Activation of Phospholipid Methyltransferase by Glucagon in Rat Hepatocytes*

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Glucagon produces a time- and dose-dependent activation of phospholipid methyltransferase activity in isolated rat hepatocytes. Half-maximal effect is caused by a dose of glucagon of 1 x 10⁻¹⁸ M. This activation is due to an increase of the V_max value of the enzyme, without affecting the K_m value for S-adenosylmethionine. Exogenous cyclic AMP added to isolated rat hepatocytes mimics the effect of glucagon, and the activation of phospholipid methyltransferase by a nonsaturating concentration of glucagon is spontaneously reversible within 40 min of incubation.

Phospholipids synthesized in liver are utilized as essential components of the membranes within the organ or are transferred into bile or lipoproteins of blood plasma (1). The synthesis of phosphatidylcholine in liver can occur by two different pathways: by the CDP-choline pathway (2) and by successive methylation of phosphatidylethanolamine utilizing S-adenosylmethionine (AdoMet) as the methyl donor (3). The enzymes required for the synthesis of phosphatidylcholine are, for both pathways, mainly located in the microsomal fraction of the liver (4, 5).

An increase in the phospholipid transmethylating pathway has been observed after treatment of the rats with phenobarbital, without change in the CDP-choline pathway (6-8). This fact, together with the rare contribution of the transmethylating pathway to the biliary phosphatidylcholine (1), suggests that the methyl transfer synthesis of phosphatidylcholine must be used preferentially for the structural requirements of phospholipids.

Recent work from Axelrod's group and other laboratories has shown that the methylation of phospholipids is a very active metabolic pathway, being related to several processes such as calcium transport (9), β-adrenergic receptor-adenylyl cyclase coupling (10) and chenotaxis (11-13). In the general context of regulation of phospholipid methylation by extracellular signals, we decided to study the possible effect of glucagon on the activity of liver phospholipid methyltransferase. Here is presented evidence of a time- and dose-dependent activation of this enzyme from isolated rat hepatocytes treated with glucagon. This activation is due to an increase in the V_max value of the enzyme and is mimicked by cAMP.

EXPERIMENTAL PROCEDURES

Isolation and Incubation of Hepatocytes—Hepatocytes were isolated from normally fed Wistar rats (250 to 300 g) as described by Castaño et al. (14). Isolated hepatocytes were incubated as follows: 2 ml of cell suspension (30 to 50 mg wet weight/ml of suspension) were shaken (150 strokes/min) in stopped 20-ml vials at 37°C in the presence of 0 mg glucose. The gas phase was 95% O₂ and 5% CO₂. After 30 min of preincubation, glucagon, cAMP, or saline (0.8% NaCl solution) were added. For the phospholipid methyltransferase assay, the suspension of hepatocytes at the time indicated was poured into precooled centrifuge tubes and immediately centrifuged at 1000 x g for 20 s. The supernatant was discarded and the pellet was immediately frozen in a dry ice-acetone bath.

Measurement of Enzyme Activity—For the determination of phospholipid methyltransferase activity, 0.2 ml of homogenate buffer (50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), 100 mM KF, 15 mM ethylene glycol bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid (EGTA), pH 7.4) were added to the frozen aliquots then vigorously shaken in a mixer until the sample was completely thawed. Further homogenization was not necessary and did not increase phospholipid methyltransferase activity. The resultant enzyme was directly used for assay in a reaction mixture that contained, in a final volume of 0.55 ml, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.3, 4 mM dithiothreitol, 6% sucrose, 5 mM MgCl₂, 100 μM S-adenosylmethionine, 2 μCi of S-adenosyl-L-[methyl-¹³C]methionine (Amersham, 15 Ci/mmol) or 0.05 μCi of S-adenosyl-L-[methyl-¹⁴C]methionine (Amersham, 86 mCi/mmol) and usually 50 μl of homogenate. The reaction was initiated by the addition of a mixture of the labeled and unlabeled S-adenosylmethionine and terminated by pipetting 100 μl of assay mixture into 2 ml of chloroform/methanol/2 N HCl (6:3:1, v/v) for lipid extraction (10). The chloroform phase was washed with 1 ml of 0.5 M KCl in 50% methanol. After washing, 0.7 ml of the chloroform phase was pipetted into a counting vial, dried at room temperature, dissolved in 5 ml of Triton X-100/toluol-based scintillator, and counted. The reaction was linear with time of incubation, up to 60 min and with the amount of homogenate added (from 10 to 200 μl), for homogenates from both control and glucagon-treated hepatocytes.

The known inhibitor of transmethylation, S-adenosyl-L-homocysteine, added to the reaction mixture at a final concentration of 0.1 mM, produced a 90% inhibition of the enzymatic reaction.

To separate and quantitate the various methylated phospholipids, the chloroform phase was dried at room temperature under a stream of N₂ gas and the residue was dissolved in 0.5 ml of chloroform. A sample, usually 0.2 ml, was then applied to a silica gel plate (Silica Gel plates 60, Merck) and developed by ascending chromatography with propionic acid/n-propyl alcohol/chloroform/water (2:2:1.1, v/v) for phospholipid separation (10). After the front migrated 14 cm, the silica gel was scraped into 5-mm bands. Each band was then eluted with 0.5 ml of the solvent used for chromatography and, after 6-h incubation at room temperature, 0.4 ml was pipetted into a counting vial, dried at 70°C, dissolved in 5 ml of scintillator, and counted.

Results were measured according to Lowry et al. (15). Specific activity of the enzyme is expressed as picomoles of [¹³C] or [¹⁴C]-methyl groups incorporated into phospholipids/min/mg of protein, at 25°C.

Biochemicals were from Sigma or Boehringer. Other reagents were of analytical reagent grade.

RESULTS AND DISCUSSION

Treatment of isolated rat hepatocytes with glucagon (10⁻⁶ M) produces a time-dependent activation of phospholipid methyltransferase activity (Fig. 1). Maximal activation is about 2-fold and is attained 5 min after the addition of the hormone.
different experiments, each one with incubations in triplicate. Assay of phospholipid methyltransferase for NaF, 10 mM CAMP, 0.1 mM Saline as described under "Experimental Procedures." Results are expressed as means of enzyme activity was carried out as described under "Experimental Procedures." At zero time, saline or glucagon were added. Values are means ± S.D. from three different experiments, each one with incubations in triplicate.

TABLE 1
Effect of cAMP, NaF, and MIX addition to isolated rat hepatocytes on phospholipid methyltransferase activity

<table>
<thead>
<tr>
<th>Additions</th>
<th>Phospholipid methyltransferase activity (pmol/min X mg protein⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>cAMP, 0.1 mM</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>cAMP, 5 mM</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>cAMP, 0.1 mM + MIX, 50 µM</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>NaF, 10 mM + MIX, 50 µM</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>NaF, 10 mM</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>MIX, 50 µM</td>
<td>18 ± 2</td>
</tr>
</tbody>
</table>

The dose-dependent activation of phospholipid methyltransferase activity by glucagon is shown in Fig. 4. Half-maximal activation is approximately observed at a dose of glucagon of 1 × 10⁻¹⁰ M, which is in the range of the physiological concentrations of the hormone.

As shown in Fig. 5, the activation of phospholipid methyltransferase by a nonsaturating dose of glucagon (3 × 10⁻¹⁰ M) is spontaneously reversible within 40 min of incubation. This result indicates that a proteolytic mechanism for the short term effect of glucagon on the enzyme activity can be discarded.

The activation of phospholipid methyltransferase activity by glucagon seems to be a cAMP-dependent process because: (a) cAMP and NaF, a known activator of adenylyl cyclase, added to isolated rat hepatocytes also activate the enzyme, (b) the dose of glucagon that produces half-maximal activation.

The abbreviation used is: MIX, 1-methyl-3-isobutylxanthine.
of the enzyme is similar to that reported for other known cAMP-dependent actions of glucagon, i.e. inactivation of phosphofructokinase and pyruvate kinase L (14). As for other CAMP-dependent actions of glucagon, mechanism of activation of phospholipid methyltransferase by glucagon remains to be established. As phospholipid methyltransferase activity has been shown to affect membrane structure (18), it can be suggested that a CAMP-dependent phosphorylation or dephosphorylation of the enzyme could be involved in the mechanism of activation of phospholipid methyltransferase by glucagon.

The possible physiological role of the activation of phospholipid methyltransferase by glucagon in isolated rat hepatocytes remains to be established. As phospholipid methyltransferase activity has been shown to affect membrane structure (18), it can be suggested that the increase of phospholipid methyltransferase activity by glucagon could probably affect processes in which membranes, especially of the endoplasmic reticulum, are involved, including membrane transport and the activity of membrane-bound enzymes.

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REFERENCES


FIG. 3. Separation of methylated phospholipids from hepatocyte homogenates. Open bars, control hepatocytes; closed bars, glucagon (10^-7 M)-treated hepatocytes. Aliquots of hepatocytes were taken 5 min after the addition of saline or glucagon. Incubation of hepatocytes and assay of phospholipid methyltransferase were carried out as described under "Experimental Procedures." The reaction was carried out for 40 min and the methylated lipids were separated and quantitated as described under "Experimental Procedures." LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PDE, phosphatidyl-N,N-dimethylethanolamine; PME, phosphatidyl-N-methylethanolamine; PE, phosphatidylethanolamine.

FIG. 4. Effect of various concentrations of glucagon on phospholipid methyltransferase activity of rat hepatocytes. Aliquots of hepatocytes for enzyme assay were taken 5 min after the addition of glucagon. Incubation of hepatocytes and assay of enzyme activity were carried out as described under "Experimental Procedures." Values are means ± S.D. of triplicate incubations from a single experiment.

FIG. 5. Time course of the action of a nonsaturating dose of glucagon on phospholipid methyltransferase activity of rat hepatocytes. O, control hepatocytes; ●, glucagon-treated hepatocytes. Incubations of hepatocytes and assay of phospholipid methyltransferase were carried out as described under "Experimental Procedures." Values are means ± S.D. from three different experiments, each one with incubations in triplicate.