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GUANOSINE 5'-TRIPHOSPHATE MODULATION OF S-ADENOSYL-L-METHIONINE-MEDIATED METHYLATION OF PHOSPHATIDYLETHANOLAMINE IN RAT LIVER PLASMA MEMBRANE

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Summary: Effect of guanosine 5'-triphosphate(GTP) on the S-adenosyl-L-methionine-mediated methylation of phosphatidylethanolamine was examined using rat liver plasma membranes. Methyltransferase I, which catalyzes methylation of phosphatidylethanolamine to phosphatidyl-N-mono-methylethanolamine was inhibited by GTP, whereas methyltransferase II, which transfers methyl groups from S-adenosyl-L-methionine to produce phosphatidyl-N,N-dimethylethanolamine or phosphatidylcholine was stimulated by GTP. d,l-isoproterenol stimulated methyltransferase II activity slightly. This stimulation was greatly augmented by GTP. d,l-isoproterenol inhibited methyltransferase I and this inhibition was enhanced by GTP. The results indicate that GTP has a regulatory role in the methylation of phospholipids in the plasma membrane through inactivation of methyltransferase I and activation of methyltransferase II by binding to these enzymes.

Phosphatidylcholine synthesis via the methylation of phosphatidylethanolamine has important roles in many membrane events such as membrane fluidity(1), lipid translocation(2) and coupling of the β-adrenergic receptor to adenylate cyclase(3). Enhancement of phospholipid methylation by l-isoproterenol decreases membrane microviscosity and that results in an increase in lateral movement of the β-adrenergic receptors and coupling with adenylate cyclase(3). The enhancement of the methylation by l-isoproterenol has been known to be stimulated by guanosine 5'-triphosphate(GTP), and this GTP stimulation is not mediated by adenylate cyclase activation(3). Although GTP-dependent β-adrenergic agonists-induced methylation of phosphatidylethanolamine may have a key role in the hormone action at the target tissues, the precise mechanism of GTP-dependent methylation is uncertain.

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In this study, GTP-induced modulation of S-adenosyl-L-methionine-mediated phosphatidylethanolamine methylation was examined by using rat liver plasma membranes. And we obtained results that GTP stimulates methylation of phosphatidyl-N-monomethylethanolamine to phosphatidylcholine but inhibits methylation of phsophatidylethanolamine to phosphatidyl-N-monomethylethanolamine in rat liver plasma membrane.

MATERIALS AND METHODS

Materials: S-adenosyl-L-[methyl-^{3}H]methionine(14 Ci/mmol) wa purchased from New England Nuclear(Boston, MA). GTP, ATP, d,l-isoproterenol, adenosine 3',5'-monophosphate(cyclic AMP), theophylline and phosphatidyl-N-monomethylethanolamine were purchased from Sigma Chemical Co.(St. Louis,MO).

Preparation of liver plasma membranes: Male Wistar rats(50-75 g) were killed by decapitation and the livers were preperfused with ice-cold saline for 10 min. The plasma membranes of the liver were prepared as previously described(4). The plasma membranes were purified by using discontinuous sucrose density gradient centrifugation. Protein concentration was determined by the method of Lowry et al. (5).

Enzyme assay: The methylation of phosphatidylethanolamine to phosphatidyl-N-monomethylethanolamine was assayed by measuring incorporation of methyl group from S-adenosyl-L-[methyl-^{3}H]methionine into phospholipids in membrane as previously described(4). The assay medium contained 4 μM S-adenosyl-L-[methyl-^{3}H]methionine, 10 mM $MgCl_2$, 0.1 mM EGTA, 50 mM sodium acetate buffer(pH 6.5) and plasma membrane fraction(0.1 mg of protein) in a total volume of 300 μl. In several experiments, reaction mixture contained GTP or ATP as indicated in each study. The reaction was performed at 37°C for 30 min. and was terminated by adding 3 ml of chloroform/methanol/hydrochloric acid (2/1/0.02, vol/vol). After the addition of 3 ml of KCl in 50 % in methanol, the tubes were vigorously shaken and centrifuged at 2,000 x g for 10 min. The chloroform phase was transferred to a vial and the solvent was evaporated. After addition of 10 ml of scintilation liquid(0.4 % 2,5-diphenyloxazol in toluene and Triton X-100(2:1)) the radioactivity was measured.

The methylation of phosphatidyl-N-monomethylethanolamine was assayed with 50 mM sodium borate buffer(pH 9.0), 1 μM S-adenosyl-L-[methyl-^{3}H]methionine and 100 μg of phosphatidyl-N-monomethylethanolamine by the procedure as described above. In several experiments the assays were performed in the presence of various concentrations of GTP or ATP.

RESULTS

As is well known, synthesis of phosphatidylcholine from phosphatidylethanolamine is mediated by S-adenosyl-L-methionine, and the reaction is catalyzed by two enzymes(2,6,7). One is stimulated at pH 6.5 and another is at pH 9.0 in liver as previously reported(4). The former enzyme catalyzes methylation of phosphatidylethanolamine to

phosphatidyl-N-monomethylethanolamine(methyltransferase I). The latter transfers two methyl groups to phosphtidyl-N-monomethylethanolamine to produce phosphatidylcholine via phosphatidyl-N,N-dimethylethanolamine(methyltransferase II). Preliminarily, these enzyme activities were measured in the presence of 100 μg of phosphatidyl-N-monomethylethanolamine. Both ATP and GTP inhibited methyltransferase activity at pH 6.5. In the absence of phosphatidyl-N-monomethylethanolamine, the results were not different from those in the presence of the phospholipid at pH 6.5. At pH 7.5, ATP inhibited the methylation. However, GTP did not show significant inhibition at pH 7.5. Furthermore, at pH 9.0, GTP stimulated methyltransferase activity though ATP slightly inhibited the methylation(Fig. 1). The results suggest that ATP inhibits both methyltransferase I and II and that GTP inhibits methyltransferase I but stimulates methyltransferase II.

Isoproterenol is known to stimulate phospholipid methylation in the reticulocyte membrane(3). The effect of GTP on the isoproterenol stimulation of phospholipid methylation in liver plasma membrane was examined. At pH 6.5, d,l-isoproterenol inhibited the methyltrans-

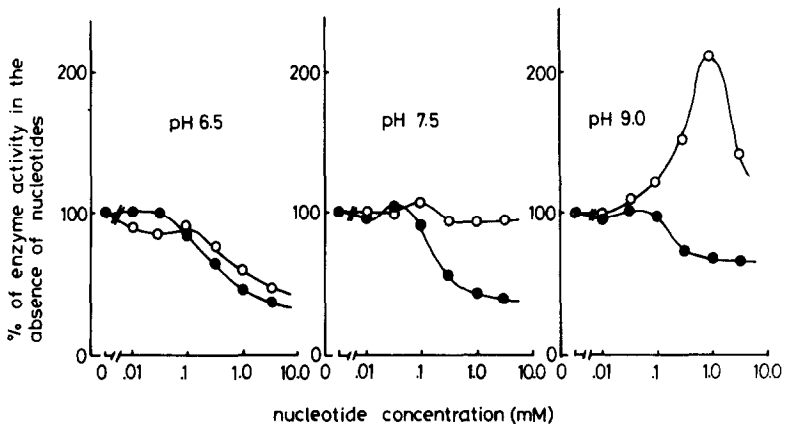

Fig. 1 Effects of purine nucleotides on the methyltransferase activities in rat liver plasma membranes.
Left panel: The reaction mixture contained 10 mM $MgCl_2$, 0.1 mM EGTA and 50 mM sodium acetate buffer. Center and right panel: The reaction mixture contained 0.1 mM EGTA and 50 mM sodium borate buffer. Each reaction was performed in the presence of 100 μg of phosphatidyl-N-monomethylethanolamine. Effect of GTP(—o—) and of ATP(—●—) were illustrated.

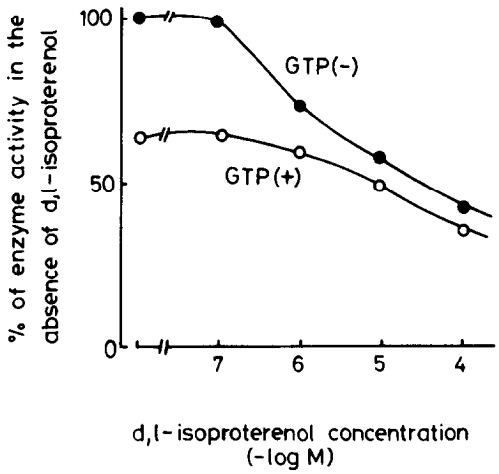

Fig. 2 Effect of d,l-isoproterenol on the methyltransferase I. Each datum indicates the mean of triplicate determinations. Concentration of GTP was 10^{-4}M.

ferase I activity with concentration dependent manner. GTP further diminished the enzyme activity as shown in Fig. 2. ATP also diminished the enzyme activity. On the contrary, GTP stimulated d,l-isoproterenol-induced methylation of phosphatidyl-N-monomethylethanolamine at pH 7.5 or at pH 9.0 as shown in Fig. 3. When ATP, instead of GTP, was used the d,l-isoproterenol-induced methylation was not modified.

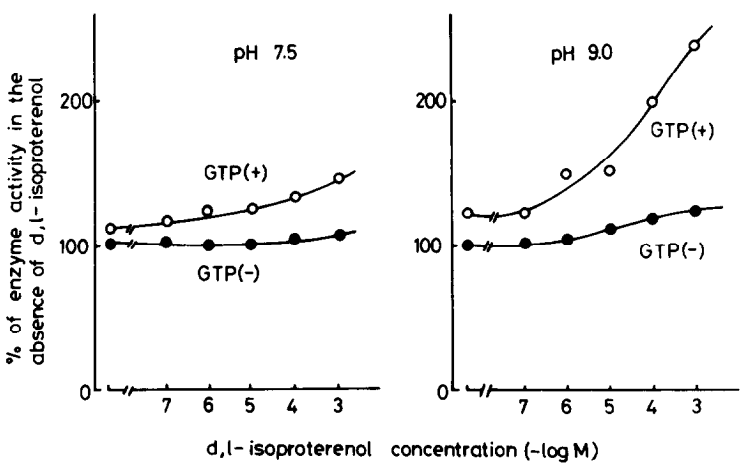

Fig. 3 Effect of d,l-isoproterenol on the methyltransferase II. Each datum indicates the mean of triplicate determinations. Concentration of GTP used was 10^{-4}M.

Table 1. Effect of cyclic AMP or theophylline in the methyltransferase II activity.

Added substance	Concentration(mM)	% of enzyme activity
Buffer	–	100.0
Cyclic AMP	10^{-5}	98.7
	10^{-4}	93.8
Theophylline	10^{-4}	96.5
	10^{-3}	92.8
Cyclic AMP + Theophylline	10^{-4}(Cyclic AMP) 10^{-5}(Theophylline)	96.4

Each result indicates the mean of triplicate determinations.

GTP has been known to be important in the coupling of receptor and adenylate cyclase activity(7). In order to investigate the effect of adenylate cyclase activation on the methyltransferase II, cyclic AMP and theophylline were employed in the experiments. In the presence of 10^{-5}-10^{-4}M of cyclic AMP or 10^{-4}-10^{-3}M of theophylline, the methyltransferase activity was not modified at pH 9.0 (table 1).

DISCUSSION

It is well established that phosphatidylcholine biosynthesis via methylation pathway is mediated by two enzymes, methyltransferase I and II(2,4,6,7). As is suggested by Hirata and Axelrod(2), the first enzyme localized on the cytoplasmic side of the membrane whereas the second faces the outside. This asymmetric arrangement of the two enzymes across the membrane seems to make possible the stepwise methylation of phosphatidylethanolamine and to facilitate the rapid transmembrane transfer of the methylated phospholipid, phosphatidylcholine. On the other hand, it is known that l-isoproterenol stimulates phospholipid methylation and that results in an acceleration of β-adrenergic receptor-adenylate cyclase coupling(3). An it is postulated that unoccupied β-receptors interacts with the methyltransferases to decrease their activity and that this suppression

is removed and phospholipid methylation proceeds at an accelerated rate when the β-adrenergic receptor is bound to its agonist. The resultant accumulation of phosphtidyl-N-monomethylethanolamine within the membrane decreases membrane viscosity, and this makes possible rapid lateral movement of the β-receptor-hormone complex.

In the present study, we have demonstrated that GTP has variable actions on phospholipid methylation. Methyltransferase I was inhibited by GTP whereas methyltransferase II was stimulated. As GTP directly interacts with both enzymes, GTP may have regulatory roles in these enzyme activities. Both enzymes may have GTP binding sites. Methyltransferase I may usually bound to GTP and this GTP-bound form of the enzyme is inactive. On the contrary, the binding sites for GTP in methyltransferase II are usually unoccupied. When β-adrenergic agent binds to its receptor, methyltransferase II is dissociated from the enzyme-receptor complex. At the same time, GTP, which has previously bound to methyltransferase I is transferred to methyltransferase II. In this situation, both enzymes are activated and accumulate a large amount of phosphatidyl-N-monomethylethanolamine, and that results in an accelration of coupling of β-adrenergic receptor and adenylate cyclase. In our study, d,l-isoproterenol directly inhibited methyltransferase I. Although the precise mechanism is uncertain, the β-adrenergic receptor may also be present as a complex with this enzyme.

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