Stimulation of Brain Synaptosome – Associated Adenylate Cyclase by Acidic Phospholipids

Stylianos Tsakiris

Department of Experimental Physiology, University of Athens, Medical School, P.O. Box 14185, GR-115 10 Athens, Greece

Z. Naturforsch. **39 c,** 1196–1198 (1984);
received August 6, 1984

Adenylate Cyclase, Vesicles, Membrane Fluidity, Neurotransmitters

Phosphatidylserine (PS), phosphatidylinositol (PIN) or phosphatidylglycerol (PGL) incubated with synaptosomal plasma membranes (SPM) of dog brain, stimulated adenylate cyclase. The enzyme activity showed a dramatic increase at around 1.6 μmol PS/mg protein, while use of higher concentrations led to inhibition of the activity with respect to the maximal percentage of stimulation. Moreover, PS stimulated the dopamine-sensitive adenylate cyclase. Solubilization of SPM by the detergent Lubrol-PX did not affect the enzyme activation induced by dopamine. The solubilization, also, showed that the enzyme activity does not change at any PS, PIN or PGL concentration used. These results indicate that acidic phospholipids do not directly act on adenylate cyclase, but indirectly, affecting the membrane fluidity probably. Such modifications of interactions through lipid-protein(s) of adenylate cyclase may have implications to physiological responses to hormones or/and neurotransmitters in the central nervous system.

A. Introduction

The most negatively charged phospholipids PS and PIN, although they are in small amounts in SPM [1], are involved in important physiological processes in the central nervous system. PIN lipids are well known to be involved in synaptic transmission and their catabolism plays an important and perhaps primary central role in the biochemical events associated with physiological activity [2, 3]. Intravenous injection of a sonicated dispersion of brain phospholipids results in a significant increase of both the dopamine-sensitive adenylate cyclase activity, and the cyclic AMP content of mouse brain [4]. Under these experimental conditions, PS liposomes stimulate the release of acetylcholine from rat cerebral cortex [5] and the release of histamine from mast-cells [6]. Moreover, PS vesicles (liposomes) interact with biological membranes causing changes of the membrane physicochemical properties [7]. Such alterations of the state of the bilayer evoke several membrane linked events such as, modifications of the activities of membrane-bound enzymes, *e.g.* Na^+,K^+-ATPase, acetylcholinesterase and adenylate cyclase [8–10].

This study was carried out in order to investigate: a) whether PS only or other acidic phospholipids as well, can induce changes in the activity of adenylate cyclase, b) which is the mechanism of action of these compounds.

B. Materials and Methods

Synaptosomal plasma membranes (SPM) from dog brain were prepared and qualitatively assessed as previously described [11]. Phosphatidylserine (PS), Phosphatidylinositol (PIN) or Phosphatidylglycerol (PGL) liposomes were prepared essentially as previously described [12]. Incubations of SPM with different concentrations of phospholipids-liposomes were carried out for 3 h at 25 °C and then the bound and free phospholipids were separated [9]. In the samples of bound phospholipids to SPM, the protein content was determined by the Lowry method as described by Miller [13] and then the enzyme activity was determined. The chloroform extracts of membrane-bound and free phospholipids were chromatographed [1]. They moved identically with those phospholipids alone, suggesting no significant metabolic conversion under the present incubation conditions.

Adenylate cyclase was assayed by incubating for 15 min at 37 °C 40 μg of SPM protein in medium containing, to a total volume of 1 ml, 50 mM Tris-HCl, pH 7.4, 0.5 mM ATP, 5 mM $MgSO_4$, 1 mM EDTA, 5 mM theophylline, 5.6 mM phosphoenolpyruvate and 2.3 units (10 μg/ml) of pyruvate kinase. The incubation reaction was stopped by boiling the mixtures for 3 min and the amount of cyclic AMP produced was determined by using techniques described previously [11, 14].

Solubilization of the membranes was performed using 0.5% w/v Lubrol-PX at 4 °C for 4 h under magnetic stirring, in a medium 0.24 M Tris-HCl, pH 7.4 and 8.4% w/v sucrose. After the solubilization, the sample was centrifuged at 150 000 × *g* for 1 h and the supernate contained solubilized adenylate cyclase [15]. In the assay mixture of the enzyme,

Reprints should be requested from S. Tsakiris.
0341-0382/84/1100-1196 $ 01.30/0

Table I. Effect of acidic phospholipids on adenylate cyclase activity.

Preparation	Membrane-bound	Solubilized
	pmol cAMP / mg protein/min	
Non-treated SPM (Control)	110.2 ± 4.4	60.0 ± 2.4
+0.1 mM DA	200.3 ± 10.4	62.4 ± 4.0
Phosphatidylserine	330.5 ± 23.1	70.9 ± 3.5
+0.1 mM DA	720.0 ± 51.8	68.1 ± 4.5
Phosphatidylinositol	270.6 ± 16.2	65.7 ± 4.1
Phosphatidylglycerol	300.8 ± 15.3	75.3 ± 4.3

Values are means ± S.D. of three independent experiments. The average value of each experiment came from duplicate determinations. Phospholipids-liposomes were at concentration 1.5 µmol/mg protein in the incubation medium. When dopamine (DA) was required, it was added 10 min before enzyme incubation in the assay medium (see Materials and Methods).

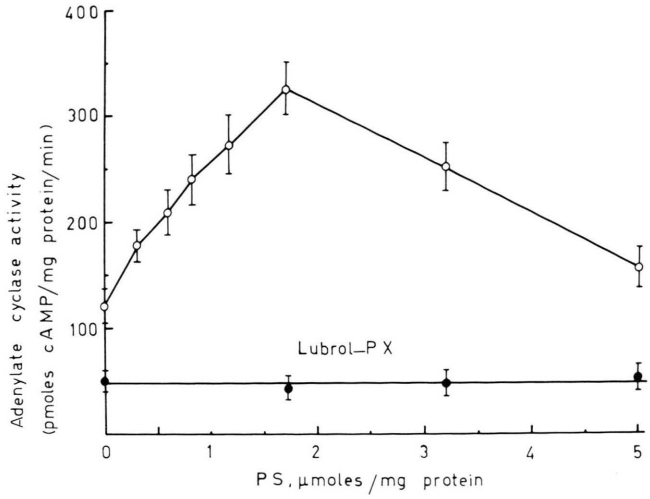

Fig. 1. Effect of different concentrations of phosphatidylserine (PS) liposomes on the activities of SPM-bound adenylate cyclase ○——○ and the solubilized one ●——●. Points and bars represent means ± S.D. respectively from three different experiments. The average value of each experiment came from duplicate determinations.

the detergent was in a final concentration less than 0.008%, which was not able to influence the measurement of enzyme activity.

C. Results and Discussion

The data in Table I show the influence of acidic phospholipids PS, PIN or PGL on the activity of the adenylate cyclase. At concentration 1.5 µmol/mg protein, these phospholipids produced an important stimulation of the entire activity, which disappeared in the experiments with solubilized enzyme. The solubilization successed with the nonionic detergent Lubrol-PX. Moreover, it was observed that PS liposomes incubated with SPM resulted in a dramatic increase in the activity of dopamine-sensitive adenylate cyclase. Solubilization of SPM by Lubrol-PX, however, did not affect the enzyme activation induced by the dopamine (DA), indicating that its receptor may not be to bind ligands [16] or/and not be found linked to others proteins of the enzyme.

The enzyme activity exhibited an important increase at around 1.6 µmol PS/mg protein, as can be seen in the Figure 1. Further increase of PS concentration above this point, however, led to inhibition of the activity with respect to the maximal percentage of stimulation. No change in the activity was found at any concentration of PS, when liposomes were incubated with solubilized enzyme.

These results indicate that acidic phospholipids do not act on adenylate cyclase directly, but indirectly, affecting the membrane fluidity probably. Changes in the viscosity of the membrane can affect the conformation, rotation, diffusion and association of enzyme proteins, and especially the ability of hormone receptor(s) to bind ligands. The increased activities of the adenylate cyclase achieved by low concentrations of phospholipids (Fig. 1), presumably due to a slight increase in fluidity of the membranes resulting to an increase of the conformational flexibility of the enzyme achieved by a relief of a physical constraint imposed by the bilayer upon the protein molecules. Further increases of the membrane fluidity induced by high concentrations of phospholipids, inhibit the activity, probably by displacing annular lipids from around the protein molecules [17]. In conclusion, modulation of SPM architecture caused by changes in lipid fluidity by acidic phospholipids, could affect the activities of other membrane-bound enzymes and the binding of neurotransmitters, events which could control the synaptic transmission.

[1] W. C. Breckenridge, G. Gombos, and I. G. Morgan, Biochim. Biophys. Acta **266,** 695–707 (1972).
[2] R. H. Michell, Biochim. Biophys. Acta **415,** 81–147 (1975).
[3] R. M. C. Dawson, R. F. Irvine, and K. Hirasawa, in Phospholipids in the Nervous System, **Vol. 1,** p. 241–249 (L. Horrocks, G. B. Ansell, and G. Procellati, eds.), Raven Press 1982.
[4] A. Leon, D. Benvegnu, G. Toffano, P. Orlando, and P. Mascori, J. Neurochem. **30,** 23–26 (1978).
[5] F. Casamenti, P. Mantovani, L. Amaducci, and G. Pepeu, J. Neurochem. **32,** 529–533 (1979).
[6] F. Hirata, J. Axelrod, and F. T. Crews, Proc. Natl. Acad. Sci. USA **76,** 4813–4816 (1979).
[7] A. Portis, C. Newton, W. Pangborn, and D. Papahadjopoulos, Biochemistry **18,** 780–790 (1979).
[8] M. Floreani, A. C. Bonetti, and F. Carpenedo, Biochem. Biophys. Res. Comm. **31,** 1337–1344 (1981).
[9] S. Tsakiris and G. Deliconstantinos, Biochem. J. **220,** 301–307 (1984).
[10] E. M. Ross and A. G. Gilman, Ann. Rev. Biochem. **49,** 533–564 (1980).
[11] A. Papaphilis and G. Deliconstantinos, Biochem. Pharmacol. **29,** 3325–3327 (1980).
[12] G. Deliconstantinos, G. Gregoriadis, G. Abel, J. Marvyn, and D. Robertson, Biochem. Soc. Trans. **5,** 1327–1329 (1977).
[13] G. L. Miller, Anal. Chem. **31,** 964–968 (1959).
[14] K. C. Tovey, K. G. Oldham, and J. A. M. Whelan, Clin. Chim. Acta **56,** 221–234 (1974).
[15] N. I. Swislocki and J. Tierney, Arch. Biochem. Biophys. **168,** 455–462 (1975).
[16] A. F. Welton, P. M. Lad, A. C. Newby, H. Yamamura, S. Nicosia, and M. Rodbell, J. Biol. Chem. **252,** 5947–5950 (1977).
[17] M. D. Houslay, I. Dipple, and L. M. Gordon, Biochem. J. **197,** 675–681 (1981).