoinositides, was reported (Koloyanides and Pastoriza-Muñoz, 1980; Marche et al., 1983). A specific interaction between neomycin and the 5'-phosphomonoester of phosphatidylinositol bisphosphate was determined by ^{31}P nuclear magnetic resonance (Hayashi et al., 1980). Finally, a series of aminoglycosides suppressed K^+ permeation in bilayer membranes formed by lyso-phosphatidylinositol bisphosphate and the degree of suppression correlated with the toxicity of the drugs (Sokabe et al., 1982).

Additional observations

While the involvement of phosphatidylinositol bisphosphate in the toxicity of aminoglycosides thus seems well established a number of additional observations remained to be incorporated into a molecular model of ototoxicity.

First, recent electrophysiological and pharmacokinetic studies from our laboratory demonstrated that aminoglycosides occupy at least two distinct cellular compartments in the course of their toxic action. When gentamicin was applied to the cochlea by perilymphatic perfusion, the loss of the cochlear microphonic potential could be prevented by the simultaneous or immediately following addition of calcium (Takada and Schacht, 1983). This was in good agreement with an aminoglycoside/calcium antagonism which we had demonstrated in natural and artificial membranes (Lodhi et al., 1976). However, if the application of calcium was delayed, the decrease of the cochlear microphonics could no longer be reversed. This differentiated an acute and reversible phase of aminoglycoside toxicity from a non-reversible one. A similar observation of a biphasic action of streptomycin had been made by Wersäll and Flock (1964) in the lateral line organ of the fish.

Pharmacokinetic studies of gentamicin uptake into and release from inner ear tissues (Tran Ba Huy et al., 1986) provided independent evidence for the occupation of at least two cellular compartments by the drug. After both short-time infusion and chronic injections, gentamicin reached similar steady-state levels in the cochlea. The short-time treatment was not associated with signs of ototoxicity and the drug was released from the tissues with a half-life of 8 days. After chronic injections ototoxicity developed and the drug persisted in the tissues with a half-life of 36 days. Clearly, this difference in half-lives indicated the release of the drug from two different binding sites or compartments, although these need not be identical to those observed in the perfusion study.

If several compartments for drug binding exist the question arises as to their location and the process by which they are linked. One possible constellation is an initial drug action on the plasma membrane (representing the acute and reversible phase) followed by entry of the drug into the cell and intracellular actions (representing the chronic

phase). Presently little is known about the mechanisms of entry into cochlear cells of aminoglycoside antibiotics. This is quite in contrast to the well-researched uptake of these drugs into bacterial cells (Hancock, 1982) where energy-dependent processes have been established and into kidney where transport processes have been somewhat characterized (Josepovitz et al., 1982; Bennett et al., 1982).

The hypothesis that an energy-dependent uptake process may be a necessary step in aminoglycoside action comes from studies of gentamicin toxicity in cochlear perfusions and mechanisms of its prevention (Takada et al., 1985). If a bolus of gentamicin is administered during a time when the endolymphatic potential is depressed by anoxia or drugs the expected loss of the cochlear microphonic potential is prevented. In the most general interpretation, this signifies that a process dependent on normal energy metabolism is required for the expression of toxicity such as an uptake driven by an active transport or sym-port mechanism or by the transmembrane potential. An energy-dependent uptake of aminoglycosides is also, at least partially, operating in the crista ampullaris. A block of metabolism by low temperature or drugs prevented a significant portion of gentamicin uptake in vitro (Williams and Schacht, in preparation).

The hypothesis (Fig. 2)

The data elaborated by our laboratory are consistent with the following multi-step model of aminoglycoside toxicity.

(1) The first step in the reaction sequence is an electrostatic interaction of the aminoglycoside with negatively charged sites on the outer plasma membrane. The resulting displacement of calcium accounts for the acute effects of the drug and the action is reversible and antagonized by cations.

(2) The drug is taken up into the cell by an energy-dependent transport process. This step can be prevented by metabolic blockers.

(3) The next and crucial step in the subsequent intracellular actions is the binding of the drug to phosphatidylinositol bisphosphate. The formation of the drug-lipid complex has two consequences: first, it prevents the hydrolysis of phosphatidylinositol bisphosphate which is the key reaction

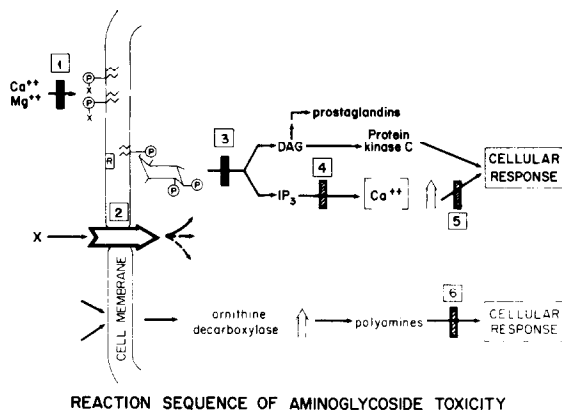


Fig. 2. Hypothesis of aminoglycoside toxicity. (1) Aminoglycosides bind to negatively charged sites on the plasma membrane. (2) They are taken up into the cell by an active transport system. (3) They form a complex with phosphatidylinositol bisphosphate and inhibit its hydrolysis. (4-5) They interfere with further steps of the phosphoinositide cascade and (6) other intracellular reactions. Solid bars and lines: documented aminoglycoside actions; hatched: postulated actions. Abbreviations as in Fig. 1. For details, see text.

in the phosphoinositide second messenger signal cascade; secondly, it disturbs membrane integrity and structure resulting in non-specific permeabilities of the membrane to ions and possibly to the antibiotic itself, causing an 'autocatalytic' entry of the drug into the cell.

(4) This binding would also inhibit other reactions regulated by polyphosphoinositides such as the polymerization of actin (Burn et al., 1985; Lassing and Lindberg, 1985) and the synthesis of prostaglandins. The inhibition of the latter has been documented recently (Escoubet et al., 1985).

(5) Once inside the cell, the aminoglycosides may interfere with further intracellular reactions as amply documented in the literature. This interference may be based on competition with divalent cations or polyamines or on binding to negatively charged compounds.

Critical considerations

To the best of our knowledge the hypothesis presented is compatible with data presented not only by our but also other laboratories to date. Nevertheless, several questions remain to be discussed.

The first question is why, in the face of such a multistep reaction, the binding to polyphosphoinositides is considered a crucial step. The most convincing answer comes from the study of physicochemical models of drug-lipid interactions. In monomolecular films containing phosphatidylinositol bisphosphate the disturbance of membrane structure (measured as an increase in surface pressure) shows an excellent correlation with the ototoxicity of various aminoglycosides (Wang et al., 1984b; Fig. 3). Such a correlation, in particular the correct ranking of netilmicin and neamine, is not achieved with models that are based on lipids other than phosphatidylinositol bisphosphate strongly suggesting a direct relationship between polyphosphoinositide binding and toxicity.

A second question is the tissue specificity of the drug action. Neural tissue (including the ear) shows an active metabolism of phosphoinositides as does the kidney, while other tissues (such as liver or lung) seem less active. In both the inner ear and the kidney polyphosphoinositides may thus be a crucial determinant of toxicity. However, for the drug to exert this and other intracellular actions an entry into the cell is required. The susceptibility of an organ to aminoglycosides then may be de-

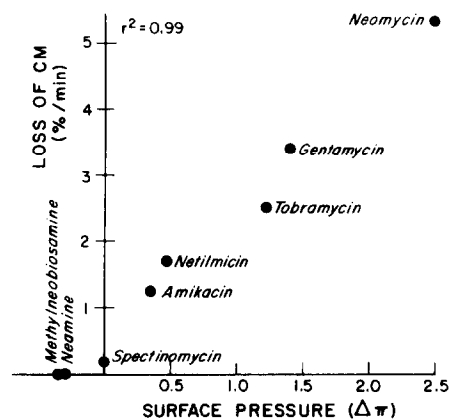


Fig. 3. Correlation of aminoglycoside ototoxicity with action on phosphatidylinositol bisphosphate. Aminoglycoside antibiotics and derivatives were tested for ototoxicity by cochlear perfusions (plotted on the ordinate) and for their interaction with a monomolecular film containing phosphatidylinositol bisphosphate (plotted on the abscissa). For details, see text and Wang et al. (1984b).

termined by a combination of at least two factors: the presence of an active uptake system and the presence of a physiologically important population of phosphoinositides.

Another question and one of utmost clinical importance is the prevention of aminoglycoside-induced hearing loss. Pharmacologic treatments for detoxification or amelioration of ototoxicity have long been tried but have largely been unconvincing. It seems that an approach to prevention or amelioration of toxicity based on the hypothesis of membrane interactions may be more promising. Reversibility of initial aminoglycoside damage is a postulate of our hypothesis and supported by experimental data (Wersäll and Flock, 1964; Shiozawa and Yanagisawa, 1979; Kroese and van den Bercken, 1982) but less so by clinical experience (Matz, 1975). It should be noted, however, that cases of recovery of patients from gentamicin-induced hearing loss have been reported (Jackson and Arcieri, 1971; Federspil, 1971; Tjernström et al., 1973) as well as from streptomycin and kanamycin damage, particularly when drug treatment was stopped shortly after the onset of symptoms (Federspil, 1979). The paucity of support for reversibility after parenteral treatment with aminoglycosides may be explained by our in-situ studies showing the reversibility limited to a narrow set of conditions determined by both duration and concentration of drug treatment (Takada et al., 1983). Under appropriate conditions, calcium should be an effective antagonist in the early phase of toxicity. Indeed, Humes et al. (1984) reported that a dietary calcium load lowered gentamicin nephrotoxicity: excess serum calcium is eliminated by the kidney and competes with renal binding of aminoglycosides. It is interesting that calcium levels in the perilymph can be elevated by intravenous CaCl_2 injections (Juhn and Youngs, 1976). Whether such transiently increased levels of cochlear calcium might affect aminoglycoside binding and ototoxicity remains to be established.

Conclusion

Some unanswered, yet important questions remain. Why is there no strict correlation between nephro- and ototoxicity? Why do some of the drugs cause more damage to the cochlea than to

the vestibular system and vice versa? Why are outer hair cells more susceptible than inner hair cells? These questions do in no way invalidate our model: they simply point to the fact that drug reactions are usually complex and models necessarily simple. Speculation about answers can first point to the combined requirement of an uptake system and phosphoinositides for the maximal expression of toxicity. If one or the other or both are absent from a cell, entirely different patterns of toxicity can be expected. In addition, calcium- or polyamine-regulated intracellular processes may be an important part of the overall mechanism and these still need to be investigated. In this context the hypothesis presented provides a model that is amenable to experimental scrutiny. The next years will show what modifications we will have to make to accommodate new and critical findings.

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