Enterostatin (VPDPR) and Its Peptide Fragment DPR Reduce Serum Cholesterol Levels after Oral Administration in Mice

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We found that enterostatin (VPDPR), an anorexigenic peptide for a high-fat diet, significantly reduces serum cholesterol levels after oral administration of 100 mg/kg for 3 days in mice fed a high cholesterol-cholic acid diet. DPR, a peptide fragment of VPDPR, also had hypocholesterolemic activity at a dose of 50 mg/kg. Food intake was not suppressed under these dietary conditions. Fecal excretion of cholesterol and bile acids was increased significantly by both VPDPR and DPR. Interestingly, DPR induced hypocholesterolemic effects just two hours after a single oral administration at a dose of 100 mg/kg.

Key words: enterostatin; hypocholesterolemic effect; bile acids

Enterostatin (VPDPR) is released from the amino terminus of procolipase during its conversion to colipase,1,2) and inhibits food intake after central or peripheral administration in animals fed a high-fat diet.3–8) Furthermore, VPDPR reduces serum triglyceride levels5) and influences energy metabolism in rats fed a high-fat diet by activating the sympathetic drive in brown adipose tissue, to increase thermogenesis.9) VPDPR reportedly reduces intake of high fat foods via stimulation of the specific μ-opioid agonist β-casomorphin (1–7),10) indicating that an anti-opioid mechanism may be responsible for the anorectic effect. Morphine reportedly elevates serum cholesterol levels.11) Furthermore, we demonstrated previously that VPDPR inhibits analgesia induced by the μ-opioid agonist morphine.12) Therefore, we investigated the effects of VPDPR on serum cholesterol levels.

Male mice of the ddY strain (Otsu Exp. Animals, Nagasaki, Japan) weighing about 14 g were used. Experimental protocols involving laboratory animals were approved by the ethical committee of the Graduate School of Agriculture, Kyoto University. The room temperature was maintained at 23°C with a 12-h light (07:00–19:00) and dark cycle. The mice were housed in groups of four per standard plastic cage and had free access to food and water. The mice were fed a commercial stock diet (MF; Oriental Yeast Co., Tokyo, Japan) for 4 days during adaptation to the new environment, and then fed a purified high cholesterol-cholic acid diet. The composition of the basal diet was based on the formula recommended by the American Institute of Nutrition, in weight percent as follows: casein, 20; DL-methionine, 0.3; corn starch, 43.50; sucrose, 10.0; cellulose, 5.0; corn oil, 5.0; mineral mixture (AIN-76), 3.5; vitamin mixture (AIN-76), 1.0; choline bitartrate, 0.20; cholesterol, 1.0; cholic acid, 0.5; coconut oil, 10. Mice were fed this high cholesterol-cholic acid diet for 3 days. Peptides were dissolved in saline adjusted to pH 7.0 and then given orally every day at 11:00. Blood was collected by cardiac puncture 24 hours after the third dose was administered. Total and HDL cholesterol in serum were measured by an enzymatic method using a commercially available kit (Cholesterol E-test Wako and HDL-C, Wako Pure Chemical Ind., Ltd., Osaka, Japan). The reported levels of LDL and VLDL were the calculated differences between total and HDL cholesterol.

VPDPR significantly reduced serum cholesterol levels after oral administration at a dose of 100 mg/kg (Fig. 1a). On the other hand, serum triglyceride levels were unaffected in mice under these high-cholesterol dietary conditions (data not shown). Both LDL and VLDL cholesterol levels were decreased, while the HDL-cholesterol level was not changed (Fig. 1b, c). It was reported that VPDPR and its fragment DPR inhibited secretion of insulin after peripheral administration in rats.13) Then the effects...
Hypocholesterolemic Effect of Enterostatin

Effects of VPDPR on Total Serum (A), LDL + VLDL (B) and HDL (C) Cholesterol Levels after Oral Administration.

The reported levels of LDL and VLDL were the calculated differences between total and HDL cholesterol. The values shown are the means ± SEM (n = 8). Statistical analysis was done with Bonferroni’s test (*P < 0.05).

Effects of DPR on Total Serum (A), LDL + VLDL (B) and HDL (C) Cholesterol Levels after Oral Administration.

The values shown are the means ± SEM (n = 8). Statistical analysis was done with Bonferroni’s test (*P < 0.05).

Table 1. Effects of VPDPR and DPR on Serum, Fecal, and Liver Cholesterol in Mice

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>VPDPR</th>
<th>DPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g)</td>
<td>1.8 ± 0.6</td>
<td>2.5 ± 0.5</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Food intake (g/2 days)</td>
<td>9.59 ± 0.83</td>
<td>10.92 ± 0.83</td>
<td>10.63 ± 0.60</td>
</tr>
<tr>
<td>Serum Total cholesterol (mg/dl)</td>
<td>289.4 ± 23.3</td>
<td>230.0 ± 15.0*</td>
<td>217.7 ± 11.9*</td>
</tr>
<tr>
<td>Feces Weight (mg/2 days)</td>
<td>600.9 ± 49.5</td>
<td>767.6 ± 75.4</td>
<td>789.6 ± 37.0**</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>34.7 ± 3.4</td>
<td>54.1 ± 5.7*</td>
<td>51.1 ± 3.3**</td>
</tr>
<tr>
<td>Bile acids (mg)</td>
<td>12.2 ± 1.9</td>
<td>22.0 ± 3.2*</td>
<td>21.0 ± 1.8**</td>
</tr>
<tr>
<td>Liver Weight (g)</td>
<td>1.53 ± 0.07</td>
<td>1.70 ± 0.05</td>
<td>1.61 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>29.6 ± 1.3</td>
<td>30.1 ± 1.9</td>
<td>28.6 ± 2.0</td>
</tr>
</tbody>
</table>

Both VPDPR and DPR were administered at a dose of 100 mg/kg (p.o.). Values are means ± SEM. *P < 0.05, **P < 0.01 compared with the control group.

Effects of DPR on serum cholesterol levels were also investigated. As shown in Fig. 2, DPR reduced total and LDL + VLDL cholesterol levels in serum after oral administration at a dose of 50 mg/kg (Fig. 2). Thus, DPR was two-fold more potent at inducing hypocholesterolemia than VPDPR on the basis of weight.

Lipid levels in serum, feces, and liver were then measured in mice housed in individual cages (Table 1). VPDPR and DPR did not induce anorectic effects in mice fed a high cholesterol-cholic acid diet; food intake and body weight gains tended to increase in the VPDPR- or DPR-treated groups, though not significantly. Feces were collected for 48 hours after the second oral dose of the peptides, and lyophilized. Fecal cholesterol and bile acids were extracted 3 times with 100 times volumes of ethanol at 75°C for 1 hour. After extraction, the supernatant was recovered by centrifugation. The supernatant was dried, and then dissolved in methanol. Fecal cholesterol and bile acids were measured by an enzymatic method using the Cholesterol E-test Wako and Total Bile Acid-test Wako (Wako Pure Chemical Ind., Ltd., Osaka, Japan). Liver cholesterol was extracted by the method of Folch et al., and measured using the Cholesterol E-test Wako. As shown in Table 1, the serum cholesterol level was significantly decreased in mice given VPDPR or DPR. Cholesterol and bile acids in feces were significantly higher in the VPDPR- or DPR-treated groups than the control group. Amount of feces were higher in VPDPR- or DPR-treated mice. This might reflect an increase of food intake in these group. It should be noted that not only the total amount of cholesterol and bile acids in feces but also their concentrates per gram of feces increased in mice given these peptides. The liver cholesterol level was unaffected by VPDPR- or DPR-treatment.

Next, we investigated how rapidly the peptides reduce serum cholesterol levels. Peptides were orally administered to mice fed a high cholesterol-cholic acid diet for 6 days. Blood was collected 2 hours after peptide administration. Interestingly, DPR induced a hypocholesterolemic effect after a single oral dose of 100 mg/kg, while VPDPR was ineffective even at the dose of 200 mg/kg (Fig. 3). The rapid hypocholesterolemic effect of DPR might be mediated, at least in part, by stimulation of bile acids secretion. In fact, DPR stimulated bile acids secretion in rats while VPDPR did not (unpublished result). On the other hand, VPDPR induced a hypocholesterole-
lemic effect after oral administration for 3 days (Fig. 1). Thus, the hypocholesterolemic effect of VPDPDR might be due to its conversion to DPR in vivo. Alternatively, VPDPDR might reduce cholesterol levels by a different mechanism. High-molecular-weight core peptides derived from soybean proteins reportedly lower serum cholesterol by binding to bile acids to inhibit their reabsorption. However, binding of VPDPDR to bile acids may be unlikely due to the repulsion between the negative charges of both molecules. Nagaoka et al. reported that the low molecular weight peptide IIAEK derived from β-lactoglobulin suppressed cholesterol absorption to induce hypocholesterolemia in rats. It is uncertain whether the observed increase in fecal cholesterol was caused by the inhibition of cholesterol absorption by VPDPDR.

We recently reported that the anti-analgesic activity of VPDPDR is mediated by corticosterone released from the adrenal cortex. However, the hypocholesterolemic effect of VPDPDR or DPR was not inhibited by the glucocorticoid receptor antagonist RU486, and was observed in adrenalectomized mice (data not shown). These results suggest that the hypocholesterolemic effect is not mediated by corticosterone.

Thus, we report here for the first time that VPDPDR and DPR have hypocholesterolemic effects in mice after oral administration of 100 mg/kg and 50 mg/kg, respectively. DPR is more potent than VPDPDR due to its lower effective dose and rapid onset of efficacy.

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References