The effect of dietary eritadenine on the plasma homocysteine concentration was investigated in methionine-induced hyperhomocysteinemic rats. The rats were fed on the control or eritadenine-supplemented (50 mg/kg) diet for 10 d. The animals were then injected with saline or methionine at a level of 100 or 300 mg/kg of body weight, and sacrificed 2 h or a more appropriate time after injection. The methionine injection increased the post-2 h concentration of plasma homocysteine in a dose-dependent manner in the control rats, this increase being significantly suppressed in the eritadenine-fed rats. This effect persisted up to 8 h after the methionine injection. The hepatic concentrations of $S$-adenosylmethionine and $S$-adenosylhomocysteine were increased by eritadenine, whereas the hepatic homocysteine concentration was inversely decreased. The cystathionine $\beta$-synthase activity in the liver was increased by eritadenine. It is suggested from these results that eritadenine might suppress the methionine-induced increase in plasma homocysteine concentration by dual mechanisms: slowing the homocysteine production from $S$-adenosylhomocysteine and increasing the removal of homocysteine due to the enhanced activity of cystathionine $\beta$-synthase.

**Key words:** homocysteine; eritadenine; *Lentinus edodes*; methionine; rat

Homocysteine is one of the intermediates of methionine metabolism. It has been established that an elevated plasma homocysteine concentration represents an independent risk factor for thrombosis and vascular disease.1-3) The normal plasma homocysteine concentration in humans is in the range of 5 to 15 $\mu$M, and a 5-$\mu$M increase in this amino acid concentration is associated with an increased risk of 60% for men and 80% for women of coronary heart disease.2) It has been reported that the plasma homocysteine concentration was affected by various types of factor; e.g., nutritional, pharmacological, hormonal, disease, lifestyle, and genetic factors.3,4) However, information about regulation of the plasma homocysteine concentration by dietary factors is limited, except for deficiencies of several vitamins such as folate, vitamin B$_{12}$ and vitamin B$_{6}$.

Eritadenine [2(R),3(R)-dihydroxy-4-(9-adenyl)-butyric acid] is a compound that has been isolated from the popular mushroom *Lentinus edodes* (shitake in Japanese), as a hypocholesterolemic factor of the mushroom.5,6) This compound has elicited a potent effect at a low dose level when added to the diet for rats; the addition of only few mg/kg of diet brought about a significant decrease in the plasma cholesterol concentration.7) At present, no adverse effects of eritadenine, e.g., growth retardation, are known when eritadenine is added to the diet at a physiological level and fed to rats. Although the mechanism by which eritadenine elicits its hypocholesterolemic action has not yet been fully elucidated, we have demonstrated that eritadenine also affected the metabolism of phospholipids7,8) and fatty acids9,10) It has been shown that, like many other adenosine analogues, eritadenine is an inhibitor of $S$-adenosylhomocysteine (SAH) hydrolase (EC 3.3.1.1),11-13) indicating that eritadenine affects the metabolism of methionine (Fig. 1). The inhibition of hepatic SAH hydrolase by eritadenine is thought to be the cause of a series of metabolic alterations, including modulation of the phospholipid and fatty acid metabolism.14) However, there is no information, to our knowledge, regarding the in vivo effect of eritadenine on the plasma homocysteine concentration, although Svardal et al.15) have shown that the export of homocysteine from rat hepatocytes into a culture medium was inhibited by eritadenine. This led us to investigate whether eritadenine could suppress the increase in plasma homocysteine concentration. One of the simple hyperhomocysteinemia models is the me-
thionine loading model. Therefore, in the present study, we investigated the effect of dietary eritadenine on the plasma homocysteine concentration by using methionine-induced hyperhomocysteinemic rats.

Male six-week-old rats (120–140 g) of the Wistar strain were obtained from Japan SLC (Hamamatsu, Japan). They were individually housed in hanging stainless-steel wire cages kept in an isolated room at a controlled temperature (23–25°C) and humidity (40–60%). Lighting was maintained on a 12-h cycle (lights on from 07.00 to 19.00h). Before starting the experiments, all rats were acclimatized to the facility for 3 d and given free access to water and a stock diet which was subsequently used as the control diet. The control diet consisted of the following ingredients (g/100 g): casein, 25; corn starch, 42.9; sucrose, 20; corn oil, 5; AIN-93G mineral mixture, 3.5; AIN-93G vitamin mixture, 1; choline bitartrate, 0.5; cellulose, 2; and lactose 0.1. Eritadenine, which was kindly supplied by Tanabe Seiyaku (Osaka, Japan), was mixed with lactose and added to the control diet at a level of 50 mg/kg at the expense of lactose. We used this dose level of eritadenine since it has been proved sufficient to elicit maximal hypocholesterolemic action without growth retardation. After the rats had been fed with the control diet or eritadenine-supplemented diet for 10 d, they were injected with saline or l-methionine dissolved in saline diet or eritadenine-supplemented diet for 10 d, they were injected with saline or l-methionine dissolved in saline.

Dietary supplementation with eritadenine did not affect the growth or food consumption of the animals during the experimental feeding period; the body weight gain and food intake of the control group (n = 36) and eritadenine group (n = 36) were 50 ± 1 vs. 50 ± 1 g/10 d and 125 ± 2 vs. 124 ± 2 g/10 d, respectively. The liver weight was no different between the control and eritadenine-fed groups; the relative liver weights for the control group and eritadenine group were 4.31 ± 0.07 vs. 4.27 ± 0.04 g/100 g of body weight. The intraperitoneal injection of methionine increased the plasma homocysteine concentration in a dose-dependent manner, this increase in plasma homocysteine concentration being significantly suppressed in the eritadenine-fed rats at both low (100 mg/kg) and high (300 mg/kg) dose levels of methionine (Fig. 2A). The suppressive effect of eritadenine on the plasma homocysteine concentration persisted up to 8 h after the injection of methionine (Fig. 2C). In contrast, the plasma cysteine concentration was little affected by either the methionine injection or eritadenine supplementation (Figs. 2B and D). Figure 3 summarizes the effects of dietary eritadenine on the hepatic concentrations of the methionine metabolites and on the cystathionine β-synthase activity in the liver after the methionine injection (experiment 2).

![Fig. 1. Participation of Eritadenine in the Metabolism of Methionine.](image-url)

**DMG, N,N-dimethylglycine; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate.**
hepatic concentrations of SAM and SAH were significantly higher or tended to be higher in the eritadenine-fed rats than in the control rats, whereas the hepatic concentration of homocysteine tended to be lower in the eritadenine-fed rats than in the control rats, although statistical significance was detected only 4 h after the methionine injection. On the other hand, the activity of cystathionine \(/C12\)-synthase, which catalyzes the formation of cystathionine from homocysteine and serine, was consistently higher in the eritadenine-fed rats than in the control rats. The hepatic concentration of cysteine was significantly lower in the eritadenine-fed rats than in the control rats before and 2 h after the methionine injection.

These results clearly demonstrate that eritadenine could suppress methionine-induced acute hyperhomocysteinemia, at least under the conditions used. There are several fates of homocysteine in the liver: (i) remethylation to methionine using either 5-methyltetrahydrofolate or betaine as a methyl-group donor, (ii) formation of cystathionine by cystathionine \(/C12\)-synthase, and (iii) export to the blood plasma. When an animal is loaded with methionine, excess sulfur must be excreted into urine mainly in the forms of taurine and sulfate. Under such a condition, cystathionine formation was estimated to be greater than remethylation. Therefore, the increased activity of cystathionine \(/C12\)-synthase observed in the eritadenine-fed rats appears to have contributed to the reduction of liver homocysteine concentration. It seems reasonable to consider that the increased activity of cystathionine \(/C12\)-synthase in the eritadenine-fed rats might be ascribed, at least in part, to the increase in hepatic SAM concentration, since SAM
is known to activate the enzyme allosterically. In addition to the increase in cystathionine β-synthase activity, the inhibition of SAH hydrolase by eritadenine is also considered to favor the decrease in hepatic homocysteine concentration as was observed in a previous study using rat hepatocytes and in the present study. Since the liver is the central organ of methionine metabolism, the hepatic homocysteine concentration is thought to reflect to some extent the plasma homocysteine concentration. Thus, it is likely that eritadenine elicited its hypohomocysteinemic effect by dual mechanisms, i.e., (i) slowing the homocysteine production from SAH, and (ii) increasing the removal of homocysteine due to the enhanced activity of cystathionine β-synthase.

An earlier report has shown that an adenosine deaminase inhibitor such as 2′-deoxycoformycin decreased the plasma homocysteine concentration in acute lymphoblastic leukemia patients. This effect was assumed to have been due to the inhibition of SAH hydrolase; the inhibitor probably indirectly inhibited the SAH hydrolase activity through an increase in adenosine concentration. The present study confirms more clearly that the inhibition of SAH hydrolase led to a decrease in the plasma homocysteine concentration under the condition of methionine loading. Since the methionine loading test has been used to assess the capacity to metabolize homocysteine in humans, it seems possible that eritadenine or the L. edodes mushroom may also be effective in humans, although this remains to be experimentally tested.

References

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