Vol. 145, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1987 Pages 631-636

PHOSPHATIDYLSERINE VESICLES INCREASE RAT BRAIN SYNAPTOSOMAL ADENYLATE CYCLASE ACTIVITY

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Received April 13, 1987

Phosphatidylserine vesicles incubated in hypotonic conditions with rat brain synaptosomes increased basal adenylate cyclase activity but did not modify the response of the enzyme to norepinephrine. Moreover, phosphatidylserine antagonized the stimulation of adenylate cyclase activity by NaF.

We suggest that in present experimental conditions the effect of phosphatidylserine vesicles is at the level of the G_s regulatory protein of adenylate cyclase.

Among the phospholipid vesicles extensively studied as carriers for biologically active molecules and drugs, phosphatidylserine (PS) liposomes possess notable pharmacological properties. The injection of PS vesicles into rats is followed by modifications in cerebral glucose metabolism (1), by increases in cAMP content of brain (2) and in acetylcholine output from cerebral cortex (3) and in norepinephrine turnover in hypothalamus (4). Moreover, the administration of PS to humans causes alterations in pituitary functions (5).

In vitro, PS liposomes increase the antigen-mediated histamine release from rat peritoneal mast-cells (6), increase Na^+/K^+ ATPase activity in rat and dog brain synaptosomes (7,8) and in BHK21 cells (9), and acetylcholinesterase activity in dog brain synaptosomal plasma membranes (8). These effects have been correlated with the incorporation of PS into the membrane (8,10), with consequent modifications of its lipid composition or microenvironments of membrane enzymes and creation of domains of altered membrane permeability.

In this paper we report that PS vesicles increase rat brain synaptosomal adenylate cyclase, possibly interacting with G_s protein.

0006-291X/87 $1.50

Materials and Methods

ATP, GTP, norepinephrine, bovine serum albumin were from Sigma. Theophylline was from Farmitalia-Carlo Erba, Milano. [^{3}H] cAMP was from Radiochemical Center, Amersham. All other reagents were of analytical grade. Phosphatidylserine (PS) was a kind gift from Fidia S.p.A. (Abano, Italy).

PS vesicles were prepared as previously described (7).

Synaptosomes were isolated from brain of male albino Wistar rats (body wt 180-250 g) according to Whittaker and Barker (11). After preparation, synaptosomes were precipitated at 150000 x g and suspended in 0.32 M sucrose, to a protein concentration of about 3 mg/ml.

Adenylate cyclase activity of synaptosomes was assayed in hypotonic conditions (12). About 30 µg synaptosomal proteins were incubated for 10 min at 37°C in the absence and presence of PS vesicles in a medium containing 40 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 100 µg bovine serum albumin, 6 mM theophylline, 0.3 mM ATP and, where present, 4 mM NaF or 1 µM norepinephrine (final volume 0.1 ml). Ca^{2+}-calmodulin stimulated adenylate cyclase activity was assayed in the presence of 10 µM $CaCl_2$ and 1.5 µg calmodulin purified according to Ho et al. (13). The incubation reaction was stopped by boiling the mixture for 3 min. The amount of cAMP formed was measured in duplicate on aliquots of supernatant by the competitive binding reaction method of Gilman (14), by using a cAMP--binding protein purified from bovine skeletal muscle by the procedure of Miyamoto et al. (15), in the presence of the protein-kinase inhibitor purified according to Gilman et al. (16) from bovine skeletal muscle. ATP final concentrations present in the binding reaction mixture did not interfere with the binding of cAMP to the protein.

Control experiments ruled out any effect of PS vesicles on the cAMP-binding protein as cAMP binding was not modified by the direct addition of 1.28 µmoles PS vesicles/mg cAMP-binding protein.

Protein content was assayed by the method of Lowry et al. (17).

Results

As in other tissues, cerebral adenylate cyclase is comprised of two components, one of which requires calcium and calmodulin for full activity (18). Synaptosomes incubated in hypotonic medium had a basal adenylate cyclase activity of 41.4±4.9 pmoles/mg protein/min, that was increased to 98.5±14.7 (+138%) by the addition of 10 µM $CaCl_2$ and 1.5 µg calmodulin. These values were very close to the data reported by Brostrom et al. (18) for both brain homogenate and brain microsomal fraction. NaF increased basal adenylate cyclase activity of synaptosomal preparation in a concentration-dependent way. Maximum stimulating activity (+245%) was observed with 4 mM NaF. Similar values of stimulation by the same concentrations of NaF have been reported for adenylate cyclase activity of cerebral tissue (12).

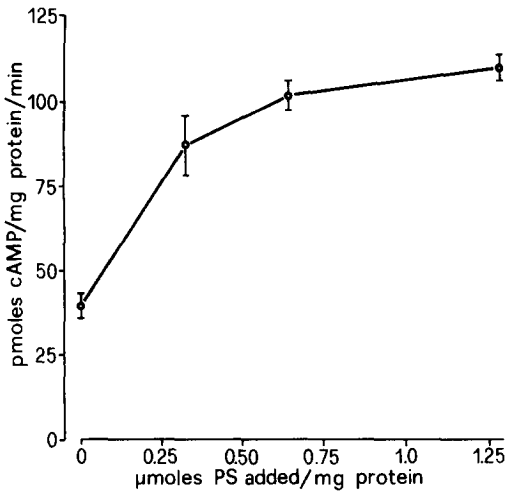

Figure 1. Effect of increasing concentrations of PS vesicles on basal adenylate cyclase activity of rat brain synaptosomes. Incubation conditions as described in the "Materials and Methods". cAMP was assayed by the competitive binding protein method of Gilman (14). For each experiment, a cAMP calibration curve was done with 1.25 pmoles [^{3}H]cAMP plus 0.5-2.5 pmoles unlabelled cAMP. The results are mean ± S.E. of 6 experiments in duplicate.

1 μM norepinephrine increased basal activity by 58%. The addition of GTP further increased the activation by norepinephrine to 81%. Stimulations of cerebral adenylate cyclase by norepinephrine ranging from 20 to 100% have been reported (19,20).

When synaptosomes were exposed to increasing concentrations of PS vesicles, a progressive increase in basal adenylate cyclase activity was observed (Fig. 1). The effect was almost maximum at 0.64 μmoles PS/mg protein (+160%) and only slightly increased at 1.28 μmoles PS/mg protein (+182%).

When PS liposomes were added to synaptosomes in the presence of norepinephrine or NaF, different effects were observed (Table 1). The addition of 0.64 μmoles PS/mg protein did not modify the stimulation of adenylate cyclase by norepinephrine, in that the increase of basal adenylate cyclase activity in the presence of PS liposomes plus norepinephrine represented the sum total of the effects determined by the two compounds added separately. In contrast, when PS vesicles were added in the presence of NaF, an antagonistic effect was observed between the two substances. In fact, the stimulating effect determined by the contemporary presence of PS and NaF was less than expected on the basis of their individual actions.

Table 1. Effect of PS vesicles on adenylate cyclase activity in the presence of norepinephrine or NaF

	pmoles cAMP/mg/min	% stimulation
-	39.1 ± 4.4	
PS 0.64 μmoles/mg	101.8 ± 3.9	160
1 μM norepinephrine	65.4 ± 5.7	67
PS 0.64 μmoles/mg + 1 μM norepinephrine	131.0 ± 7.5	235
4 mM NaF	145.8 ±18.7	273
PS 0.64 μmoles/mg + 4 mM NaF	154.8 ± 9.8	296

Incubation conditions as described in Materials and Methods. The results are mean ± S.E. of 5 experiments in duplicate.

Discussion

This paper reports that the addition of PS vesicles to synaptosomes in a hypotonic incubation medium increases basal adenylate cyclase activity in a concentration-dependent way, does not modify the adenylate cyclase stimulation by norepinephrine and antagonizes that by NaF. These results suggest that, in these experimental conditions, PS liposomes and norepinephrine increase adenylate cyclase activity by interacting with two different regulatory sites. A lack of PS vesicles effect on the adenylate cyclase stimulation by norepinephrine was unexpected because acidic phospholipids play an important role in adenylate cyclase activity and in its response to hormones and drugs. Partial delipidation of liver plasma membranes by phospholipase C (21) or by mild solvent extraction (22) reduces basal adenylate cyclase activity and abolishes its sensitivity to epinephrine, glucagon and NaF. PS vesicles almost completely restore the sensitivity of adenylate cyclase to epinephrine and only partially restore the basal activity and its stimulation by glucagon and NaF (22). Adenylate cyclase activity is regulated by its lipid environment even when inserted in a complete membrane structure (23-25). A decreased bilayer fluidity decreases adenylate cyclase activity and its response to agonists by impairing the coupling of different proteins forming adenylate cyclase system. Rat liver plasma membranes enriched in synthetic saturated phosphatidylcholine lose their sensitivity to glucagon and NaF (23) and rat kidney fibroblasts treated with cholesterol-containing phosphatidylcholine liposomes exhibit decreased adenylate cyclase activity (24).

Similarly, a significant decrease in adenylate cyclase activity, related to the cholesterol incorporation, has been observed in dog brain synaptosomal membranes (25).

As far as we know, no results have been presented regarding the effect of an increased bilayer fluidity on adenylate cyclase activity. But an increase in membrane fluidity as a consequence of the fusion of PS with the membrane (26) has been invocated to explain the increase of Na^+/K^+ ATPase and acetylcholinesterase activities after the interaction of PS with synaptosomes (7,8). We have previously reported that PS fusion with the membranes occurs when hypertonic conditions are employed (10). Because in present experiments an hypotonic incubation medium was used, it is likely that fusion does not occur. Therefore, the lack of PS effect on nor epinephrine response can be explained by conditions of non-fusion of PS vesicles with synaptosomal membrane.

How PS vesicles increase basal adenylate cyclase activity can be inferred by the evidence that the stimulation of the enzyme activity by PS liposomes and NaF added together is lower than the sum of the actions of the two substances added separately. This result demonstrates a kind of antagonism between PS liposomes and NaF and can indicate that the two agents stimulate adenylate cyclase activity possibly by interacting with the same site. Since it is acknowledged that NaF stimulates adenylate cyclase by interacting with regulatory G_s protein (27), this site of action for PS is suggested.

Therefore PS vesicles are able to modify adenylate cyclase even if they are only bound to the cell membrane and not inserted in its phospholipid matrix. If the serine head of the phospholipid or the entire phospholipid molecule is responsible for this effect has to be ascertained.

In summary, in a hypotonic incubation medium PS vesicles increase basal adenylate cyclase activity of rat brain synaptosomes possibly through an interaction with G_s regulatory protein. Moreover, they do not modify the adenylate cyclase stimulation by norepinephrine probably because they do not fuse with the synaptosomal membrane.

Modulation of synaptosomal AC activity may account for the pharmacological effects of PS vesicles.

Acknowledgement : This research was supported by a grant from MPI.

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