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# Eritadenine-induced alteration of hepatic phospholipid metabolism in relation to its hypocholesterolemic action in rats

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*The hypocholesterolemic effect of dietary supplementation with eritadenine, a hypocholesterolemic factor present in the* Lentinus edodes *mushroom, was investigated in relation to its effect on hepatic phospholipid metabolism in rats. The plasma total cholesterol level was significantly decreased by eritadenine supplementation at levels above 8 μmol/kg of diet in a dose-dependent manner, accompanying decreases in both VLDL + LDL and HDL cholesterol levels. Eritadenine supplementation significantly increased the phosphatidylethanolamine (PE) content and inversely decreased the phosphatidylcholine (PC) content of liver microsomes in a dose-dependent manner. There was a highly significant correlation between plasma cholesterol levels and the content or proportion of PC and PE of liver microsomes. Eritadenine supplementation did not decrease the activity of PE* N*-methyltransferase in liver microsomes but rather increased the activity, possibly because of the increased PE content of liver microsomes. On the one hand, eritadenine had no direct inhibitory effect on the enzyme activity when added to the assay mixture. On the other hand, eritadenine supplementation increased the hepatic* S*-adenosylhomocysteine (SAH) level and decreased the ratio of* S*-adenosylmethionine (SAM) to SAH in a dose-dependent manner. The in vivo incorporation of radioactivity of [methyl-$^3$H]methionine into the PC of liver microsomes and blood plasma was also markedly depressed by dietary eritadenine supplementation at a level of 200 μmol/kg of diet. These results suggest that the hypocholesterolemic action of eritadenine might be elicited through an alteration of the hepatic phospholipid metabolism that resulted from an inhibition of PE* N*-methylation due to a decreased SAM/SAH ratio in the liver.* (J. Nutr. Biochem. 6: 80–87, 1995.)

**Keywords:** eritadenine; hypocholesterolemic action; plasma cholesterol; phosphatidylcholine; phosphatidylethanolamine; phosphatidylethanolamine *N*-methylation

## Introduction

Certain species of mushrooms are known to have a plasma cholesterol–lowering effect when added to the diet of experimental animals.[1–5] As a potent hypocholesterolemic factor, eritadenine (2(*R*),3(*R*)-dihydroxy-4-(9-adenyl)-butyric acid) (*Figure 1*) was isolated and identified by several groups of investigators from *Lentinus edodes*,[6–8] a mushroom that has been abundantly consumed in Japan for many years. Previous studies on the hypocholesterolemic action of eritadenine have shown that eritadenine did not inhibit cholesterogenesis in the liver[9] nor stimulate steroid excretion into feces.[10] Previous studies have also suggested that *L. edodes* or eritadenine might exert its hypocholesterolemic action through depressed secretion of lipoprotein cholesterol from the liver into blood circulation[11,12] and/or through increased uptake of plasma cholesterol by tissues.[9] However, the detailed mechanism is not yet fully understood. Recently, we found that dietary supplementation with a powder of *L. edodes* evoked a marked alteration of hepatic phospholipid composition, especially the ratio of

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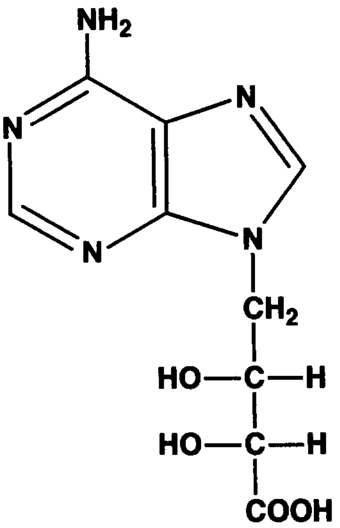

**Figure 1** Structure of eritadenine.

phosphatidylcholine (PC) to phosphatidylethanolamine (PE) with a significant correlation with plasma cholesterol levels, suggesting that the hypocholesterolemic action of *L. edodes* might be evoked through an alteration of hepatic phospholipid metabolism.[13]

In the present study, we investigated the dose-dependent effect of dietary eritadenine on the profile of microsomal phospholipids in the rat liver in order to further examine whether or not an alteration of hepatic phospholipid metabolism is involved in the hypocholesterolemic action of *L. edodes*. The mechanism by which dietary eritadenine altered the hepatic phospholipid composition was also investigated.

## Methods and materials

### Materials

The eritadenine used in the present study was isolated from dried *L. edodes* according to the method of Tokita et al.[8] *S*-[methyl-$^{14}$C]adenosyl-L-methionine and L-[methyl-$^{3}$H]methionine were obtained from Amersham (USA), and [1,2-$^{14}$C]choline chloride was obtained from New England Nuclear (USA). Mineral and vitamin mixtures were obtained from Nihon Nosan Kogyo Co. (Tokyo, Japan).

### Animals and diets

Male rats of the Wistar strain, weighing 90 to 100 g (5 weeks of age), were obtained from Japan SLC (Hamamatsu, Japan). They were housed individually in stainless wire-mesh cages in a temperature (24 ± 1°C)- and humidity (50 to 60%)-controlled room with a 12-hr cycle of light (6:00 a.m. to 6:00 p.m.) and dark. Animals were allowed free access to food and water. After feeding a stock (25% casein) diet for 6 to 7 days, rats were divided into experimental groups. The basal diet contained (per kg of diet) 250 g of casein, 432.5 g of corn starch, 200 g of sucrose, 50 g of corn oil, 35 g of mineral mixture (AIN-76 composition), 10 g of vitamin mixture (AIN-76 composition), 2.5 g of choline chloride, and 20 g of cellulose powder.

In Experiment 1, 56 rats were fed the basal diet or diets supplemented with eritadenine at levels of 4 to 200 μmol/kg of diet for 14 days. The amount of 4 μmol of eritadenine corresponds to 1 mg. Animals were killed by decapitation under light anesthesia with diethylether between 11:00 a.m. and 12:00 a.m. to obtain blood and livers. In Experiment 2, 10 rats were fed the basal diet or a diet supplemented with eritadenine at a level of 200 μmol/kg of diet for 14 days. On the 15th day, food was removed at 7:00 a.m. and rats were injected intraperitoneally with 0.5 ml of saline that contained 814 kBq of [methyl-$^{3}$H]methionine (3,138 MBq/mol) and 163 kBq of [1,2-$^{14}$C]choline chloride (2.22 MBq/mol) at around 11:00 a.m. The animals were killed just 2 hr later in a similar manner as Experiment 1.

### Analyses

Blood plasma was obtained from heparinized whole blood by centrifugation at 2,000*g* for 20 min and stored at 4°C until subsequent lipid analyses. The whole liver was quickly excised, rinsed in ice-cold saline, blotted on filter paper, cut into three portions, and weighed. Two portions of the liver were quickly frozen in liquid nitrogen and stored at −80°C until analyses for lipids and metabolites of methionine. The residual portion of the liver was homogenized in 4 vol (v/w) of an ice-cold 10 mmol/L Tris-HCl buffer (pH 7.4) containing 150 mmol/L of KCl. The homogenates of the liver were centrifuged at 10,000*g* for 10 min at 4°C, and the resulting supernatants were further centrifuged at 105,000*g* for 60 min at 4°C to obtain a microsomal fraction as a precipitate. The microsomal fraction was resuspended in the homogenizing buffer and stored at −80°C until analyses for phospholipids, protein, and enzyme activity were performed.

The plasma concentrations of total cholesterol, HDL cholesterol, free cholesterol, triglycerides, and phospholipids were measured enzymatically with kits: Cholesterol C-Test, HDL Cholesterol Test, Free Cholesterol C-Test, Triglyceride G-Test, and Phospholipid B-Test, respectively (Wako Pure Chemical Ind., Osaka, Japan). The difference between total cholesterol and HDL cholesterol was assumed to be cholesterol associated with VLDL + LDL. Esterified cholesterol was estimated by subtracting free cholesterol from the total cholesterol. The lipids of liver homogenates and liver microsomes were extracted according to Folch et al.[14] The cholesterol, triglycerides, and phospholipids in the liver extracts were measured according to Zak,[15] Fletcher,[16] and Bartlett,[17] respectively. The phospholipids in the extracts of liver microsomes were separated into each class by TLC with silica gel 60 (Merck, Darmstadt, Germany), using chloroform-methanol-water (65:25:4, v/v) as a developing solvent. The bands of each phospholipid class in the silica gel plate were visualized with iodine vapor, scraped off, and directly analyzed for inorganic phosphorus.[17] In Experiment 2, PC in liver microsomes and blood plasma was likewise separated, scraped off, and directly counted for radioactivity with a liquid scintillation counter.

*S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) in the liver were estimated by HPLC essentially according to Cook et al.[18] with some modifications. Briefly, the frozen liver was thawed and homogenized in 4 vol (v/w) of an ice-cold perchloric acid solution (0.5 mol/L), and the homogenates were centrifuged at 10,000*g* for 20 min at 4°C. The resultant supernatants were filtered through a 0.45 μm Millipore filter and applied to an HPLC column (Shim-pack CLC-ODS, 6 × 150 mm; Shimadzu Seisakusho, Kyoto, Japan). The mobile phase was 100 mmol/L of $KH_2PO_4$ solution containing 3% methanol (v/v) and 10 mmol/L of sodium heptanesulfonate. The flow rate was 1.5 ml/min, and the elution was monitored at 254 nm. The PE *N*-methyltransferase activity of liver microsomes was measured according to Tanaka et

**Table 1** Body weight gain, food intake, liver weight, and liver lipid contents in rats fed the basel diet or diets supplemented with eritadenine at graded levels*

| Eritadenine addition | Weight gain (g/14 days) | Food intake (g/14 days) | Liver weight (g/100 g of body weight) | Liver lipids (μmol/g of liver) | | |
|---|---|---|---|---|---|---|
| | | | | Cholesterol | Triglycerides | Phospholipids |
| 0 μmol/kg | 72 ± 3 | 181 ± 8 | 4.80 ± 0.11 | 9.7 ± 0.1 | 23.6 ± 1.3 | 32.5 ± 0.4 |
| 4 μmol/kg | 71 ± 3 | 180 ± 5 | 4.77 ± 0.08 | 9.6 ± 0.1 | 23.5 ± 0.7 | 34.4 ± 0.4$^b$ |
| 8 μmol/kg | 72 ± 2 | 177 ± 4 | 4.63 ± 0.06 | 10.1 ± 0.2 | 22.6 ± 1.2 | 34.7 ± 0.3$^c$ |
| 20 μmol/kg | 72 ± 2 | 183 ± 5 | 4.70 ± 0.06 | 9.6 ± 0.1 | 21.2 ± 1.1 | 35.7 ± 0.7$^c$ |
| 40 μmol/kg | 73 ± 2 | 179 ± 4 | 4.56 ± 0.07 | 10.1 ± 0.2 | 21.0 ± 1.7 | 37.4 ± 0.3$^c$ |
| 80 μmol/kg | 73 ± 1 | 182 ± 2 | 4.68 ± 0.05 | 11.4 ± 0.2$^c$ | 38.1 ± 1.5$^c$ | 37.8 ± 0.4$^c$ |
| 200 μmol/kg | 71 ± 3 | 175 ± 5 | 4.58 ± 0.09 | 14.8 ± 0.7$^c$ | 103.0 ± 11.5$^c$ | 39.9 ± 0.9$^c$ |

*Values are mean ± SEM for 8 rats. $^{a,b,c}$A significant difference from the eritadenine-unsupplemented control group is indicated at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

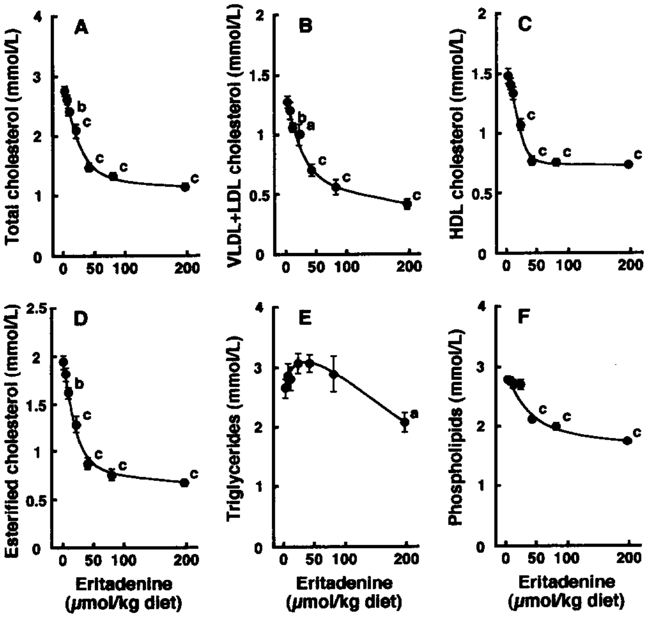

**Figure 2** Effects of dietary supplementation with eritadenine on the plasma concentrations of total cholesterol (A), VLDL + LDL cholesterol (B), HDL cholesterol (C), esterified cholesterol (D), triglycerides (E), and phospholipids (F) in rats. The circle and its bar represent mean and SEM, respectively, for 8 rats. The letters a, b, and c attached to circles indicate a significant difference from the control value at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

al.[19] except that a higher substrate concentration (200 μmol/L of S-[methyl-$^{14}$C]SAM) was used. Protein was measured according to Lowry et al.[20] using bovine serum albumin as a standard.

### *Statistical analysis*

Statistical analysis was carried out using Student's *t*-test to examine the significance between the eritadenine-unsupplemented control group and eritadenine-supplemented test groups.

## Results

*Table 1* shows the effects of dietary eritadenine on the growth, food intake, liver weight, and liver lipid levels in rats. Eritadenine supplementation did not affect the growth, food intake, and relative liver size of rats. The contents of cholesterol and triglycerides in the liver were not influenced by eritadenine supplementation up to 40 μmol/kg of diet, but they were significantly increased by 80 and 200 μmol/kg of diet of eritadenine. The hepatic phospholipid content was slightly but significantly increased by eritadenine supplementation in a dose-dependent manner.

*Figure 2* summarizes the effects of dietary eritadenine on plasma lipid levels. The plasma total cholesterol level was significantly decreased by eritadenine supplementation at levels above 8 μmol/kg of diet in a dose-dependent manner, accompanying decreases in both VLDL + LDL and HDL cholesterol levels. The plasma phospholipid level was also significantly decreased by eritadenine at levels above 40 μmol/kg of diet, whereas the plasma triglyceride level was decreased only by the highest addition level of eritadenine.

*Table 2* shows the effects of dietary eritadenine on the content and composition of each phospholipid class in liver microsomes. Eritadenine supplementation significantly increased the PE content and inversely decreased the PC content of liver microsomes at levels above 20 μmol/kg of diet in a dose-dependent manner. Consequently the ratio of PC to PE was more clearly decreased by eritadenine supplementation in a dose-dependent manner (*Figure 3*). The proportion of PC and PE to the total phospholipids was likewise affected by eritadenine supplementation. The content and proportion of the other phospholipid classes, however, were little or only slightly affected by eritadenine. As shown in *Figure 4*, there was a highly significant correlation between the plasma total cholesterol level and the PC/PE ratio of liver microsomes. A significant correlation was also observed between the plasma total cholesterol level and the PC content ($r = 0.995$, $P < 0.001$), the proportion of PC ($r = 0.998$, $P < 0.001$), the content of PE ($r = -0.990$, $P < 0.001$), or the proportion of PE ($r = -0.992$, $P < 0.001$).

*Figure 5* shows the effects of dietary eritadenine on the levels of SAM, SAH, and SAM/SAH ratio in the liver. The hepatic level of SAH was significantly increased by eritadenine supplementation at levels above 40 μmol/kg in a dose-dependent manner. The hepatic SAM level was also significantly enhanced by eritadenine at levels above 80

**Table 2** Effects of dietary supplementation with eritadenine on the content and proportion of microsomal phospholipids in the liver of rats*

| Eritadenine addition | Phospholipids in liver microsomes | | | | |
|---|---|---|---|---|---|
| | PC | PE | PS + PI | SM | Others |
| | (nmol/mg protein) | | | | |
| 0 µmol/kg | 220.0 ± 6.7 | 57.1 ± 1.1 | 31.8 ± 0.7 | 12.8 ± 0.6 | 4.6 ± 0.2 |
| 4 µmol/kg | 220.2 ± 9.2 | 59.5 ± 2.9 | 34.9 ± 1.6 | 13.7 ± 0.5 | 6.0 ± 0.4$^{b}$ |
| 8 µmol/kg | 208.8 ± 6.2 | 60.7 ± 1.5 | 33.7 ± 1.3 | 17.0 ± 0.9$^{b}$ | 8.4 ± 0.3$^{c}$ |
| 20 µmol/kg | 200.8 ± 5.5$^{a}$ | 72.5 ± 2.1$^{c}$ | 36.5 ± 1.5$^{a}$ | 15.8 ± 1.1 | 7.2 ± 0.4$^{c}$ |
| 40 µmol/kg | 186.4 ± 2.8$^{b}$ | 95.6 ± 1.6$^{c}$ | 35.6 ± 0.9$^{b}$ | 15.4 ± 1.1 | 7.6 ± 0.5$^{c}$ |
| 80 µmol/kg | 180.5 ± 3.9$^{c}$ | 101.3 ± 1.9$^{c}$ | 36.6 ± 1.0$^{b}$ | 16.0 ± 0.6$^{b}$ | 6.8 ± 0.4$^{c}$ |
| 200 µmol/kg | 176.3 ± 3.2$^{c}$ | 102.9 ± 2.3$^{c}$ | 35.7 ± 0.8$^{b}$ | 15.8 ± 0.5$^{b}$ | 6.2 ± 0.2$^{c}$ |
| | (% of total phospholipids) | | | | |
| 0 µmol/kg | 67.4 ± 0.4 | 17.5 ± 0.2 | 9.8 ± 0.1 | 3.9 ± 0.2 | 1.4 ± 0.1 |
| 4 µmol/kg | 65.9 ± 0.4$^{a}$ | 17.8 ± 0.3 | 10.4 ± 0.2$^{a}$ | 4.1 ± 0.2 | 1.8 ± 0.1$^{b}$ |
| 8 µmol/kg | 63.5 ± 0.3$^{c}$ | 18.5 ± 0.2$^{b}$ | 10.2 ± 0.3 | 5.2 ± 0.3$^{b}$ | 2.6 ± 0.1$^{c}$ |
| 20 µmol/kg | 60.4 ± 0.7$^{c}$ | 21.9 ± 0.7$^{c}$ | 11.0 ± 0.3$^{b}$ | 4.6 ± 0.3 | 2.2 ± 0.1$^{c}$ |
| 40 µmol/kg | 54.7 ± 0.4$^{c}$ | 28.1 ± 0.3$^{c}$ | 10.5 ± 0.3 | 4.5 ± 0.3 | 2.2 ± 0.2$^{b}$ |
| 80 µmol/kg | 52.9 ± 0.3$^{c}$ | 29.7 ± 0.1$^{c}$ | 10.7 ± 0.2$^{c}$ | 4.7 ± 0.2$^{b}$ | 2.0 ± 0.1$^{c}$ |
| 200 µmol/kg | 52.3 ± 0.2$^{c}$ | 30.5 ± 0.1$^{c}$ | 10.6 ± 0.1$^{c}$ | 4.7 ± 0.1$^{b}$ | 1.8 ± 0.1$^{c}$ |

*Values are mean ± SEM for 8 rats. $^{a,b,c}$A significant difference from the eritadenine-unsupplemented control group is indicated at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; SM, sphingomyelin.

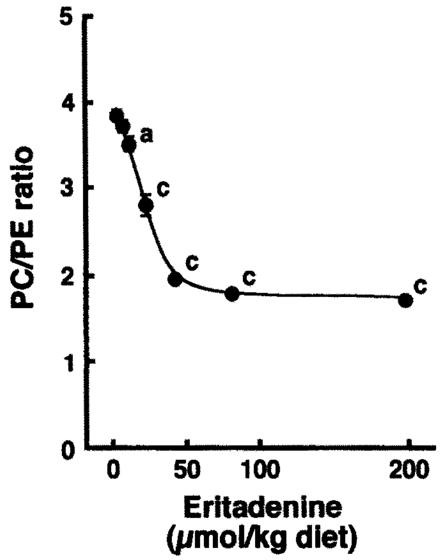

**Figure 3** Effect of dietary supplementation with eritadenine on the ratio of phosphatidylcholine to phosphatidylethanolamine in the liver microsomes of rats. The circle and its bar represent mean and SEM, respectively, for 8 rats. The letters a and c attached to circles indicate a significant difference from the control value at $P < 0.05$ and $P < 0.001$, respectively. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

µmol/kg of diet. Since the extent of the increase in the SAH
level was greater than that in the SAM level, the ratio of
to SAH was consequently decreased by eritadenine
ntation at levels above 40 µmol/kg of diet. As
*ure 6*, the in vitro activity of PE *N*-methyl-
er microsomes was significantly increased
lementation at levels above 8 µmol/kg
dent manner.

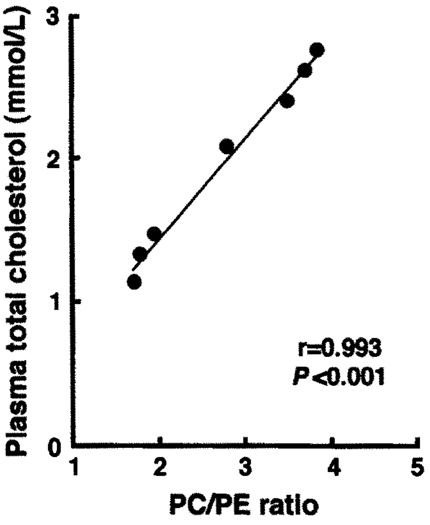

**Figure 4** Correlation between the plasma total cholesterol level and the ratio of phosphatidylcholine to phosphatidylethanolamine in liver microsomes of rats fed diets containing none or different amounts of eritadenine. Each circle denotes the mean value of each group. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine.

*Table 3* shows the effects of dietary eritadenine on the in vivo incorporation of radioactivity of [1,2-$^{14}$C]choline chloride and [methyl-$^{3}$H]methionine into the PC of liver microsomes and blood plasma over a 2 hr period. Dietary supplementation with eritadenine at a level of 200 µmol/kg of diet did not interfere with the incorporation of radioactivity of [1,2-$^{14}$C]choline chloride into the PC of both liver microsomes and blood plasma, but rather stimulated the

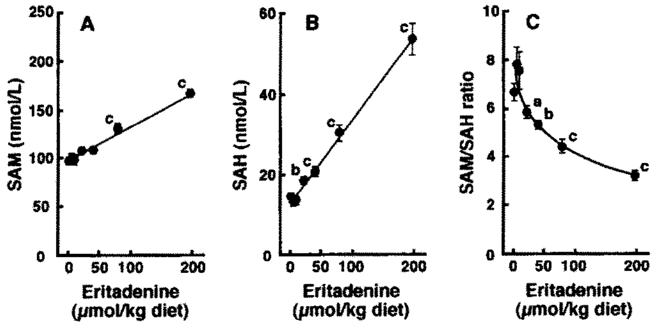

**Figure 5** Effect of dietary supplementation with eritadenine on the hepatic levels of *S*-adenosylmethionine (A), *S*-adenosylhomocysteine (B) and the ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine (C) in rats. The circle and its bar represent mean and SEM, respectively, for 8 rats. The letters a, b, and c attached to circles indicate a significant difference from the control value at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Abbreviations: SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

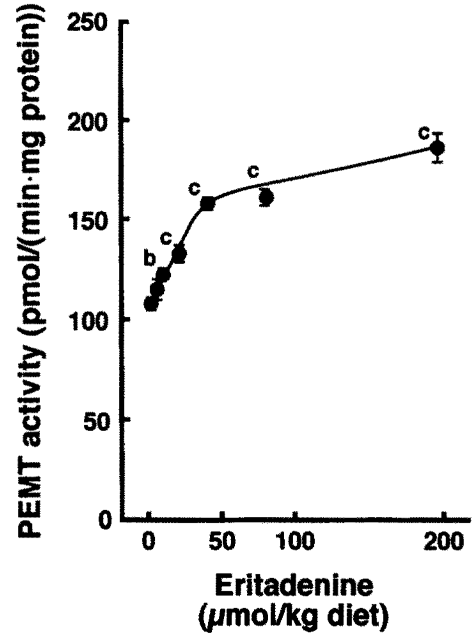

**Figure 6** Effect of dietary supplementation with eritadenine on the activity of phosphatidylethanolamine *N*-methyltransferase in liver microsomes of rats. The circle and its bar represent mean and SEM, respectively, for 8 rats. The letters b and c attached to circles indicate a significant difference from the control value at $P < 0.01$ and $P < 0.001$, respectively.

incorporation into plasma PC when expressed in terms of specific radioactivity. In contrast eritadenine supplementation markedly inhibited the incorporation of radioactivity of [methyl-$^3$H]methionine into the PC of both liver microsomes and blood plasma irrespective of the basis of expression.

## Discussion

The results obtained here clearly demonstrate that dietary supplementation with eritadenine could drastically decrease the PC/PE ratio of liver microsomes in addition to the level of plasma cholesterol in rats. These results are essentially in accordance with those obtained with a powder of *L. edodes* mushroom as reported previously in a preliminary form,[13] indicating that the effects of *L. edodes* on the two parameters are mainly attributable to the eritadenine included in the mushroom.

The significant effect of dietary eritadenine on the profile of phospholipids in liver microsomes could be anticipated to some extent, since eritadenine was shown to be a potent inhibitor of SAH hydrolase[21] and therefore to increase the SAH level in isolated rat hepatocytes when added to the incubation medium.[22] *S*-adenosylhomocysteine is known to inhibit the reaction of various methyltransferases, which require SAM as the donor of a methyl group, including PE *N*-methyltransferase.[23] Further, it was demonstrated that in rats PE *N*-methylation was strongly inhibited by 3-deazaadenosine, another adenosine analog that inhibits SAH hydrolase.[24] It is well confirmed that PE *N*-methylation catalyzed by PE *N*-methyltransferase plays a significant role in the phospholipid metabolism, especially in the liver, by converting PE to PC.[25] These facts suggested that eritadenine would inhibit PE *N*-methylation also in vivo and thereby modify the hepatic phospholipid profile. The present study could demonstrate, as expected, that dietary supplementation with eritadenine significantly increased the level of hepatic SAH and markedly depressed the incorporation of label of [methyl-$^3$H]methionine into the PC of liver microsomes and blood plasma. However, the data for radioisotope experiment must carefully be interpreted, since the incorporation of $^{14}$C and $^3$H into PC dose do not directly represent the amounts of choline and methyl group of methionine incorporated into PC. Unfortunately the extent of the dilution of administered label compounds within the body could not be measured. Since eritadenine supplementation (200 µmol/kg of diet) increased the hepatic SAM level to a value 1.7 fold of the control value, the free methionine level is also anticipated to be increased by eritadenine more or less. Hence another confirmation is required for an accurate estimation of the depression of PE *N*-methylation by eritadenine in vivo.

On the contrary the PE *N*-methyltransferase activity of liver microsomes was found to be rather increased in rats fed eritadenine-supplemented diets. This increase in the in vitro activity of the enzyme is possibly ascribed to the increase in the PE content of liver microsomes due to eritadenine supplementation because PE *N*-methyltransferase activity of liver microsomes was shown to be influenced by the levels of enzyme substrates SAM and PE rather than the enzyme mass.[26] Eritadenine had little inhibitory effect on the PE *N*-methyltransferase activity when added to the assay mixture at concentrations of up to 100 µmol/L (data not shown). Thus it seems possible to conclude that eritadenine, as well as 3-deazaadenosine, can inhibit in vivo PE *N*-methylation indirectly through an increase in the hepatic SAH level and thereby lead to an enhancement of the PE content of liver microsomes.

Vance et al.[27] have shown that 3-deazaadenosine had no effect on the amount of PC of cultured rat hepatocytes when added to the culture medium, although the compound dou-

**Table 3** Effects of dietary supplementation with eritadenine on the in vivo incorporation of radioactivity of [1,2-$^{14}$C]choline and [methyl-$^{3}$H]methionine into the PC of liver microsomes and blood plasma*

| Eritadenine addition | Incorporation of [1,2-$^{14}$C]choline | | Incorporation of [methyl-$^{3}$H]methionine | |
|---|---|---|---|---|
| | Microsomes | Plasma | Microsomes | Plasma |
| | Bq/mg protein | Bq/mL | Bq/mg protein | Bq/mL |
| 0 μmol/kg | 47 ± 2 | 163 ± 9 | 99 ± 5 | 549 ± 27 |
| 200 μmol/kg | 48 ± 7 | 179 ± 24 | 6 ± 4$^{c}$ | 17 ± 10$^{c}$ |
| | | Bq/μmol PC | | |
| 0 μmol/kg | 207 ± 8 | 89 ± 4 | 435 ± 18 | 299 ± 6 |
| 200 μmol/kg | 266 ± 38 | 160 ± 20$^{a}$ | 32 ± 21$^{c}$ | 15 ± 9$^{c}$ |

*Values are mean ± SEM for five rats. $^{a,c}$A significant difference from the eritadenine-unsupplemented control group is indicated at $P < 0.05$ and $P < 0.001$, respectively.

bled the pool size of cellular PE. In contrast the present study showed that dietary supplementation with eritadenine decreased the PC content of liver microsomes by a maximum of 20% (*Table* 2). One of the reasons for this discrepancy may be the difference in the experimental conditions employed, i.e., in vivo or cells in culture. In the liver PC is synthesized mainly either by the CDP-choline pathway or by the PE *N*-methylation pathway. These two pathways are thought to contribute to the total PC biosynthesis in a manner of mutual compensation. For instance, it is well known that feeding a diet devoid of choline does not necessarily cause the development of fatty liver due to PC deficiency unless the methionine content of the diet is also lowered, indicating that a decrease in PC biosynthesis via the CDP-choline pathway can be compensated by the PE *N*-methylation pathway.

In supporting this, it was suggested that choline deficiency results in an increased utilization of SAM.[28] However, Pritchard et al.[29] have shown that the inhibition of PC biosynthesis via the PE *N*-methylation pathway by 3-deazaadenosine increased PC biosynthesis via the CDP-choline pathway through an increase in the activity of CTP: phosphocholine cytidyryltransferase, the rate-limiting enzyme for the CDP-choline pathway, in cultured rat hepatocytes. Hence, PC biosynthesis via the CDP-choline pathway in rats fed eritadenine-supplemented diets is considered to be stimulated. Nonetheless, eritadenine supplementation resulted in a decrease in the PC content of liver microsomes and higher levels of eritadenine caused fat accumulation in the liver, suggesting that the PC requirement may not be fully met by PC biosynthesis via the CDP-choline pathway under the experimental conditions employed. This implies that the choline supply from the diet (2.5 g of choline chloride/kg of diet) was insufficient to meet a choline requirement, or that PC biosynthesis via the CDP-choline pathway was impaired by eritadenine either directly or indirectly. It seems reasonable to consider that the requirement for dietary choline should be augmented by eritadenine supplementation, so the first possibility cannot be excluded. With regard to the latter possibility, an increased competitive inhibition by ethanolamine of phosphorylation of choline catalyzed by choline/ethanolamine kinase can be considered since various types of treatment to increase hepatic PE are shown to increase the free ethanolamine concentration in the liver.[30–32] However, it was also reported that there was a large difference in the Ki values of choline and ethanolamine for a purified choline/ethanolamine kinase from the rat liver, i.e., 0.014 mmol/L and 2.0 mmol/L, respectively, indicating that ethanolamine is a weak inhibitor for the phosphorylation of choline while choline is a strong inhibitor for the phosphorylation of ethanolamine.[33] It is not known though whether or not eritadenine has a direct inhibitory effect on some step(s) in the CDP-choline pathway. Thus the mechanism by which dietary eritadenine decreased the PC content of liver microsomes remains to be further elucidated.

The present study clearly showed a significant correlation between plasma cholesterol levels and the content or proportion of PC and PE in liver microsomes, suggesting that the hypocholesterolemic action of eritadenine might be elicited through an alteration of hepatic phospholipid metabolism. In supporting this idea, several reports have shown that the PC/PE ratio of liver microsomes decreased in response to certain types of hypocholesterolemic treatment such as dietary supplementation with PE,[34] ethanolamine,[32,34] or glycine,[32] and feeding a soybean protein diet.[35] Of these hypocholesterolemic substances, eritadenine appears to have the most potent effect in lowering both the plasma cholesterol level and the PC/PE ratio of liver microsomes. Feeding a diet deficient in choline and methionine is also known to cause a decrease in the PC/PE ratio of the liver.[36] Additionally, an earlier report pointed out the possibility that several kinds of hypolipidemic drugs elicit their action through the inhibition of PC biosynthesis in the liver.[37]

Taken together, these results are considered to suggest the existence of a general rule that a variety of treatments to decrease the PC/PE ratio of liver microsomes necessarily result in a reduction of plasma cholesterol but not vice versa. Since PC is the major phospholipid class of plasma lipoproteins, the depression of PC biosynthesis causes an impaired secretion of VLDL but not HDL from liver cells.[38] Therefore, it is possible that eritadenine supplementation brought about a shortage of PC biosynthesis and thereby decreased the secretion of VLDL from the liver. This appears to be partly supported by the fact that higher levels of eritadenine caused fat accumulation in the liver (*Table 1*). However, it should be noted that lower levels of eritadenine (up to 40 μmol/kg of diet) significantly decreased the plasma cholesterol level without a concomitant increase in the liver fat. Further, as compared with the plasma cholesterol level, the plasma triglyceride level was less sensitive to eritadenine supplementation. These results can be taken to suggest that some mechanism(s) other than the decreased secretion of triglyceride-rich lipoprotein from the liver also participates in the hypocholesterolemic action of eritadenine.

The activation of CTP:phosphocholine cytidyryltransferase by converting an inactive cytosol form to an active membrane-bound form was shown to be regulated by the ratio of bilayer-forming lipids (e.g., PC) to nonbilayer-forming lipids (e.g., PE) of hepatocyte membranes rather than the PC content itself.[39] Likewise, there existed a significant correlation between the PC/PE ratio of liver microsomes and plasma cholesterol levels. However, unlike the definite role of PC, the role of PE in the regulation of plasma cholesterol level is little understood. Currently there is no direct evidence for that microsomal PE content itself has any significant effect on the assembly and secretion of lipoproteins in the liver. Further studies on the effect of dietary eritadenine on the molecular species of phospholipids in the liver microsomes and plasma lipoproteins should help to explain the more detailed mechanism of the hypocholesterolemic action of *L. edodes* mushroom.

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