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POLYAMINES

BIOLOGICAL MODULATORS OF MEMBRANE FUSION

KEELUNG HONG [a], FRANCIS SCHUBER [a,*] and DEMETRIOS PAPAHADJOPOULOS [a,b]

[a] *Cancer Research Institute and* [b] *Department of Pharmacology, University of California, San Francisco, CA 94143 (U.S.A.)*

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The effect of polyamines on the kinetics of Ca^{2+}- and Mg^{2+}-mediated membrane fusion was studied by following the intermixing of the contents of vesicles composed of phosphatidate/phosphatidylserine/phosphatidylethanolamine/cholesterol (1:2:3:2). Addition of polyamines at specific concentration ranging from 40 to 400 μM promoted aggregation of the vesicles. In addition, low levels of spermine (50–100 μM) enhanced both Ca^{2+}- and Mg^{2+}-mediated fusion. The initial fusion rate of this membrane system increased more than 200-fold when fusion was initiated by Ca^{2+} after 5 min pre-incubation of vesicles with 50 μM spermine. These results indicate that in addition to their other known effects on cellular metabolism, polyamines may be involved in modulating intracellular membrane fusion.

Polyamines (spermine, spermidine and putrescine) are present in most living cells and have been implicated as biochemical regulators in several cellular processes [1–4]. Increased polyamine levels are found in rapidly growing and neoplastic cells and in bodily fluids of animals with tumors [5,6]. In several microorganisms, polyamines are required for growth [3,7]. Available evidence indicates that polyamines, as polycations at physiological pH, bind to major cellular anionic sites including DNA, RNA and cell membranes [8,9]. The few effects of polyamines on membrane properties which have been noted are stabilization of membrane against lysis [10,11] and inhibition of some enzymes associated with membranes [12,13]. However, the interaction of polyamines with biomembranes and the possible involvement of polyamines in membrane functions remains largely unexplored. The present research aims to delineate the role of polyamines in membrane functions, specifically membrane fusion, which is a key event in many biological processes.

Liposomes have been studied extensively as models for biological membranes to elucidate the physical properties of membranes and the mechanism of membrane fusion [14–18]. In this study, we have used a multicomponent membrane system of large unilamellar vesicles composed of phosphatidate/phosphatidylserine/phosphatidylethanolamine/ cholesterol (PA/PS/PE/cholesterol) (1 : 2 : 3 : 2). The effects of polyamines were studied by following the aggregation and fusion of these vesicles.

Phosphatidylserine from bovine brain, and both transesterified phosphatidylethanolamine and phosphatidate from egg phosphatidylcholine were purchased from Avanti Polar Lipids (Birmingham, AL). Hydrochloride of spermidine and spermine

Abbreviation: Tes, 2-([2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino)ethanesulfonic acid.

* Present address: Laboratoire de Biochimie Végétale, Institut de Botanique, Université Louis Pasteur 6700, Strasbourg, France.

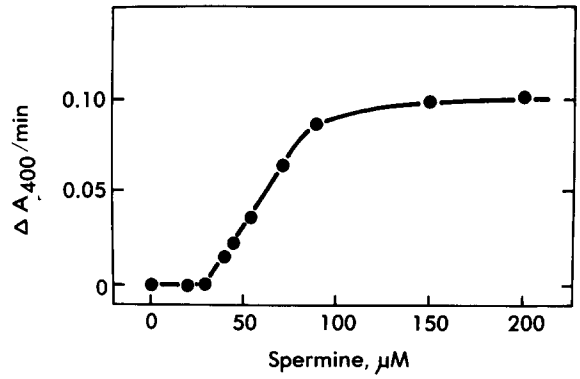

Fig. 1. Aggregation of large unilamellar PA/PS/PE/cholesterol (1:2:3:2) vesicles induced by spermine. The initial rate of absorbance change of vesicles at 400 nm (ΔA_{400}/min) was measured at various concentrations of spermine. The vesicles (74 μM phospholipids) were in 100 mM NaCl/5 mM Tes (pH 7.4). t = 25°C.

were obtained from Sigma (St. Louis, MO). Cholesterol from Sigma was recrystalized twice before use. Large unilamellar vesicles encapsulating the desired aqueous content were prepared following published procedures [19,20]. Vesicle aggregation resulting in a close apposition of vesicle surfaces, and fusion of the membranes involving local bilayer destabilization and resulting in the mixing of the encapsulated vesicle contents were monitored by methods previously described [20]. The fusion assay is based on the interaction of Tb^{3+} encapsulated in one population of vesicles with dipicolinic acid in another. When fusion occurs, the vesicles' aqueous contents are mixed and a highly fluorescent Tb-dipicolinate complex is formed inside the fusing vesicles. The increase of fluorescence is measured as fusion rate (see Ref. 20 for details).

Fig. 1 shows a typical aggregation of vesicles monitored by the increase in turbidity following addition of spermine. The concentrations of polyamines required to reach half the maximum turbidity change (0.06 mM spermine and 0.8 mM spermidine) were much lower than that of divalent cation (2 mM and 3 mM for Ca^{2+} and Mg^{2+}, respectively). Addition of polyamines up to 1 mM did not have any effect on agrregration of vesicles containing no acidic phospholipid, e.g., phosphatidylcholine vesicles.

These results indicate that polyamines interact with the negative charges on the vesicles' surface. However, the effectiveness of polyamines in reducing the repulsive forces between the negatively charged vesicles cannot be interpreted simply as charge neutralization. Polyamines could interact with anionic membrane surfaces in three ways: (1) loose, long-range interactions through the diffuse ion layers; (2) tight, localized binding; or (3) tight but delocalized binding in which the polyamines bind to the membrane surface, but are still free to diffuse along the membrane surface. The experimental analysis to determine the possible modes of polyamine-membrane interaction is in progress.

The fact that polyamines promote vesicle aggregation indicates that polyamines reduce the repulsive force among vesicles allowing them to come to close apposition, without resulting either in fusion between these vesicles, or in changes in permeabil-

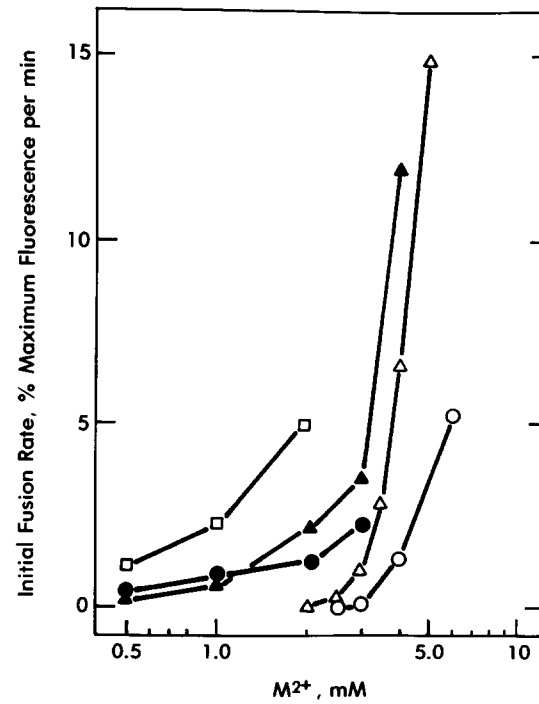

Fig. 2. Initial rate of fusion of PA/PS/PE/cholesterol (1:2:3:2) vesicles at various divalent cation concentrations. Divalent cation and spermine was added to the vesicle suspension (50 μM phospholipid, 25 μM Tb-containing vesicles, 25 μM dipicolinic acid-containing vesicles) at zero time with constant stirring. Experiments were carried out at 25°C. ○, Mg^{2+} only; ●, Mg^{2+}/50 μM spermine; △, Ca^{2+} only; ▲, Ca^{2+}/50 μM spermine; □, Ca^{2+}/1 mM Mg^{2+}/50 μM spermine.

ity of vesicles, as was indicated by lack of mixing or release of their contents. However, we have found that in the presence of polyamines, significantly less Ca^{2+} or Mg^{2+} is required for fusion to occur. As can be seen in Fig. 2, inclusion of 50 μM spermine in divalent cation solution, reduced the Ca^{2+} concentration needed to obtain a fusion rate of 2% maximum fluorescence per min from 3.2 mM to 1.8 mM. With 1 mM Mg^{2+} also present in the medium, spermine reduced the Ca^{2+} threshold to less than 1 mM (see Fig. 2).

When vesicles were briefly incubated with polyamines before the addition of divalent cations, the initial rate of fusion increased drastically, as shown in Fig. 3. The fusion rate increased more than 200-fold when subthreshold Ca^{2+} (1 mM Ca^{2+} in this case) was added to vesicles following a 5 min preincubation with 50 μM spermine (see curve d vs. curve a in Fig. 3). For comparison, the effect of spermidine and spermine on the fusion rate of vesicles is tabulated in Table I. This table reveals three significant findings. First, the levels of polyamines (0.02 mM spermine, 0.5 mM spermidine) which will reduce the Ca^{2+} threshold required for vesicle fusion are in the physiological range found in some tissues. Second, the enhancement of the fusion rate by polyamines is time-dependent. This indicates that the mode of interaction of polyamines with negatively-charged phospholipid vesicles is different from that of Ca^{2+}. Third, small fluctuations in polyamine concentrations can have profound effects on the rate of membrane fusion.

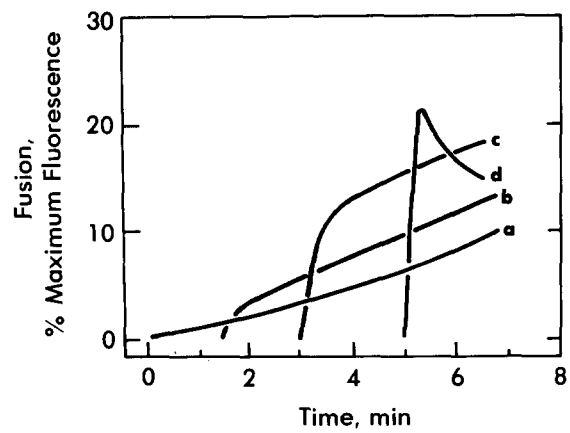

Fig. 3. Time-course of PA/PS/PE/cholesterol (1:2:3:2) vesicle fusion. Curve a: 50 μM spermine was added together with 1 mM Ca^{2+} to vesicle suspension (50 μM phospholipid) at zero time. Curves b, c, and d: 1 mM Ca^{2+} was added to vesicles, 1.5 min, 3 min, and 5 min, respectively, after the addition of 50 μM spermine.

The dramatic increase of the fusion rate after brief incubation of vesicles with polyamines has an important biological implication: assuming that the intracellular membrane surfaces (i.e., inner surface of plasma membrane and outer surface of intracellular vesicles) will interact with polyamines, a small fluctuation of polyamine levels could have profound effect on fusogenic activity inside cells. Exocytotic events associated with secretion are dependent on Ca^{2+}, but it is not clear how Ca^{2+} stimulates the fusion of the secretory

TABLE I

THE EFFECT OF SPERMIDINE AND SPERMINE ON Ca^{2+}-INDUCED FUSION OF PA/PS/PE/CHOLESTEROL (1:2:3:2) VESICLES

Calcium was added to all mixtures at 1.0 mM. Lipid concentration was at 50 μM. Spermidine and spermine were added either concomitantly with or 5 min before the calcium, at indicated concentrations (mM). k is the rate of fusion expressed as percent of maximum fluorescence per minute. k_0 is obtained with concomitant addition of calcium and k_5 obtained with polyamine added 5 min before calcium. Each k value represents the mean of two experiments. There were differences of less than 10% except for the very fast fusion in which the lower value is listed.

Spermidine (mM)	k_0	k_5	Spermine (mM)	k_0	k_5
0.5	0.7	0.6	0.02	0.1	0.2
0.6	1.2	2.0	0.03	0.1	5.2
0.7	2.0	> 200	0.04	0.4	82
			0.05	0.9	> 300

vesicle membrane with the plasma membrane. The threshold Ca^{2+} concentrations which induce fusion of vesicles made of acidic phospholipids are much higher than intracellular Ca^{2+} levels necessary for exocytosis [21,22]. Our observation that polyamines reduce the Ca^{2+} threshold and increase the rate of vesicle fusion suggests that in addition to synexin, a Ca^{2+}-binding protein found in several secretory tissues [23], polyamines could modulate Ca^{2+}-mediated membrane fusion.

The link between polyamines and membrane fusion may explain some earlier observations related to polyamines in cultured cells. Normally, intracellular membrane flow, i.e., endocytic vesicle-lysosome fusion and Golgi vesicle-plasma membrane fusion, is required for cell growth. Addition of inhibitors of ornithine decarboxylase, an enzyme necessary for polyamine biosynthesis, causes an accumulation of intracellular vacuoles and a decline of growth rate, both of which are reversed upon the addition of exogenous putrescine to the culture medium [24]. By controlling membrane fusion rates, polyamines may regulate intracellular membrane flow, i.e., that limitation of polyamine synthesis may cause a buildup of intracellular membrane due to the lack of fusogenic activity necessary for normal membrane flow.

In summary, we have shown that polyamines, at concentrations comparable to those found intracellularly, facilitate the fusion of phospholipid membranes. This facilitation is partly due to an effective enhancement of vesicle aggregation by polyamines and depends also on the presence of calcium and magnesium. From our results on model membranes, we suggest that in addition to their involvements in cellular metabolism, polyamines may modulate intracellular membrane fusion events, thus affecting cell growth and other phenomena dependent on fusion.

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