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Blood Pressure Lowering Effect of a Pea Protein Hydrolysate in Hypertensive Rats and Humans

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ABSTRACT: The blood pressure lowering effect of a pea protein hydrolysate (PPH) that contained <3 kDa peptides, isolated by membrane ultrafiltration from the thermolysin digest of pea protein isolate (PPI), was examined using different rat models of hypertension as well as hypertensive human subjects. The PPH showed weak *in vitro* activities against renin and angiotensin converting enzyme (ACE) with inhibitory activities of 17 and 19%, respectively, at 1 mg/mL test concentration. Oral administration of the PPH to spontaneously hypertensive rats (SHR) at doses of 100 and 200 mg/kg body weight led to a lowering of hourly systolic blood pressure (SBP), with a maximum reduction of 19 mmHg at 4 h. In contrast, orally administered unhydrolyzed PPI had no blood pressure reducing effect in SHR, suggesting that thermolysin hydrolysis may have been responsible for releasing bioactive peptides from the native protein. Oral administration of the PPH to the Han:SPRD-*cy* rat (a model of chronic kidney disease) over an 8-week period led to 29 and 25 mmHg reductions in SBP and diastolic blood pressure, respectively. The PPH-fed rats had lower plasma levels of angiotensin II, the major vasopressor involved in development of hypertension, but there was no effect on plasma activity or renal mRNA levels of ACE. However, renal expression of renin mRNA levels was reduced by approximately 50% in the PPH-fed rats, suggesting that reduced renin may be responsible for the reduced levels of angiotensin II. In a 3-week randomized double blind placebo-controlled crossover human intervention trial (7 volunteers), significant ($p < 0.05$) reductions (over placebo) in SBP of 5 and 6 mmHg were obtained in the second and third weeks, respectively, for the PPH group. Therefore, thermolysin derived bioactive peptides from PPH reduced blood pressure in hypertensive rats and human subjects, likely via effects on the renal angiotensin system.

KEYWORDS: pea protein hydrolysate, renin, spontaneously hypertensive rats, angiotensin converting enzyme (ACE), chronic kidney disease, SHR rats, Han:SPRD-*cy* rats, human intervention trial

INTRODUCTION

The renin−angiotensin system (RAS) plays a central role in the regulation of blood pressure and electrolyte metabolism in mammals.[1,2] Renin (EC 3.4.99.19) is the first and rate-determining enzyme in RAS, catalyzing the hydrolytic release of angiotensin I from the N-terminal end of angiotensinogen.[3,4] Angiotensin I is subsequently converted into a potent pressor octapeptide, angiotensin II, by angiotensin I-converting enzyme (ACE). ACE is one of the most extensively studied enzymes, and its predominant physiological function in cardiovascular homeostasis is well documented.[5−8] Blockade of angiotensin II accumulation through the inhibition of ACE has been proven as a validated treatment approach for hypertension.[6,9] However, the long-term treatment by ACE inhibitors seems not to completely suppress the circulating RAS as plasma angiotensin II and aldosterone levels tend to return toward pretreatment values. The presence of non-RAS enzymes, including tonin and cathepsin, also is capable of generating angiotensin II directly from angiotensinogen, which contributes to the elevated angiotensin II and aldosterone levels after ACE inhibitor treatment.[2] ACE is an enzyme with broad substrates and inhibitor specificities.[9] In addition to the inhibition of conversion of angiotensin I into angiotensin II, the metabolism of bradykinin also is affected, which may be related to the side effects observed with all ACE inhibitors such as rare cases of angioneurotic edema and the more frequent occurrence of dry cough.[9−11] In contrast to ACE activity, renin is a highly specific enzyme, having angiotensinogen as its only known physiological substrate.[12] Inhibiting renin at this step would be expected to block the classic RAS cascade and, therefore, would have a blood pressure lowering effect and avoid ACE inhibitor-related side effects.[13] Although very attractive, the lack of oral bioavailability or efficacy of renin inhibitors has precluded them from being as clinically useful as ACE inhibitors.[14,15]

Rapid advances in biomedical research have revealed that certain proteins and their hydrolytic products such as peptides may possess regulatory activities and can be useful in the prevention and/or treatment of certain diseases.[16] Different ACE-inhibitory peptides have been widely demonstrated to be present in various food proteins.[17] ACE-inhibitory activities were reported in digested pea protein hydrolysate.[18] However, to the best of our knowledge, there has been scant reporting on protein-derived renin inhibiting peptides. Recent reports have shown production of flaxseed protein hydrolysates[19] and isolation of three pea protein-derived peptides[30] that had *in vitro* inhibitory activities against ACE and renin. Pea protein is an important but cheap vegetable protein with high nutritional and functional values with

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great potential as an ingredient for the production of bioactive peptides.[20−22] Though several reports have shown antihypertensive effects of food protein hydrolysates, there is scant reporting on how the peptides impact plasma angiotensin II levels, and we are not aware of any report on kidney disease modulation. Therefore, the objectives of this study were to determine the potential antihypertensive effects of an enzymatic pea protein hydrolysate (PPH) in rat models of systemic hypertension and chronic kidney disease (CKD)-related hypertension, the two most common forms of human hypertension. We also determined hypotensive effects using a human intervention trial. Potential mechanisms of PPH action were determined by measuring plasma angiotensin II as well as renal production of renin and ACE in the CKD rats.

MATERIALS AND METHODS

Materials. Pea protein isolate (85% protein content) was a gift from Nutri-Pea Ltd. (Portage la Prairie, Manitoba, Canada). Renin assay buffer, renin protein, and renin substrate were provided in the Renin Inhibitor Screening Assay Kit purchased from Cayman (Cayman Chemical, Ann Arbor, MI). Purified rabbit lung angiotensin I-converting enzyme (ACE), thermolysin (type X, *Bacillus thermoproteolyticus rokko*, 39 units/mg solid), hippuryl-histidyl-leucine, and hippuric acid were purchased from Sigma (St. Louis, MO). HPLC-grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA), and trifluoroacetic acid (TFA) was obtained from Fluka (Buchs, Switzerland). HPLC-grade water generated by Milli-Q system (Millipore, Bedford, MA, USA) was used for the preparation of the HPLC mobile phase; all other chemicals were of reagent grade and obtained from Sigma (St. Louis, MO).

Preparation of PPH. Pea protein hydrolysate was prepared as follows according to a previously described method.[23] A 6.0% (w/v) aqueous slurry of pea protein isolate (PPI) was heated to 55 °C and adjusted to pH 8.0 followed by addition of thermolysin (0.5%, w/w pea protein basis). The temperature and pH of the slurry were maintained at constant values for 3 h, after which hydrolysis was stopped by heating at 95 °C for 15 min and cooling to room temperature. The cooled reaction mixture hydrolysate was centrifuged at 10000*g* for 25 min, and the clear supernatant was further passed through an ultrafiltration membrane with 3 kDa molecular weight cutoff. The resulting permeate was collected, freeze-dried and labeled as PPH for further use. Protein content of PPH was determined by a modified Lowry protein assay method.[24] Inhibitory activities of the PPH against renin and ACE were determined using methods previously described.[20]

Determination of Antihypertensive Activity of PPH in SHR. The rat experiments were carried out according to protocols approved by the University of Manitoba Animal Protocol and Management Review Committee. Male SHR (10 weeks old) weighing 160−180 g were kept in the Animal Housing Facility at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba under a 12-h day and night cycle at 21 °C and were fed a regular chow diet and tap water, *ad libitum*. The SHR were divided into 5 groups of 6 rats each that received the following treatments: PPH dissolved in phosphate buffered saline (PBS), pH 7.2 (100 and 200 mg/kg body weight), PPI in PBS (200 mg/kg body weight), captopril in PBS (3 mg/kg body weight), and PBS only. The sample (1 mL) treatments were administered to the rats by oral gavage followed by measurement of systolic blood pressure (SBP) at 2, 4, 6, 8, and 24 h by the tail-cuff method in rats that have been slightly anesthetized with isofluorane. Prior to sample administration, the SBP at time zero was determined. In order to mitigate the blood pressure depression effect of isofluorane, the gas flow was optimized such that rats became conscious usually within 3−4 min after removal from the chamber, which provided enough time to perform the blood pressure measurement. Rats were first anesthetized in a chamber (maintained at about 40 °C) with 4% isofluorane for 4 min. They were then removed from the isofluorane chamber, and tail-cuff measurement of blood pressure was performed in the unconscious state. The change in SBP (ΔSBP, mmHg) was determined by subtracting the data for the different time points from their respective data at time zero.

Determination of Antihypertensive Activity of PPH in Polycystic Kidney Disease (Han:SPRD-*cy*) Rats. The Han:SPRD-*cy* rat is characterized by renal cyst development and is a model of human chronic kidney disease (CKD).[25] Rats were obtained from our breeding colony in the Department of Human Nutritional Sciences, University of Manitoba, Winnipeg. Weanling male heterozygote Han:SPRD-*cy* rats were randomly divided into 3 groups that each contained 13 diseased rats. Diets (total protein of 20%, w/w of feed) were formulated based on the AIN (American Institute of Nutrition)-93G guidelines for growing rodents. The 3 diets differed only in the ratio of casein to PPH. Rats were fed one of the following diets for 8 weeks: casein (20%), 0.5% PPH (19.5% casein + 0.5% PPH), or 1.0% PPH (19% casein + 1% PPH). Rats were housed individually in hanging transparent plastic cages and kept in a controlled environment with temperature at 21−23 °C, 55% humidity, and 14 h light:10 h dark cycle. Food and water were provided *ad libitum*, and rats were weighed weekly. In each group, 5 rats were randomly selected to measure blood pressure from week 4 to 8 (same time period each week) using the tail-cuff method. At the end of the experiment (8th week), rats were anesthetized and blood was collected into heparinized tubes through heart puncture and centrifuged at 1500*g* for 15 min, and plasma was stored at −80 °C. Kidneys were also collected and stored at −80 °C until required for analysis. Plasma angiotensin II level was determined as follows using a modified method of Fruitier-Arnaudin et al.[26] A 200 μL aliquot of the plasma was injected into a Waters Symmetry 5 μm C18 column (3.0 × 150 mm), which was coupled to a Delta 600 HPLC system (Waters, Mississauga, ON). Elution was carried out with a linear gradient of acetonitrile (20−40% over 20 min containing 0.05% v/v TFA). Absorbance intensity was monitored and recorded at 210 nm; pure angiotensin II was used to identify the peak.

RNA Extraction and Quantification. Total RNA was isolated from kidney tissue, using an RNA isolation kit (Life Technologies, ON, Canada) according to the manufacturer's procedures. Briefly, kidney tissue was mixed with 4 mL of TRIzol reagent and homogenized. The homogenate was transferred into four 1 mL Eppendorf tubes, and 0.2 mL of chloroform was added. The tubes were shaken and then centrifuged at 10000*g* for 15 min at 4 °C; the upper aqueous phase contained the RNA, which was then transferred into a clean Eppendorf tube. A 0.5 mL aliquot of isopropanol was added to the transferred aqueous phase and allowed to stand at room temperature for 10 min followed by centrifugation at 10000*g* for 10 min at 4 °C. The supernatant was discarded and the pellet washed with 1 mL of 75% ethanol, vortexed and centrifuged at 5000*g* for 5 min at 4 °C. The washed RNA was air-dried and concentration determined by spectrophotometry. The iScript One-Step RT-PCR kit with SYBR Green was used for real-time qPCR analysis. cDNA synthesis and PCR amplifications were carried out in the same tube. A total RNA of 500 ng was used for reverse transcription. The Superscript First-Strand Synthesis System for RT-PCR (Bio-Rad, Hercules, CA, USA) was used according to the instructions of the manufacturer. Primer sequences used are as follows. Renin: TTCAGGAACGATGACCTGTG (forward) and GAACCCGATGCGATTGTTAT (reverse). ACE: CATGTCACTTTCTGCAGCTACC (forward) and ACC ATC CAC CTC CAC TTC TCT A (reverse). GAPDH: CATGACAACTTTGGCATCGT (forward) and GGATGCAGGGATGATGTTCT (reverse). Quantitative real time PCR (qRT-PCR) was performed using the Bio-Rad iCycler detection System. For analysis, cycle threshold (Ct) values were calculated for each sample; this value

represents the value at which the fluorescent signal rises above background levels. Gene expression was further analyzed by the $2^{-\Delta\Delta Ct}$ method.[27]

Determination of Antihypertensive Activity in Human Subjects. The human intervention trial was conducted according to a protocol approved by the University of Manitoba Research Ethics Board, and all participants provided written consent. A randomized double-blind placebo-controlled crossover design was utilized in the oral feeding of 7 human volunteers (4 females and 3 males, ages 30−55 years, 145−185 lb) with SBP ranging from 125 to 170 mmHg. All participants were nonsmokers and were not taking any antihypertensive prescription medication or similar nonprescription natural health products. Each phase consisted of 3 weeks with 2 weeks of washout period in between the phases and orange juice (50 mL) used as the carrier for PPH. There were 3 treatments as follows: placebo (50 mL of orange juice), 1.5 and 3 g of PPH per day divided into 3 doses 0.5 or 1 g each and taken at breakfast, lunch and dinner. Three consecutive blood pressure readings were taken daily at the same time of the day, and mean change was determined for each week. All participants completed each of the 3 phases.

Statistical Analysis. The experimental data were subjected to one way analysis of variance using Statistical Analysis System Software (SAS version 9.2, SAS Institute, Cary, NC). For the rat experiments, significant differences were determined by Duncan's multiple range test and accepted at $p < 0.05$. However, the Student's paired t test was used to determine significant differences ($p < 0.05$) between placebo and treatments for the human intervention trial.

RESULTS AND DISCUSSION

Short-Term Hypotensive Effects of PPH in SHR. SHR are a good model of human systemic hypertension arising from excessive production of angiotensin II through increased activity of ACE and renin. Therefore, the SHR allows testing of antihypertensive agents for the treatment of non-kidney related hypertension, which is a major cause of mortality. The inhibitory values for the *in vitro* activities of PPH (1 mg/mL) against ACE and renin were determined to be 19% and 17%, respectively. The ACE-inhibitory level of the PPH is similar to some reported values (13−23%) for wheat gliadin hydrolysate fractions[28] as well as the pepsin (16.8%) and molsin F (19.8%) digests of wheat gliadin.[29] However, the ACE-inhibitory level of the PPH was much lower than that of wheat gliadin protein hydrolysates produced by digestion with proteases such as Rapidase (42.4%), protease M (45.6%), and pepsin-protease M (51.6%).[29] Therefore, the ability of the PPH to act *in vivo* and reduce blood pressure was determined and results are shown in Figure 1. Though the *in vitro* inhibition values were not as high as reported for other food protein-derived peptides, there was effective lowering of SBP with a maximum of −19 mmHg after 4 h and −13 mmHg at 8 h after oral administration to SHR. This could be due to conversion of some inactive peptides in the PPH preparation to active peptides after passage through the gastrointestinal tract (GIT), which is typical of prodrug peptides as defined by Fujita et al.[30] Or, the strong blood pressure lowering effect could mean that the active peptides in PPH were efficiently absorbed from the GIT into the blood circulatory system. From 2 to 8 h post-oral administration, the PPH reduced SBP when compared to ordinary saline or the unhydrolyzed pea proteins (PPI). In contrast, captopril (3 mg/kg body weight) was a more effective hypotensive agent at 2−4 h postoral administration when compared to the PPH. Thus, it is evident that the enzyme hydrolysis was responsible for generating hypotensive peptides (contained in the 3 kDa permeate) from the PPI. The reductions

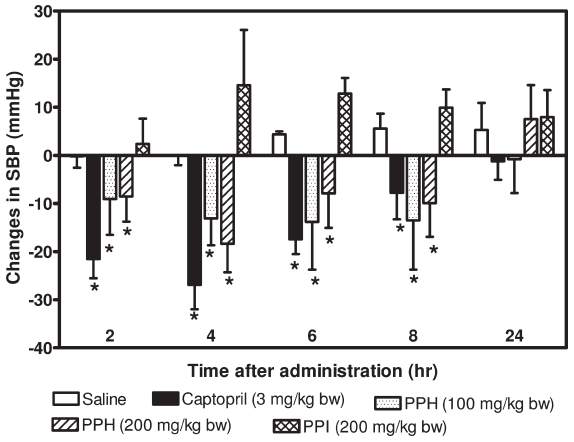

Figure 1. Short-term (24 h) changes in systolic blood pressure (SBP) of spontaneously hypertensive rats (average baseline SBP of 150 mmHg) after oral administration of saline, captopril, pea protein isolate (PPI) and the pea protein hydrolysate (PPH). *Significantly different from saline and PPI at $p < 0.05$.

in SBP are within the range that has been reported for other food protein hydrolysates (at similar doses) such as −11 mmHg for those derived from spinach[31] and −15 mmHg for those from rapeseed.[32] The blood pressure reducing effect of the PPH is less than the value of −29 mmHg reported for buckwheat protein hydrolysate[33] after 4 h of oral administration to SHR. Kodera and Nio[34] also reported higher reductions (>−20 mmHg) in SBP of SHR up to 4 h following oral administration of various food protein hydrolysates from soy, wheat gluten, casein and whey proteins. The hypotensive effect of PPH at the 100 mg/kg body weight dose was better than the effect reported for similar concentrations of apricot almond meal protein hydrolysate[35] and bonito protein hydrolysate.[36] However, the hypotensive effects of the PPH (100 and 200 mg/kg rat body weight) were lost 24 h after oral administration, which was similar to the result obtained for captopril (antihypertensive drug used at 3 mg/kg body weight) and other previously reported food protein hydrolysates. There were no differences in the hypotensive effects at either level of PPH (100 and 200 mg/kg body weight).

Long-Term Hypotensive Effects in the Han:SPRD-*cy* Rat Model of Chronic Kidney Disease (CKD). Hypertension is a common and frequent complication that is associated with CKD due to increased activity of RAS. In the Han:SPRD-*cy* rat, RAS is activated during cyst expansion and plays an important role in the development hypertension, a known important risk factor for cardiovascular morbidity and mortality in CKD patients.[25] Therefore, the Han:SPRD-*cy* rat provides an excellent model to test potency of PPH in reducing blood pressure that is associated with CKD. During the 8-week period, there were no differences in feed consumption (average 25−30 g/day) and growth rate (325−340 g at week 8) of rats in the control and PPH-fed groups. Results of the 8-week feeding experiment on blood pressure of Han:SPRD-*cy* rats are shown in Figure 2, for SBP and diastolic blood pressure (DBP), respectively. There were reductions in SBP starting at week 6 (up to −35 mmHg), and they continued to the end of the study (−29 mmHg) in week 8 (Figure 2A). By weeks 7 and 8, both levels of PPH reduced SBP by as much as −30 mmHg, indicating that the product can mitigate development of hypertension that is normally associated with CKD. Similar effects also were found with DBP, which was

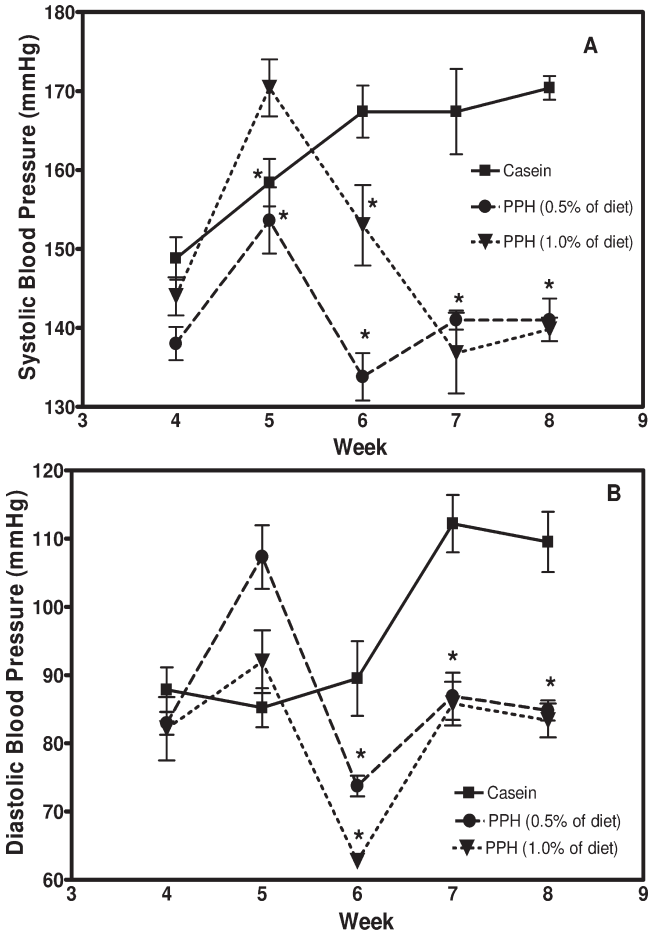

Figure 2. Long-term (8 weeks) changes in (A) systolic blood pressure (SBP) and (B) diastolic blood pressure (DBP) of Han:SPRD-*cy* rats (model of chronic kidney disease) fed casein or casein supplemented with pea protein hydrolysate (PPH). *Significantly different from casein at $p < 0.05$.

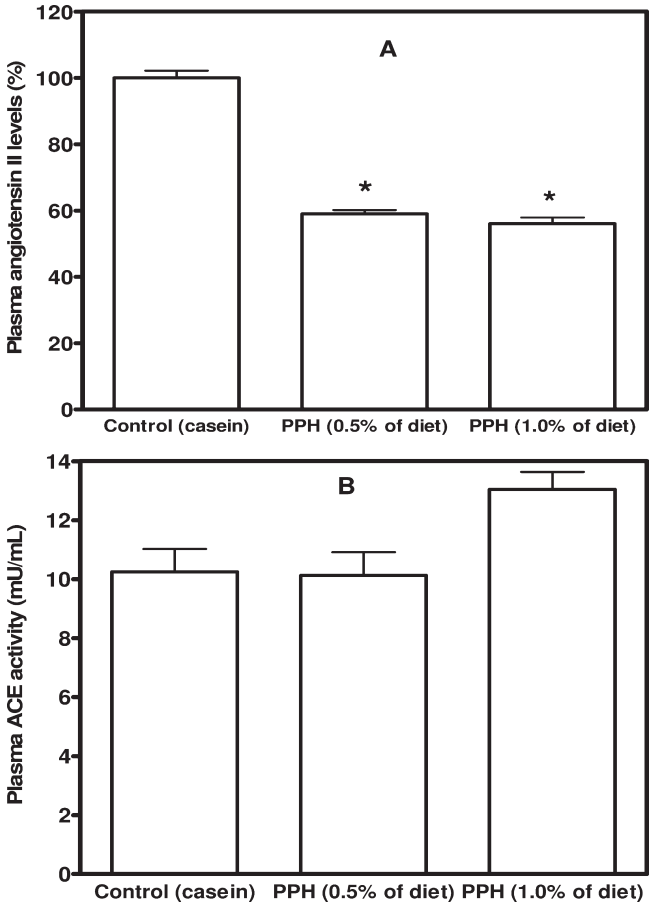

Figure 3. Effect of pea protein hydrolysate (PPH) on (A) plasma angiotensin II levels in treated rats expressed as percentage of level present in control rats and (B) angiotensin converting enzyme (ACE) activity in Han:SPRD-*cy* rats. Diets were formulated to contain 20% protein (casein) in the control rats but partially substituted with PPH (19.5% casein + 0.5% PPH or 19% casein + 1% PPH) in treated rats. *Significantly different from casein at $p < 0.05$.

also reduced by about −25 mmHg from week 6 to week 8 (Figure 2B). The importance of these results lies in the fact that cardiovascular complications, including hypertension, are often associated with CKD and are the most common cause of fatality.[37] This is the first report demonstrating the ability of a food protein hydrolysate to ameliorate a high blood pressure condition in an experimental animal model of CKD. Therefore, the PPH could find multiuse as an effective therapeutic agent against systemic and renal disease-related hypertension.

Effect of PPH on Angiotensin II Concentration and ACE Activity in Plasma of Han:SPRD-*cy* Rats. The vasopressor effect of angiotensin II is responsible for blood pressure regulation, and its plasma concentration plays an important role in the development and pathology of hypertension. Reductions in plasma angiotensin II concentration are usually achieved through inhibition of RAS, and this approach has proved to be a critical component in the successful therapeutic management of hypertension.[6,9] Figure 3A shows effective reductions (approximately 45%) in plasma angiotensin II concentrations in Han:SPRD-*cy* rats after 8 weeks of oral administration of PPH at 0.5 and 1.0% of diet. Thus the drop in plasma angiotensin II concentrations may have been responsible for the observed decreases in SBP and DBP of the Han:SPRD-*cy* rats. Apart from contributing to decreased blood pressure, lower levels of plasma angiotensin II may reduce renal production of TGF-β, a compound that could lead to increased renal injury and functional failure.[38] ACE is one of the major targets for therapeutic interventions aimed at blood pressure reduction. Therefore, ACE inhibitors are routinely used to control blood pressure associated with CKD, and this approach has been reported to reduce pathological symptoms such as left ventricular hypertrophy.[39] To investigate the cause of the reduced angiotensin II in PPH-treated rats, we tested the plasma for ACE activity. There were no differences between the ACE activity levels in plasma of control (casein) rats and those fed casein that contained PPH (Figure 3B). In contrast to these findings, a previous study demonstrated that oral administration of soluble cocoa fiber to SHR led to decreased activity of ACE in the plasma.[40] Thus, decreased plasma ACE activity is possible with nutritional intervention as seen in the case of soluble cocoa fiber but not with the PPH, suggesting differences in the mechanisms responsible for the observed hypotensive effects. The differences between the observed effects of PPH and those with soluble cocoa fiber on plasma ACE level also could be due to the type of animal model used: Han:SPRD-*cy* rats in the former and SHR in the latter.

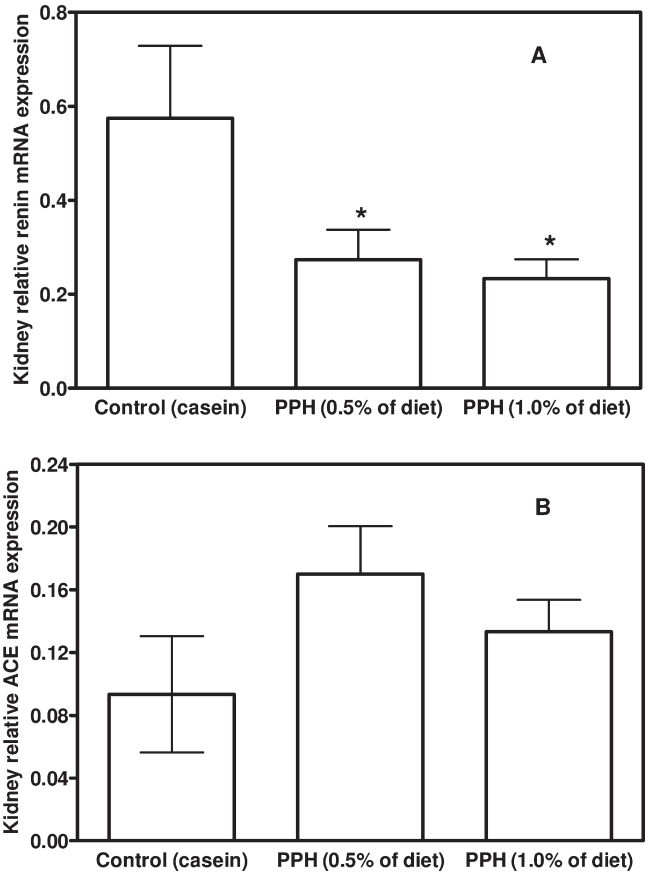

Figure 4. Effect of pea protein hydrolysate (PPH) on renal tissue level of renin (A) and angiotensin converting enzyme, ACE (B) mRNA in Han:SPRD-*cy* rats. Diets were formulated to contain 20% protein (casein) in the control rats but partially substituted with PPH (19.5% casein + 0.5% PPH or 19% casein + 1% PPH) in treated rats. *Significantly different from casein at $p < 0.05$.

Renin and ACE mRNA Expression Levels in Kidneys. The lack of effect on ACE activity suggests that other factors such as a decrease in renin production by the kidneys or decreased chymase activity may have contributed to the observed decreases in plasma angiotensin II levels in the PPH-fed rats. Chymase is an enzyme that also can produce angiotensin II while renin produces the substrate, angiotensin I. The kidney is the main organ responsible for producing renin, and decreased expression could provide beneficial effects by reducing the activity of RAS, which is compatible with blood pressure reduction. Therefore, the level of renin mRNA expression by the kidneys may be used for indirect determination of the effects of inhibitors on RAS. Figure 4A shows that inclusion of PPH in the Han:SPRD-*cy* rat diet led to decreases in kidney renin mRNA expression. In contrast there was no significant increase in ACE mRNA expression (Figure 4B), suggesting that the observed decreases in plasma angiotensin II were likely due to the effect of PPH on renin production in the kidneys. This is important because kidney disease and hypertension promote each other in a vicious cycle that worsens both diseases. Therefore, to break this cycle, it is desirable for an inhibitor to have a direct effect on the kidneys, which could be in the form of reduced production of renin mRNA as shown for PPH. By controlling the output of renin from the kidneys, the PPH may attenuate blood pressure increases through lowering

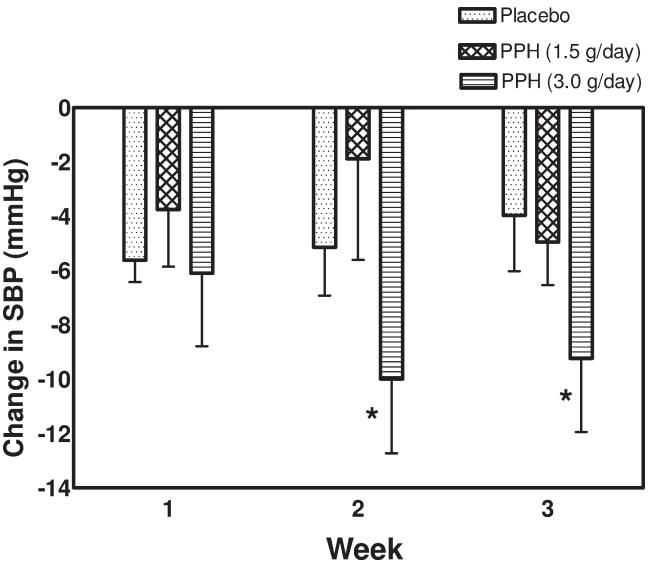

Figure 5. Mean changes in systolic blood pressure (SBP) of human subjects during 3 weeks of oral consumption of pea protein hydrolysate (PPH) or placebo. *Significantly different from the placebo treatment ($p < 0.05$).

the activity of plasma RAS and level of angiotensin II. It is important to note that the approximately 50% decrease in renal renin mRNA expression is similar to the observed 45% decrease in plasma angiotensin II concentration. Potential mechanism of action of PPH could involve suppression of β-adrenergic receptor activation of the renin secretory pathway, which leads to decrease in level of plasma renin activity.[41] As expected the renal levels of renin mRNA were much higher than those of ACE mRNA because the kidneys are the major sites for renin production while ACE is produced mainly in the lungs.

Effect of PPH on Human Blood Pressure. Since this is a crossover study with each participant completing all 3 phases, this is equal to a parallel human intervention study involving 21 participants. Results from the human intervention study demonstrated that 3 mg/day of PPH compared to placebo resulted in reductions in SBP of 5 and 6 mmHg, respectively, in human subjects at weeks 2 and 3 but not in the first week (Figure 5). In contrast, at a dosage of 1.5 g/day there was no significant effect on blood pressure when compared with the placebo group. The overall maximum SBP reduction of about 10 mmHg with the 3 g/day PPH dose is similar to the average effect on blood pressure usually obtained for a single dose of an antihypertensive drug.[42] Although the dose of drugs is usually several times lower than the typical dose for PPH, the outcome presents an opportunity to use a food protein-derived product as an intervention agent to control human hypertension. Toxicity of food protein-derived peptides has not been reported even at the very high doses sometimes used in animal experiments and human clinical trials, which is an advantage over typical drugs that can produce various toxic effects. During the 3 week duration of this experiment, none of the participants reported any adverse side effect. Similar hypotensive effects of 5−7 mmHg decreases (over the placebo group) in human SBP were reported following 2−5 weeks of oral consumption of 1.5 g/day bonito protein hydrolysate.[36] Various reports also have shown SBP-lowering effects of fermented milk that contained bioactive peptides; results ranged from −2 to up to −7 mmHg over the placebo group.[43−46] Thus, the current

results show that the hypotensive effect of the PPH is well within the range that has been reported in the literature for food protein-derived peptides. Similar to our current report, the bonito protein hydrolysate human intervention trial also reported no abnormal symptoms in the human subjects.[36]

This study has demonstrated that PPH can effectively lower blood pressure in rat models of hypertension as well as in human subjects. The higher hypotensive effects observed in the animal experiments are consistent with the genetic homogeneity of laboratory rats in comparison to the human trial where diversity in human genetics can lead to lesser responses to therapeutic interventions. Also, the animal models consisted of only male rats while male and female human subjects participated in the clinical study. Importantly, we have shown that PPH exerts a direct effect on renal renin mRNA levels which may be responsible for the observed decreases in plasma angiotensin II concentrations and resultant hypotensive effects.

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