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INTERACTION OF SPERMINE WITH POLYPHOSPHOINOSITIDES CONTAINING LIPOSOMES AND MYO-INOSITOL 1,4,5 TRIPHOSPHATE

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The interaction of spermine with liposomes containing 2% phosphatidylinositol, phosphatidylinositol 4 phosphate and phosphatidylinositol 4,5 biphosphate was inferred from the ability of these liposomes to interfere with spermine binding to the resin heparin-Sepharose. The inositol phospholipids tested showed different affinities for spermine: the order of binding strength appear to be phosphatidylinositol phosphatidylinositol 4 phosphate phosphatidylinositol 4,5 biphosphate. The ability of vesicles containing 2% polyphosphoinositides to interact with spermine is comparable to that of either single stranded RNAs or highly negatively charged liposomes. Myo-inositol 1,4,5 triphosphate has a much lower ability to bind spermine.

The polyphosphoinositides, phosphatidylinositol 4 phosphate and phosphatidylinositol 4,5 biphosphate are, likely, quantitatively minor constituents of all eukaryote cells (1,2). It now appears that these compounds play a central role in signal transmission for a wide variety of hormons, growth factors and neurotransmitters. Hydrolysis of membrane phosphoinositides represents a fundamental transducing mechanism that gives two products (diacylglycerol and inositol 1,4,5 triphosphate) both of which may function as second messenger to initiate the signal cascade (3).

Neomycin, streptomycin, gentamicin and related amino glycosidic antibiotics strongly interact with polyphosphoinositides (4,5) and this binding is exploited for the purification of these lipids (6). It has been suggested that this interaction alters specific renal transport functions and induces clinically significant degree of nephrotoxicity (7,8). Spermine, a normal constituent of all eukaryotic cells (9) appears to compete with gentamicin under many aspects:

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in vivo in the rat it inhibits renal uptake of the antibiotic (10); in vitro it inhibits gentamicin uptake by brush border membrane vesicles (11); it duplicates the action of gentamicin on lisosomal membrane stabilization (12). In vitro studies also showed that spermine and gentamicin interact with comparable intrinsic association constant with phosphatidylcholine liposomes containing either phosphatidylserine or phosphatidylinositol (13). These similarities suggest that spermine too may interact with phophatidylinositides. We have therefore studied the binding of this amine to liposomes containing phosphatidylinositol and polyphosphoinositides and in this report we present the results obtained.

Materials and methods

Egg yolk phosphatidylcholine (PC) and phosphatidylinositol (PI) were obtained from Lipid Products, Redhill, Surrey, U.K. Phosphatidylinositol 4 phosphate (PI-P), phosphatidylinositol 4,5 biphosphate (PI-P2), myo-inositol 1,4,5 triphosphate, spermine tetrahydrochloride, ATP and ADP were purchased from Sigma Chemical Co. St. Louis Mo, USA; [^{14}C] spermine was from the Radiochemical Centre, Amersham, Bucks, U.K.; heparin-Sepharose CL-6B was from Pharmacia Fine Chemical, Uppsala Sweden, polyribonucleotides from Miles Laboratories, Inc. Ind., USA.

Unilamellar phospholipid vesicles were prepared by the method of Huang (14) in 5 mM Mops buffer, pH 7. Phosphorus determination was by the method of Marinetti (15).

The binding of spermine to heparin-Sepharose in the absence or presence of phospholipid vesicles was measured by incubating heparin-Sepharose, that is known to interact with spermine (16) with labeled spermine in 3 ml of 5 mM Mops pH 7 for 30 min at room temperature (17). The incubation mixture was then layered on 3 ml of 1 M sucrose in the same buffer and centrifuged at 3000 g for 5 min. The heparin-Sepharose/ [^{14}C] spermine complex was collected at the bottom of the tube and the radioactivity content of the pellet was determined.

Results

The ability of phospholipid vesicles of different composition to complex spermine was inferred from their ability to remove spermine from the heparin-Sepharose/spermine complex. Liposomes composed of 49:1 molar ratio of PC and either phosphatidylinositol or polyphosphoinositides were assayed.

Addition of increasing concentration of PI-containing vesicles slightly interferes with spermine binding to heparin-Sepharose (Fig.1). On the contrary substitution of PI with either PI-P or PI-P2 greatly affects the formation of heparin-Sepharose/spermine complex (Fig.1).

Zwitterionic phospholipid vesicles made of 100% PC were unaffective in removing spermine also when assayed at total phospholipid concentrations much higher than those utilized in these experiments (data not shown).

Spermine binding ability of vesicles containing 2% polyphosphoinositides is equal or higher than that of liposomes containing 20 and 50% phosphatidylinositol (Fig.2).

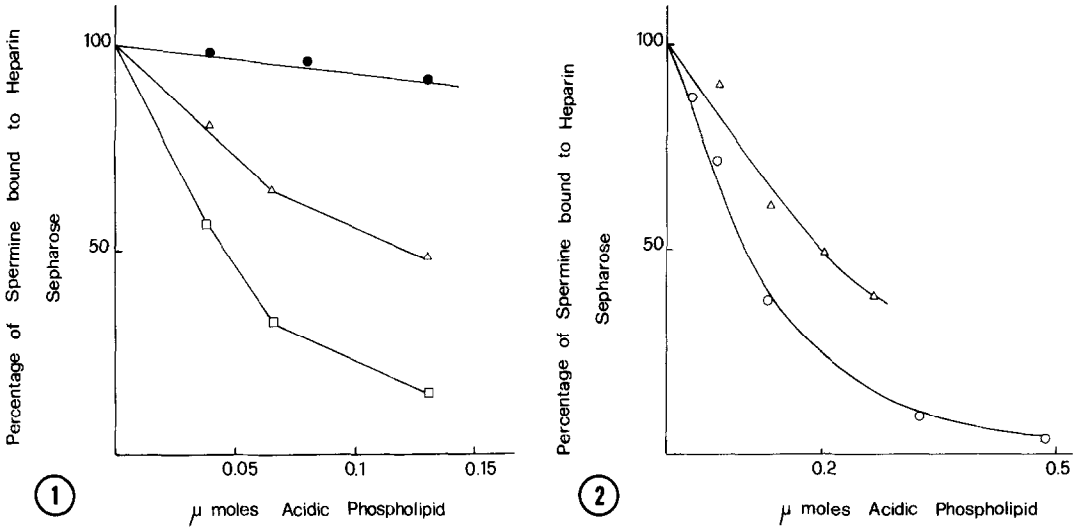

Fig.1: Inhibition of spermine binding to heparin-Sepharose by increasing concentration of vesicles composed of phosphatidylcholine mixed with phosphoinositides.
Labeled spermine (25 nmoles, 1 x 10^5 dpm) was incubated with heparin-Sepharose (10 λ swollen resin) and increasing concentration of ● PC/PI (49:1), △ PC/PI-P (49:1), □ PC/PI-P2 (49:1) vesicles. In the absence of liposomes 80% of spermine was bound to the resin.

Fig.2: Inhibition of spermine binding to heparin-Sepharose by increasing concentration of vesicles composed of different ratio of phosphatidylcholine and phosphatidylinositol.
The binding of spermine to heparin-Sepharose in the presence of increasing concentration of ○ PC/PI (1:1) and △ PC/PI (4:1) vesicles was measured as described in Figure 1.

TABLE I

Inhibition of spermine binding to heparin-Sepharose by polyanions

	Spermine bound (n moles)	Inhibition (percentage)
Control	20	—
+ myo-inositol 1,4,5 triphosphate 0.12 /umoles	17.6	12
+ " " 0.24 /umoles	16	20
+ ATP 0.25 /umoles	18.4	8
+ " 0.50 /umoles	17.2	14
+ ADP 0.25 /umoles	19.6	2
+ " 0.5 /umoles	19.2	4
+ Poly (G) 0.125/umoles	4	80
+ " 0.25 /umoles	1.8	91
+ Poly (C) 0.125/umoles	10	50
+ " 0.25 /umoles	5.8	71
+ Poly (U) 0.25 /umoles	3.6	82
+ Poly (I) 0.25 /umoles	3.6	82

The binding of spermine to heparin-Sepharose was measured as described in Figure 1. The concentration of single stranded RNAs is expressed as /umoles of nucleotide.

Other polyanions were tested to compare their effectiveness in removing spermine from the heparin-Sepharose/spermine complex (Table I). Whereas single stranded RNAs compete efficiently with heparin to bind spermine, myo-inositol 1,4,5 triphosphate, ADP and ATP poorly interact with the polycation.

Discussion

During the study of aminoglycoside antibiotic side effect it was frequently utilized the natural polycation spermine. This amine showed similarities with these drugs in many experimental systems (12,13,18). Spermine itself has a

marked renal toxicity (19) and the damage to the auditory system frequently observed in uremic patients (20) might be related to the strikingly elevated levels of this and other polyamines (21) in the blood of these patients. Spermine, spermidine and their diamine precursor putrescine are naturally occurring cations. Their specific function is still obscure, but their ubiquitous distribution and the complexity of the mechanisms that regulate their intracellular concentrations suggest for these compounds important roles in normal cellular growth and differentiation (9). It is generally believed that, because of their polycationic nature, their physiological functions might be related to same intercation with cellular polyanions.

Spermine binding to nucleic acid (9), natural and model membranes (17,22), polysaccarides (16), acid domains of proteins (23) has been studied. The data presented indicate that spermine binds polyphosphoinositides. The ability of these phospholipids to interact with this amine is comparable to that of either single stranded RNAs or highly negatively charged liposomes.

The binding of neomycin to polyphosphoinositides has marked effect on their metabolism (24,25) and on calcium binding to membranes (4). A similar effect exerted by spermine might involve this amine in the polyphosphoinositide mediated transducing mechanism.

It was shown that polyamines regulate calcium fluxes in a rapid plasma membrane response by increasing calcium influx and mobilizing intracellular calcium via a cation-exchange reaction (26,27). The observation (25) that spermine produces changes in lipid labelling like those produced by neomycin may indeed give a rational to that report. The lack of effect of spermine on myo-inositol 1,4,5 triphosphate mediated release of calcium from non mitochondrial intracellular store (28), by contrast suggests that this amine

does not affect the mobilization of calcium from this compartment. We present here data showing that spermine has a very low affinity for myo-inositol 1,4,5 triphosphate and this may explain why spermine does not affect the action of this second messanger.

Acknowledgements

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