The effect of polar head group substitution on phospholipid methylation and the β -adrenergic response in C6 glial cells

Roderick C. McKENZIE,* C. Stewart GILLESPIE and Peter J. BROPHY†

Department of Biological Science, University of Stirling, Stirling FK9 4LA, U.K.

The membranes of intact C6 cells were enriched with phosphatidyl-dimethylethanolamine or phosphatidyl-monomethylethanolamine. These cells showed enhanced rates of phospholipid methylation but this was not accompanied by an increased β -adrenergic response. We conclude that phospholipid methylation is not coupled to the activation of adenylate cyclase in the β -adrenergic response of C6 glial cells.

INTRODUCTION

Hirata & Axelrod (1980) have proposed that phospholipid methylation is a general mechanism for transducing receptor-mediated events in the plasma membrane of a variety of cells (for a more recent review, see Mato & Alemany, 1983). Many ligand–receptor interactions are associated with increased rates of phospholipid methylation; these include the β -adrenergic response of C6 glial cells (Strittmatter *et al.*, 1979), the action of nerve growth factor on neurites (Pfenninger *et al.*, 1982) and the stimulation of adipocytes by insulin (Kelly *et al.*, 1984). Nevertheless, a functional association between phospholipid methylation and such a diversity of receptor-mediated events remains elusive and controversial (Moore *et al.*, 1982, 1984; Vance & de Kruijff, 1980).

We have previously confirmed that stimulation of C6 glial cells with the β -adrenergic agonist isoproterenol enhances the rate of incorporation of methyl groups from [*methyl*- 3 H]methionine into lipid extracts (McKenzie & Brophy, 1983). The physical properties of the plasma membrane were unaffected although modulation of lipid fluidity has been proposed as one possible role for methylation in receptor-mediated events (Hirata & Axelrod, 1980). We wished to know whether we could affect the rate of accumulation of cyclic AMP in response to isoproterenol stimulation by simply increasing the flux through the phospholipid methylation pathway. PtdMeEtn and PtdMe₂Etn are intermediates in the methylation of PtdEtn to PtdCho (Bremer & Greenberg, 1961) and Maeda *et al.* (1980) have shown that the pathway is much more active in cells enriched with PtdMeEtn and PtdMe₂Etn. Growth of C6 cells in media containing *N*-monomethylethanolamine or *NN*-dimethylethanolamine causes the accumulation of the corresponding phospholipids to about 30% of total membrane phospholipid at the expense of PtdCho and PtdEtn (McKenzie & Brophy, 1984). The modified cells have normal growth characteristics. We demonstrate that these cells do show increased rates of phospholipid methylation but that this does not enhance the extent of adenylate cyclase activation by isoproterenol.

MATERIALS AND METHODS

C6 cells from a rat glioma (passages 46–52) were maintained in culture and their membrane phospholipid composition modified as described previously (McKenzie & Brophy, 1984). Measurements of methyl group incorporation into lipids from [*methyl*- 3 H]methionine (Amersham International), lipid extractions, conditions for β -adrenergic stimulation with isoproterenol and cyclic AMP assays have all been described in detail (McKenzie & Brophy, 1983). Incorporation of methyl groups from [*methyl*- 3 H]methionine into phospholipids that had been separated by two-dimensional t.l.c. (McKenzie & Brophy, 1984) was determined by scraping the silica gel into scintillation vials and measuring the radioactivity in 10 ml of Fiso-fluor MPC (Fisons Ltd).

A membrane fraction was prepared by scraping the cells from Petri dishes and homogenizing them with a Potter–Elvehjem homogenizer in phosphate-buffered saline at 4 °C. The homogenate was centrifuged at 10000 *g* for 10 min at 4 °C and the membrane fraction was obtained from the supernatant after centrifugation at 100000 *g* for 45 min at 4 °C. Membranes were resuspended in 50 mM-Tris/HCl buffer, pH 8.5, and stored at –70 °C. Methyltransferase assays were conducted in a 50 μ l reaction mixture containing 1 mM-L-cysteine, 100 μ M-S-adenosyl-L-[*methyl*- 3 H]methionine (142 Ci/mol; Amersham International) and 125 mM buffer as indicated in the legend to Fig. 1. Both non-radioactive (from Sigma) and radioactive S-adenosyl-L-methionine were purified by Dowex 1X8 and Cellex P column chromatography as recommended by Audubert & Vance (1983). T.l.c. in the solvent system suggested by these authors showed the compounds to be free of impurities. Reactions were started by the addition of membrane protein (100 μ g) and were stopped after 30 min at 37 °C by the addition of 3 ml of chloroform/methanol/2 M-HCl (6:3:0.5, by vol.). The lipid extract was washed twice with methanol/water/2 M-HCl (5:4.5:0.5, by vol.) and once with methanol/water (1:1, v/v). The radioactivity of aliquots of the lipid extract was measured by scintillation counting in 10 ml of Fiso-fluor MPC.

Abbreviations used: PtdCho, phosphatidylcholine; PtdMe₂Etn, phosphatidyl-dimethylethanolamine; PtdMeEtn, phosphatidyl-monomethylethanolamine; PtdEtn, phosphatidylethanolamine.

* Present address: Cancer Research Group, Department of Pathology, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

† To whom correspondence and reprint requests should be addressed.

Table 1. Effect of enriching C6 cells with PtdMe₂Etn and PtdMeEtn on the rate of phospholipid methylation

C6 cells were grown for 24 h in the presence of *NN*-dimethylethanolamine or *N*-monomethylethanolamine (5 mM). The growth medium was then replaced with 1 ml of Medium 199 (Flow Laboratories) containing [*methyl*-³H]methionine (20 μ Ci) for 55 min at 37 °C. The distribution of radioactivity in the phospholipids of the methylation pathway was then determined after extraction and separation of the lipids by two-dimensional t.l.c. Values are means of duplicates.

Phospholipid enrichment	[³ H]Methyl groups incorporated (pmol/mg of protein)			
	PtdEtn	PtdMeEtn	PtdMe ₂ Etn	PtdCho
None	2.4	1.8	4.3	12.2
PtdMe ₂ Etn	1.3	1.3	8.0	80.0
PtdMeEtn	8.4	8.4	75.6	59.0

RESULTS AND DISCUSSION

Enrichment of C6 cell membranes with PtdMe₂Etn or PtdMeEtn increases the flux through the phospholipid methylating pathway as shown in Table 1. As might be expected, the increase in methylation is more marked for PtdMeEtn-enriched cells. Fig. 1 shows that the increased rates of methylation are a result of elevated methyltransferase activity in C6 cell membranes, presumably the result of the presence of more lipid substrate. The pH-dependency of the methyltransferase reaction is similar to that reported by Audubert & Vance (1983) who found that methyltransferase activity in rat liver microsomal preparations was highest between pH 10 and 10.5.

Table 2 shows that the increased rate of phospholipid methylation did not enhance the response of C6 cells to β -adrenergic stimulation; on the contrary, isoproterenol appeared to be less effective in raising intracellular cyclic AMP levels in the modified cells. The extent of cyclic AMP accumulation in modified cells in the presence of theophylline was close to control values, which suggested that their decreased response might be due to enhanced phosphodiesterase activity. However, it cannot be excluded that theophylline might have had effects other

Fig. 1. pH dependence of lipid methyltransferase activity in membranes from C6 cells enriched in PtdMeEtn or PtdMe₂Etn

Membrane protein (100 μ g) was added to 1 mM-L-cysteine, 100 μ M-S-adenosyl-L-[*methyl*-³H]methionine (142 Ci/mol) and 125 mM-buffer in a total volume of 50 μ l. Reactions were for 30 min at 37 °C. Buffer: sodium phosphate, pH 5.5–7.0; Tris/HCl, pH 7.5–9.5; glycine/KOH, pH 10.0–11.0. $\circ$, Control; $\triangle$, PtdMe₂Etn; $\bullet$, PtdMeEtn. All values are means of duplicates.

than the inhibition of cyclic AMP phosphodiesterase activity. There was no difference in the time course of cyclic AMP accumulation in normal cells versus modified cells in response to isoproterenol (results not shown).

Houslay & Gordon (1983) have reviewed the role of phospholipid head groups in modulating the activity of adenylate cyclase. They concluded that the evidence for an effect of polar head group substitution on adenylate cyclase activity was equivocal because most studies have failed to distinguish the specific effects of a change in the polar head groups of phospholipids on adenylate cyclase activity from a general response to an altered bilayer fluidity. We have shown previously that the physical properties of the modified C6 cell membranes are unchanged, as measured by spin-label e.s.r. spectroscopy (McKenzie & Brophy, 1984). Therefore it is unlikely that the increased activity of the phospholipid *N*-methylation

Table 2. Effect of enriching C6 cell membranes with PtdMe₂Etn and PtdMeEtn on basal and isoproterenol-stimulated cyclic AMP levels

C6 cells were grown in the wells of Linbro trays and incubated for 30 min in Medium 199 with or without isoproterenol (10 μ M) in the absence or presence of theophylline (1 mM). Values are means $\pm$ S.E.M. except for the values in column 4 which are means of duplicates.

Phospholipid enrichment	Cyclic AMP (pmol/mg of protein)			
	Basal	+ Isoproterenol	Basal + theophylline	+ Isoproterenol + theophylline
None	14.4 $\pm$ 3.0(3)	806 $\pm$ 150(3)	45.0(2)	685 $\pm$ 58(3)
PtdMe ₂ Etn	22.3 $\pm$ 0.6(3)	288 $\pm$ 34(3)	89.0(2)	616 $\pm$ 179(3)
PtdMeEtn	50.0 $\pm$ 13.0(3)	473 $\pm$ 58(3)	133.0(2)	595 $\pm$ 50(3)

pathway in modified cells is accompanied by alterations in the physical properties of the lipid bilayer. The fact that the β -adrenergic response of modified cells is not enhanced argues against an important role for the *N*-methylation pathway in modulating the coupling between receptor, guanine nucleotide binding protein and adenylate cyclase.

We thank the SERC for a studentship to R. C. McK.

REFERENCES

- Audubert, F. & Vance, D. E. (1983) *J. Biol. Chem.* **258**, 10695–10701
- Bremer, J. & Greenberg, D. M. (1961) *Biochim. Biophys. Acta* **46**, 205–216
- Hirata, F. & Axelrod, J. (1980) *Science* **209**, 1082–1090
- Houslay, M. D. & Gordon, L. M. (1983) *Curr. Top. Membr. Transport* **18**, 179–231
- Kelly, K. L., Kiechle, F. L. & Jarett, L. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1089–1092
- Maeda, M., Tanaka, Y. & Akamatsu, Y. (1980) *Biochem. Biophys. Res. Commun.* **96**, 876–881
- Mato, J. M. & Alemany, S. (1983) *Biochem. J.* **213**, 1–10
- McKenzie, R. C. & Brophy, P. J. (1983) *FEBS Lett.* **164**, 244–246
- McKenzie, R. C. & Brophy, P. J. (1984) *Biochim. Biophys. Acta* **769**, 357–362
- Moore, J. P., Smith, G. A., Hesketh, T. R. & Metcalfe, J. C. (1982) *J. Biol. Chem.* **257**, 8183–8189
- Moore, J. P., Johannsson, A., Hesketh, T. R., Smith, G. A. & Metcalfe, J. C. (1984) *Biochem. J.* **221**, 675–684
- Pfenninger, K. H. & Johnson, M. P. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7797–7801
- Strittmatter, W. J., Hirata, F., Axelrod, J., Mallorga, P., Tallman, J. F. & Henneberry, R. C. (1979) *Nature (London)* **282**, 857–859
- Vance, D. E. & de Kruijff, B. (1980) *Nature (London)* **288**, 277–279

Received 28 May 1985/6 August 1985; accepted 14 August 1985