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Casein-derived tripeptide Ile–Pro–Pro improves angiotensin-(1–7)- and bradykinin-induced rat mesenteric artery relaxation

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ABSTRACT

Aims: Milk casein-derived bioactive tripeptides isoleucine–proline–proline (Ile–Pro–Pro) and valine–proline–proline (Val–Pro–Pro) lower blood pressure in animal models of hypertension and humans. In some studies, their angiotensin-converting enzyme (ACE)-inhibitory effect has been demonstrated. Besides classical ACE-angiotensin II-AT_1-receptor pathway (ACE-Ang II- AT_1), the significance of ACE2-angiotensin-(1–7)-Mas-receptor (ACE2-Ang-(1–7)-Mas) axis in the blood pressure regulation has now been acknowledged. The present study was aimed to further evaluate the renin–angiotensin system (RAS)-related vascular effects of Ile–Pro–Pro in vitro using rat mesenteric arteries.

Main methods: Superior mesenteric arteries of spontaneously hypertensive rat (SHR) were isolated, cut into rings and mounted in standard organ bath chambers. Endothelium-intact arterial rings were incubated in Krebs solution either with Ile–Pro–Pro, proline–proline (Pro–Pro), isoleucine (Ile), proline (Pro) or captopril for 6 h at +37 °C and vascular reactivity was measured.

Key findings: In the presence of AT_1-antagonist valsartan, Ang II induced vasodilatation, which was more pronounced in the arteries incubated with Ile–Pro–Pro ($P<0.05$) compared to the other compounds. Ang-(1–7)-induced vasodilatation was augmented by Ile–Pro–Pro or Pro ($P<0.001$ *vs.* control). Mas-receptor antagonist A-779 did not alter the responses. Ile–Pro–Pro and Pro augmented also bradykinin-induced relaxations ($P<0.001$ *vs.* control). Control arteries and arteries incubated with captopril showed only slight relaxations at higher bradykinin concentrations.

Significance: Casein-derived tripeptide Ile–Pro–Pro and amino acid Pro enhance the vasodilatory effect of Ang-(1–7) and bradykinin. The role of ACE2-Ang–(1–7)-Mas axis in the modulation of vascular tone by these compounds seems probable.

Introduction

Milk protein contains a considerable number of bioactive peptides, which are released from the parent protein during fermentation of milk with proteolytic starter cultures, by gastrointestinal digestion or enzymatic hydrolysis (Korhonen 2009). Fermentation of milk with *Lactobacillus helveticus* strain produces tripeptides isoleucine–proline–proline (Ile–Pro–Pro) and valine–proline–proline (Val–Pro–Pro), which inhibit angiotensin-converting enzyme (ACE) in vitro (Nakamura et al. 1995; Lehtinen et al. 2010).

The antihypertensive effect of milk casein-derived Ile–Pro–Pro and Val–Pro–Pro or milk products containing them has been shown both in experimental (Jauhiainen et al. 2005a; Jäkälä et al. 2009a,b) and in clinical studies (Aihara et al. 2005; Jauhiainen et al. 2005b; van der Zander et al. 2008; Turpeinen et al. 2009). The mechanism is thought to be based at least partly on the inhibition of renin–angiotensin system (RAS) (Sipola et al. 2002, Jäkälä et al. 2009a,b). However, not all clinical studies have supported the role of RAS in the effects of tripeptides (Jauhiainen et al. 2005b; Engberink et al. 2008; Wuerzner et al. 2009). Therefore, the role of other targets of RAS in the cardiovascular effects of the tripeptides needs further evaluation.

In the classical RAS, angiotensin II (Ang II) produced from angiotensin I (Ang I) by ACE, binds to its receptors, AT_1 and AT_2 (Fyhrquist and Saijonmaa 2008). AT_1-receptors mediate vasoconstriction, aldosterone release, fibrosis and cellular growth while partly opposite functions are mediated by AT_2-receptor. However, a new axis of RAS has been established recently (Santos et al. 2008). In this axis, Ang I and Ang II are eventually converted to angiotensin-(1–7) [Ang-(1–7)] by angiotensin-converting enzyme 2 (ACE2). In blood vessels, Ang-(1–7) induces endothelium-dependent vasodilatation through a newly discovered receptor Mas (Santos et al. 2003), has antiproliferative effects (Tallant and Clark 2003) and antagonizes the pressor effect of AT_1-receptor stimulation (Benter et al. 1995). Thus, ACE2-Ang-(1–7)-Mas axis may act as a counterregulatory system against classical ACE-Ang II-AT_1 pathway. In addition, Ang-(1–7) potentiates the effects of bradykinin (Tom et al. 2001; Greco et al.

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2006), either through the inhibition of ACE or by inhibiting the desensitization of bradykinin receptors. Also vasodilatory AT_2-receptors may be involved (Gorelik et al. 1998).

In our previous in vitro studies, Ile–Pro–Pro and Val–Pro–Pro protected endothelial function of isolated SHR mesenteric arteries in a time-dependent manner (Jäkälä et al. 2009c). Acetylcholine-induced endothelium-dependent relaxation was better preserved after 12 or 24 h incubation with a tripeptide than without it. The role of endothelium-derived hyperpolarizing factor (EDHF) in the relaxation responses was also demonstrated. However, the direct effects of Ile–Pro–Pro on vascular function have been rather minor (unpublished data). Considering the ACE-inhibitory activity of Ile–Pro–Pro and Val–Pro–Pro and the previous findings from in vitro studies, the present study was aimed to further evaluate the RAS-related vascular effects of Ile–Pro–Pro in vitro using mesenteric arteries of SHR.

Materials and methods

Animals

The protocols were approved by the National Animal Experimentation Committee of Finland according to EC Directive 86/609/EEC and Finnish Experimental Animal Act 62/2006. Thirty male spontaneously hypertensive rats (SHR, age 9–11 weeks, weight 235 ± 2 g) were purchased from Charles River, Sulzfeld, Germany. The rats were housed five to a cage in a standard experimental animal laboratory (illuminated from 7.00 a.m. to 7.00 p.m., temperature 22 ± 2 °C, humidity 55 ± 15%). The rats received tap water ad libitum and had free access to standard rat chow (Harlan Teklad Global 16% Protein Rodent Diet 2016, Madison, WI, USA), which contained 0.6% salt. After one week's adaptation, systolic blood pressure was measured by a tail-cuff blood pressure analyzer (Apollo-2AB Blood Pressure Analyzer, Model 179-2AB, IITC Life Science, Woodland Hills, CA, USA) to demonstrate the hypertensive state of the animals (151 ± 2 mm Hg). Before the measurements, rats were warmed for 20 min at +32 °C to make the pulsations of the tail artery detectable. After obtaining three consecutive and successful measurements without disturbance of the signal, the mean of the values was regarded as the systolic blood pressure.

The rats weighed 235 ± 2 g on average in the time of the experiments. The systolic blood pressure was 151 ± 2 mm Hg.

Arterial preparations

Rats were rendered unconscious with CO_2/O_2 (30%/70%, AGA, Riihimäki, Finland) and decapitated. Superior mesenteric arteries were excised and placed in ice-cold Krebs buffer (pH 7.4–7.6, composition in mM: NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2) and carefully cleaned of adherent connective tissue on a Petri dish under a light microscope. A 5 mm section from the proximal end of mesenteric artery-aorta junction was cut off and the two following 3 mm sections were used in the experiments. The endothelium-intact rings were placed between stainless steel hooks (diameter 0.15 mm) and mounted in an organ bath chamber (volume 10 ml) in Krebs solution (composition, as above, +37 °C), and oxygenated with O_2/CO_2 (95%/5%, AGA).

Arterial rings were incubated in the organ baths without tension for 6 h (+37 °C oxygenated with O_2/CO_2, 95%/5%) either with 1 mM Ile–Pro–Pro, proline–proline (Pro–Pro), isoleucine (Ile), proline (Pro), 10 μM captopril or without these agents (control) in Krebs solution. The incubation times and concentrations were chosen on the basis of pilot experiments and our previous studies (Jäkälä et al. 2009c), in which 12 h (at +4 °C) was needed to show beneficial effect on endothelial function. Compounds were diluted in a freshly prepared Krebs solution. After 5 h of incubation, a pretension of 1.0 g was adjusted to the rings. The force of contraction was measured with an isometric force–displacement transducer using a computerized system (EMKA Technologies, Paris, France).

Vascular responses

After 6 h of incubation, the solutions containing a peptide, an amino acid or captopril were removed and arterial rings washed 2–3 times with Krebs solution. The vascular reactivity was studied by adding vasoactive agents directly into the chambers either cumulatively [acetylcholine, angiotensin II, angiotensin–(1–7), bradykinin] or as a single concentration (phenylephrine, KCl, angiotensin II). The concentrations henceforth are the final concentrations in the organ chamber. The rings were equilibrated for 20–30 min between different experiments and washed 2–3 times with Krebs solution during this period.

Before the experiments, arterial ring viability was assessed by exposing them to 60 mM KCl (Krebs solution with equimolar substitution of NaCl by KCl). To study the effect of 6 h incubation on the endothelium-dependent relaxation, arterial rings were precontracted with 1 μM phenylephrine (PE) and cumulative concentration–response curves to acetylcholine (ACh, 1 nM–10 μM) were constructed.

The following vascular reactivity measurements were performed in the presence of either 10 μM Ile–Pro–Pro, Pro–Pro, Ile, Pro or captopril corresponding to the compound used in the 6 h incubation. Different compounds were added into the chambers 10 min prior to each response study.

To study the possible effect of Ile–Pro–Pro, its components and captopril on AT_1-receptors, arteries were contracted with 0.1 μM angiotensin II (Ang II). After washing, the rings were pretreated with 10 μM valsartan (AT_1-receptor antagonist) for 15 min, precontracted with 1 μM PE and cumulative concentration–response curves to Ang II (1 nM–1 μM) were constructed. To elucidate the role of AT_2-receptors, the former was repeated but in the presence of PD123319 (AT_2-receptor antagonist, 10 μM) as well.

Using another set of arterial rings, Mas-receptor-mediated effects were studied by precontracting the arterial rings with PE and constructing cumulative concentration–response curves to angiotensin-(1–7) [Ang-(1–7), 0.1 nM–1 μM] thereafter. The same was performed also after 15 min pretreatment with A-779 (Mas-receptor antagonist, 1 μM). Bradykinin-related effects were elucidated by precontracting the arterial rings with PE and constructing cumulative concentration–response curves to bradykinin (0.1 nM–1 μM).

Compounds

Angiotensin II acetate salt, angiotensin-(1–7) trifluoroacetate salt, A-779 [(D-Ala^7)-Angiotensin I/II (1–7) trifluoroacetate salt], bradykinin acetate salt, isoleucine, isoleucine–proline–proline, proline–proline and proline were purchased from Bachem AG (Bubendorf, Switzerland). Acetylcholine chloride, captopril, (*R*)-(−)-phenylephrine hydrochloride and PD123319 di(trifluoroacetate) salt hydrate were from Sigma-Aldrich (St Louis, MO, USA).

Statistical analysis

Results are presented as mean ± SEM (standard error of the mean). Statistical analysis was performed using GraphPad Prism® software (version 4.02). Repeated measures analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test was used to compare concentration–response curves obtained in mesenteric arterial rings. Cumulative concentration–response curves for acetylcholine were fitted to a logistic function by using a non-linear regression analysis (sigmoidal dose–response model, standard slope) to estimate EC_{50} values. The EC_{50} values are expressed as the negative logarithm of the molar concentration (pD_2-values) at which 50% of the maximal relaxation effect has been reached. The relaxation

responses of different compounds are expressed as percentage of 1 μM phenylephrine-induced precontraction. Differences were considered significant if $P<0.05$.

Results

Endothelium-dependent relaxation

Endothelium-dependent relaxation to ACh was studied after 6 h incubation with Ile–Pro–Pro, Pro–Pro, Pro, Ile or captopril. All arteries showed almost maximal relaxations and no differences in pD_2—values were detected between the compounds (Table 1).

AT_1- and AT_2-receptors

Ang II induced only minor contractions when added as a single dose to the rings at basal state (<0.2 g *vs.* PE ca. 1 g, data not shown). However, Ang II induced concentration-dependent relaxations in the PE-precontracted mesenteric arteries (Fig. 1A and B). In the presence of AT_1-antagonist valsartan, arteries incubated with Ile–Pro–Pro showed more pronounced relaxation responses than the control arteries ($P<0.05$, Fig. 1A). Arteries incubated with captopril did not differ from the control. AT_2-receptor antagonist PD123319 (in the presence of valsartan) did not affect the relaxation responses (Fig. 1B). However, no statistically significant differences were observed between arteries incubated with any of the compounds after pretreatment with both the inhibitors (valsartan and PD123319).

Mas-receptors

The effect of Ile–Pro–Pro and its components on Ang-(1–7)-induced responses was studied in PE-precontracted arterial rings. Ang-(1–7) produced concentration-dependent relaxations, which were more prominent in the arteries incubated either with Ile–Pro–Pro or Pro ($P<0.001$ Ile–Pro–Pro or Pro vs. other groups, Fig. 2A). Mas-receptor antagonist A-779 did not significantly alter these relaxation responses (Fig. 2B). Incubation of the arteries with Ile or Pro–Pro did not influence the responses when compared to control (data not shown).

Bradykinin-related responses

As cumulative concentration–response curves to bradykinin were constructed to PE-precontracted arterial rings at the beginning of the study protocol, only weak contractions followed by relaxations of a comparable magnitude were seen. However, if Ang-(1–7) responses were studied prior to bradykinin, distinct responses were observed by bradykinin as well. In the control arterial rings, bradykinin induced contractions at lower concentrations followed by relaxation at higher concentrations (over 0.1 μM) (Fig. 3). A similar pattern was seen with

Table 1
pD_2-values for acetylcholine-induced endothelium-dependent relaxations of spontaneously hypertensive rat (SHR) mesenteric arteries.

	pD_2
Control	6.72 ± 0.05
Captopril	6.77 ± 0.06
Ile–Pro–Pro	6.92 ± 0.05
Pro–Pro	6.69 ± 0.06
Ile	6.73 ± 0.11
Pro	7.00 ± 0.07

Values are mean ± SEM, n = 7–12. pD_2: negative logarithm of molar concentration of acetylcholine causing half-maximal relaxation (EC_{50}) to phenylephrine-precontracted mesenteric artery rings. Prior to experiment, mesenteric arterial rings were incubated with the given compound for 6 h at +37 °C. *P* ns.

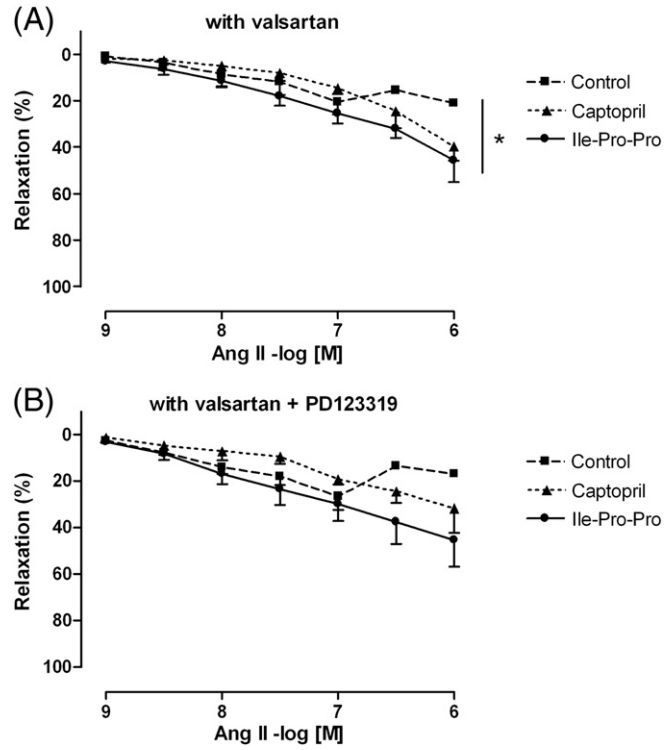

Fig. 1. Angiotensin II induced concentration–response curves for SHR mesenteric artery rings after precontraction with 1 μM phenylephrine. Rings were incubated without (control) or with the given compound for 6 h at +37 °C. Values are mean ± SEM, n = 4–7. (A) Pretreatment with AT_1-receptor antagonist valsartan (10 μM) for 15 min. *$P<0.05$ Ile–Pro–Pro *vs.* control. (B) Pretreatment with valsartan and AT_2-receptor antagonist PD123319 (10 μM) for 15 min. *P* ns.

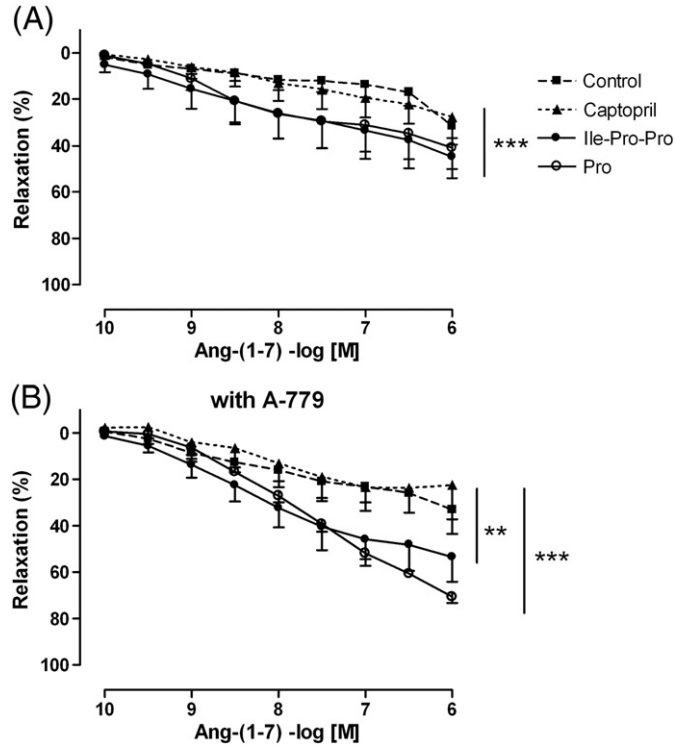

Fig. 2. Angiotensin-(1–7)-induced concentration–response curves for SHR mesenteric artery rings after precontraction with 1 μM phenylephrine. Rings were incubated with the given compound for 6 h at +37 °C. Values are mean ± SEM, n = 3–6. (A) ***$P<0.001$ Ile–Pro–Pro and Pro *vs.* other groups. (B) Pretreatment with Mas-receptor antagonist A-779 (1 μM) for 15 min. ***$P<0.001$ Ile–Pro–Pro and Pro *vs.* captopril, **$P<0.01$ Ile–Pro–Pro and Pro *vs.* control.

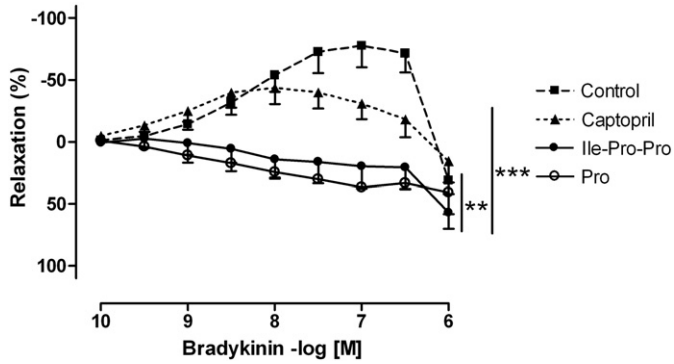

Fig. 3. Bradykinin-induced concentration–response curves for SHR mesenteric artery rings after precontraction with 1 μM phenylephrine. Rings were incubated with the given compound for 6 h at +37 °C. Values are mean ± SEM, n = 3–6. ***$P<0.001$ Ile–Pro–Pro and Pro *vs.* control, **$P<0.01$ Ile–Pro–Pro and Pro *vs.* captopril.

the arteries incubated with captopril. In contrast, arteries incubated either with Ile–Pro–Pro or Pro showed distinct relaxations to bradykinin already at low concentrations (less than 1 nM, $P<0.001$ and $P<0.01$ vs. control and captopril, respectively). Arteries incubated with Ile or Pro–Pro did not differ from the control or captopril groups (data not shown).

Discussion

In the present study, the effects of milk casein-derived tripeptide Ile–Pro–Pro and its components on the receptors of renin–angiotensin system (RAS) were studied in vitro using isolated rat mesenteric arteries. The main finding was that after 6 h incubation with Ile–Pro–Pro, Ang-(1–7)-induced relaxation was improved and the bradykinin responses were enhanced. Similar effects were seen with the amino acid Pro as well.

The involvement of RAS in the antihypertensive effect of tripeptides has been demonstrated in animal studies by reporting of ACE-inhibition, decrease in serum aldosterone and increase in plasma renin activity (Sipola et al. 2002; Jäkälä et al. 2009a,b). Our previous in vitro studies with isolated rat mesenteric arteries showed that tripeptides Ile–Pro–Pro and Val–Pro–Pro protect the endothelial function in a time-dependent manner up to 24 h (Jäkälä et al. 2009c). The present study was aimed to further investigate the mechanisms underlying the beneficial effects of tripeptides and their components on vascular function.

Incubation for 6 h at +37 °C did not affect the viability of the arteries in general. As shown in Table 1, acetylcholine-induced endothelium-dependent relaxations remained unaltered independently of incubation either with Ile–Pro–Pro, its components or captopril compared to control. This is contrary to our previous study, where we showed significantly better preservation of endothelial function by Ile–Pro–Pro and Val–Pro–Pro after 24 h incubation at +4 °C (Jäkälä et al. 2009c). The most probable reason for this discrepancy is that in the present incubation conditions (shorter period at higher temperature) the endothelial function of the arteries did not impair to the same extent than in the longer incubation and thus could not be further improved. Furthermore, in the present study the rats, although clearly hypertensive, did not possess severe endothelial dysfunction as the responses to acetylcholine remained almost normal.

In the present study, Ang II metabolite Ang-(1–7) produced concentration-dependent relaxations, which were significantly augmented in the arterial rings incubated with Ile–Pro–Pro or Pro. The vasodilatory activity of Ang-(1–7) is reported to be endothelium-dependent (Santos et al. 2003). In the present study A-779 did not inhibit the Ang-(1–7)-induced relaxation responses. Although only one concentration of A-779 was used, there are also other studies demonstrating that A-779 does not necessarily affect Ang-(1–7)-mediated vasodilatation. Silva et al. (2007) observed that A-779 in concentrations up to 10 μM did not affect aortic vasodilatation induced by Ang-(1–7) in Sprague–Dawley rats, whereas D-Pro^7-Ang-(1–7), another Ang-(1–7) receptor antagonist, abolished the vasodilator effect. Thus, this may suggest the existence of different Ang-(1–7) receptor subtypes or that the effects of Ang-(1–7) were mediated by a mechanism independent of Ang–(1–7) receptors in the present study.

Interestingly, in the present study, bradykinin induced relaxations in certain arteries only when Ang-(1–7) responses were studied before the bradykinin responses. Apparently, the effect of Ang-(1–7) carried over the washes and caused a potentiation of bradykinin responses, which were almost undetectable, if performed in the beginning of the study protocol without prior exposure to other agents. The enhanced responses to bradykinin were seen in the arteries incubated with Ile–Pro–Pro or Pro. By inhibiting bradykinin inactivation, ACE-inhibitors can potentiate the actions of bradykinin and enhance signalling via bradykinin receptors 1 and 2 (B_1 and B_2) (Erdös et al. 2010). Ile–Pro–Pro has been shown to inhibit ACE with IC_{50} value of 5 μM (Nakamura et al. 1995) or lower (Lehtinen et al. 2010). To our knowledge, there are no reports of the ACE-inhibitory activity of Pro, but it is known to inhibit arginase (Dabir et al. 2006), thus providing more L-arginine for NO production. The IC_{50} for captopril, depending on the method used, is reported to be in nanomolar range (Vermeirssen et al. 2002). Consequently, it seems obvious that the observed differences in the responses cannot be explained by ACE-inhibition, as both the compounds were present at sufficient concentrations in the incubation bath, but no potentiation of bradykinin was seen with captopril. Although captopril is reported to be transformed to its disulphide dimer quite easily (Migdalof et al. 1984; Zhou and Li Wan Po 1994), the dimer has a similar kind of antihypertensive and bradykinin-potentiating effect as captopril homomer (Drummer and Kourtis 1988). Therefore, the possible dimerization does not seem to play a role in this respect. As Ang-(1–7) has been reported to potentiate the responses to bradykinin (Oliveira et al. 1999; Tom et al. 2001), we suggest that the augmentation of bradykinin response by Ile–Pro–Pro and Pro was mediated by Ang–(1–7) and not by ACE.

Under physiological conditions, vascular actions of bradykinin are mediated via B_2-receptor, which is constitutively expressed by many cell types (Bhoola et al. 1992). B_2-receptor localization in different parts of the vasculature is dependent on the artery size (Figueroa et al. 2001). In rat mesenteric arteries, B_2-receptors are mainly expressed in the endothelial cell layer. In contrast to B_2-receptors, B_1-receptors are inducible and involved in certain cardiovascular disorders, such as inflammation, atheromatous disease and myocardial ischemia (McLean et al. 2000). B_1-receptor-mediated responses may be upregulated as a consequence of tissue isolation and incubation (Bouthillier et al. 1987; Deblois et al., 1993; Levesque et al. 1995). Our preliminary results show that hardly any B_2-receptor expression can be found from aorta and renal artery of the animals used in the present study (unpublished data). It has been reported that bradykinin induces relaxation in 2-month old SHR mesenteric vascular bed via stimulation of B_2-receptors (Mantelli et al. 1995). Consequently, if B_2-receptors do not exist, B_1-receptors (induced by tissue incubation and/or hypertension) may play a dominant role and bradykinin exert mainly contractile effects (McLean et al. 2000). This explains why bradykinin-induced relaxations were seen only after preceding Ang-(1–7) exposure, again providing evidence from the potentiating effect of Ang-(1–7) on bradykinin. To go more in detail, the bradykinin-induced relaxations should be studied in the presence of Ang-(1–7) antagonist A-779 to clarify the crosstalk between bradykinin and Ang-(1–7) and possibly also in the presence of bradykinin receptors antagonists.

Angiotensin II, a highly potent vasoconstrictor, exerts its effects via AT_1- and AT_2-receptors (de Gasparo et al. 2000). The pattern of distribution of the receptors in the rat vasculature depends on the age and strain of the animals. AT_1-receptor expression seems to be lower in mesenteric vasculature of young SHR than older rats (Touyz et al. 1999). In contrast, the expression of AT_2-receptors is upregulated in young SHR, but in adult SHR the vascular expression of AT_2-receptor is strongly reduced (Touyz et al. 1999; Diniz et al. 2007). The vasodilator effect mediated via AT_2-receptor has been repeatedly described in the literature and has been linked e.g. to the involvement of bradykinin (Carey et al. 2001; Soares de Moura et al. 2004). However, there are only few studies concerning the vasodilator action of Ang II in SHR (Ognibene et al. 2009). In the present study, Ang II produced relaxations in phenylephrine-precontracted mesenteric arteries, which were neither dependent on AT_1- nor AT_2-receptor, as the blockade of these receptors first by valsartan and then by valsartan and PD123319 did not affect the relaxation. Thus, it remains questioned, how the relaxations were mediated then. If neither AT_1- nor AT_2-receptors were involved in the observed relaxations, possible conversion of Ang II to Ang-(1–7) by ACE2 and following binding of Ang-(1–7) to Mas- or some other receptors, or Ang II binding to AT_4, still rather poorly known receptor, could explain the findings. Arteries incubated with Ile–Pro–Pro relaxed more by Ang II in the presence of valsartan compared to the other groups. The difference was not significant after addition of PD123319 anymore, but even so this in line with the above described finding that Ile–Pro–Pro potentiates the Ang-(1–7)-mediated relaxation.

Interesting structural similarities between casein-derived Ile–Pro–Pro and snake venom-derived bradykinin-potentiating peptides can be found. Namely, these proline-rich, ACE-inhibitory peptides (e.g. *Bj*-BPP-7a and *Bj*-BPP-10c) contain Ile–Pro–Pro-sequence in their C-terminal (Ianzer et al. 2007). They show antihypertensive effects, which are reported to be unrelated to their ability to inhibit ACE or potentiate bradykinin. This raises a question if also other compounds possessing an ACE-inhibitory effect lower blood pressure by mechanisms independent of ACE-inhibition. The antihypertensive effect of casein-derived tripeptides has been repeatedly shown, but clinical studies have failed to support ACE-inhibition as a mechanism of action. A weak ACE-inhibition may partly explain the antihypertensive effect of the tripeptides, but considering the findings from the present study, although in vitro, we propose that casein-derived tripeptide Ile–Pro–Pro and possibly also Pro enhance the effect of Ang-(1–7) and bradykinin, thus increasing the importance of ACE2-Ang–(1–7)-Mas axis in the regulation of vascular tone and blood pressure.

Conclusions

In the present study, effects of casein-derived tripeptide Ile–Pro–Pro and its components on RAS-related vascular effects were studied in vitro using isolated rat mesenteric arteries. Captopril was used as a reference ACE-inhibitor. Acetylcholine-induced endothelium-dependent relaxation was not affected by the compounds studied. However, tripeptide Ile–Pro–Pro and amino acid Pro enhanced the responses to Ang–(1–7) and bradykinin. The previously shown ACE-inhibitory activity of Ile–Pro–Pro did not explain the findings and it is concluded that Ile–Pro–Pro and Pro positively affect ACE2-Ang–(1–7)–Mas axis, which may play an important role in the previously shown antihypertensive actions of Ile–Pro–Pro.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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