Effects of Methyl-Group Acceptors on the Regulation of Plasma Cholesterol Level in Rats Fed High Cholesterol Diets

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(Received July 21, 1989)

Summary The effects of methyl-group acceptors such as glycine, guanidinoacetic acid, and nicotinamide on cholesterol metabolism and phosphatidylcholine (PC) biosynthesis were investigated with rats fed a 25% casein diet containing cholesterol with or without methionine supplement. The effect of ethanolamine, an indirect methyl-group acceptor via phosphatidylethanolamine (PE) formation, was also compared with those of methyl-group acceptors. The methyl-group acceptors and ethanolamine decreased or tended to decrease plasma total cholesterol level when added to the 25% casein diet. These compounds also significantly depressed the methionine-induced enhancement of plasma cholesterol level. The activity of PE N-methyltransferase was decreased by the addition of glycine, guanidinoacetic acid, and nicotinamide, but not ethanolamine, to the reaction mixture when assayed using the postmitochondrial fraction of liver homogenate, suggesting that PE N-methyltransferase activity can be depressed by glycine N-methyltransferase, guanidinoacetic acid N-methyltransferase, and nicotinamide N-methyltransferase systems. The PE N-methyltransferase activity in liver microsomes, however, did not decrease in response to the dietary addition of methyl-group acceptors. The in vitro incorporation of [CH$_3$-$_{14}$C]methionine into PC of liver slices was also significantly inhibited by the addition of glycine and nicotinamide, but not guanidinoacetic acid and ethanolamine, to the incubation medium. It is suggested that methyl-group acceptors can decrease plasma cholesterol level at least in part through the depression of PC biosynthesis via PE N-methylation pathway, and that the mechanism for the plasma cholesterol-lowering effect of ethanolamine is different from that of methyl-group acceptors.

Key Words cholesterol, methionine, glycine, guanidinoacetic acid, nicotinamide, ethanolamine, methyl-group acceptor, phosphatidylcholine, methyltransferase

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The plasma cholesterol level is influenced by various dietary components including certain kinds of amino acid. Previously we reported the differential effects of sulfur-containing amino acids in rats fed a 25% casein diet containing cholesterol; methionine increased plasma cholesterol level while cystine and taurine decreased it (1). The plasma cholesterol-elevating effect of methionine could be effectively prevented by the concurrent addition of glycine to the diet (2). Although this interacting effect of methionine and glycine on the plasma cholesterol level was assumed to be associated with the metabolism of methyl-group of methionine (2, 3), the detailed mechanism is not fully elucidated. A variety of biological effects of methionine arise either from methyl-group or from sulfur portion of the amino acid. It is well confirmed that phosphatidylethanolamine (PC) can by synthesized via phosphatidylethanolamine (PE) N-methylation pathway in addition to via cytidine 5'-diphosphate (CDP)-choline pathway (4). Since PC is a major phospholipid of plasma lipoproteins including very-low-density lipoprotein (VLDL), the PC biosynthesis has a significant role in the assembly and secretion of VLDL from the liver and therefore in the regulation of plasma cholesterol level (5, 6). Hence, it can be expected that dietary supplementation with methionine stimulates the PC biosynthesis via PE N-methylation pathway and thereby stimulates the synthesis and secretion of VLDL. In fact, it was shown that methionine supplementation could increase plasma cholesterol level in rats fed a choline-deficient low casein diet containing cholesterol (7). Thus, methionine can replace choline in the effect on the plasma cholesterol level. However, it remains to be confirmed whether the methionine-induced hypercholesterolemia observed in rats fed diets containing adequate choline can be accounted for also by the stimulation of PC biosynthesis, since it is reported that inhibition of the methylation of PE for the synthesis of PC does not affect the amount of apoproteins or PC secreted into the lipoproteins when CDP-choline pathway is functional in cultured rat hepatocytes (8).

On the other hand, methionine rather decreases plasma cholesterol level when added to certain kinds of diet, e.g., low protein (2, 7), soy protein (9), and glycine-supplemented (2, 3) diets containing cholesterol. Hence, it appears likely that the effect of methionine on the plasma cholesterol level might be influenced by dietary conditions that affect the metabolism of methionine, especially methyl-group of methionine.

In this study, we investigated the effects of methyl-group acceptors such as glycine, guanidinoacetic acid, and nicotinamide on the cholesterol metabolism and PC biosynthesis in rats fed a high cholesterol diet with or without methionine supplement in order to obtain insight into the mechanism of plasma cholesterol-elevating effect of methionine. For comparison, the effect of ethanolamine, an indirect methyl-group acceptor via PE formation, was also investigated.
MATERIALS AND METHODS

Male rats of the Wistar strain (Japan SLC Inc., Hamamatsu) weighing about 140 g (Expt. 1) or 120 g (Expt. 2) were fed the experimental diets for 3 (Expt. 1) or 2 (Expt. 2) weeks in a temperature-controlled (24 ± 1°C) room with a 12-h cycle of light (06:00 to 18:00) and dark. The basal diet (25C) contained 25% casein, 15% sucrose, 15% lard, 2% corn oil, 5% salt mixture (10), 1% vitamin mixture (10), 0.2% choline chloride, 2% cellulose powder, 1% cholesterol, 0.25% sodium cholate, and α-corn starch to make 100%. The salt and vitamin mixtures were purchased from Oriental Yeast Co., Tokyo. The addition to the basal diet was made at the expense of starch. Glycine and methionine were added to the basal diet at levels of 3% and 0.8%, respectively, according to our previous study (3). Guanidinoacetic acid, nicotinamide, and ethanolamine were added at the same level as that of methionine. At the end of the feeding period, food was removed at 24:00, and animals were killed to obtain blood plasma and liver between 11:00 and 12:00 on the following day. Feces were collected for the last 3 days in the experiment 1. The plasma concentrations of total cholesterol, high-density-lipoprotein (HDL) cholesterol, triglycerides, and phospholipids were measured enzymatically with kits (Wako Pure Chemical Ind., Osaka). The total lipids in the liver and steroids in the feces were extracted by the methods of Folch et al. (11) and Van Beresteyn et al. (12), respectively. The cholesterol, triglycerides, and phospholipids in the liver extracts were measured colorimetrically by the methods of Zak (13), Fletcher (14), and Bartlett (15), respectively. Neutral steroids, i.e., cholesterol and coprostanol, were measured by gas-liquid chromatography using 5α-cholestan as an internal standard. The total bile acids in the fecal extracts were measured enzymatically using 3α-hydroxysteroid dehydrogenase (16).

For enzyme assays, about 2.5 g of the liver was homogenized with 4 volumes (v/w) of 0.01 M sodium phosphate buffer (pH 7.4) containing 0.15 M KCl, then the homogenates were centrifuged at 10,000 × g for 10 min. The supernatants (postmitochondrial fraction) were centrifuged at 105,000 × g for 60 min to precipitate microsomal fraction. The activities of cholesterol 7α-hydroxylase (7α-hydroxylase), PE N-methyltransferase and CTP: cholinephosphate cytidyltransferase (cytidyltransferase) in liver microsomes were measured according to the methods of Ogishima and Okuda (17), Tanaka et al. (18), and Sleight and Kent (19), respectively. In one experiment, postmitochondrial fraction was also employed for the assay of PE N-methyltransferase activity. The incorporation of [CH$_3$-14C]methionine into PC of liver slices prepared from rats fed 25C and 25C + 0.8% methionine diets was measured according to the method of Case et al. (20) with slight modifications of incubation conditions; about 0.2 g of liver slices prepared with a hand slicer of Stadie-Riggs type were incubated in 4 ml of Krebs-phosphate buffer containing 1 mM [CH$_3$-14C] methionine (18.5 kBq/μmol) and 15 mM glucose at 37°C for 60 min under atmosphere of O₂. The incorporation of [CH$_3$-14C]methionine into phospholipid choline was assumed as the incorporation into PC, since the content
of choline-containing phospholipids other than PC is considerably low (21). S-Adenosyl-t-[CH$_3$-$^{14}$C]methionine, [CH$_3$-$^{14}$C]cholinephosphate, and [CH$_3$-$^{14}$C]-methionine used in the enzyme assay and incorporation experiment were purchased from Amersham Japan Co., Tokyo.

Protein was measured by the method of Lowry et al. (22) using bovine serum albumin as a standard. Experimental data were statistically analyzed using Duncan's multiple-range test (23).

RESULTS

Table 1 shows the effects of methyl-group acceptors and ethanolamine on growth, food intake, liver weight, and liver lipid content in rats fed a 25% casein diet with or without methionine supplement (Expt. 1). The addition of 0.8% nicotinamide markedly depressed growth and food intake. The slight but significant depression by 0.8% methionine of growth was prevented by the addition of glycine and guanidinoacetic acid. Methionine supplementation slightly decreased liver cholesterol content. Although the addition of guanidinoacetic acid to the 25C diet significantly increased liver triglyceride content, this increase was partially prevented by methionine supplementation. The addition of nicotinamide caused lower liver levels of cholesterol and triglyceride probably due in part to the reduction of food intake.

Table 2 shows plasma lipid concentrations. The plasma total cholesterol level significantly decreased or tended to decrease when methyl-group acceptors or ethanolamine were added to the 25C diet. Addition of methionine to the 25C diet markedly enhanced plasma total cholesterol level, but this rise was depressed in rats fed diets supplemented with methyl-group acceptors or ethanolamine. The plasma HDL-cholesterol level was considerably increased by the addition of nicotinamide. Although there was no significant difference in the plasma triglyceride level among groups, the plasma phospholipid level changed in a similar manner to that of plasma total cholesterol level; there was a significant correlation between plasma phospholipid level and plasma total cholesterol level ($p < 0.01$).

Table 3 shows fecal steroid excretion and the activities of certain enzymes which participate in the biosynthesis of bile acids and PC in liver microsomes. The excretion of neutral steroids and bile acids into feces was expressed as percentages of the amounts of cholesterol and sodium cholate ingested during collection of feces on a molar basis, respectively. There was no large difference in the excretion of neutral steroids among groups, whereas methionine supplementation increased fecal bile acid excretion as compared with diets without methionine supplement. Nicotinamide increased fecal bile acid excretion irrespective of dietary methionine level. The activity of 7α-hydroxylase, the rate-limiting enzyme for the biosynthesis of bile acids, tended to be enhanced by methionine supplementation; there was a significant correlation between the enzyme activity and fecal bile acid excretion ($p < 0.05$), but not between the enzyme activity and plasma total cholesterol level.
Table 1. Body weight gain, food intake, liver weight, and liver lipid content in rats fed experimental diets (Expt. 1).

| Diets               | Body wt. gain (g/21 day) | Food intake (g/21 day) | Liver wt. (g/100 g body wt.) | Liver lipids (mg/g) |
|---------------------|--------------------------|------------------------|-------------------------------|---------------------|
|                     |                          |                        | CHOL                          | TG                  | PL                  |
| 25C                 | 116 ± 6\(^1\)\(^a\)     | 282 ± 7\(^b\)         | 5.02 ± 0.07\(^a\)            | 81 ± 1\(^a\)        | 73 ± 4\(^a\)        | 23.5 ± 0.3\(^a\)    |
| 25C + 3.0% Gly\(^2\) | 120 ± 8\(^c\)           | 286 ± 11\(^b\)        | 4.84 ± 0.19\(^a\)            | 75 ± 2\(^b\)        | 74 ± 7\(^a\)        | 23.4 ± 0.6\(^b\)    |
| 25C + 0.8% GA       | 118 ± 3\(^c\)           | 297 ± 5\(^a\)         | 5.89 ± 0.10\(^c\)            | 81 ± 2\(^a\)        | 136 ± 5\(^b\)       | 20.0 ± 0.3\(^c\)    |
| 25C + 0.8% NA       | 52 ± 1\(^d\)            | 213 ± 5\(^c\)         | 4.85 ± 0.09\(^a\)            | 59 ± 2\(^c\)        | 45 ± 5\(^c\)        | 26.6 ± 0.4\(^d\)    |
| 25C + 0.8% EA       | 111 ± 2\(^b\)           | 275 ± 6\(^ab\)        | 5.07 ± 0.10\(^a\)            | 81 ± 2\(^a\)        | 67 ± 3\(^a\)        | 22.7 ± 0.3\(^b\)    |
| 25C + 0.8% Met (25CM)| 97 ± 6\(^e\)             | 260 ± 8\(^d\)         | 5.07 ± 0.13\(^a\)            | 71 ± 2\(^b\)        | 80 ± 5\(^a\)        | 23.0 ± 0.3\(^ab\)   |
| 25CM + 3.0% Gly     | 113 ± 3\(^b\)           | 270 ± 6\(^bd\)        | 4.57 ± 0.04\(^bd\)           | 60 ± 2\(^c\)        | 74 ± 4\(^a\)        | 23.9 ± 0.9\(^a\)    |
| 25CM + 0.8% GA      | 131 ± 2\(^e\)           | 284 ± 6\(^ab\)        | 4.86 ± 0.08\(^ab\)           | 64 ± 2\(^c\)        | 93 ± 3\(^d\)        | 22.1 ± 0.3\(^b\)    |
| 25CM + 0.8% NA      | 52 ± 3\(^d\)            | 220 ± 5\(^c\)         | 4.35 ± 0.11\(^d\)            | 46 ± 4\(^d\)        | 50 ± 2\(^c\)        | 27.4 ± 0.3\(^d\)    |
| 25CM + 0.8% EA      | 104 ± 5\(^be\)          | 264 ± 8\(^bd\)        | 4.65 ± 0.11\(^bd\)           | 58 ± 1\(^c\)        | 76 ± 5\(^a\)        | 23.9 ± 0.4\(^a\)    |

\(^1\) Values are mean ± SE for 6 rats; values in a column not sharing the same superscript letter are significantly different at \(p < 0.05\).

\(^2\) Abbreviations: Gly, glycine; Met, methionine; GA, guanidinoacetic acid; NA, nicotinamide; EA, ethanolamine hydrochloride; CHOL, cholesterol; TG, triglyceride; PL, phospholipid.
Table 2. Concentrations of plasma lipids in rats fed experimental diets (Expt. 1).

| Diet              | Plasma lipid concentration (mg/100ml) |
|-------------------|---------------------------------------|
|                   | Total CHOL (a) | HDL-CHOL (b) | (b/a) × 100 | TG | PL |
| 25C               | 183 ± 13b      | 23 ± 1b      | 13.0 ± 1.1b | 184 ± 10b | 171 ± 4bd |
| 25C + 3.0% Gly    | 132 ± 5bc      | 23 ± 1b      | 16.7 ± 2.0bc | 186 ± 18b | 151 ± 6cd |
| 25C + 0.8% GA     | 134 ± 7bc      | 28 ± 2ab     | 21.2 ± 2.0bc | 157 ± 13b | 158 ± 5ae |
| 25C + 0.8% NA     | 153 ± 11ab     | 39 ± 4c      | 26.5 ± 3.2cd | 167 ± 12b | 181 ± 6b  |
| 25C + 0.8% EA     | 139 ± 5b       | 23 ± 1a      | 15.9 ± 1.2ab | 159 ± 16b | 152 ± 7ed |
| 25C + 0.8% Met (25CM) | 392 ± 22d   | 32 ± 1b      | 16.7 ± 1.0ab | 167 ± 8a  | 245 ± 8e  |
| 25CM + 3.0% Gly   | 97 ± 4e        | 27 ± 1ab     | 27.6 ± 1.2d  | 157 ± 12a | 136 ± 4d  |
| 25CM + 0.8% GA    | 126 ± 8bc      | 30 ± 1b      | 23.9 ± 1.7cd | 170 ± 20a | 153 ± 6cd |
| 25CM + 0.8% NA    | 158 ± 10ab     | 54 ± 3d      | 35.1 ± 2.6c  | 154 ± 10a | 184 ± 6b  |
| 25CM + 0.8% EA    | 105 ± 5c       | 29 ± 1ab     | 27.6 ± 1.4d  | 151 ± 7a  | 142 ± 3cd |

1 See footnote 1 in Table 1. Abbreviations: see footnote 2 in Table 1; HDL, high density lipoprotein.
Table 3. Fecal steroid excretion and activities of selected enzymes participating in the biosynthesis of bile acids and phosphatidylcholine in liver microsomes in rats fed experimental diets (Expt. 1).

| Diet              | Fecal steroid excretion (% of ingestion) | Enzyme activities |
|-------------------|----------------------------------------|-------------------|
|                   | NS                                     | BA                | 7α-Hydroxylase² | PE N-methyltransferase² | Cytidyltransferase³ |
| 25C               | 52.8 ± 2.0¹,a                          | 145 ± 8³         | 6.6 ± 1.0³     | 91 ± 3³                 | 1.09 ± 0.03³       |
| 25C + 3.0% Gly    | 51.8 ± 2.2²ab                         | 144 ± 6³         | 12.2 ± 2.1³bc  | 104 ± 7³ab              | 1.19 ± 0.09³ab     |
| 25C + 0.8% GA     | 46.4 ± 0.9b                           | 124 ± 2²b        | 9.3 ± 1.2³b    | 126 ± 6³cd              | 1.46 ± 0.07³c      |
| 25C + 0.8% NA     | 52.9 ± 2.4³                          | 161 ± 5³e        | 14.6 ± 1.7³bcd | 109 ± 7³b               | 1.28 ± 0.11³b      |
| 25C + 0.8% EA     | 49.9 ± 2.3³ab                         | 126 ± 3³b        | 7.5 ± 0.8³     | 108 ± 8³b               | 1.14 ± 0.05³ab     |
| 25C + 0.8% Met (25CM) | 51.4 ± 1.8³ab                       | 160 ± 5³e        | 27.3 ± 3.9³e   | 91 ± 4³                 | 1.18 ± 0.04³ab     |
| 25CM + 3.0% Gly   | 48.8 ± 1.3³ab                         | 176 ± 3³de       | 16.7 ± 2.0³cd  | 107 ± 4³b               | 1.02 ± 0.05³a      |
| 25CM + 0.8% GA    | 46.8 ± 0.6³e                         | 164 ± 5³e        | 20.8 ± 2.4³d   | 114 ± 4³bcd              | 1.18 ± 0.03³b      |
| 25CM + 0.8% NA    | 49.2 ± 1.8³ab                         | 181 ± 6³d        | 31.2 ± 2.7³e   | 129 ± 4³d               | 1.12 ± 0.07³ab     |
| 25CM + 0.8% EA    | 50.4 ± 1.6³ab                         | 165 ± 4³e        | 17.0 ± 1.5³ed  | 112 ± 3³bc              | 1.02 ± 0.05³a      |

¹ See footnote 1 in Table 1. ² Activity was expressed as pmol/min/mg of protein. ³ Activity was expressed as nmol/min/mg of protein. Abbreviations: NS, neutral steroids; BA, bile acids; for others, see footnote 2 in Table 1.
Fig. 1. In vitro effects of methyl-group acceptors and ethanolamine on PE
N-methyltransferase activity measured with postmitochondrial (A) or microsomal
(B) fraction (Expt. 1). Aliquots of postmitochondrial or microsomal fraction
prepared from livers to rats fed a 25C+0.8% methionine diet were incubated with
0.2 mM [CH$_3$-14C]-S-adenosylmethionine at 37°C for 30 min in the absence (control)
or presence of methyl-group acceptors and ethanolamine at concentrations indicated.
Values are mean of triplicate (control) or duplicate assays. The values of controls
in A and B were 23.8 and 98.5 (pmol/min/mg of protein), respectively, and they
were designated at 100%.

The activity of PE N-methyltransferase tended to increase in rats fed diets
supplemented with methyl-group acceptors or ethanolamine, although the extent
of increase was small. Unexpectedly, methionine supplementation did not increase
PE N-methyltransferase activity. There was no large difference in the activity of
cytidyltransferase, the regulatory enzyme for PC biosynthesis via CDP-choline
pathway, among groups.

Figure 1 shows the effects of methyl-group acceptors and ethanolamine on PE
N-methyltransferase activity in both postmitochondrial and microsomal fractions
of the liver obtained from rats fed a 25C+0.8% methionine diet in the experiment
1. When postmitochondrial fraction was used as enzyme and endogenous PE sources,
the enzyme activity was depressed by the addition of glycine (about 80% depression),
guanidinoacetic acid (about 30%), and nicotinamide (about 20%) to the reaction
mixture. Ethanolamine had not effect on the enzyme activity. On the other hand,
the depression of PE N-methyltransferase activity by glycine was only slight when
microsomal fraction was employed, indicating that the cytosol fraction is necessary
for the depression of the enzyme activity by glycine and probably other methyl-group
Figure 2 shows the effects of methyl-group acceptors and ethanolamine on the incorporation of [CH$_3$-¹⁴C]methionine into PC of liver slices prepared from rats fed a 25C or 25C+0.8% methionine (25CM) diet (Expt. 2). Liver slices were incubated in the absence (control) or presence of methyl-group acceptors and ethanolamine at a concentration of 5 mM. The column and its bar represent mean value and SE, respectively, for 5 rats. The values of controls in rats fed 25C and 25CM diets were 27.7±3.0 and 22.9±1.7 ($\times 10^3$ dpm/h/g of liver), respectively, and they were designated as 100%.

**DISCUSSION**

The plasma cholesterol-lowering effect of glycine was first demonstrated by Hermus and Dallinge-Thie (24) with rabbits. Katan et al. (25) have shown that glycine has a plasma cholesterol-lowering efficacy also in rats fed a high cholesterol diet. On the other hand, we have shown that the plasma cholesterol-lowering effect of glycine is reinforced by methionine supplement in rats fed high cholesterol diets (2), and that although glycine, as well as taurine, can be a substrate for bile acid conjugation in the liver, the effect of glycine cannot be fully explained in terms...
of augmentation of bile acid excretion into feces (26). We assumed that the plasma cholesterol-lowering effect of glycine might be elicited at least in part through influence on the metabolism of methyl-group of methionine (2, 3, 26), since glycine is known to function as a methyl-group acceptor in the liver. The validity of such an assumption would be justified if many other methyl-group acceptors which can significantly affect the metabolism of methyl-group of methionine had a similar effect to that of glycine. As a whole, the results obtained here appear to support the above assumption. Because, not only glycine but also guanidinoacetic acid and nicotinamide could depress the methionine-induced enhancement of plasma cholesterol level, although the effect of nicotinamide must be carefully interpreted since the compound caused marked depression of growth and food intake. These findings, on the contrary, support that the plasma cholesterol-elevating effect of methionine might be mainly due to its capability of donating methyl-group to PE via S-adenosylmethionine (SAM) formation.

Several reports have shown a significant role of PC biosynthesis in lipoprotein assembly and its secretion from the liver cells and hence in the regulation of plasma cholesterol level (5, 6). It is well confirmed that the reduction of PC biosynthesis due to dietary choline-deficiency results in significant decrease in plasma lipids including cholesterol (5, 7, 27, 28). Yao and Vance (29) have demonstrated that the addition of choline or methionine to the medium of cultured hepatocytes isolated from rats fed a choline-deficient diet increases VLDL secretion into the medium. Consistent with this, we have demonstrated that the plasma cholesterol level is markedly enhanced by dietary supplementation with methionine (1-3) or choline chloride (3, 30, 31) in rats fed a high cholesterol diet. Furthermore, the present study confirmed a possibility that methionine-induced hypercholesterolemia can be prevented by methyl-group acceptors which can depress PE N-methyltransferase activity. From these results, it is likely that the plasma cholesterol-elevating effect of methionine might be elicited, if not entirely, through the stimulation of PC biosynthesis via PE N-methylation pathway, although direct evidence remains to be offered.

There are a number of methyltransferases which catalyze the transfer of methyl-group of SAM to various acceptors including glycine, guanidinoacetic acid, nicotinamide, and PE in the liver of higher animals. Most of these methyltransferases exist in the cytosol of cells, whereas PE N-methyltransferase is localized to the microsomal fraction (32). Kerr (33) has shown that tRNA methyltransferase activity is decreased by glycine and glycine N-methyltransferase (glycine N-methyltransferase system) in the liver of rabbits; she referred to these two methyltransferases as competing methyltransferases. Further, tRNA methyltransferase activity was also shown to be decreased by nicotinamide N-methyltransferase system (34). It is assumed that the decrease in tRNA methyltransferase activity by other methyltransferase system is attributable not only to the competition for SAM but also to the differential sensitivity of methyltransferases to S-adenosylhomocysteine, a reaction product which inhibits methyltransferases (33). Similarly, the results of the
present study showed a possibility that glycine $N$-methyltransferase, guanidinoacetic acid $N$-methyltransferase, and nicotinamide $N$-methyltransferase systems in the cytosol fraction can decrease the activity of PE $N$-methyltransferase in the microsomal fraction. It appears unlikely that PE $N$-methyltransferase activity is inhibited directly by glycine and other methyl-group acceptors since the depression of the enzyme activity by glycine was only slight when microsomal fraction was employed for the enzyme assay. The slight depression observed with microsomal fraction seems to be due to contamination with cytosol fraction since microsomal fraction was not washed in the present study.

Although the activities of methyltransferases for glycine, guanidinoacetic acid, and nicotinamide were not measured in the present study, the extent of depression of PE $N$-methyltransferase activity by methyl-group acceptors seems to indicate roughly the order of intensity of respective methyltransferase activity. This assumption is consistent with results on the depression of incorporation of $[\text{CH}_3{}^{14}\text{C}]$methionine into PC of liver slices except for the effect of guanidinoacetic acid. Although the reason for disparity between the effects of guanidinoacetic acid on PE $N$-methyltransferase activity and $[\text{CH}_3{}^{14}\text{C}]$methionine incorporation is not known, one possibility is that guanidinoacetic acid was not fully taken up by tissues of liver slices. In any event, it is evident that glycine $N$-methyltransferase system has the most potent effect on PE $N$-methyltransferase activity.

On the other hand, PE $N$-methyltransferase activity was essentially insensitive to dietary methionine supplement while it tended to be increased by the addition of methyl-group acceptors to the diet. This, however, does not mean that PC biosynthesis via PE $N$-methylation pathway is also insensitive to dietary methionine level, since the concentration of substrates such as SAM and PE for the enzyme is considered to be an important factor in the PC biosynthesis via PE $N$-methylation pathway (6). It is known that the concentration of SAM in the liver increases in response to dietary methionine content (35) and decreases in the presence of sufficient glycine (36). Ridgway et al. (37) have recently shown that the enzyme protein of PE $N$-methyltransferase exists in a constitutive manner, and that changes in enzyme activity are related to altered PE levels in the rat liver. The hepatic levels of SAM in the regulation of PC biosynthesis and plasma cholesterol level remains to be further elucidated.

The serum cholesterol-lowering effect of ethanolamine has been demonstrated in rats (38) and mice (39) fed cholesterol-free diets. The present study also showed that ethanolamine could decrease plasma cholesterol level in rats fed a high cholesterol diet and prevent methionine-induced hypercholesterolemia. Of interest is that the effects of glycine and ethanolamine on plasma cholesterol level were apparently similar while the effects of these compounds on PE $N$-methyltransferase activity and incorporation of $[\text{CH}_3{}^{14}\text{C}]$methionine into PC were quite different. The mechanism by which ethanolamine elicits its plasma cholesterol-lowering effect is not known. But, it is noteworthy that the PC formed by methylation of PE derived from ethanolamine is not required for lipoprotein secretion in rat...
hepatocytes (40). This might be related to the preventive effect of ethanolamine on methionine-induced hypercholesterolemia.

It has been reported that nicotinic acid has plasma total cholesterol-lowering (41) and HDL-cholesterol-elevating (42) effects in humans, although the mechanism is not fully understood. It is not known at present whether the effect of nicotinic acid on cholesterol metabolism is related to the effect of nicotinamide as shown in the present study. Nicotinic acid is known to cause considerable side effects when administered at high levels (43). The addition of 0.8% nicotinamide also caused relatively severe growth depression in the present study. The effect of nicotinamide on lipid metabolism at lower addition levels and the comparison of the effects of nicotinic acid and nicotinamide remain to be further studied.

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