AUTOREGULATION OF PHOSPHOLIPID *N*-METHYLATION BY THE MEMBRANE PHOSPHATIDYLETHANOLAMINE CONTENT

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1. Introduction

The step-wise methylation of phosphatidylethanolamine is catalyzed by membrane-bound enzyme(s), with high activity in liver endoplasmic reticulum [1]. The rate of methylation is influenced by the concentration of one of the reactants, Ado-Met (*S*-adenosylmethionine) in experiments with subcellular fractions [1] and on the availability of methionine in isolated hepatocytes [2] and in intact rats [3]. Nothing is known about the influence of the other substrate, phosphatidylethanolamine, since phosphatidylethanolamine added to microsomal systems does not stimulate methylation, in contrast to phosphatidyl-*N*-methylethanolamine and phosphatidyl-*NN*-dimethylethanolamine [1,4].

We have recently shown that the amount of phosphatidylethanolamine in hepatocytes can be manipulated by maintaining them in primary culture in the presence and absence of ethanolamine [5]. This report deals with the several-fold variation in phospholipid methylation in such cells.

2. Materials and methods

Livers were perfused with collagenase and the hepatocytes were isolated and plated in medium L-15 on Petri dishes as in [5]. The first change of medium was after 1–2 h and then the medium was changed once a day. After incubation with different agents, the medium was aspirated and replaced by new medium and [^{14}C]methionine (0.5 mM, which is the concentration in medium L-15). After incubation for 1–2 h at 37°C, the medium was aspirated and lipids were extracted from the cells [5]. Total phospholipid content in each lipid extract was determined [6] and the incorporation of ^{14}C from [*methyl*-^{14}C]methionine was expressed as nmol/lipid P. Phosphatidylethanolamine was determined with trinitrobenzenesulphonic acid [7].

Alternatively the cells in the Petri dish were washed with 2 ml of cold NaCl (9 mg/ml) containing 1 mM Tris–HCl, pH 7.4. The cells were then transferred with four 0.5 ml portions of the same solution to centrifuge tubes and were disrupted by sonication for 30 s.

The activity of phosphatidylethanolamine methyltransferase (EC 2.1.1.17) was measured with a scaled-down modification of the method in [4]. The assay mixture contained 0.05 M Tris–HCl (pH 8.2) and cell homogenate (0.5 mg protein) in total vol. 0.5 ml. The reaction was started by the addition of 0.04 mM [*methyl*-^{3}H]Ado-Met (Radiochemical Centre, Amersham, Bucks) and the samples were incubated by shaking for 30 min or 60 min. The reaction was interrupted by the addition of 3 ml chloroform/methanol (1:1). After 30 min 1 ml water and 1.5 ml chloroform were added and then the chloroform phase was washed twice with 3 ml methanol/water (1:1, saturated with chloroform). Total lipid radioactivity was routinely measured with liquid scintillation counting. Occasional checks by thin-layer chromatography [5] showed that 90% lipid radioactivity was always in phosphatidylcholine.

3. Results

When hepatocytes were maintained in the absence of ethanolamine, the methylation of phospholipid decreased with time, when measured as methyl group incorporation either from [^{14}C]methionine into lipids in intact cells or from [^{3}H]Ado-Met in cell homogenates (fig.1). The decrease was prevented if ethanolamine was added to the culture medium. In addition, the methylation rate was rapidly brought back to its original level if ethanolamine was added to hepatocytes which had been kept in its absence for 1 day. The effect of ethanolamine did not involve synthesis of protein, since it persisted in the presence of actinomycin D (0.04–0.4 μM) or cycloheximide (5–25 μM).

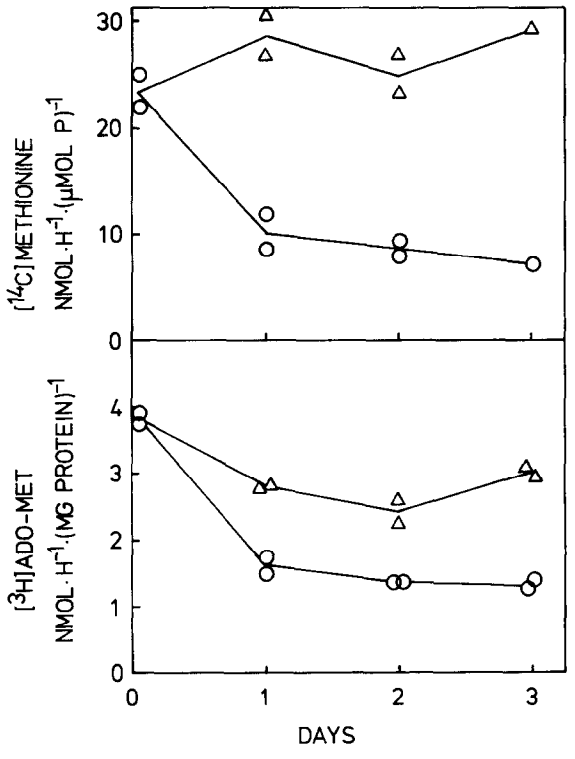

Fig.1. Phosphatidylethanolamine methyltransferase in hepatocyte cultures. Hepatocytes were maintained in medium L-15 and 2% fetal calf serum in the presence (△) or absence (○) of 1 mM ethanolamine. At different times, the cells were assayed for methyl-group incorporation from [^{14}C]methionine into total lipids (upper panel) or homogenized and assayed for phosphatidylethanolamine methyltransferase activity (lower panel).

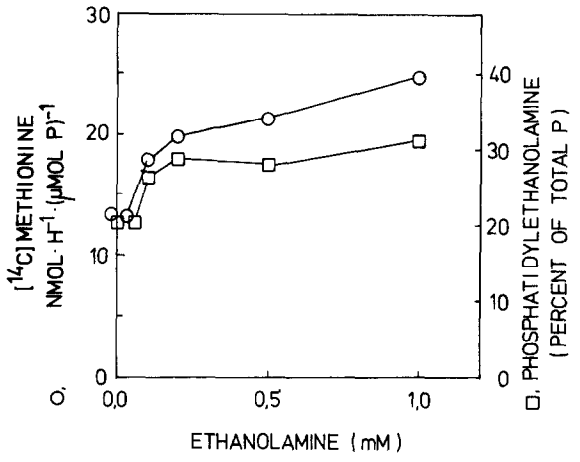

Fig.2. Effect of preincubation with different concentrations of ethanolamine on phospholipid methylation. Hepatocytes were maintained as described in fig.1 for 24 h with different concentrations of ethanolamine. Then the cells were assayed for incorporation of [^{14}C]methionine in the absence of ethanolamine (circles). In the lipid extracts from cells, the amount of phosphatidylethanolamine was determined (squares).

This indicated that ethanolamine exerted a substrate effect and that the higher proportion of phosphatidylethanolamine in hepatocytes maintained in the presence of ethanolamine [5], resulted in a higher methylation rate. This is further supported by the fact that the increase in phosphatidylethanolamine amount caused by preincubation with different concentrations of ethanolamine, was parallelled by an increased phospholipid methylation (fig.2). This is the first demonstration that the amount of phosphatidylethanolamine is important for its own methylation and it is also the first time that the amount of membrane-bound substrate in this reaction has been varied.

In another series of experiments, hepatocytes were maintained in 'methyl-group-deficient' L-15, lacking methionine, choline and folate. Preincubation in this medium resulted in a higher proportion of phosphatidylethanolamine and after 24 h the methylation of phospholipid from [^{14}C]methionine was increased. Preincubation with ethanolamine in the presence of methionine stimulated phospholipid methylation, as described above (fig.1,2), but this effect disappeared in the absence of methionine. This indicates that the enrichment of phosphatidylethanolamine substrate

Table 1
Influence of methionine and ethanolamine on phospholipid methylation in cultured hepatocytes

Preincubation conditions	nmol [^{14}C] methionine/ h and μmol P	nmol [^{14}C] methionine/ h and μmol PE
Control	34.3	113
Ethanolamine (1 mM)	34.3	102
Methionine (0.5 mM)	13.0	63
Ethanolamine plus methionine	24.9	79

Hepatocytes were maintained in methyl-group-deficient L-15 with 2% fetal calf serum for 24 h with the additions indicated. Then the incorporation of ^{14}C from 0.5 mM [^{14}C]methionine into lipids was measured and related to total phospholipid or phosphatidylethanolamine (PE) content. Data are means of duplicates

became maximal already when methyl-group-deficient L-15 was substituted for L-15.

When the incorporation of ^{14}C from [^{14}C]methionine into lipids was related to total cellular phosphatidylethanolamine, the differences became smaller (table 1), but the incorporation was not constant, as would have been expected if the methylation rate just reflected the total amount of phosphatidylethanolamine. Possibly the incorporation of methyl groups is more closely related to a specific subcellular pool of phosphatidylethanolamine.

4. Discussion

The present data suggest a mechanism for the autoregulation of the relative amounts of phosphatidylethanolamine and phosphatidylcholine in rat liver. The amount of phosphatidylethanolamine can be increased either by addition of ethanolamine or by the absence of methionine in the preincubation medium. Then phospholipid methylation is increased, which tends to lower the phosphatidylethanolamine/phosphatidylcholine ratio. On the other hand ethanolamine has no immediate effects on phosphatidylethanolamine methylation in isolated hepatocytes [2].

The physiological importance of keeping the phosphatidylethanolamine/phosphatidylcholine ratio constant is difficult to evaluate at present. In growing fibroblasts large changes in phospholipid polar headgroup composition can be induced without gross functional derangement [8,9]. An increased phosphatidylethanolamine/phosphatidylcholine ratio is also observed in methyl-group deficiency [10,11] and in vitamin B_{12} deficiency [3].

In liver from methyl-group-deficient rats, the measured activity of phosphatidylethanolamine methyltransferase is increased [12–14]. This increase must largely reflect the concomitant rise in phosphatidylethanolamine content [10,11]. Also, a decreased phospholipid methylation in choline deficiency has been suggested based on the decreased conversion of phosphatidylethanolamine to phosphatidylcholine [11]. This probably reflects a limited supply of methionine, which is another regulator of phospholipid methylation [2]. Thus in assays of phospholipid methylation, the availability of both phospholipid substrates and methionine (Ado-Met) must be controlled for a proper interpretation of the results. Data on ^{14}C incorporation from [^{14}C]methionine into phosphatidylcholine in vivo are difficult to interpret, since the increase in choline deficiency [15,16] may reflect both increased available phosphatidylethanolamine and differences in dilution with endogenous methionine.

The measured activity of liver phosphatidylethanolamine methyltransferase changes after treatment of animals with different agents, such as phenobarbital [14]. It is possible that also such changes can be explained by the mechanism advanced in the present study, namely that the availability of phosphatidylethanolamine is of major importance for the rate of its methylation. Although partially methylated phosphatidylethanolamines can be added in the assay to saturate the requirement for lipid substrate, it is uncertain whether previously reported measurements accurately reflect the true amount of enzyme activity or enzyme protein(s).

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