Angiotensin-(1–7) modulates vascular resistance and sympathetic neurotransmission in kidneys of spontaneously hypertensive rats

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Abstract

Objective: Angiotensin (Ang)-(1–7) generated from Ang I and II is reported to act as an endogenous angiotensin-converting enzyme (ACE) inhibitor and angiotensin type 1 (AT1)-receptor antagonist in vitro and in vivo. Ang-(1–7) has been suggested to play an important role in hypertension. Methods and results: Therefore, we tested whether Ang-(1–7) differentially modulates vascular resistance and neurotransmission in isolated kidneys of spontaneously hypertensive rats stroke prone (SHR-SP) and Wistar–Kyoto rats (WKY). Ang-(1–7) was administered in three concentrations (0.1, 1 and 10 µmol/l) to prevent Ang I- and Ang II-induced pressor responses and facilitation of noradrenaline release. There were indeed concentration-dependent strain differences. Ang-(1–7) prevented Ang I- and Ang II-mediated changes in vascular resistance more potently in SHR-SP than in WKY by inhibiting ACE and by blocking AT1-receptors. Ang-(1–7) interacted with the renal tissue in both strains. Ang-(1–7) inhibited Ang I-mediated facilitation of noradrenaline release more potently than Ang II-mediated facilitation of noradrenaline release. Ang-(1–7) by itself enhanced noradrenaline release from SHR-SP, but not from WKY kidneys. Conclusion: Ang-(1–7) had a greater impact on Ang I and Ang II modulation of renal resistance in SHR-SP than in normotensive rats. Furthermore, Ang-(1–7) by itself has facilitatory presynaptic effects on noradrenaline release but no postsynaptic effects on renal vascular resistance in SHR-SP. Since plasma levels of Ang-(1–7) accumulate during ACE-inhibitor or AT1-receptor antagonist therapy, Ang-(1–7) could contribute to antihypertensive effects of these agents.

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Keywords: Angiotensin-(1–7); Renin-angiotensin-system; Noradrenaline release; Vascular resistance; Experimental hypertension

1. Introduction

Angiotensin II (Ang II) is believed to be the major effector molecule of the renin–angiotensin system (RAS) [1,2]. Most of its known actions, i.e. regulation of vascular resistance, sympathetic neurotransmitter release, cell proliferation and differentiation, as well as osmo- and volume homeostasis, are mediated via AT1- and AT2-receptors [3–5]. However, Ang II is not the only active metabolite of the RAS. Other degradation products of Ang I and Ang II, such as the heptapeptide Ang-(1–7), have received more and more attention as active peptides of the RAS [6,7]. Ang-(1–7), which can be formed from either Ang I or Ang II by endopeptidases and carboxypeptidases, is further metabolized by angiotensin-converting enzyme (ACE). Recent studies suggest that Ang-(1–7) acts as an endogenous antagonist of Ang I and II and blocks Ang II-mediated vasoconstriction and cell proliferation [8–11]. In contrast, others have reported that Ang-(1–7) seemed to induce vasoconstriction in human forearm [12] and rat renal microvessels [13]. However, these effects are not easily attributable to a single mode of action. Thus, it was proposed that besides activation of a new Ang II (Ang-(1–7)-receptor), ACE-inhibition and AT1-receptor blockade could be responsible for the effects mediated by Ang-(1–7) [9,14–17]. Another important observation was that Ang-(1–7) plasma levels increase during antihypertensive therapy involving ACE-inhibitors alone or in combination with AT1-receptor antagonists [18–20]. Thus, two major areas of research interest have developed. First, a lack of the endog-
ous antagonist Ang-(1–7) may be one pathophysiological mechanism for the development and maintenance of hypertension. Accordingly, Benter et al. [21] and Kost et al. [22] reported that intravenous infusion of Ang-(1–7) decreases blood pressure in spontaneously hypertensive rats (SHR), but not in normotensive Wistar–Kyoto rats (WKY). The second area of interests concerns the possibility that Ang-(1–7) contributes to beneficial effects of antihypertensive treatment with inhibitors of the RAS. For instance, several studies have demonstrated that ACE inhibitor therapy does not totally prevent Ang II formation [23–25] and data supporting a primary role of bradykinin in mediating its antihypertensive and antihypertrophic effects remain controversial [7]. Thus, one could speculate that Ang-(1–7) is involved in preventing target organ failure in hypertension during chronic ACE inhibitor therapy. In chronic renal failure, hypertension is a result of both an activated RAS and activated sympathetic nervous system [26]. As many studies demonstrated that Ang II is more potent in kidneys of SHR, the primary aim of our study was to investigate whether Ang-(1–7) is capable to prevent Ang I- and Ang II-mediated renal vasoconstriction and enhancement of noradrenaline release in spontaneously hypertensive kidneys. Moreover, we explored the possibility that Ang-(1–7) by itself contributes to hypertension by facilitating renal sympathetic neurotransmission.

2. Materials and methods

Male Wistar–Kyoto rats (Charles River, Sulzfeld, Germany) and 16-week-old spontaneously hypertensive stroke prone rats (SHR-SP) (Max Delbrück Institut, Berlin) weighing between 220 and 310 g were used. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Rats were anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneally) and then the kidneys were isolated and perfused with Krebs–Henseleit solution according to a method described previously [27]. Bipolar platinum electrodes were placed around the renal arteries to stimulate the renal sympathetic nerves. Perfusion pressure was monitored continuously with a Statham P23 Db pressure transducer (Gould, Oxnard, CA) coupled to a Watanabe pen recorder (Graphitc, Tokyo, Japan).

2.1. Experimental protocol (Part A): effects of Ang I, Ang II and Ang-(1–7) on renal vascular resistance

The kidneys were perfused with drug-free Krebs–Henseleit solution at 37 °C with a constant flow rate of 4.2 ml/min g kidney weight. Kidney wet weights were calculated according to data of previously published experiments [27], showing that they correspond to approximately 0.5% of whole body weight. The perfusion solution was continuously gassed with carbogen (5% CO2/95% O2) and passed through a 0.45-μm filter before it reached the kidneys. Immediately after preparation, a bolus injection of ANG II (3 nmol/l) was delivered to test the viability of the preparation followed by a stabilization period of 30 min. The agonists, Ang I, Ang II and Ang-(1–7) were added to the perfusion solution in a cumulative manner expressed in a dose response curve. The time interval between application of the different agonist concentrations was 2.5 min. The perfusion of drugs was stopped when the pressor responses had reached a maximum, or when no effects were observed respectively. When dose response curves for Ang I and Ang II were determined in the presence of Ang-(1–7) (acting as an antagonist), Ang-(1–7) was added to the perfusion solution 10 min before determination of the second dose response curve.

2.2. Calculations

The pressor responses of Ang I, Ang II and Ang-(1–7) were measured as the maximum increase of perfusion pressure above basal perfusion pressure (ΔP max = P max − P basal). This increase was expressed as a percentage of ΔP max (ΔP max set as 100%). The increase in the perfusion pressure induced by either Ang I or Ang II in the presence of Ang-(1–7) was calculated as percentage to the control dose response curve (ΔP max(antagonist)/ΔP max(control)*100).

![Fig. 1. Enhancement of noradrenaline (NA) release after RNS (S1–5) induced by Ang-(1–7) in SHR (n=4) isolated perfused kidneys, but not in WKY (n=4). The RNS-induced noradrenaline release was calculated as a percentage of control stimulation S0 (S0 as % of S0) as described in Section 2. Ang-(1–7) was added to the perfusion solution in a cumulative manner 10 min before the next stimulation. In SHR, Ang-(1–7) induces a significant greater enhancement of noradrenaline release compared to WKY (two-factorial ANOVA for repeated measurements P<0.05); mean ± S.E.M.](https://academic.oup.com/cardiovascres/article-abstract/61/2/352/425371)
pK$_{B}$-values for Ang I and Ang II against Ang-(1–7) were determined according a method of Furchgott as described elsewhere [28].

2.3. Experimental protocol (Part B): renal nerve stimulation (RNS) and sympathetic neurotransmission

The kidneys were perfused as described in Section 2.2. Immediately after preparation, a priming stimulation of 5 Hz for 30 s (1 ms pulse width, 40 mA) was delivered to renal nerves to test the viability of the preparation. After a stabilization period of 30 min, cocaine (10 µmol/l) and corticosterone (20 µmol/l) were added to the perfusion solution in order to prevent neuronal and extraneuronal uptake of released noradrenaline, respectively. After another 20 min, 1-min fractions of the effluent were collected by a fraction collector (LKB, Bromma, Sweden) into vials con-

![Fig. 2. Absolute noradrenaline (NA) release after RNS in the absence (control; S$_{0}$) of Ang-(1–7) and presence of Ang-(1–7) (10 µmol/l) (S$_{5}$) (WKY: control, n=3; Ang-(1–7) (10 µmol/l), n=4; SHR control, n=4; Ang-(1–7) (10 µmol/l) n=4). The absolute RNS-induced noradrenaline release was calculated as the total RNS-induced noradrenaline release per gram kidney. Shows a significant greater RNS-induced noradrenaline release for the control as well as for Ang-(1–7)-induced noradrenaline release in SHR than in WKY. + Indicates a significant greater absolute increase of noradrenaline release induced by Ang-(1–7) compared to the control in SHR than in WKY kidneys (ANOVA, P<0.05).](https://academic.oup.com/cardiovascres/article-abstract/61/2/352/425371)

![Fig. 3. Influence of Ang-(1–7), tested in three different concentrations (0.1, 1 and 10 µmol/l) on Ang I (A)- and Ang II (B)-mediated renal vascular resistance (Ang I, n=17; Ang I + Ang-(1–7) (0.1 µmol/l), n=4; Ang I + Ang-(1–7) (1 µmol/l), n=4; Ang I + Ang-(1–7) (10 µmol/l), n=5; Ang II, n=30; Ang II + Ang-(1–7) (0.1 µmol/l), n=5; Ang II + Ang-(1–7) (1 µmol/l), n=8; Ang II + Ang-(1–7) (10 µmol/l), n=10) in WKY isolated perfused kidneys. Ang-(1–7) was added to the perfusion solution 10 min before measuring the second renal dose response curve. The increase in the renal resistance was calculated as a percentage of $\Delta P_{\text{max}}$ ($\Delta P_{\text{max}}$ set as 100%) as described in Section 2. Ang-(1–7) (1 and 10 µmol/l) shifted the dose response–curve of Ang I and Ang II significantly to the right (two-factorial ANOVA for repeated measurements, $P<0.05$); mean ± S.E.M. The lowest concentration of Ang-(1–7) showed no inhibitory potency in WKY kidneys.](https://academic.oup.com/cardiovascres/article-abstract/61/2/352/425371)
containing 167 μl of 1 M HCl, 13.3 μl of 0.067 M EDTA and 3.3 μl of 1 M Na₂SO₃. Six electrical RNS (S₀–S₅) each at 2 Hz for 30 s (1 ms pulse width, 40 mA) were applied 2, 16, 30, 44 and 58 min after the start of fraction collection. Cumulative concentrations response curves for Ang I, Ang II and Ang-(1–7) were determined. The substances were directly added to the perfusion solution in a cumulative manner starting 10 min before S₁, S₂, S₃, S₄ and S₅.

When Ang-(1–7) (used as an antagonist) was present throughout the experiment, it was added to the perfusion solution 8 min before the start of fraction collection.

2.4. Determination of endogenous noradrenaline

The noradrenaline in the isolated kidney samples was extracted (adsorption onto alumina, elution with HClO₄). Noradrenaline content was determined by reversed-phase HPLC detection [29]. The amount of noradrenaline (pg/g kidney) present in each samples was corrected for recoveries (average percent recovery of noradrenaline–HCl was 0.562 ± 0.2; n = 33).

2.5. Calculation of data

The RNS-induced outflow of noradrenaline was determined in four 1-min samples collected immediately after the onset of stimulation. S₀ served as a control/reference stimulation for the following stimulations. The RNS-induced noradrenaline outflow during S₁, S₂, S₃, S₄ and S₅ was expressed as a percentage of that during S₀ (% of S₀). For further evaluations, the effects of Ang I, Ang II and Ang-(1–7) in the absence or presence of Ang-(1–7), the Sn/S₀ ratios were calculated as a percentage of values, which were determined in the corresponding control experiments (% of control).

2.6. Statistical analysis

All data were expressed as mean ± S.E.M. Differences between dose response curves were analyzed by two-factorial ANOVA for repeated measurements followed by unpaired Student’s t-test. Probability levels of P < 0.05 were considered statistically significant. The number of experiments indicates the number of individual kidneys.

2.7. Drugs and vehicles

The Krebs–Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 0.45, NaHCO₃ 25, KH₂PO₄ 1.03, d-(+)-glucose 11.1, Na₂EDTA 0.067 and ascorbic acid 0.07. The following drugs were employed: (+)-noradrenaline HCl, corticosterone, Ang I (Sigma, Deisenhofen, Germany); cocaine HCl (Merk, Darmstadt, Germany); EXP 3174 (DU PONT, Wilmington, DE); Ang II (Angiotensinamid, Ciba-Geigy, Wehr, Germany); Ang-(1–7) (Bachem, Heidelberg, Germany). Drugs...
were dissolved in distilled water before being diluted with Krebs–Henseleit solution, except of corticosterone that was dissolved in absolute ethanol.

3. Results

SHR-SP and WKY rat kidneys were isolated and perfused with Krebs–Henseleit solution. The effects of Ang-(1–7) alone and against Ang I, Ang II on renal vascular resistance and RNS-induced noradrenaline release were analyzed.

3.1. Effects of Ang-(1–7) on pressor responses and RNS-induced noradrenaline release

Ang-(1–7) failed to induce vasodilation or vasoconstriction by itself as well as in noradrenaline preconstricted kidneys of WKY and SHR-SP (data not shown). In contrast, Ang-(1–7) significantly enhanced noradrenaline release in SHR-SP and not in WKY kidneys (Figs. 1). The maximal absolute RNS-induced noradrenaline release (pg/g kidney weight) in the absence of Ang-(1–7) was greater in SHR-SP than in WKY (Fig. 2). Moreover, the absolute increase of noradrenaline release induced by Ang-(1–7) was also significantly greater in SHR-SP than in WKY kidneys (Fig. 2) (control: WKY: 1837 ± 129, SHR: 2308 ± 192; Ang-(1–7): WKY 1597 ± 61, SHR 3292 ± 311 pg/g kidney).

3.2. Effects of Ang-(1–7) on pressor responses to Ang I and Ang II

Ang-(1–7) inhibited pressor responses to Ang I and Ang II in WKY and SHR-SP kidneys in a competitive manner (Figs. 3 and 4). However, there were different inhibitory potencies of Ang-(1–7) in WKY and SHR-SP kidneys. Furthermore, concentration-dependent differences in the effects of Ang-(1–7) against Ang I and Ang II were observed. Thus, the lowest concentration of Ang-(1–7) (0.1 µmol/l) shifted the dose response curves of Ang I and Ang II significantly to the right in SHR-SP but not in WKY (Figs. 3 and 4). Higher concentrations of Ang-(1–7) (1 and 10 µmol/l) shifted the dose response curves of Ang I and Ang II in both strains. Calculated pK\textsubscript{B}-values (Table 1A and B) for Ang-(1–7) (1 µmol/l) against Ang I and Ang II suggest a similar inhibitory potency in WKY. In contrast, in SHR-SP Ang-(1–7) (1 µmol/l) appears to be more potent against Ang I than against Ang II (Table 1A and B). The highest concentration of Ang-(1–7) (10 µmol/l) shifted the dose response curves of Ang I more effectively to the right than the dose response curve of Ang II for both WKY (Fig. 3A and B) and SHR-SP (Fig. 4A and B). The corresponding pK\textsubscript{B}-values for Ang-(1–7) were similar (Table 1A and B).

Table 1

<table>
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(A) and (B) shows pK\textsubscript{B}-values of Ang 1–7 in two different concentrations (1 and 10 µmol/l) against Ang I and Ang II in WKY and SHR kidneys. In WKY, Ang 1–7 (1 µmol/l) was equipotent against Ang I and II. In all other groups, Ang-(1–7) was more potent against Ang I than against Ang II. pK\textsubscript{B} values (– log K\textsubscript{B}) were obtained according to the following equation: K\textsubscript{B}=([A]/(concentration ratio − 1)) where [A] is the concentration of the antagonist and concentration ratio is the EC\textsubscript{50} in the presence of the antagonist divided by the EC\textsubscript{50} in the absence of antagonist.

Fig. 5. Influence of Ang-(1–7) (10 µmol/l) on the Ang I-mediated induced noradrenaline (NA) release after RNS (WKY: Ang I, \textit{n}= 3; Ang I + Ang-(1–7), \textit{n}= 4; SHR: Ang I, \textit{n}=4; Ang I + Ang-(1–7), \textit{n}=5) in isolated perfused kidneys of WKY and SHR. Ang-(1–7) was added to the perfusion solution 10 min before the first RNS. The RNS-induced noradrenaline release was calculated as a percentage of control stimulation S\textsubscript{0} (Sn as % of S\textsubscript{0}) as described in Section 2. Ang-(1–7) shifted the dose response–curve of Ang I in both strains significantly to the right (two-factorial ANOVA for repeated measurements, \textit{P}<0.05); mean ± S.E.M. *Indicates in the presence of Ang-(1–7) (10 µmol/l) a significant smaller maximal Ang I-induced enhancement of noradrenaline release in SHR than in WKY.
release was calculated as a percentage of control stimulation S0 (Sn as % of solution 10 min before the first RNS. The RNS-induced noradrenaline (NA) release after RNS (WKY: Ang II, SHR: Ang II, n = 4; SHR: Ang II + Ang-(1–7), n = 6) in isolated perfused kidneys of WKY and SHR. Ang-(1–7) was added to the perfusion solution 10 min before the first RNS. The RNS-induced noradrenaline release was calculated as a percentage of control stimulation S0 (Sn as % of S0) as described in Section 2. Ang-(1–7) shifted the dose response curve to Ang I than against Ang II. This greater shift of the Ang I as compared to the Ang II dose response curve by Ang-(1–7) is most likely due to simultaneous AT1-receptor blockade and ACE inhibition [17]. The identical potency of the intermediate concentration (1 μmol/l) of Ang-(1–7) against Ang I and II gives indirect evidence that in this concentration Ang-(1–7) blocks AT1-receptors only. The situation in SHR-SP was different. In the hypertensive strain the low concentration of Ang-(1–7) shifted the dose response of Ang I and II significantly to the right. This more potent effect of Ang-(1–7) in SHR-SP is consistent with results of Kost et al.[22], Benter et al.[21], Fernandes et al.[32] and Iyer et al.[14,19]. They suggested that the more potent effect of Ang-(1–7) in SHR was due to the release of prostanoids, kinines and/or nitric oxide by activation of putative Ang-(1–7)-receptors [6]. However, this is not a likely explanation for our findings in SHR-SP isolated kidneys, since Ang-(1–7) had no vasodilatory effect in noradrenaline preconstricted kidneys even in subnanomolar concentrations considered to be selective for Ang-(1–7)-receptors [9,32]. There seem to be also species differences, since Ang-(1–7) has been shown to dilate rabbit renal arteries in vitro [9]. In contrast to the almost identical inhibitory potency of an intermediate concentration of Ang-(1–7) (1 μmol/l) against Ang I and II in WKY, in SHR-SP Ang-(1–7) (1 μmol/l) was more potent against Ang I than against Ang II. This may suggest a higher affinity of Ang-(1–7) for ACE in SHR-SP than in WKY kidneys. The inhibitory effect of Ang-(1–7) in the highest concentration (10 μmol/l) showed no differences between both strains with respect to Ang I- and Ang II-induced pressor responses.

In the present study, Ang-(1–7) neither induced vasodilatation nor vasoconstriction. Thus, our results do not provide evidence for a vasoactive potency of Ang-(1–7) by itself in the renal vasculature of normotensive and hypertensive rats. These results are in contrast to findings

\[ 4.1. \text{Effects of Ang-(1–7) on Ang I- and Ang II-induced increases of renal vascular resistance} \]

Previous studies showed that Ang-(1–7) blocks AT1-receptors and inhibits ACE [10,15,16,30,31]. Such an inhibitory effect of Ang-(1–7) on pressor responses to Ang I and II has been previously demonstrated in kidneys of normotensive rats. An involvement of specific Ang-(1–7)-receptors was excluded [17]. In the present study, several concentrations of Ang-(1–7) (0.1, 1 and 10 μmol/l) were tested to detect possible differences in the inhibitory potency of Ang-(1–7) on Ang I- and II-mediated pressor responses elicited in WKY versus SHR-SP. Ang-(1–7) in the lowest concentration (0.1 μmol/l) failed to shift the Ang I- and Ang II-mediated dose response curve significantly to the right in WKY kidneys, whereas the intermediate and highest concentrations of Ang-(1–7) caused a significant shift to the right. The intermediate concentration was equipotent against Ang I and II, however, the highest concentration of Ang-(1–7) revealed a more potent inhibitory effect against Ang I than against Ang II. The greater shift of the Ang I as compared to the Ang II dose response curve by Ang-(1–7) is most likely due to simultaneous AT1-receptor blockade and ACE inhibition [17]. The identical potency of the intermediate concentration (1 μmol/l) of Ang-(1–7) against Ang I and II gives indirect evidence that in this concentration Ang-(1–7) blocks AT1-receptors only. The situation in SHR-SP was different. In the hypertensive strain the lowest concentration of Ang-(1–7) shifted the dose response of Ang I and II significantly to the right. This more potent effect of Ang-(1–7) in SHR-SP is consistent with results of Kost et al.[22], Benter et al.[21], Fernandes et al.[32] and Iyer et al.[14,19]. They suggested that the more potent effect of Ang-(1–7) in SHR was due to the release of prostanoids, kinines and/or nitric oxide by activation of putative Ang-(1–7)-receptors [6]. However, this is not a likely explanation for our findings in SHR-SP isolated kidneys, since Ang-(1–7) had no vasodilatory effect in noradrenaline preconstricted kidneys even in subnanomolar concentrations considered to be selective for Ang-(1–7)-receptors [9,32]. There seem to be also species differences, since Ang-(1–7) has been shown to dilate rabbit renal arteries in vitro [9]. In contrast to the almost identical inhibitory potency of an intermediate concentration of Ang-(1–7) (1 μmol/l) against Ang I and II in WKY, in SHR-SP Ang-(1–7) (1 μmol/l) was more potent against Ang I than against Ang II. This may suggest a higher affinity of Ang-(1–7) for ACE in SHR-SP than in WKY kidneys. The inhibitory effect of Ang-(1–7) in the highest concentration (10 μmol/l) showed no differences between both strains with respect to Ang I- and Ang II-induced pressor responses.

In the present study, Ang-(1–7) neither induced vasodilatation nor vasoconstriction. Thus, our results do not provide evidence for a vasoactive potency of Ang-(1–7) by itself in the renal vasculature of normotensive and hypertensive rats. These results are in contrast to findings
of Ueda et al. [12], Abbas et al. [33] and van Rodijnen et al. [13] who showed a weak but significant AT1-receptor-mediated vasoconstriction of high Ang-(1–7) doses in the human forearm, anesthetized rats and rat renal microvessels. On the other hand recent studies showed that Ang-(1–7) induces AT1-/AT2-receptor independent relaxation in different tissues and species, including coronary arteries of canines and pigs [34,35] and isolated heart and aorta of rats [36,37]. Osei et al. [38] found apparently opposing vascular responses to Ang-(1–7) in mesenteric and hindlimb vascular beds, suggesting tissue selectivity.

4.2. Effects of Ang-(1–7) as a modulator of renal sympathetic neurotransmission

Ang-(1–7) (10 μmol/l) inhibited Ang I- and Ang II-mediated enhancement of RNS-induced noradrenaline release in both strains. There was a greater rightward shift of the dose response curve for Ang I than for Ang II by about one log unit in both strains. This greater shift of the Ang I dose response curve may be attributed to a simultaneous AT1-receptor blockade and ACE inhibition by the high concentration of Ang-(1–7) [17]. The inhibitory potency of Ang-(1–7) (10 μmol/l) against Ang I and Ang II was not different between SHR-SP and WKY. Thus, obvious differences of Ang-(1–7) as an antagonist of Ang I- and II-mediated modulation of renal sympathetic neurotransmitter release appear not to play a role for hypertension in the SHR-SP. Ang-(1–7) reduced the maximal Ang I- but not the maximal Ang II-mediated enhancement RNS-induced noradrenaline release in SHR-SP. This may suggest that Ang-(1–7) acts as a non competitive inhibitor in SHR-SH kidneys as previously described for Ang II-induced pressor effects in rabbit aortic rings by Mahon et al. [10].

It is noteworthy that Ang-(1–7) by itself enhanced RNS-induced noradrenaline release in SHR-SP but not in WKY kidneys. One explanation could be that Ang-(1–7) especially in high concentrations has an intrinsic activity at AT1- or AT2-receptors as suggested for rat atria and hypothalamic tissues [39–41]. However, this explanation seems not likely for several reasons. Even the highest concentration of Ang-(1–7) (10 μmol/l) did not have any faciliatory effects on RNS-induced noradrenaline in WKY kidneys. Furthermore, as outlined above, Ang-(1–7) had a similar inhibitory effect against AT1-receptor-mediated enhancement of noradrenaline release in WKY and SHR. Thus, there has to be an alternative explanation. One could speculate that there are presynaptic high affinity binding sites for Ang-(1–7) in SHR-SP, which might be responsible for these observed differences. Thus, the facilitatory effect of Ang-(1–7) in SHR-SP may play a pathophysiological role in this model of hypertension. In conclusion, Ang-(1–7) acts as an endogenous antagonist of ACE and AT1-receptors in WKY and SHR-SP kidneys and has a greater impact on Ang I- and Ang II-mediated modulation of renal vascular resistance in SHR-SP than in WKY. Furthermore, Ang-(1–7) by itself shows differential pre- and postsynaptic effects in SHR-SP, e.g. it facilitates noradrenaline release presynaptically but has no direct vasoactive effects. Since plasma levels of Ang-(1–7) accumulate during therapy with ACE-inhibitors and/or AT1-receptor antagonists [18–20,30,42], Ang-(1–7) could contribute to beneficial effects of this antihypertensive therapy. This view is supported by our findings that Ang-(1–7) inhibits Ang I- and II-mediated pressor responses more potently in a genetic model of hypertension. Admittedly, plasma levels of Ang-(1–7) reported even under therapy with ACE-inhibitors and/or AT1-receptor antagonists are lower than concentrations used in this study. Nevertheless, it may be that Ang-(1–7) tissue levels exceed Ang-(1–7) plasma levels in analogy to the situation with Ang II itself.

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