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INTERACTIONS OF NEOMYCIN AND CALCIUM IN SYNAPTOSOMAL MEMBRANES AND POLYPHOSPHOINOSITIDE MONOLAYERS

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Summary

Neomycin and related aminoglycosidic antibiotics displace calcium from synaptosomes of guinea pig cerebral cortex and from preparations of phosphatidylinositol diphosphate. At low drug concentrations, inhibition of synaptosomal calcium binding is competitive ($K_i = 3 \cdot 10^{-5}$ M), at high concentrations it is non-competitive ($K_i = 4 \cdot 10^{-4}$ M). Monomolecular films of phosphatidylinositol diphosphate are contracted by low concentrations of neomycin in the subphase, and are expanded at high concentrations. This expansion persists even at the collapse pressure indicating a strong interaction between the drug and the lipid.

Neomycin, streptomycin and related aminoglycosidic antibiotics exert two types of toxic actions on mammalian tissues. Neuromuscular and ganglionic toxicity are acute effects antagonized by Ca^{2+}, while chronic application of these drugs leads to irreversible cochlear and to renal damage [1]. Concentration-dependent dual effects of aminoglycosides on some tissues have been reported. In the small intestine of the rat, 6 mM neomycin reversibly increases glucose absorption while 18 mM drug irreversibly inhibits it [2]. Streptomycin suppresses the microphonic output of the fish lateral line organ reversibly at concentrations around $2 \cdot 10^{-5}$ M and irreversibly at concentration tions above 10^{-2} M [3].

We have recently demonstrated an inhibition by neomycin of polyphosphoinositide metabolism in vivo in the inner ear [4] of chronically treated guinea pigs. In vitro, this antibiotic was shown to interfere with polyphosphoinositide metabolism and calcium binding in inner ear tissues [4] and with lipid labeling in synaptosomes from cerebral cortex [5]. We, therefore, investigated possible correlations between the neomycin action on calcium binding and on polyphosphoinositides with synaptosomal membranes and with monomolecular films of phosphatidylinositol diphosphate.

Neomycin which inhibits calcium binding at the vascular smooth muscle [6] is also a potent inhibitor of calcium binding to synaptosomal membranes (Table I). The structurally related antibiotics tobramycin, gentamicin and kanamycin show similar activity. Of the various drug fragments tested, the most effective were neamine and neobiosamine, which are moieties of neomycin. At low concentrations neomycin inhibits calcium binding competitively but at higher concentrations the inhibition is non-competitive (Fig. 1). This was confirmed in several independent experiments with different synaptosomal preparations. From these experiments constants were calculated [7] for calcium binding ($K_m = 6 \cdot 10^{-4}$ M), for the competitive inhibition by neomycin ($K_i = 3 \cdot 10^{-5}$ M) and the non-competitive inhibition ($K_i = 4 \cdot 10^{-4}$ M).

Both proteins and lipids are calcium binding sites in biological membranes but drug-calcium interactions generally involve calcium bound to phospholipids [8]. In view of the neomycin action on polyphosphoinositide metabolism and the known capability of phosphatidylinositol diphosphate to form calcium salts in vitro [9], we investigated neomycin interactions with this lipid. Calcium bound to a phosphatidylinositol diphosphate preparation is readily displaced by neomycin (Table II). In phosphatidylinositol diphosphate rich monolayers, the addition of 10^{-2} M Ca^{2+} to the subphase results in a condensation of the film (Fig. 2). Neomycin, at 10^{-3} M, produces a strong condensation at low film pressures. As the surface pressure is increased, the condensation effect diminishes and the curve becomes almost superimposable with the standard curve at about 30 dynes/cm. The presence of 10^{-2} M neomycin in the subphase causes a significant expansion of the film which is evident even at the collapse pressure. This indicates a strong interaction between the lipid and neomycin at this drug concentration.

TABLE I

INHIBITION OF CALCIUM BINDING BY AMINOGLYCOSIDIC ANTIBIOTICS AND FRAGMENTS

Lysed synaptosomes (0.5 mg protein) prepared from guinea pig cerebral cortex as described previously [10], were incubated for 15 min at 37° C in 100 mM sodium-HEPES, pH 7.4, and 0.1 μCi (0.1 mM) $^{45}CaCl_2$, volume 0.5 ml. Bound calcium was assayed by centrifugal sedimentation [4]. Numbers are means of at least duplicate determinations. In all experiments: neomycin = neomycin B.

	Calcium bound (% of control)	
	10^{-4} M drug	10^{-3} M
Neomycin	52	42
Tobramycin	56	48
Gentamicin	61	47
Kanamycin	72	42
Streptomycin	77	55
Dihydrostreptomycin	81	61
Bluensomycin	110	98
Neamine	69	54
Neobiosamine	84	56
Neosamine	82	63
Dihydrostreptobiosamine	89	60
Streptobiosamine	92	67
Methyl-neobiosamine	81	82
2-Deoxystreptamine	89	76
Streptidine	90	77
Streptamine	99	91
3-Deoxy-3-aminoglucose	107	100
N-Methyl-L-glucosamine	100	102

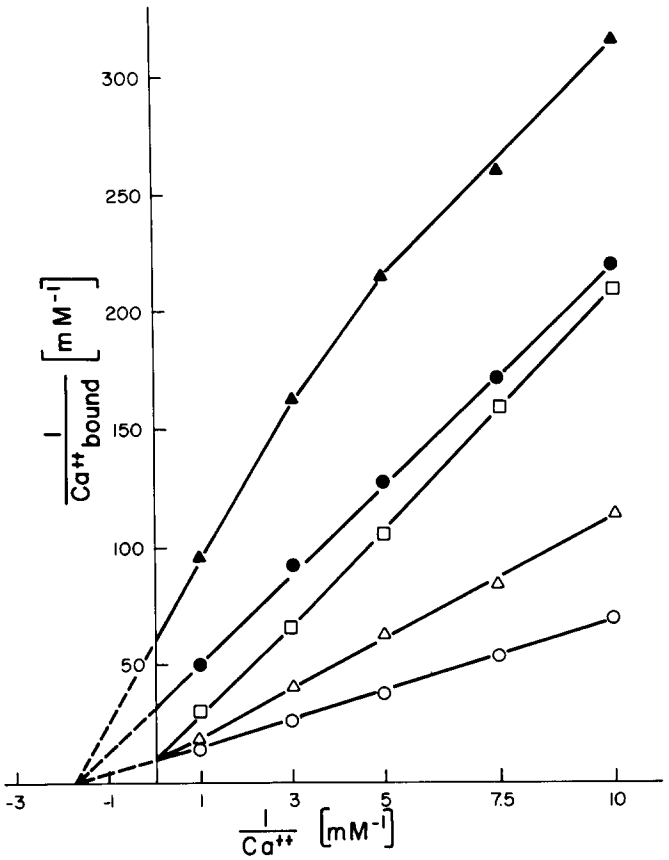

Fig. 1. Inhibition by neomycin of calcium binding to synaptosomal membranes. Lysed synaptosomes [10] (0.6 mg protein) were incubated for 30 min at 37°C in 70 mM sodium-HEPES, pH 7.4, with $CaCl_2$ (0.2 μCi $^{45}CaCl_2$) and neomycin as indicated; volume 0.5 ml. Neomycin was added 10 min prior to the addition of Ca^{2+}. Bound calcium was assayed on filter discs (Metricel GN-6). Each point is the mean of duplicate determinations; filter blanks (incubations without protein) were subtracted. ○—○, no neomycin; △—△, $3 \cdot 10^{-5}$ M; □—□, 10^{-4} M; ●—●, $3 \cdot 10^{-4}$ M; △—△, 10^{-3} M neomycin.

TABLE II

INHIBITION BY NEOMYCIN OF CALCIUM BINDING TO PHOSPHATIDYLINOSITOL DIPHOSPHATE

Approximately 0.1 μmol phosphatidylinositol diphosphate (prepared after Hendrickson and Ballou [11], purity by thin layer chromatography [4]: > 70% phosphatidylinositol diphosphate, 15—20% phosphatidylinositol phosphate) was dissolved in 0.2 ml chloroform/methanol (2 : 1) and vigorously mixed for 1 min with 0.2 ml sodium-HEPES, pH 7.4, containing $^{45}CaCl_2$ and neomycin. After centrifugation, calcium in the chloroform phase was measured. Numbers are means of four determinations with S.D. less than 5%.

	Calcium bound (nmol)	
	0.5 mM $CaCl_2$	1.0 mM
0 neomycin	66	78
0.2 mM neomycin	48	56
0.5 mM neomycin	33	38

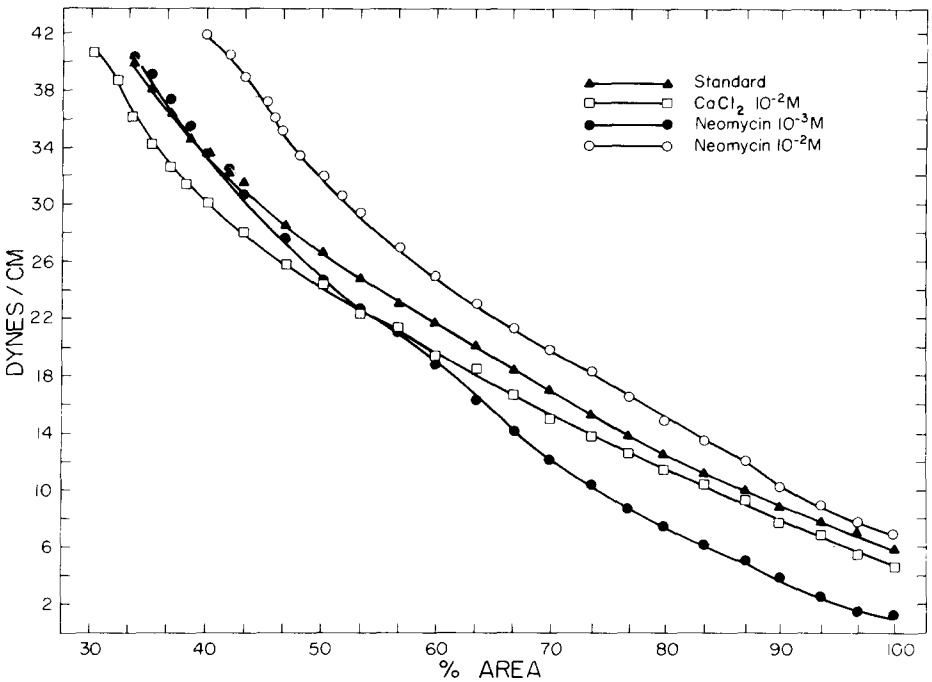

Fig. 2. Effects of calcium and neomycin on phosphatidylinositol diphosphate rich monomolecular films. The film balance assembly has been described previously [12]. The subphase consisted of 0.1 M Tris chloride, pH 7.4, and NaCl to give an ionic strength of 0.2. Approximately 40 μg phosphatidylinositol diphosphate (see legend to Table II) in hexanol/ethanol (9 : 1, by vol.) were spread over the subphase with an Agla micrometer syringe. Surface area of the trough was 15 cm × 30 cm (100% area on x-axis). 45 min were allowed for equilibration prior to compression of the film, and one minute was allowed between each area change. $CaCl_2$ or neomycin were added to the subphase in the concentrations indicated. Each point is the mean of duplicate determinations.

In summary, we have demonstrated an interaction of neomycin with calcium binding in synaptosomal membranes and with monomolecular films of phosphatidylinositol phosphate. It is intriguing that in both systems the drug exerts a dual effect, as its pharmacological actions also seem to show two distinct mechanisms. It remains to be established, however, how the in vitro effects on calcium binding and on polyphosphoinositides relate to each other and to the in vivo toxicity of neomycin.

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