EFFECT OF LYSOLECITHIN ON GUANYLATE AND ADENYLATE CYCLASE ACTIVITIES IN NEUROBLASTOMA CELLS IN CULTURE

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1. Introduction

In most mammalian cells, guanylate cyclase activity is found in both soluble and particulate fractions. Stimulation by Triton X-100 of both types of guanylate cyclase is well known [1]. In rat small intestines guanylate cyclase is stimulated and adenylate cyclase inhibited by the same concentration of Triton X-100 [2]. Lysolecithin, which is a widely distributed and naturally occurring detergent [3], and has been shown to induce cell fusion [4] is known to stimulate membrane-associated syalyltransferase [5] and galactosyltransferase activities [6] in a manner similar to Triton X-100. Guanylate cyclase in rat lung is stimulated by lysolecithin and phospholipase A [7]. In the other hand, Fujimoto and Okabayashi [8] have reported that guanylate cyclase in guinea pig tracheal muscle homogenate is stimulated by phospholipase A. Studies on cultured neuroblastoma cells have contributed evidence indicating a role for cyclic nucleotides in the regulation of cell differentiation [9–10]. Thus it can be expected that either lysolecithin or phospholipase A (producing lysolecithin) play a regulatory role in cell differentiation.

We have observed that lysolecithin stimulates neuroblastoma cells homogenate guanylate cyclase up to 11-fold. Membrane-bound as well as soluble guanylate cyclase is stimulated. Simultaneously adenylate cyclase is inhibited by the same concentration of lysolecithin. Phospholipase A also induces a doubling guanylate cyclase activity.

2. Materials and methods

In this study an adrenergic neuroblastoma clone, M1 [11] was used. Cells were grown to confluency in Falcon plastic dishes (75 cm^2) in Dulbecco's modified Eagle's medium, supplemented with 10% foetal calf serum. Cultres were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Cultures were harvested during confluence in Tris–HCl buffer (10 mM, pH 7.5).

Guanylate cyclase activity was determined on aliquots containing 80–180 μg protein by the method of Goridis and Reutter [12]. 3-Isobutyl-1-methylxanthine was used as phosphodiesterase inhibitor. Adenylate cyclase activity was determined essentially by the method of Ramachandran and Lee [13]; 0.5 mM ATP as substrate was used. Cyclic nucleotide phosphodiesterase activity was determined following the two steps isotopic procedure as described by Coquil et al. [14]; 10 μM each cyclic nucleotide was used as substrate. Protein was determined by the method of Lowry et al. [15].

For lysolecithin treatment, cell homogenate was preincubated with the indicated amount of lysolecithin (Egg Yolk-Sigma). The alcoholic solution of the phospholipid was brought to dryness under a stream of N_2 before adding cell homogenate. After 10 min preincubation at 0°C, the mixture was diluted three times with

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Abbreviations used: cGMP, cyclic guanosine 3',5'-monophosphate; cAMP, cyclic adenosine 3',5'-monophosphate.

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Tris–HCl buffer (10 mM, pH 7.5). An aliquot of 40 μl was taken for immediate determination of enzyme activities. A similar procedure is used for testing other phospholipids. Triton X-100 (1%) was also used during 10 min preincubation at 0°C.

For phospholipase treatment, cell homogenate was incubated with phospholipase A (*Crotalus* venom–Boehringer),in a medium containing 50 mM Tris–HCl (pH 7.5) and 1 mM $CaCl_2$ at 37°C for 7 min. After incubation and dilution of the mixture, guanylate cyclase activity was immediately assayed.

3. Results

As shown in table 1, lysolecithin stimulate guanylate cyclase activity in homogenate of M1 neuroblasts 11-fold. The effect appeared specific since neither phosphatidylcholine nor lysophosphatidylethanolamine nor phosphatidylethanolamine produce a stimuation of guanylate cyclase. The effect was at a maximum after 6 min of preincubation at 0°C (fig.1). It was dose-dependent up to 2 mg/ml lysolecithin during the preincubation (fig.2). At a higher dosage, the stimulation effect decreased. At the maximum response of guanylate cyclase, adenylate cyclase was almost completely inhibited (fig.2). Lysophosphatidylethanolamine was also without effect on adenylate cyclase activity as shown in table 2. Triton X-100 (1%) had a similar effect on adenylate cyclase activity as lysolecithin, when homogenate was tested (table 2).

Table 1
Effect of several phospholipids on guanylate cyclase activity

	(pmoles cGMP formed/min/mg prot.) ± S.D.
Control	2.87 ± 0.38
Lysolecithin	32.0 ± 2.8
Lecithin	2.80 ± 0.10
Lysophosphatidyl-ethanolamine	2.86 ± 0.31
Phosphatidyl-ethanolamine	2.84 ± 0.32

Aliquots of M1 cells homogenate were preincubated with each phospholipid (2 mg/ml) as described in Methods. The mean ± S.D. of 4 determinations is given.

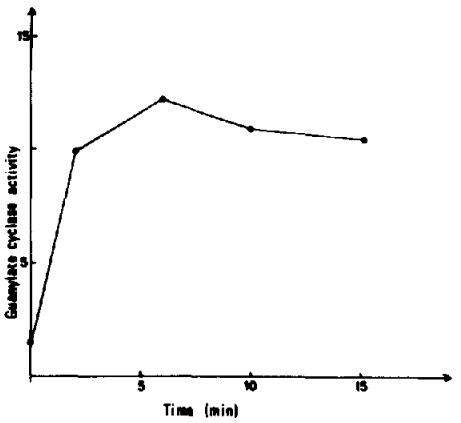

Fig.1. Time-course of guanylate cyclase stimulation by lysolecithin (2 mg/ml during the preincubation) in neuroblastoma M1 cells. Guanylate cyclase specific activity is expressed as pmoles/min/mg protein. Each point is the mean of at least 3 determinations. Standard deviation is less than 13%.

Cyclic GMP phosphodiesterase activity remained unchanged with each of both treatment while cAMP phosphodiesterase was slightly stimulated (36%) when treated with lysolecithin but not with Triton (table 3).

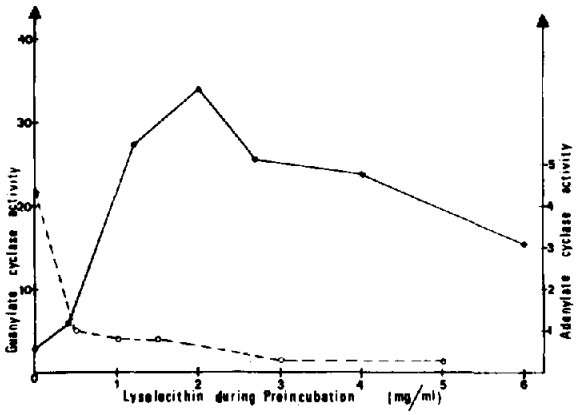

Fig.2. Dose–response curves of stimulation of guanylate cyclase (●——●) and of inhibition of adenylate cyclase (○- - -○) by lysolecithin. Concentrations of lysolecithin indicated are those present in 10 min preincubation. Specific activities are expressed as pmoles/min/mg protein. The mean value is given for 4 determinations. Standard deviation is less than 11% for guanylate cyclase and 21% for adenylate cyclase activity.

Table 2
Effect of several detergents on adenylate cyclase specific activity

	(pmoles cAMP formed/min/mg protein)
Control	2.38 ± 0.18
Lysolecithin (2 mg/ml)	0.65 ± 0.21
Triton X-100 (1%)	0.55 ± 0.15
Lysophosphatidyl-ethanolamine (2 mg/ml)	1.80 ± 0.35

Concentrations of detergents indicated were present during preincubation (10 min; 0°C). Each value is the mean ± S.D. of 4 determinations.

When M1 cells were treated in parallel with lysolecithin and with Triton, and then centrifuged (100 000 × g, 1 h), 47% of the guanylate cyclase activity was found in the soluble fraction with the former treatment, while 91% of the activity was solubilysed with the latter treatment (table 4). However, Triton as well as lysolecithin stimulation of the homogenate guanylate cyclase were similar. In the particulate fraction of lysolecithin treated cells, the specific activity of guanylate cyclase was lower.

In order to characterize the effect of lysolecithin, the apparent K_m and V were measured. It appears that under the effect of lysolecithin, there is a decrease of the apparent K_m and a doubling of V (table 5).

Table 3
Effect of lysolecithin and Triton X-100 on cyclic nucleotide phosphodiesterase activity

	cGMP phosphodiesterase (nmoles/min/mg prot.)	cAMP phosphodiesterase (nmoles/min/mg prot.)
Control	1.84 ± 0.26	0.42 ± 0.06
Lysolecithin (2 mg/ml)	1.74 ± 0.14	0.57 ± 0.05
Triton X-100 (1%)	1.80 ± 0.15	0.36 ± 0.08

Enzymatic activity was measured with respectively 10 μM each cyclic nucleotide as substrate. Each value represents the mean ± S.D. of 3 to 5 determinations.

Table 4
Effect of lysolecithin and Triton X-100 on the sedimentation of guanylate cyclase at 100 000 g

Treatment	Percent of total enzyme activity in the soluble fraction	Guanylate cyclase specific activity		
		Homogenate	Soluble	Particulate
Control	21	2.57	2.20	3.70
Lysolecithin (2 mg/ml)	47	29.2	15.8	17.5
Triton X-100 (1%)	91	27.6	35.1	5.08

Aliquots of M1 cells homogenate were incubated 10 min at 0°C with lysolecithin (2 mg/ml) or Triton (1%). The mixtures were then centrifuged at 100 000 × g for 1 h at 4°C. The supernatants (soluble fractions) were analysed directly and the pellets were resuspended in the same volume of Tris 10 mM, pH 7.5 (particulate fraction). Specific activities of guanylate cyclase are expressed as pmoles cGMP formed/min/mg protein.

Table 5
Effect of lysolecithin on the kinetic parameters of guanylate cyclase of M1 neuroblastoma cells

	Control	Lysolecithin
K_m (mM)	1.56	0.31
V (pmoles/min/mg protein.)	66	125

M1 cells homogenate was preincubated with lysolecithin (2 mg/ml) as described in methods. Guanylate cyclase activity was determined with various concentrations of GTP (0.025–1.6 mM). Mu^{2+} concentration was always 7.7 mM.

Taking in account a possible activity of phospholipase A in vivo, producing lysolecithin, we tested the effect of treatment with this enzyme on M1 cells homogenate; a 100% increase of guanylate cyclase activity was observed in this condition (table 6).

4. Discussion

The effect of lysolecithin on the enzymic formation and degradation of cyclic GMP and cyclic AMP in neuroblastoma cells was examined. Guanylate cyclase was stimulated and adenylate cyclase inhibited by the same concentrations of lysolecithin. Therefore, our data are in agreement with the Yin Yang hypothesis of Goldberg [16] implying an antagonistic regulation of cyclic AMP and cyclic GMP in cellular systems. The fact that both cyclase activities were altered by lysolecithin only and not by lysophosphatidylethanolamine indicates that the stimulatory activity on guanylate cyclase and the inhibitory activity on adenylate cyclase could be related to the choline moiety of the molecule. The effect of lysolecithin on guanylate cyclase involved substrate affinity as well as V. The effects were observed under non-stimulated adenylate cyclase conditions, but nevertheless can be compared to some extent to the results obtained by Shier et al. [17]. These authors recently reported a stimulation of guanylate cyclase activity by lysolecithin in 3T3 mouse fibroblasts. In that system, only fluoride-stimulated by not unstimulated adenylate cyclase was inhibited by lysolecithin.

Table 6
Effect of phospholipase a on guanylate cyclase activity

	pmoles cyclic GMP formed/ min/mg protein
Control	1.23 ± 0.10
Phospholipase A (1U)	2.50 ± 0.36
Phospholipase A (2U)	2.78 ± 0.12

Aliquots of M1 cell homogenate were incubated with phospholipase A as described in Materials and methods. Control values were done in the same conditions with boiled phospholipase A. Each value represents the mean ± S.D. of 4 determinations.

The effect of lysolecithin was found not to be identical to that of Triton X-100. Solubilization of guanylate cyclase activity was much lower when lysolecithin was used. It is probable that, in addition to its detergent effect similar to that of Triton, lysolecithin may play a specific regulatory role. The fact that phospholipase A also induces a stimuation of guanylate cyclase further supports this hypothesis. It might act by generating lysolecithin which in turn may serve as a transducer altering the general properties of the membrane and play a role in the regulation of cellular metabolism in neuroblastoma cells.

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References

[1] Kimura, H. and Murad, F. (1975) Metabolism 24, 439–445.
[2] Ishikawa, E., Ishikawa, S., Davis, J. W. and Sutherland, E. W. (1969) J. Biol. Chem. 244, 6371–6376.
[3] Helenius, A. and Simons, K. (1975) Biochim. Biophys. acta 415, 29–79.
[4] Poole, A. R., Howell, J. I. and Lucy, J. A. (1970) Nature 227, 810–813.
[5] Shier, W. T. and Trotter, J. T. III (1976) FEBS Lett. 62, 165–168.
[6] Kirschbaum, B. B. and Bosman, H. B. (1973) FEBS Lett. 34, 129–132.
[7] White, A. A. and Lad, P. J. (1975) Fed. Proc. 34, 232.
[8] Fujimoto, M. and Okabayashi, T. (1975) Biochem. Biophys. Res. Commun. 67, 1332–1336.

[9] Prasad, K. N. and Hsie, A. W. (1971) Nature New Biol. 233, 141–142.
[10] Prasad, K. N. (1972) Nature New Biol. 236, 49–52.
[11] Ciesielski-Treska, J., Warter, S. and Mandel, P. (1975) Neurobiology 5, 382–392.
[12] Goridis, C. and Reutter, W. (1975) Nature 257, 698–700.
[13] Ramachandran, J. and Lee, W. (1970) Biochem. Biophys. Res. Commun. 41, 358–366.
[14] Coquil, J. F., Virmaux, N., Mandel, P. and Goridis, C. (1975) Biochim. Biophys. Acta 403, 425–437.
[15] Lowry, O. H., Rosebrough, N. I., Farr, A. L. and Randall, R. J. (1951) J Biol. Chem. 193, 265–275.
[16] Goldberg, N. D., Haddox, M. K., Nicol, S. E., Glass, D. B., Sanford, C. H., Kuehl, F. A. and Estensen, R. (1975) in: Advances in cyclic nucleotide research (Drummond, G. J., Grengard, P. and Robison, G. A., eds), Vol. 5, pp. 307–330, Raven Press, New York.
[17] Shier, W. T., Baldwin, J. H., Nilsen-Hamilton, M., Hamilton, R. T. and Thanassi, N. M. (1976) Proc. Natl. Acad. Sci. USA 73, 1586–1590.