Antihypertensive activities of the plasteins derived from casein hydrolysates in spontaneously hypertensive rats

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ABSTRACT

Two casein plasteins were prepared from the Neutrase-catalyzed plastein reaction of casein hydrolysates in the presence of proline and phenylalanine, respectively, and assessed for their antihypertensive activities at 300 mg/kg body using rat model. After being oral administered, the two plasteins at initial time of 2–6 h resulted in the rats decreased systolic and diastolic blood pressures of 10–23 and 8–19 mmHg, respectively; however, the measured antihypertensive activities showed decreasing trends as oral administrating time prolonged. The two plasteins also decreased serum levels of angiotensin II, angiotensin I converting enzyme, and aldosterone, but had unclear effect on serum angiotensinogen level. The two plasteins showed same antihypertensive activities without statistical difference (p > 0.05), and were more efficient than casein hydrolysates but less effective than captopril (p < 0.05) to exert antihypertensive effect. It is concluded that the plastein reaction can confer casein hydrolysates with enhanced in vivo antihypertensive activities.

Introduction

High blood pressure (or hypertension) is one of leading risk factors for the cardiovascular diseases (Gu, Burt, Poulose-Ram, Yoon, & Gillum, 2008). In the body, the etiology of hypertension is complex. The well-known renin–angiotensin system (RAS) is the most critical hormonal system to regulate cardiovascular physiology. Angiotensin I converting enzyme (ACE) catalyzes the RAS reaction to produce a potent vasoconstrictor (angiotensin II) (Livi, Adrian, & Goldsmith, 2007), which performs critical role in the etiology of hypertension. Blood pressure can thus be effectively decreased via ACE inhibition (i.e. using so-called ACE inhibitors). Captopril is a synthetic ACE inhibitor for hypertension therapy (Cushman & Connor, 1997). In recent years, ACE inhibitory (ACEI) peptides have been verified in several studies to exert in vivo activities to decrease blood pressure (Balti et al., 2015; Chen et al., 2014; Deng et al., 2014; Ngo et al., 2015; Yamada et al., 2015). Val-Pro-Pro and Ile-Pro-Pro are two typical peptides found in fermented milk with good in vitro ACE inhibition (Nakamura, Yamamoto, Sakai, & Takano, 1995). Protein hydrolysates and purified peptides have been verified in several studies to exert in vivo activities to decrease blood pressure (Balti et al., 2015; Chen et al., 2014; Deng et al., 2014; Ngo et al., 2015; Yamada et al., 2015).

In recent years, the classic plastein reaction is suggested to be an effective approach to enhance multiple bioactivities especially ACEI activities of protein hydrolysates and peptides (Gong, Mohan, Gibson, & Udenigwe, 2015; Udenigwe &...
Casein and soy protein hydrolysates after their plastein reaction are considered very important to the enhanced activities (Li, Li, & Zhao, 2010; Udenigwe & Rajendran, 2016). Transpeptidation occurred in the plastein reaction is considered to be the enhanced activities (Li, Li, & Zhao, 2010; Udenigwe & Rajendran, 2016). Casein and soy protein hydrolysates after their plastein reaction thus show increased in vitro ACEI activities (Gao & Zhao, 2012; Sun & Zhao, 2012; Xu, Kong, & Zhao, 2014; Xu, Li, & Zhao, 2013; Zhao & Li, 2009). In the presence of some hydrophobic amino acids, in vitro ACEI activities of casein hydrolysates can also be enhanced by the plastein reaction (Li et al., 2010). However, it is still not assessed if the plastein reaction can also confer these protein hydrolysates with enhanced in vivo antihypertensive activities.

In this study, casein hydrolysates generated by a commercial protease Neutrase were subjected to a Neutrase-catalyzed plastein reaction in the presence of proline and phenylalanine, respectively. After that, in vivo antihypertensive activities of the two modified casein hydrolysates (i.e., two plasteins) were evaluated using spontaneously hypertensive rats (SHR) and captopril (as a positive control). Six indices in terms of systolic and diastolic blood pressures, serum angiotensinogen (AGT), ACE, angiotensin (Ang) II, and aldosterone of the rats after oral administration of casein hydrolysates and the two plasteins were measured, and compared with those indices of the control rats. The aim of this study was to assess in vivo antihypertensive activities of the two plasteins, verifying the potential of the plastein reaction to enhance antihypertensive activities of protein hydrolysates.

Materials and methods

Materials and chemicals

Casein with a protein concentration of 89.1% (on dry basis) was bought from Beijing Aoboxing Biotechnology Co. (Beijing, China). Neutrase 0.8 L with measured activity of 3100 units (U) per milliliter was obtained from Novozymes Co. (Bagsvaerd, Denmark). Rabbit lung acetone powder and phenylalanine used in the plastein reaction were bought from Beijing Aoboxing Biotechnology Co. (Bagsvaerd, Denmark). Rabbit lung acetone powder and phenylalanine were purchased from Beijing Aoboxing Biotechnology Co. (Bagsvaerd, Denmark). Rabbit lung acetone powder and phenylalanine used in the plastein reaction were purchased from Beijing Aoboxing Biotechnology Co. (Bagsvaerd, Denmark). Rabbit lung acetone powder and phenylalanine were purchased from Beijing Aoboxing Biotechnology Co. (Bagsvaerd, Denmark). Rabbit lung acetone powder and phenylalanine used in the plastein reaction were purchased from Beijing Aoboxing Biotechnology Co. (Bagsvaerd, Denmark). 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Degree of hydrolysis (DH) of the CH was calculated as per the method (Adler-Nissen, 1979).

In vitro ACEI activities of the CH and two modified products were assessed using HHL as substrate and rabbit lung acetone powder as ACE source as previously described (Cushman & Cheung, 1971). Serum AGT, ACE, Ang II, and aldosterone levels were detected by the respective kits with the guidelines from the kit manufacturer. In brief, all assays employed a quantitative sandwich enzyme immunoassay technique, in which the polyclonal antibodies specific for AGT, ACE, Ang II, and aldosterone were pre-coated onto the microplates. AGT, ACE, Ang II, and aldosterone in the standards and collected sera were sandwiched by the immobilized antibodies and biotinylated polyclonal antibodies, which was then recognized by a streptavidin-peroxidase conjugate. After that, 3,3',5,5'-tetramethylbenzidine (TMB) as peroxidase enzyme substrate was added. The intensity of the developed color was measured by a microplate reader (Bio Rad Laboratories, Hercules, CA, USA) within 10 min after the reaction was terminated. Serum AGT, ACE, Ang II, and aldosterone levels were thus determined using the generated standards curves.

Statistical analysis

The chemical or biochemical assays for the prepared specimens were carried out in triplicate, whilst the SBP and DBP assays for the rats were conducted at six different times. The reported data were expressed as mean values ± standard deviations. All data were analyzed by one-way analysis of variance (ANOVA), using Duncan’s multiple range tests to determine the differences between the mean values of multiple groups. The SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used in this analysis.

Results and discussion

In vitro and in vivo antihypertensive activities of the two plasteins

The prepared CH was detected to have a DH value of 13.6%, and exerted in vitro antihypertensive activity with an IC\textsubscript{50} value of (39.35 ± 1.72) µg/mL. The two plasteins CHPRO and CHPHE both showed in vitro antihypertensive activities with IC\textsubscript{50} values of (22.10 ± 3.19) and (24.31 ± 4.24) µg/mL, respectively. CHPRO and CHPHE both had lower IC\textsubscript{50} values than the CH. This indicated that the applied plastein reaction conferred CHPRO and CHPHE with higher in vitro ACEI activities. This result was consistent with those previously reported results (Li et al., 2010; Sun & Zhao, 2012; Xu et al., 2014; Zhao & Li, 2009), proving again that the plastein reaction could enhance in vitro ACEI activities of protein hydrolysates.

In the prior in vivo experiment, the CH, CHPRO, and CHPHE at three doses (100, 300, and 500 mg/kg body) were applied on the SHR. The results showed that the dose level of 100 mg/kg body only decreased SBP and DBP by about 5 mmHg in the SHR. However, another two dose levels could bring much decreases of SBP and DBP (ΔSBP and ΔDBP) in the SHR. Dose level of 300 mg/kg body was thus used in this study. The obtained results (Table 1) indicated that CHPRO and CHPHE both had in vivo antihypertensive activities in the SHR. SBP and DBP values of the SHR administrated with CHPRO and CHPHE were decreased, as the calculated ΔSBP and ΔDBP both were shown as negative values. CHPRO and CHPHE exerted stronger antihypertensive effects when the SHR were monitored at initial time of 2–6 h, as SBP and DBP were decreased by about 10–23 and 8–19 mmHg, respectively. However, the monitored antihypertensive effects decreased rapidly in the SHR when the monitor time prolonged. In general, CHPRO and CHPHE did not show any statistical difference in the measured antihypertensive effects \((p > 0.05)\), and they were two more potential than the CH (but less effective than captopril) to decrease SBP and DBP of the SHR \((p < 0.05)\). It is thus concluded that it was the plastein reaction (but not the types of the exogenous hydrophobic amino acids used in the plastein reaction) that enhanced in vivo antihypertensive activities of the two plasteins.

Other studies have assessed in vivo antihypertensive activities of protein hydrolysates or peptides using rat model, and reported conclusion similar to this study. Bovine casein hydrolysates at 400 mg/kg body can decrease SBP and DBP by 20 mmHg at 2 h after administration (Miguel, Contreras, Recio, & Aleixandre, 2009). Administration of a peptide with sequence of Val-Glu-Leu-Tyr-Pro at 10 mg/kg body decreased SBP by 20 mmHg at 6 h after the administration (Balti et al., 2015). In a reported study of Deng et al. (2014), if the SHR were given a peptide with sequence of Arg-Ser-Pro at 400 mg/kg body, SBP value

### Table 1. Antihypertensive effects of casein hydrolysates (CH) and two plasteins (CHPRO, and CHPHE) in the rats.

| Indices | Groups | 2 | 4 | 6 | 8 | 24 |
|---------|--------|---|---|---|---|----|
| ΔSBP (mmHg) 0.9% NaCl | 5.59 ± 2.85<sup>a</sup> | 7.04 ± 3.45<sup>d</sup> | 6.47 ± 4.73<sup>c</sup> | 4.22 ± 5.91<sup>b</sup> | 5.25 ± 6.85<sup>a</sup> |
| CH | −11.41 ± 4.56<sup>a</sup> | −7.98 ± 5.67<sup>c</sup> | −7.62 ± 4.55<sup>b</sup> | −3.82 ± 5.67<sup>a</sup> | −2.52 ± 5.77<sup>a</sup> |
| CHPRO | −23.37 ± 5.28<sup>d</sup> | −19.14 ± 4.70<sup>b</sup> | −12.40 ± 5.86<sup>b</sup> | −7.32 ± 8.21<sup>a</sup> | −3.99 ± 5.56<sup>a</sup> |
| CHPHE | −22.72 ± 5.12<sup>d</sup> | −18.08 ± 4.01<sup>b</sup> | −10.29 ± 6.22<sup>b</sup> | −8.11 ± 6.92<sup>a</sup> | −0.99 ± 8.91<sup>a</sup> |
| Captopril | −36.09 ± 5.03<sup>c</sup> | −46.97 ± 10.14<sup>a</sup> | −36.32 ± 8.20<sup>a</sup> | −23.49 ± 6.15<sup>b</sup> | −5.53 ± 4.32<sup>d</sup> |
| ΔDBP (mmHg) 0.9% NaCl | 4.37 ± 6.82<sup>a</sup> | 2.02 ± 3.08<sup>d</sup> | 5.22 ± 2.93<sup>c</sup> | 4.96 ± 9.88<sup>c</sup> | 3.26 ± 6.30<sup>d</sup> |
| CH | −10.64 ± 3.61<sup>c</sup> | −9.57 ± 4.64<sup>c</sup> | −6.43 ± 3.59<sup>c</sup> | −3.57 ± 5.63<sup>c</sup> | −1.51 ± 5.80<sup>c</sup> |
| CHPRO | −19.58 ± 3.18<sup>d</sup> | −16.98 ± 4.77<sup>b</sup> | −8.78 ± 4.28<sup>b</sup> | −7.95 ± 6.92<sup>c</sup> | −3.55 ± 6.00<sup>c</sup> |
| CHPHE | −18.09 ± 5.12<sup>d</sup> | −16.71 ± 3.41<sup>d</sup> | −7.93 ± 4.83<sup>b</sup> | −4.99 ± 6.32<sup>b</sup> | −2.47 ± 6.25<sup>d</sup> |
| Captopril | −29.11 ± 5.97<sup>c</sup> | −32.56 ± 10.96<sup>c</sup> | −17.97 ± 6.73<sup>c</sup> | −12.58 ± 7.92<sup>d</sup> | −4.86 ± 8.88<sup>d</sup> |

CHPRO and CHPHE: two plasteins of CH modified by plastein reaction in the presence of proline and phenylalanine, respectively. Different lowercase letters after the values of one index as the superscripts within the same column indicate that the values are significant differences \((p < 0.05)\).

CHPRO y CHPHE, dos plasteinas de CH modificadas con reacción plasteina con presencia de prolina y fenilalanina, respectivamente. Las diferentes letras minúsculas después de los valores de un índice como los superíndices en la misma columna indican que los valores presentan diferencias significativas \((p < 0.05)\).
of the SHR was reduced by about 30 mmHg. However, in vivo antihypertensive activities of bovine casein hydrolysates reported by Miguel et al. (2009) might be somewhat lower than those of CHPRO and CHPHE.

Serum AGT, ACE, Ang II, and aldosterone levels of the rats

Blood pressure is regulated by a well-known mechanism. Renin converts AGT into Ang I, followed by cleavage of Ang I into Ang II by the ACE. Ang II as a potent vasoconstrictor induces the release of aldosterone, and therefore, increases blood pressure. This study also measured four biochemical indices in the sera from the SHR (Table 2), as these indices could be used to support the observed in vivo antihypertensive activities of CHPRO and CHPHE. The results (Table 2) demonstrated that the CH, CHPRO, and CHPHE all were able to decrease serum ACE, Ang II, and aldosterone levels of the SHR. CHPRO and CHPHE both showed the same ability to decrease ACE, Ang II, and aldosterone levels ($p > 0.05$), but were more powerful than the untreated CH ($p < 0.05$). However, captopril was detected the most effective to decrease serum ACE, Ang II, and aldosterone levels. Although serum AGT level was detected with different values among the SHR of different groups, these values were found to have no statistical difference ($p > 0.05$). It is thus concluded that the plastein reaction of CH led to CHPRO and CHPHE enhanced capacities to decrease serum levels of ACE, Ang II, and aldosterone; as a consequence, decreased blood pressures were observed for the SHR.

In other in vivo studies, the effects of protein hydrolysates or peptides on these serum biochemical factors involved in the regulation of blood pressure have also been verified. Lactoferrin hydrolysates have in vivo antihypertensive activities, and can reduce serum ACE, Ang II and aldosterone levels (Fernández-Musoles, Manzanares, Burguete, Alborch, & Salom, 2013). When the SHR are given two peptides (Ile-Gly-Pro and Arg-Val-Pro-Ser-Leu) with antihypertensive activities, lower ACE, Ang II, and aldosterone levels are detected in sera (Lu et al., 2011; Yu, Yin, Zhao, Chen, & Liu, 2014). For example, respective ACE and Ang II levels are decreased by about 30% and 60% (Lu et al., 2011). These results supported the present results, proving in vivo antihypertensive activities of CHPRO and CHPHE once again. The two modified products perhaps reduced vascular Ang II formation in the rats via inhibiting ACE activity. This led to decreased vascular peripheral resistance; on the other hand, this also enabled a reduced aldosterone production, which then affected sodium and water storage to bring lower blood pressure (Fernández-Musoles et al., 2013).

Conclusion

When casein hydrolysates were treated by the mentioned plastein reaction, the two plasteins generated had enhanced in vitro and in vivo antihypertensive activities. In the rat model, the two plasteins demonstrated greater ability than casein hydrolysates to decrease SBP and DBP as well as serum levels of ACE, Ang II, and aldosterone. The plastein reaction is thus verified to be an effective approach to enhance in vivo antihypertensive activities of protein hydrolysates.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 2

| Groups | NaCl | CH | CHPRO | CHPHE | Captopril | Aldosterone |
|--------|------|----|-------|-------|-----------|-------------|
| 0.9%   | 2.81 ± 0.50a | 17.63 ± 0.41b | 62.92 ± 4.86b | 281.51 ± 5.74b | 120.91 ± 5.70b | 3.84 ± 0.84 |
| 2.75   | 14.28 ± 0.68b | 47.84 ± 0.84b | 215.93 ± 4.18b | 155.23 ± 5.11b | 154.24 ± 4.85b | 10.10 ± 0.14 |
| 2.53   | 11.05 ± 0.81c | 35.52 ± 2.68c | 154.24 ± 4.85c | 14.28 ± 0.68c | 154.24 ± 4.85c | 3.84 ± 0.84 |
| 2.44   | 10.10 ± 0.14d | 38.01 ± 3.79d | 154.24 ± 4.85d | 10.10 ± 0.14d | 154.24 ± 4.85d | 3.84 ± 0.84 |
| 2.09   | 8.60 ± 0.55e | 21.37 ± 3.01e | 120.91 ± 5.70e | 10.10 ± 0.14e | 154.24 ± 4.85e | 3.84 ± 0.84 |

Different lowercase letters after the values as the superscripts within the same column indicate that the values are significant differences ($p < 0.05$).
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