Antihypertensive Peptides Derived from Egg Proteins

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ABSTRACT There have been studies of antihypertensive peptides derived from food proteins, but very few described the production of bioactive peptides from egg proteins. The first 2 antihypertensive peptides isolated in egg were obtained by enzymatic hydrolysis of ovalbumin. They correspond to the sequences Phe-Arg-Ala-Asp-His-Pro-Phe-Leu (ovokinin) and Arg-Ala-Asp-His-Phe-Leu (ovokinin 2-7). Both exhibited endothelium-dependent vasodilatory activity. Ovokinin (2-7) had higher antihypertensive potency than ovokinin in spontaneously hypertensive rats (SHR). Modifications in the sequence of ovokinin (2-7) improved the bioavailability of this peptide. It was also demonstrated that different ovalbumin hydrolysates can inhibit angiotensin I–converting enzyme (ACE). We recently obtained an egg white hydrolysate that inhibited the enzyme in vitro. It was obtained by treating egg white with pepsin and it exhibited antihypertensive activity in SHR. Some ACE-inhibitory peptides obtained from this hydrolysate (Tyr-Arg-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, Arg-Ala-Asp-His-Pro-Phe-Leu, and Ile-Val-Phe) also showed antihypertensive activity in these rats. The egg products mentioned could be used as functional food ingredients with potential therapeutic benefit in the prevention and treatment of hypertension. J. Nutr. 136: 1457–1460, 2006.

KEY WORDS: bioactive peptides • egg proteins • hypertension • angiotensin I–converting enzyme

In recent years, society has become increasingly aware of the close relation that exists between diet and health. As a result, functional foods, beneficial to health, are having a marked effect on the food sector. Research carried out to produce these foods has paid special attention to the study of the physiological role played by dietary proteins. There are certain fragments within the sequence of food proteins that may show biological activity once released by hydrolysis. These fragments, known as bioactive peptides, can be produced in vivo by the action of gastrointestinal enzymes and can also be obtained in vitro using specific enzymes, or during the preparation of certain foods.

Since their discovery in 1979 (1), bioactive peptides with different biological activities have been described. Some have the ability to reduce arterial tone and control hypertension, which is the primary cause of death in developed countries. Although this condition is relatively easy to detect and control, many hypertensive patients are unaware of their illness, whereas others are diagnosed but receive inappropriate treatment. It is therefore not surprising that great interest exists at present in research into antihypertensive peptides found in food. Functional foods containing these peptides may represent a new strategy for the prevention and treatment of hypertension.

Antihypertensive peptides are obtained from proteins of both animal (2–6) and plant (7–9) origin. Egg proteins are a very important source of dietary nitrogen, and this food plays a vital role in human nutrition. Egg white has a particularly high protein content (Tables 1 and 2); yet very few studies have examined the production of bioactive peptides from egg proteins. This paper attempts to bring together the most important findings of the current research in this field, concentrating in particular on studies conducted in the last decade using egg white–derived antihypertensive products and peptides (Table 3). Below we give detailed information about the most important peptides.

Egg products and antihypertensive peptides. The first peptides with antihypertensive effects to be obtained from egg proteins were 2 peptides with direct vascular effects. Fujita et al. (10) isolated an antihypertensive and vasorelaxing octapeptide with the amino acid sequence Phe-Arg-Ala-Asp-His-Pro-Phe-Leu (FRADHPFL).1 This sequence corresponds to fragment 358–365 of egg albumen, which is the chief protein present in egg white. The protein in question showed partially endothelium-dependent vasodilatory activity in canine mesenteric arteries, and was given the name ovokinin. Its effect was mediated in part by B1 receptors, which stimulated the release of prostacyclin. Ovokinin displayed antihypertensive effects when administered in high doses to spontaneously hypertensive rats (SHR), an effect that increased when the peptide was administered orally in the form of an emulsion in egg yolk. It was postulated that the phospholipids in the egg yolk increased the oral availability of ovokinin because they improved its intestinal absorption and protected the peptide from digestion by intestinal peptidases (11).

The second egg white–derived peptide with vasorelaxing properties was a hexapeptide, characterized as fragment 2-7 of ovokinin. It was given the name ovokinin (2-7) and its sequence was as follows: Arg-Ala-Asp-His-Phe-Leu (RADHPF) (12). In 1999, Matoba et al. (12) purified this peptide from an egg albumen hydrolysate using chymotrypsin, and observed that it corresponded to residues 359–364 of this protein. The peptide caused endothelium-dependent relaxation in mesenteric arteries of SHR rats, and this relaxation was mediated principally by nitric oxide. It did not, however, produce relaxation in the arteries of normotensive Wistar-Kyoto rats (WKY).

Footnotes:
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2 To whom correspondence should be addressed. E-mail: amaya@med.ucm.es.

Abbreviations used: ACE, angiotensin I–converting enzyme; F, fraction with a molecular mass <3000 Da; FRADHPFL, Phe-Arg-Ala-Asp-His-Pro-Phe-Leu; IC50, concentration that inhibits 50% of the enzyme activity; IVF, Ile-Val-Phe; LW, Leu-Trp; RADHPF, Arg-Ala-Asp-His-Phe-Leu; RADHPFL, Arg-Ala-Asp-His-Pro-Phe-Leu; RPHFHP, Arg-Pro-Asp-His-Pro-Phe; RPLKPW, Arg-Pro-Leu-Lys-Pro-Tyr; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; YAEERYPIL, Tyr-Arg-Glu-Glu-Arg-Tyr-Pro-Ile-Leu.
In addition, the arterial blood pressure of SHR rats decreased when doses of ovokinin (2-7) 10 times smaller than effective doses of ovokinin were administered orally, but the blood pressure of WKY rats was not affected when the same doses of this peptide were administered in the same way. Moreover, i.v. administration of ovokinin (2-7) in the aforementioned doses did not affect the rats’ arterial blood pressure. Administering very high i.v. concentrations of the peptide caused only a slight decrease in this variable in SHR rats (13). Recently, Scruggs et al. (14) in their studies of vascular reactivity in isolated arteries showed that ovokinin (2-7) produces its effects by activation of bradykinin B2 vascular receptors.

A range of factors may condition the potential antihypertensive activity of peptides when administered orally (15). Technological factors, such as processing, may condition their oral activity, but a range of physiological factors, such as digestion via gastrointestinal enzymes, may also condition their bioavailability and activity. On occasion, attempts were made to improve the oral activity of peptides by means of structural modifications. For example, several products similar to ovokinin (2-7) were synthesized to improve its oral antihypertensive activity. Of particular interest among these products are the peptides Arg-Pro-Phe-His-Pro-Phe (RPFPHF) and Arg-Pro-Leu-Lys-Pro-Trp (RPLKPW), which showed 10 and 100 times more activity, respectively, than ovokinin (2-7) when administered orally to SHR rats. The substitution of amino acids would probably make these sequences more resistant to digestive tract proteases (13,15). In most cases, the inhibitory activity of the prohormone 1-converting enzyme (ACE) explains the action of antihypertensive peptides of food origin.

### TABLE 1

<table>
<thead>
<tr>
<th>Egg component</th>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White or albumen</td>
<td>9.7–10.6</td>
<td>0.03</td>
<td>0.4–0.9</td>
</tr>
<tr>
<td>Yolk</td>
<td>15.7–16.6</td>
<td>31.8–35.5</td>
<td>0.2–1.0</td>
</tr>
<tr>
<td>Whole egg</td>
<td>12.8–13.4</td>
<td>10.5–11.8</td>
<td>0.3–1.0</td>
</tr>
</tbody>
</table>

1 Values are ranges.

In addition to the aforementioned antihypertensive activity, several studies carried out by Miguel et al. in 2004 (17), also showed that hydrolysis of egg white proteins with different enzymes of digestive origin produces hydrolysates with a large degree of ACE-inhibitory activity. The most potent hydrolysates were obtained after hydrolysis of egg white with pepsin, but the incubation time of the enzyme was significant. After >30 min of incubation, active hydrolysates with relatively low IC50 values were obtained. Hydrolysis of egg white with pepsin over a 3-h period produced a hydrolysis with potent ACE-inhibitory activity and an IC50 value of 55 mg/L. The ultrafiltration of this hydrolysate made it possible to obtain a fraction with a molecular mass of <3 kDa (F), which exhibited much more ACE-inhibitory activity than the hydrolysate itself. The IC50 value of this fraction was 34 mg/L, and it contained six ACE-inhibitory peptides with IC50 values of 4.7, 6.2, and 33.1 mg/L respectively. Six ACE-inhibitory peptides were isolated in the ovumubulin hydrolysate that was obtained using pepsin. These peptides had IC50 values between 0.4 and 15 μmol/L. However, only one of them, the dipeptide Leu-Trp (LW), showed antihypertensive activity in SHR rats. According to these researchers, treatment with trypsin and chymotrypsin did not produce active hydrolysates. The IC50 values for these hydrolysates were in fact >1000 mg/L.

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The hydrolysate obtained by Miguel et al. (17) from egg white by treatment with pepsin for 3 h, the fraction F, and the peptide sequences YAEYPIL, RADHPFL, and IVF ex-

### TABLE 2

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amount</th>
<th>Isoelectric point</th>
<th>Molecular mass</th>
<th>Td°C</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovalbumin</td>
<td>54</td>
<td>4.5</td>
<td>45,000</td>
<td>84.0</td>
<td>Phosphoglycoprotein</td>
</tr>
<tr>
<td>Ovotransferrin</td>
<td>12</td>
<td>6.1</td>
<td>76,000</td>
<td>61.0</td>
<td>Binds metallic ions</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>11</td>
<td>4.1</td>
<td>28,000</td>
<td>79.0</td>
<td>Binds trypsin</td>
</tr>
<tr>
<td>Ovomucin</td>
<td>3.5</td>
<td>4.5−5.0</td>
<td>5.5−8.3 × 10⁶</td>
<td>—</td>
<td>Sialoprotein, viscous</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>3.4</td>
<td>10.7</td>
<td>14,300</td>
<td>75.0</td>
<td>Destroys some bacteria</td>
</tr>
<tr>
<td>G2 globulin</td>
<td>4.0</td>
<td>5.5</td>
<td>3.0−4.5 × 10⁴</td>
<td>92.5</td>
<td>—</td>
</tr>
<tr>
<td>G3 globulin</td>
<td>4.0</td>
<td>4.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ovothien</td>
<td>1.5</td>
<td>5.1</td>
<td>49,000</td>
<td>—</td>
<td>Inhibits serine-proteases</td>
</tr>
<tr>
<td>Ovoglycoprotein</td>
<td>1.0</td>
<td>3.9</td>
<td>24,400</td>
<td>—</td>
<td>Sialoprotein</td>
</tr>
<tr>
<td>Ovothioprotein</td>
<td>0.8</td>
<td>4.0</td>
<td>32,000</td>
<td>—</td>
<td>Binds riboflavin</td>
</tr>
<tr>
<td>Ovomacroglobulin</td>
<td>0.5</td>
<td>4.5</td>
<td>7.7 × 10⁵</td>
<td>—</td>
<td>Strongly antigenic</td>
</tr>
<tr>
<td>Cystatin</td>
<td>0.05</td>
<td>5.1</td>
<td>12,700</td>
<td>—</td>
<td>Inhibits thiol-proteases</td>
</tr>
<tr>
<td>Avidin</td>
<td>0.05</td>
<td>10</td>
<td>68,300</td>
<td>85</td>
<td>Binds biotin</td>
</tr>
</tbody>
</table>

1 Td is the denaturation temperature in the water or buffer.
hindered clear antihypertensive effects. These products produced a significant decrease in both systolic and diastolic arterial blood pressure when administered orally in 1 single dose to SHR rats. In contrast, this administration did not affect the arterial blood pressure of normotensive WKY rats. The antihypertensive potency of the fraction F was greater than that of the hydrolysate, and the antihypertensive potency of the 3 aforementioned sequences was similarly greater than that of the fraction F (18). Later studies simulating gastrointestinal digestion indicate that the sequences YAEEYRIP and RADHPF hydrolyze when administered orally (19). It is therefore highly likely that the products of this hydrolysis are in fact responsible for the antihypertensive effect observed. However, the IVF sequence, which exhibited less antihypertensive effect than the other 2 sequences, would be able to act directly when administered orally (18). In addition, the hydrolysate obtained from egg white by treatment with pepsin for 3 h attenuated the onset of arterial hypertension in SHR rats when administered orally after weaning. Treatment finished when the rats were 20 wk old, and this caused a complete reversal of the effect. Two weeks later, the rats that we had stopped treating with the hydrolysate showed arterial blood pressure values similar to those of the untreated rats in the control group (20).

Some peptides with antihypertensive activity were also produced by the enzymatic hydrolysis of egg yolk. Yoshii et al. (21) showed that ACE-inhibitory oligopeptides may be produced when egg yolk is hydrolyzed with different enzymes. Oral administration of different doses of these oligopeptides to SHR rats produced a significant drop in both systolic and diastolic blood pressure.

Finally, it should be mentioned that some researchers succeeded recently in improving antihypertensive activity by oral administration of some peptides derived from food proteins, incorporating them into liposomes to protect them as they passed through the gastrointestinal tract. The activity of peptides has also on occasion been improved by modifying their structure to obtain a cyclical shape, achieved by constructing a disulfuric bridge between the amino acids of the extreme N- and C-terminal (22). Other researchers used genetic modification to incorporate peptide sequences with antihypertensive activity into the proteins of some foodstuffs. Using controlled mutagenesis, it has been possible, for example, to incorporate some egg peptide sequences with potent antihypertensive activity into soy proteins. It is also possible to modify residues close to the active peptide to facilitate its release in vivo (13,23,24).

In conclusion, the results of the research discussed above suggest the possibility of using antihypertensive peptides derived from egg proteins for the prevention and treatment of hypertension. The idea of including them as functional food ingredients is particularly attractive. It is clear, however, that clinical studies should first be conducted with these peptides, or with the foods that contain them, to guarantee their safety and effectiveness in both healthy subjects and hypertensive patients.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


