time is limited to the cases where the dissolution data are well described by Eq. 1, *Approach II* provides a simple, rapid, and reliable tool for prediction of disintegration profiles. The computation involved is simple and can be carried out manually without the use of computer programs as required by other techniques.

REFERENCES

(1) K. G. Nelson and L. Y. Wang, *J. Pharm. Sci.*, **66**, 1758 (1977).
(2) *Ibid.*, **67**, 86 (1978).
(3) A. El-Yazigi, *ibid.*, **70**, 535 (1981).

Lysine and Polylysine: Correlation of their Effects on Polyphosphoinositides *In Vitro* with Ototoxic Action *In Vivo*

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Abstract □ Low concentrations of poly-L-lysine, a polycationic hydrophilic molecule, caused a large expansion of polyphosphoinositide monolayers and produced a significant loss of cochlear microphonic potentials in perilymphatic perfusions in the guinea pig. In contrast, the monomeric L-lysine had only slight effects on polyphosphoinositide monolayers and did not affect cochlear microphonic potentials even at concentrations as high as 10 m*M*. These data substantiate the hypothesis that the expansion of polyphosphoinositide monolayers by a drug is an indicator of its ototoxicity.

Keyphrases □ Polyphosphoinositides—correlation of effects of lysine and polylysine *in vitro* with ototoxic action *in vivo* □ Lysine—correlation of effects on polyphosphoinositides *in vitro* with ototoxic action *in vivo*, polylysine □ Polylysine—correlation of effects on polyphosphoinositides *in vitro* with ototoxic action *in vivo*, lysine

Evidence has previously been provided in *in vivo* and *in vitro* studies for an involvement of polyphosphoinositides in the toxic actions of aminoglycoside antibiotics (1). Monolayer studies have shown that polyphosphoinositides are unique among various anionic phospholipids with respect to both the type and magnitude of the interactions with neomycin and Ca^{2+} (2). The observed increase in surface pressure was indicative of a very strong preference of the polyphosphoinositide film for neomycin over Ca^{2+} and other cations. It was further shown that the degree of interaction of eight aminoglycoside antibiotics and fragments with polyphosphoinositides correlated well with their ototoxicity in the guinea pig (3).

In order to test further the hypothesis that the degree of expansion of polyphosphoinositide monolayers is correlated with ototoxicity, monolayer studies, and ototoxicity tests for two compounds unrelated to aminoglycosides, poly-L-lysine and L-lysine, are reported here. Poly-L-lysine represents a polycationic hydrophilic compound, capable of occupying an area-determining position in lipid films (4), and L-lysine represents the corresponding singly-charged hydrophilic monomer.

EXPERIMENTAL

Materials and Methods—Poly-L-lysine had a molecular weight of 3400 (polymerization grade, 16)[1]. Polyphosphoinositides were purified from ox brain by chromatography on immobilized neomycin (5).

A polytef beaker (8-cm diameter) held 100 ml of subphase containing 50 m*M* sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate, pH 7.0, 1 m*M* $CaCl_2$, and sufficient sodium chloride to adjust the ionic strength to 0.2. A stationary syringe, with the needle immersed in the subphase throughout the experiment, delivered the additions of lysine and polylysine. The solutions were stirred with a magnetic bar at slow speed without the film being disturbed. The polyphosphoinositide mixtures (phosphatidylinositol phosphate–phosphatidylinositol bisphosphate, 1:2 molar ratio) were spread as a solution in *n*-hexane–ethanol–chloroform (80:5:15, v/v/v). Approximately 0.2 μg of lipid/cm^2 was spread to obtain the desired surface pressure (14–15 dyne/cm) which was read with a balance[2] after the film had stabilized for 10 min. Polylysine or L-lysine was then injected into the subphase for final concentrations of 10^{-7}–10^{-4} *M*. After each addition, the subphase was mixed for 15 min before measurements were taken. Experiments were performed at 25±2° in duplicate, and surface tension readings always agreed within 0.1 dyne/cm.

Perilymphatic Perfusions—Perilymphatic perfusions were carried out in male albino guinea pigs (200–300 g). An animal with a positive Preyer hearing reflex was anesthetized with pentobarbital (20 mg/kg of body weight ip), atropine sulfate (0.05 mg/kg sc), and 0.5 ml/kg of body weight im of a solution containing 0.4 mg of fentanyl and 20.0 mg of droperidol/ml (6). The animal's body temperature was maintained at 37 ±1° with a heating pad and artificial respiration was provided through a tracheal cannula. Surgical and perfusion techniques were similar to that described previously (3).

The perilymphatic spaces of the cochlea were perfused at a rate of ~30 μl/min. Cochlear microphonic potentials were measured in response to a sound stimulus of white noise, 20–4000 Hz, delivered through an earphone. The sound intensity level was adjusted to give an initial microphonic potential of 200–400 μV. After the potential had stabilized for 30 min (control period), ototoxicity was determined as its loss after 30 min of a subsequent perfusion with added drug.

[1] Poly-L-lysine and L-lysine were obtained from Sigma Chemical Co., St. Louis, Mo.
[2] Wilhelmy balance.

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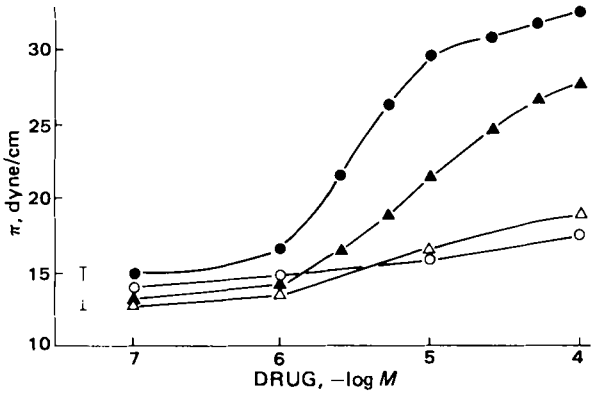

Figure 1—*Effects of cationic agents on surface pressure (π) of a Ca^{2+}/polyphosphoinositide film. Increasing amounts of drug were added to the subphase of a monomolecular film of polyphosphoinositides as described in* Experimental. *Each point represents multiple measurements agreeing within 0.1 dyne/cm. Bar on left indicates range of surface pressure before additions. Key: (○) lysine; (△) neamine; (●) poly-L-lysine; (▲) neomycin.*

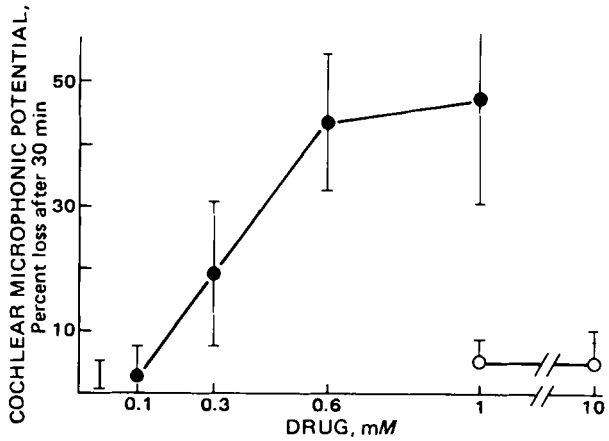

Figure 2—*Effects of cationic agents on cochlear microphonic potentials in the guinea pig. Numbers are means ±SD for five to eight animals at each condition. Bar on left indicates range of microphonic potentials without drug. Toxicity of poly-L-lysine at 0.3, 0.6, and 1 mM is significant at $p < 0.01$. Key: lysine (○); poly-L-lysine (●).*

Perilymphatic perfusions could usually be carried out for up to 2 hr without significant detrimental effect on cochlear microphonic potentials. Animals with an initial potential of <200 μV or with an unstable potential during the control period (loss of >10%) were excluded from the studies.

RESULTS AND DISCUSSION

It has previously been suggested that the specific interaction of a drug with polyphosphoinositides that leads to an expansion of the lipid monolayer in the presence of Ca^{2+} is an indicator of its ototoxicity. This interaction is apparently based on the configuration of the positive charges on the drug and not only on their number. For instance, kanamycin and neamine both have four primary amino groups, yet the first is more interactive with polyphosphoinositide films and also more ototoxic (3).

The cationic polymer, poly-L-lysine, can form complexes with acidic lipids (4) including polyphosphoinositides (7) and induce structural damage in biological membranes (8). Its action on the polyphosphoinositide film was strikingly different from that of the lysine monomer (Fig. 1). While low concentrations of the polymer dramatically increased the surface pressure ($\Delta\pi$ at 10^{-4} M, > 14 dyne/cm) the monomer showed only little effect (~2 dyne/cm). For comparison, the curves for the highly ototoxic compound, neomycin, and the nontoxic compound, neamine, (3) are shown.

In perilymphatic perfusions, effects on the cochlear microphonic potential were seen within 2–8 min after the introduction of polylysine into the perfusion fluid. The rate of loss was dependent on the drug concentrations up to 0.6 mM when an apparent maximum of toxicity was reached (Fig. 2). In contrast to polylysine, lysine did not affect the microphonic potential even at concentrations as high as 10 mM.

It should be noted that the molarity given for the polymer in these experiments refers to lysine equivalents for better comparison to the monomer, *i.e.*, 1 mM poly-L-lysine represents 1 mM lysine residues in the polymer corresponding to an actual concentration of 0.06 mM polymer. This consideration further accentuates the discrepancy between the action of the two compounds.

The correlation between polyphosphoinositide interaction and ototoxicity was demonstrated for a compound unrelated to aminoglycoside antibiotics. This finding further supports the hypothesis that the polyphosphoinositides play a crucial role in drug-induced ototoxicity.

REFERENCES

(1) N. D. Weiner and J. Schacht, in "Aminoglycoside Toxicity," S. A. Lerner, G. J. Matz, and J. E. Hawkins, Jr., Eds., Little, Brown, Boston, Mass., 1981, p. 113.

(2) S. Lodhi, N. D. Weiner, and J. Schacht, *Biochim. Biophys. Acta*, **557**, 1 (1979).

(3) S. Lodhi, N. D. Weiner, I. Mechigian, and J. Schacht, *Biochem. Pharmacol.*, **29**, 597 (1980).

(4) W. Hartmann and H. J. Galla, *Biochim. Biophys. Acta*, **509**, 474 (1978).

(5) J. Schacht, *J. Lipid Res.*, **19**, 1063 (1978).

(6) E. F. Evans, *Arch. Otolaryngol.*, **165**, 185 (1979).

(7) J. G. Fullington and H. S. Hendrickson, *J. Biol. Chem.*, **241**, 4098 (1966).

(8) M. W. Seiler, M. A. Venkatachalam, and R. S. Cotran, *Science*, **189**, 390 (1975).

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