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Effects of the bioactive peptides Ile-Pro-Pro and Val-Pro-Pro upon autonomic neurotransmission and blood pressure in spontaneously hypertensive rats

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ABSTRACT

The bioactive peptides Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP) are believed to improve blood pressure and arterial function. To gain a better understanding of the mechanisms underlying the action of these peptides, we investigated their effects upon autonomic neurotransmission and blood pressure in spontaneously hypertensive rats (SHR). Both IPP and VPP caused a significant reduction in cutaneous arterial sympathetic nerve activity (CASNA) and reduced mean arterial pressure (MAP); however, both of these effects were eliminated following sub-diaphragmatic vagotomy. On the other hand, captopril, an angiotensin-converting enzyme inhibitor, reduced MAP without changing CASNA, and maintained this hypotensive effect following vagotomy. Moreover, the effects of IPP and VPP upon CASNA were observed following gastric administration but not by duodenal administration. These results suggest that IPP and VPP reduce CASNA via the stomach and afferent vagus nerve, thus causing reductions in MAP in SHR.

1. Introduction

Hypertension is a prevalent disease related to lifestyle and represents a primary risk factor for cardiovascular (CV) diseases. Approximately 30% of adults are hypertensive and over 50% of the hypertensive population are associated with poorly-controlled blood pressure (Yoon et al., 2015). Consequently, the treatment and prevention of hypertension have become critical issues for public health. Many clinical practice guidelines for hypertension recommend lifestyle modifications to diet, exercise, and the cessation of smoking (Mancia et al., 2013; Shimamoto et al., 2014). In addition, several studies have reported that some nutrients and food components are beneficial in controlling blood pressure (Binia et al., 2015; Miller et al., 2014).

Ile-Pro-Pro (IPP) and Val-Pro-Pro (VPP) are milk-derived peptides with inhibitory activity against angiotensin-converting enzyme (ACE) (Nakamura et al., 1995). These amino acid sequences are found in bovine β-casein and are released by proteases found in microorganisms such as *Lactobacillus helveticus*. A number of clinical trials have demonstrated the hypotensive effects of foods containing IPP and VPP (Fekete et al., 2015). In addition, casein hydrolysate containing these peptides, has been shown to improve both arterial stiffness (Nakamura et al., 2011) and vascular endothelial function (Hirota et al., 2007) These findings indicate that the administration of IPP and VPP could improve both blood pressure and arterial properties.

The inhibition of ACE (Nakamura et al., 1995; Masuda et al., 1996), and the induction of nitric oxide (NO) production (Hirota et al., 2011; Nonaka et al., 2014), have been proposed as mechanisms underlying the biological action of IPP and VPP. However, IC_{50} values for ACE of IPP (5 μM) and VPP (9 μM) were determined to be much higher than those of ACE inhibitory drugs (Cushman et al., 1989), and a pharmacokinetic study estimated the bioavailability of these peptides to be only 0.1% in pigs (van der Pijl et al., 2008). In addition, following the consumption of a milk beverage containing these peptides, the maximum concentration of IPP in human plasma was < 1 nM (Foltz et al., 2007), and the plasma noradrenaline response to the tilt test was reduced (Usinger et al., 2010). These findings raise the question of whether IPP and VPP directly affect the arteries or act in an indirect manner via the autonomic nervous system. However, there is no direct evidence for the effects of IPP and VPP upon autonomic neurotransmission. Consequently, the present study aimed to investigate the effects of IPP and VPP upon autonomic neurotransmission and blood pressure in spontaneously hypertensive rats (SHR).

Abbreviations: CV, cardiovascular; IPP, Ile-Pro-Pro; VPP, Val-Pro-Pro; ACE, angiotensin-converting enzyme; NO, nitric oxide; SHR, spontaneously hypertensive rats; ASNA, adrenal sympathetic nerve activity; GVNA, gastric vagal nerve activity; CASNA, cutaneous arterial sympathetic nerve activity; MAP, mean arterial pressure; BP, blood pressure; ANOVA, analysis of variance

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Table 1
Basal values of adrenal sympathetic nerve activity (ASNA), gastric vagal nerve activity (GVNA), cutaneous arterial sympathetic nerve activity (CASNA) and mean arterial pressure (MAP) in spontaneously hypertensive rats (SHR).

Groups	ASNA (spike/5 s)	GVNA (spike/5 s)	CASNA (spike/5 s)	MAP (mm Hg)
Water	292.1 ± 8.8	280.6 ± 7.5	267.8 ± 15.4	152.3 ± 7.0
IPP	265.4 ± 31.2	279.5 ± 8.2	228.0 ± 26.8	176.4 ± 21.6
VPP	299.8 ± 48.2	270.2 ± 7.5	251.1 ± 26.4	188.0 ± 13.7
Captopril	299.6 ± 52.4	270.4 ± 7.9	246.5 ± 27.0	174.2 ± 6.7

2. Materials and methods

2.1. Animals

Male SHR/Izm rats (18 weeks old, 300–350 g body weight) were obtained from Japan SLC (Shizuoka, Japan) and acclimatized for at least 1 week in a room maintained at 24 ± 1 °C under a 12 h light and 12 h dark cycle (lights on at 8:00). Animals with given free access to a commercial diet (MF, Oriental Yeast, Tokyo, Japan) and tap water and all animal experiments were approved by the Institutional Animal Care and Use Committee of ANBAS Corporation.

2.2. Reagents

Chemically-synthesized IPP and VPP were purchased from Bachem (CA, USA). Captopril, an ACE inhibitor, was purchased from Sigma-Aldrich (MO, USA).

2.3. Autonomic nerve activity

Adrenal sympathetic nerve activity (ASNA), gastric vagal nerve activity (GVNA), and cutaneous arterial sympathetic nerve activity (CASNA), were determined as described previously (Tanida et al., 2005). In brief, access to food was prevented from 3 h prior to surgery, and the rats (n = 3/nerve/group) were anesthetized with urethane (1 g/kg). A polyethylene tube was then inserted to allow either the gastric or duodenal administration of sample solutions. In some rats, the gastric tube was inserted following pylorus ligation. Each nerve (ASNA, GVNA and CASNA) was then exposed, ligated and connected to a pair of silver wire electrodes which could then be used to determine changes in nerve activity. Body temperature was maintained at 35.0 ± 0.5 °C using a heat pad and a thermistor. After stabilizing nerve activity for 30–60 min, we then administered 16 μmol/1 mL/rat of either IPP, VPP, captopril, or water. After administration, nerve activity was amplified, monitored by an oscilloscope and recorded for 60 min. In some rats (n = 3/group), sub-diaphragmatic vagotomy was performed prior to recording CASNA, as previously described (Horii et al., 2014).

2.4. Blood pressure (BP)

After being deprived of food for 3 h, rats (n = 4/group) were anesthetized with ketamine hydrochloride, and a polyethylene catheter was inserted into the left femoral artery. The catheter was then connected to a BP transducer, and amplified in a BP amplifier (Tanida et al., 2005). Mean arterial pressure (MAP) was determined for 60 min in conscious rats following the administration of test solutions. In some rats (n = 3/group), sub-diaphragmatic vagotomy was performed prior to the determination of MAP.

2.5. Data analyses

ASNA, GVNA, CASNA and MAP were measured every 5 min for a total period of 60 min following the administration of test and vehicle solutions. Resultant data were evaluated by digital signal processing and statistical analyses. Data are expressed as means ± standard error of the mean (SE). The Mann-Whitney *U* test was used to compare baseline values of nerve activities and MAP. Due to inter-individual variability in the pre-injection state, the proportional change from baseline was calculated for ASNA, GVNA, CASNA and MAP. Analysis of variance (ANOVA), with repeated measures, was also used to compare group responses relating to autonomic nerve activity and MAP.

3. Results

3.1. The effect of IPP, VPP and captopril upon autonomic nerve activity and MAP when administered via a gastric catheter

Table 1 shows absolute baseline values (t = 0 min) for ASNA, GVNA, CASNA, and MAP. There were no significant differences in these baseline values in each determination. Relative changes in ASNA, GVNA, CASNA, and MAP following the gastric administration of test solutions were determined as a proportion (%) of the baseline values (Fig. 1A–D).

Maximal ASNA values were observed 55 min after the administration of water (124.2 ± 17.7%), 60 min after IPP (166.1 ± 28.1%), 55 min after VPP (165.3 ± 45.6%), and 60 min after captopril (124.9 ± 15.2%) (Fig. 1A). Grouped statistical analysis of the differences between ASNA values from 5 to 60 min using ANOVA with repeated measures showed significant differences between water and IPP ($P < 0.001$; $F = 42.8$) and between water and VPP ($P < 0.001$; $F = 14.6$) but not between water and captopril ($F = 1.18$).

In contract, maximal values of GVNA were observed 30 min following the administration of water (84.6 ± 5.0%), 60 min after IPP (48.5 ± 12.0%), 55 min after VPP (106.9 ± 7.4%) and 60 min after captopril (52.2 ± 7.4%) (Fig. 1B). ANOVA with repeated measures showed a significant difference between GVNA values from 5 to 60 min between water and IPP ($P < 0.001$; $F = 95.8$), water and VPP ($P < 0.001$, $F = 15.2$) and between water and captopril ($P < 0.001$, $F = 130.2$).

In terms of CASNA, maximal values were observed 30 min after the administration of water (107.8 ± 11.8%), 60 min after IPP (67.3 ± 5.5%), 55 min after VPP (77.8 ± 1.8%), and 60 min after captopril (106.9 ± 9.0%) (Fig. 1C). ANOVA with repeated measures showed a significance between CASNA values from 5 to 60 min when analyzed as a group between water and IPP ($P < 0.001$, $F = 30.5$) and between water and VPP ($P < 0.001$, $F = 13.8$) but not between water and captopril ($F = 0.07$) (Fig. 1C).

Finally, maximal values of MAP were observed 55 min after the administration of water (102.5 ± 3.8%), 60 min after IPP (87.1 ± 2.5%), 60 min after VPP (83.6 ± 2.3%) and 60 min after captopril (82.0 ± 4.8%) (Fig. 1D). ANOVA with repeated measures revealed significant differences between MAP values from 5 to 60 min when analyzed as a group between water and IPP ($P < 0.001$, $F = 44.1$), water and VPP ($P < 0.001$, $F = 111.9$) and between water and captopril ($P < 0.001$, $F = 78.7$) (Fig. 1D).

3.2. The effect of sub-diaphragmatic vagotomy upon CASNA and MAP

Table 2 shows absolute baseline values (t = 0 min) of CASNA and