Note

Potentiation of the Antihypertensive Activity of Orally Administered Ovokinin, \( \text{a peptide derived from Ovalbumin, by Emulsification in Egg Phosphatidylcholine} \)

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Received June 30, 1995

Ovokinin, a vasorelaxing octapeptide derived from ovalbumin, significantly lowered the systolic blood pressure of spontaneously hypertensive rats (SHR) when orally administered as an emulsion in 30% egg yolk at a dose of 25 mg/kg, this effect being larger than that of the peptide administered as a solution at a dose of 100 mg/kg. Egg phospholipid, especially phosphatidylcholine, showed essentially the same effect as egg yolk. However, egg neutral lipid was ineffective. Soybean phospholipid was less effective than egg phospholipid in potentiating the antihypertensive activity of ovokinin.

Many kinds of biologically active peptides are released from food proteins by enzymatic digestion. Inhibitor peptides of the angiotensin I-converting enzyme lowered the blood pressure in spontaneously hypertensive rats.\(^1-4\) We screened for a vasorelaxing peptide in the enzymatic digests of food proteins as another candidate for an antihypertensive peptide.

Ovokinin, Phe-Arg-Ala-Asp-His-Pro-Phe-Leu, is a vasorelaxing peptide that has been isolated from a pepsin digest of ovalbumin.\(^5-6\) Ovokinin bound to the bradykinin B\(_1\)-receptor stimulates the release of prostacyclin and relaxes the arteries. Ovokinin lowered the blood pressure in spontaneously hypertensive rats (SHR) after its oral administration at a dose of 100 mg/kg.\(^7\) Suetuna and Osajima have reported that the antihypertensive effect of the orally administered angiotensin I-converting enzyme inhibitor peptide with a molecular weight of 1000–2000 was potentiated by emulsification in 30% of egg yolk.\(^8\)

In this paper, the effect of emulsification in egg yolk on the antihypertensive activity of orally administered ovokinin is examined, and the component in the egg yolk essential for the potentiation is investigated.

Blood pressure was measured by the tail cuff method using a UR-5000 instrument (Ueda Seisakusho, Tokyo, Japan). Male SHR, 15–25 weeks old, with a blood pressure of about 210–230 mmHg were used in groups of five animals each. The test compounds were orally administered in SHR by oral zonde in a volume of 0.5 ml. The egg phospholipid fraction, Egg Lecithin L-95 (95% phospholipid, 79% phosphatidylcholine) and neutral lipid fraction, Yolk Oil N (no phosphatidylcholine) were kindly presented by Taiyo Chemical (Mie, Japan). Phosphatidylcholine was obtained from Sigma Chemical Co. (product No. P 3556, St. Louis, MO, U.S.A.). The soybean phospholipid fraction, Soybean Lecithin SLP-pastes SP (65% phospholipid, 18% phosphatidylcholine) was kindly presented by True Lecithin Manufacturing Co. (Mie, Japan). Ovokinin was synthesized according to the conventional \(\text{-Boc}\) strategy.

Ovokinin orally administered to SHR as a solution lowered the systolic blood pressure by 14.5 mmHg after 2 h at a dose of 100 mg/kg, but was ineffective at a dose of 25 mg/kg (Figs. 1a and 1b). Ovokinin was then administrated as an emulsion in 30% egg yolk. The peptide was dissolved in saline and then emulsified with egg yolk using ultrasonic cleaner (B-72, Branson Cleaning Equipment Co. Shelton, CONN, U.S.A.) for 5 min. The antihypertensive effect of ovokinin was potentiated by the emulsion; at a dose of 25 mg/kg, a maximum decrease of systolic blood pressure of 20 mmHg was obtained.

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**Fig. 1.** Effect of Emulsification in Egg Yolk on the Antihypertensive Activity of Ovokinin.

Ovokinin was administered as a solution in saline or as an emulsion in 30% egg yolk. Control groups (*) received the same amounts of saline or egg yolk as the experimental groups (\(\Delta\)). Changes in systolic blood pressure from zero time are expressed as mean ± S.E. * * * indicate significant differences against the control (*) \(p<0.05\), ** \(p<0.01\) \(t\)-test, \(n=5\).

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17.6 mmHg was observed 4 h after the oral administration (Fig. 1c). This decrease was larger than that observed after the administration of 100 mg/kg of the peptide dissolved in saline (Fig. 1b). Furthermore, a maximum decrease in systolic blood pressure of 25.0 mmHg was observed 4 h after the administration of 100 mg/kg of the emulsified peptide, and a considerable decrease was observed even after 8 h (Fig. 1d). The egg yolk itself had no effect on the blood pressure.

The egg yolk lipid consists of neutral lipids and phospholipid, about 70% and 30%, respectively. We then examined which component was effective for emulsification of the peptide. Ovokinin was administered in egg phospholipid or egg phosphatidylcholine as already described. Ovokinin was also emulsified in egg neutral lipid or soybean phospholipid in an syringe connected with a double-ended locking hub connector to another syringe, apparatus which is usually used for emulsification of antigen, because the peptide couldn’t be emulsified in these lipid fractions by the ultrasonic device. As shown in Fig. 2b, ovokinin emulsified with 3% of phospholipid significantly lowered the blood pressure at a dose of 25 mg/kg, the maximum decrease being 14.8 mmHg 4 h after the administration. On the other hand, ovokinin given as an emulsion of 6% of neutral lipids failed to lower the blood pressure at the same dose (Fig. 2c). About 80% of egg phospholipid is phosphatidylcholine. Ovokinin orally given as an emulsion in 3% of egg phosphatidylcholine lowered the systolic blood pressure, the maximum decrease being 17.0 mmHg 4 h after its administration at a dose of 25 mg/kg (Fig. 2d). These results suggest that phosphatidylcholine in the egg yolk played an important role in potentiating the antihypertensive activity of ovokinin. At a dose of 25 mg/kg, the antihypertensive effect of ovokinin emulsified with egg yolk was larger than that emulsified with egg phospholipid or phosphatidylcholine. Lipoproteins in egg yolk might be responsible for this difference.

The effect of soybean phospholipid was also examined. As shown in Fig. 2e, ovokinin emulsified with soybean phospholipid significantly lowered the blood pressure at a dose of 25 mg/kg, but this effect was less than that when emulsified with egg phospholipid.

The observed improvement in the oral availability of ovokinin after its emulsification may reflect the improved intestinal absorption of the peptide. As another possibility, ovokinin might be protected from peptidases by emulsification. For example, the Arg2-Ala3 bond of ovokinin is sensitive to trypsin.

When we take whole egg, ovalbumin will be released by action of pepsin in the stomach, and the peptide will be instantantly emulsified with egg yolk in the gastrointestinal tract. Thus, ovokinin will be effectively absorbed from the intestines.

References