Short-term effect of egg-white hydrolysate products on the arterial blood pressure of hypertensive rats

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Abstract

In the present study we evaluate the blood pressure-lowering effect of the following products: the hydrolysate obtained from egg white (EW) by enzymatic treatment with pepsin (HEW), the peptide fraction of HEW with molecular mass lower than 3000 Da (HEW2999Da), and three peptide sequences isolated from HEW < 3000 Da (Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu: YAEERYPIL; (Arg-Ala-Asp-His-Pro-Val-Leu: RADHPFL); and (Ile-Val-Phe (IVF)). These peptides, and also HEW and HEW < 3000 Da, had been characterized previously in vitro as potent inhibitors of angiotensin-converting enzyme (ACE). EW and the products mentioned earlier were orally administered by gastric intubation, to 17–20-week-old male spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY) rats. We measured the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) of the rats by the tail cuff method before administration and also 2, 4, 6, 8 and 24 h post-administration. Distilled water served as negative control, and we used captopril (50 mg/kg) as positive control to carry out similar experiments with a known ACE inhibitor. HEW, HEW < 3000 Da and the three peptide sequences decreased SBP and DBP in SHR but they did not modify these variables in WKY rats. The peptide sequences YAEERPIL, RADHPFL and IVF showed a potency to decrease blood pressure greater than HEW or HEW < 3000 Da. The results obtained suggest that the studied products could be used as a functional food with potential therapeutic benefit in the prevention and treatment of hypertension.

Hypertension: Angiotensin-converting enzyme: Bioactive peptides: Spontaneously hypertensive rats

Angiotensin-converting enzyme (ACE) is a multifunctional enzyme, located in different tissues, able to regulate several systems that affect blood pressure, as it is responsible for the generation of the vasopressor agent, angiotensin II, and for the inactivation of the vasodepressor agent bradykinin. The enzymatic hydrolysis of food proteins can release peptides that may exhibit different biological activities. Among the bioactive peptides known so far, those with ACE-inhibitory properties are receiving special attention due to their potential beneficial effects in the treatment of hypertension. In fact, some studies have demonstrated the antihypertensive properties of hydrolysates and peptides with ACE-inhibitory activity derived from food proteins in hypertensive animals (Nakamura et al. 1996; Wu & Ding, 2001; Fujita et al. 2001; Sipola et al. 2002; Miguel et al. 2005) and patients (Kawasaki et al. 2002; R. Nimmaguda, unpublished results; Seppo et al. 2003; Mizushima et al. 2004).

Certain egg white (EW) derived peptides and synthetic analogues are known to play a role in controlling the development of hypertension by exerting vasorelaxing effects (Fujita et al. 1995a,b; Matoba et al. 1999, 2001). Our research group has recently shown that the hydrolysate obtained from EW by enzymatic treatment with pepsin (HEW) possessed in vitro ACE-inhibitory and antioxidant properties (Miguel et al. 2004; Dávalos et al. 2004). The fraction of the hydrolysate with molecular mass lower than 3000 Da (HEW < 3000 Da) exhibited a higher ACE-inhibitory activity. Several very active peptide sequences that derive from hydrolysis of ovalbumin were identified from HEW < 3000 Da by tandem mass spectrometry (reverse phase-HPLC–MS/MS). Among them, the fragments Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu (YAEERPIL), Arg-Ala-Asp-His-Pro-Val-Leu (RADHPFL) and Ile-Val-Phe (IVF) were characterized as potent ACE inhibitors in vitro (Miguel et al. 2004).

It is important to test the in vivo effect of the ACE-inhibitory compounds to establish their possible usefulness. In fact, we have to bear in mind that their antihypertensive activity is dependent on their ability to reach the target site without being degraded and inactivated by intestinal or plasma peptidases. Therefore the aim of the present study was to evaluate the blood pressure-lowering effect of HEW, HEW < 3000 Da and the three peptide sequences in hypertensive rats.

Methods

Products

EW, HEW, HEW < 3000 Da and the peptides YAEERPIL, RADHPFL and IVF were used in the present study. EW was

Abbreviations: ACE, angiotensin-converting enzyme; DBP, diastolic blood pressure; EW, egg white; HEW, hydrolysate obtained from egg white by enzymatic treatment with pepsin; IC50, protein concentrations necessary to inhibit 50 % of enzyme activity; IVF, peptide sequence Ile-Val-Phe; RADHPFL, peptides sequence Arg-Ala-Asp-His-Pro-Val-Leu; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto, YAEERPIL, peptides sequence Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu.

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obtained in our laboratory from chicken fresh shell eggs, lyophilized and stored frozen until used. HEW and HEW < 3000 Da were prepared as explained by Miguel et al. (2004). Synthetic peptides were obtained by conventional Fmoc solid phase synthesis with a 431A peptide synthesizer (Applied Biosystem Inc., Überlingen, Germany) according to the method described by Atherton & Sheppard (1989). They were synthesized and purified by the Unitat de Peptids of Barcelona University, and their purity (>90%) was verified in our laboratory by reverse phase-HPLC–MS/MS (Gómez-Ruiz et al. 2003). Captopril (Sigma, St Louis, MO, USA) was also used in the present study. All the products used were dissolved in distilled water to be administered to the rats.

Experimental procedure in rats

In the present study we have used 17–20-week-old male spontaneously hypertensive rats (SHR) weighing 314 (SEM 3) g and 17–20-week-old male normotensive Wistar–Kyoto (WKY) rats weighing 337 (SEM 6) g. All these animals were obtained from Charles River Laboratories España S.A. (Barcelona, Spain). The animals remained at a temperature of 23°C with 12 h light–dark cycles, and consumed ad libitum tap water and a standard diet for rats (A04; Panlab, Barcelona, Spain) during the experiments. All the products were orally administered to the animals by gastric intubation between 09.00 and 10.00 hours. Distilled water served as negative control, and captopril (50 mg/kg), a known ACE inhibitor, served as positive control. We always administered 1 ml water per rat, and when a compound was orally given, 1 ml per rat of an appropriate solution of this compound was also administered. We measured the systolic blood pressure (SBP) and the diastolic blood pressure (DBP) of the rats by the tail cuff method before administration and also 2, 4, 6, 8 and 24 h post-administration. Before the measurement, the rats were kept at 30°C for 10 min to make the pulsations of the tail artery detectable. The original method for measuring arterial blood pressure using the tail cuff provides only SBP values (Buñag, 1973), but the equipment used in the present study, LE 5001 (Letica, Hospitalet, Barcelona, Spain), has a high-sensitivity pulse transducer coupled with an accurate microprocessor program, and allows us to distinguish between SBP and DBP.

Results

Before the administration of the different products, the SBP and DBP values of the SHR (248 (SEM 4) and 219 (SEM 4) mm Hg, respectively) were higher than the SBP and DBP values of the WKY rats (173 (SEM 5) and 123 (SEM 5) mm Hg, respectively) (P<0.001 with the Student’s t test). The oral administration of distilled water or EW to SHR did not change the values of SBP and DBP (Fig. 1). However, HEW and, particularly, HEW < 3000 Da caused a significant dose-dependent decrease in SBP and DBP in these animals. The lowest effective dose of HEW was 150 mg/kg and the lowest effective dose of HEW < 3000 Da was 50 mg/kg. The greatest decreases were obtained when we administered 50 mg/kg of captopril to the SHR. The maximal decreases in the SBP and in the DBP were observed, respectively, 6 and 4 h after the administration of the different products, and both variables returned to baseline 24 h after the administration (Fig. 1).

Fig. 1. Decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused in spontaneously hypertensive rats by different products: (a), water (C), captopril (50 mg/kg, L), egg white (EW; 400 mg/kg, x) and the hydrolysate obtained from EW by enzymatic treatment with pepsin (HEW; 100 (A), 150 (A), 200 (A), 400 (A) mg/kg); (b), water (C), captopril (50 mg/kg, L) and the peptide fraction of HEW with molecular mass lower than 3000 Da (HEW < 3000 Da, 25 (A), 50 (A), 100 (A) mg/kg). For details of procedures, see p. 732. Values are means with their standard errors depicted by vertical bars. The experimental groups always had eight animals except in the case of water where a group of ten animals was used.

Statistical analysis

The results are expressed as means with their standard errors for a minimum of eight rats, and were analysed by one-way ANOVA. Differences between the groups were assessed by the Bonferroni test and we always consider the differences between the means to be significant when P<0.05.
The three peptides used in the present study also exhibited a significant antihypertensive effect (Fig. 2). They decreased SBP and DBP in SHR, in a dose-dependent manner. The minimum effective doses were about 2 mg/kg YAEERYPIL and RADHPFL and 4 mg/kg IVF. The maximum decrease in SBP and DBP was observed 6 h after their administration and the effect lasted for, at least, 8 h. The values of SBP and DBP 24 h post-administration of the different fragments were always very similar to those obtained before the administration. The potency to decrease the arterial blood pressure of these peptide sequences was greater than the potency of HEW or HEW

Fig. 2. Decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused in spontaneously hypertensive rats by different products: (a), water (○), captopril (50 mg/kg, □) and the peptide sequence Tyr-Ala-Glu-Arg-Tyr-Pro-Ile-Leu (YAEERYPIL; 0·5 (●), 1 (△), 2 (■) mg/kg); (b), water (○), captopril (50 mg/kg, □) and the peptide sequence Arg-Ala-Asp-His-Pro-Phe-Leu (RADHPFL; 0·5 (●), 1 (△), 2 (■) mg/kg); (c), water (○), captopril (50 mg/kg, □) and the peptide sequence Ile-Val-Phe (IVF; 1 (●), 2 (△), 4 (■) mg/kg). For details of procedures, see p. 732. Values are means with their standard errors depicted by vertical bars. The experimental groups always had eight animals except in the case of water where a group of ten animals was used.

The present results show that HEW exerts antihypertensive effects in SHR. HEW

Discussion
The SHR strain has usually been used to carry out initial studies on the antihypertensive effect of functional food products and bioactive peptides derived from food proteins (Karaki et al. 1990; Nakamura et al. 1995, 1996; Saito et al. 1994; Nurminen et al. 2000; Fujita et al. 2001; Sipola et al. 2002; Wu & Ding, 2001). The development of hypertension in SHR is very similar to that in man. In both cases hypertension appears at an early age, there is a family history of this pathology and it is worsened by a sodium-rich diet (Okamoto & Aoki, 1963).

The present results show that HEW exerts antihypertensive effects in SHR. HEW < 3000 Da was more active than HEW

Fig. 3. Decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused in Wistar–Kyoto rats by different products: water (○), captopril (50 mg/kg, □), egg white (EW; 400 mg/kg, ⌂), the hydrolysate obtained from EW by enzymatic treatment with pepsin (HEW; 400 mg/kg, △), the peptide fraction of HEW with molecular mass lower than 3000 Da (HEW < 3000 Da; 100 mg/kg, ◆), the peptide sequence Tyr-Ala-Glu-Arg-Tyr-Pro-Ile-Leu (YAEERYPIL; 2 mg/kg, ●), the peptide sequence Arg-Ala-Asp-His-Pro-Phe-Leu (RADHPFL; 2 mg/kg, △) and the peptide sequence Ile-Val-Phe (IVF; 4 mg/kg, ⌂). For details of procedures, see p. 732. Values are means with their standard errors depicted by vertical bars. The experimental groups always had eight animals except in the case of water where a group of nine animals was used.
Table 1. Decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused in spontaneously hypertensive rats (SHR) at different times after the administration of different products§

(Mean values with their standard errors)

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<td>10††† 2.2</td>
<td>2.4 6.3†††  4.6</td>
<td>7.7††† 1.8</td>
<td>27††† 1.7</td>
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<td>-19.2***†‡</td>
<td>2.2 1.3†</td>
<td>23***†‡‡ 3.4</td>
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<td>YAEERYPIL (2 mg/kg)</td>
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<td>1.3 1.6</td>
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<td>IVF (4 mg/kg)</td>
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<td>0.9 0.8</td>
<td>-12.8***†‡</td>
<td>4.4 3.0†‡</td>
<td>-31.7†† 3.6</td>
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EW, egg white; HEW, hydrolysate obtained when EW was treated with pepsin; HEW < 3000 Da, fraction lower than 3000 Da obtained from HEW; IVF, peptide sequence Ile-Val-Phe; RADHPFL, peptide sequence Arg-Ala-Asp-His-Pro-Phe-Leu; YAEERYPIL, peptide sequence Tyr-Ala-Glu-Arg-Tyr-Pro-Ile-Leu.

Mean values were significantly different: *P < 0.05, **P < 0.01, ***P < 0.001 vs. water; †P < 0.05, ††P < 0.01, †††P < 0.001 vs. captopril; †††P < 0.001 vs. EW.

§ The experimental groups always had eight animals except in the case of water where a group of ten animals was used. For details of procedures, see p. 732.
Decreases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) caused in Wistar–Kyoto rats (WKY) at different times after the administration of different products§

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<td>HEW (400 mg/kg)</td>
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<td>1.4</td>
<td>2.7</td>
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<td>RADHPFL (2 mg/kg)</td>
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<td>2.2</td>
<td>1.9</td>
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<tr>
<td>IVF (4 mg/kg)</td>
<td>5.3</td>
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<td>4.8</td>
<td>3.8</td>
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<tr>
<td>HEW &lt; 3000 Da, fraction lower than 3000 Da obtained from HEW</td>
<td>2.3</td>
<td>2.4</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>HEW 3000 Da</td>
<td>0.9</td>
<td>1.0</td>
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<tr>
<td>YAEERYPIL</td>
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<td>IVF</td>
<td>5.3</td>
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§ The experimental groups always had eight animals except in the case of water where a group of nine animals was used. For details of procedures, see p. 732.

The antihypertensive products from egg white exerted antihypertensive activity on SHR at a very high dose (2 g/kg).

In a previous paper we reported that four of the fourteen peptide sequences identified in HEW < 3000 Da were potent ACE inhibitors in vitro. In fact, they showed IC₅₀ values lower than 34 μM (Miguel et al. 2004). One of these peptides corresponded to Ovokinin, a vasorelaxing octapeptide derived from ovalbumin (FRADHPFL; Fujita et al. 1995b). It had already been described that Ovokinin significantly lowered the SBP in SHR when orally administered in solution at a dose of 100 mg/kg or at a dose of 25 mg/kg, in the form of an emulsion in 30% egg yolk (Fujita et al. 1995a). Therefore, we focused our study on evaluating the antihypertensive effect of the other three novel sequences, YAEERYPIL, RADHPFL and IVF.

The antihypertensive effect of the three synthetic peptides followed a time course similar to that observed when we administered HEW or HEW < 3000 Da. It should be noticed that a prolonged duration of the pressure-lowering effect is usually desirable for bioactive peptides (Chen et al. 2003). The antihypertensive potency of the peptides studied could be related to their in vitro efficacy to inhibit ACE (Miguel et al. 2004). The amount of these peptides that was required to show antihypertensive effects was relatively small, bearing in mind the efficient doses of ACE-inhibitory peptides described by other researchers. For instance, 6 h after the administration of 5 mg/kg of the potent ACE inhibitors Val-Pro-Pro and Ile-Pro-Pro to SHR, the SBP of SHR decreased 20-1 and 18-3 mm Hg, respectively (Nakamura et al. 1995).

It was not surprising to find a clear decrease in SBP and DBP when we administered 50 mg/kg of captopril to the SHR, because this drug is a potent ACE inhibitor with an IC₅₀ value of 0.02 μM (Fujita & Yoshikawa, 1999). It should also be noticed that, although the studied peptides had IC₅₀ values higher than captopril (Miguel et al. 2004), they also caused a clear decrease in arterial blood pressure when they were administered at low doses (Fig. 2). In this context, Fujita & Yoshikawa (1999) suggested that, as compared with captopril, the ACE inhibitors derived from food proteins possess higher in vivo activities than those expected by their in vitro activities, and this could be explained by their higher affinity for the different tissues and by their slower elimination. Another possibility is that ACE inhibition might not be the only mechanism of action of these peptides. Yamada et al. (2002) postulated that RADHPF, an
antihypertensive peptide obtained from ovalbumin, named Ovokinin (2 – 7), lowered the arterial blood pressure of SHR through the interaction with receptors expressed in the gastrointestinal tract and/or through effects on the central nervous system. Moreover, we have demonstrated the radical scavenging activities of HEW, HEW < 3000 Da and some peptides isolated from these products, particularly YAEERYPIL (Dávalos et al. 2004), and it is known that antioxidant-rich diets significantly reduce the arterial blood pressure in SHR (Akpaftiøng & Taylor, 1998; Soares de Moura et al. 2002; Rodríguez-Iturbe et al. 2003).

In the present study, the values of SBP and DBP of the WKY rats were somewhat high, but similar to those reported by other authors (Zicha & Kunes, 1999). The values of arterial blood pressure could be overestimated when the tail cuff is used (Bazil et al. 1993). Furthermore, some researchers believe that the WKY rats are not a pure normotensive strain (Wright & Rankin, 1982). However, these animals are always used as the normotensive control in the studies carried out with SHR. In the present study, significant differences were obtained when the values of SBP and DBP in the WKY rats were compared with the correspondent values of SBP and DBP in SHR. It is therefore important to highlight that the egg products and the peptides used in the present study did not modify SBP and DBP in the WKY rats. This suggests that their effects are specific to the hypertensive state, and we could therefore expect a lack of arterial blood pressure effects in normotensive human subjects.

In conclusion, we have demonstrated the antihypertensive properties of the egg products and peptides studied in SHR. The results obtained suggest that the studied products could be used as functional food ingredients with potential therapeutic benefit in the prevention and treatment of hypertension. At the moment, we are conducting experiments aimed at establishing their long-term effect on the arterial blood pressure in animals, and clarifying whether other mechanisms different from ACE inhibition could be implicated in the antihypertensive activity of these egg products and peptides. Moreover, before routine clinical use of these substances, it would be necessary to carry out clinical studies to demonstrate their long-term antihypertensive efficiency in man.

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References


