The therapeutic potential of adenoviral gene therapy and angiotensine-(1-7) in proteinuric kidney disease

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Chapter 4

The role of angiotensin-(1-7) in renal vasculature of the rat

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Abstract

Background

Angiotensin-(1-7) [Ang(1-7)] is an active component of the renin-angiotensin-aldosterone system. Its exact role in renal vascular function is unclear. We therefore studied the effects of Ang(1-7) on the renal vasculature *in vitro* and *in vivo*.

Methods

Isolated small renal arteries were studied in an arteriograph system by constructing concentration-response curves to angiotensin II (Ang II), without and with Ang(1-7). In isolated perfused kidneys (IPK), the response of Ang II on renal vascular resistance was measured without and with Ang(1-7). The influence of Ang(1-7) on Ang II-induced glomerular afferent and efferent constriction was assessed with intravital microscopy *in vivo* under anaesthesia. In freely moving rats, we studied the effect of Ang(1-7) on Ang II-induced reduction of renal blood flow (RBF) with an electromagnetic flow probe.

Results

Ang(1-7) alone had no effect on the renal vasculature in any of the experiments. *In vitro*, Ang(1-7) antagonised Ang II-induced constriction of isolated renal arteries (9.71±1.21% and 3.20±0.57%, for control and Ang(1-7) pretreated arteries, respectively; $p<0.0005$). In IPK, Ang(1-7) reduced the Ang II response (100±16.6 versus 72.6±15.6%, $p<0.05$) and shifted the Ang II dose-response curve rightward (pEC_{50} 6.69±0.19 and 6.26±0.12 for control and Ang(1-7) pretreated kidneys, respectively; $p<0.05$). Ang(1-7), however, was devoid of effects on Ang II-induced constriction of glomerular afferent and efferent arterioles and on Ang II-induced RBF reduction in freely moving rats *in vivo*.

Conclusion

Ang(1-7) antagonises Ang II in renal vessels *in vitro*, but does not appear to have a major function in normal physiological regulation of renal vascular function *in vivo*.

Introduction

Angiotensin II (Ang II) is the main effector peptide of the renin-angiotensin-aldosterone system. In the renal vasculature, it is a potent vasoconstrictor and it regulates kidney function by modulating the glomerular filtration rate, renal blood flow (RBF), and renal vascular resistance (RVR). There is increasing evidence, however, that apart from Ang II, other components of the renin-angiotensin-aldosterone system are biologically active. In particular, angiotensin-(1-7) [Ang(1-7)], which consists of the first seven amino acids of angiotensin I (Ang I) and Ang II, is thought to play a role in counteracting the response to Ang II[1]. It is produced through cleavage of Ang I and Ang II by neutral endopeptidases, and from the recently discovered angiotensin-converting enzyme (ACE) homologue ACE2[2].

The pharmacological mechanisms and the receptors involved in the effects of Ang(1-7) are diverse. First, because Ang(1-7) is a substrate for ACE, it acts as an ACE inhibitor[3]. Second, Ang(1-7) is an antagonist of the angiotensine type 1 receptor (AT_1R)[4;5], but in higher doses AT_1R agonistic effects are also observed[6]. Third, there is some evidence for angiotensin type 2 receptor (AT_2R) agonism for Ang(1-7)[7], although the main receptor for Ang(1-7) appears to be the newly discovered Ang(1-7) receptor, Mas[8]. Through Mas receptor stimulation, Ang(1-7) potentiates bradykinin-induced effects[9], stimulates release of prostanoids[10] and releases nitric oxide (NO)[11].

Ang(1-7) may play an important role; not only in normal physiology, but it may also contribute to the therapeutic effects of ACE inhibition, as plasma levels of Ang(1-7) are increased during therapy with ACE inhibitors[12;13]. Moreover, Ang(1-7) has been shown to attenuate the development of heart failure[14] and to inhibit neointimal formation after stent implantation[15], features which are similar to those of ACE inhibitors. As ACE inhibitors are widely applied in various forms of renal disease and hypertension, Ang(1-7) may be applied as a new pharmacological agent in renovascular diseases.

The role of Ang(1-7) in the (patho)physiological regulation of kidney function is still not completely understood, however, and the current literature shows conflicting data. Diuresis and natriuresis have been documented[10;16-19], in which the release of prostanoids[10], inhibition of tubular Na^+ reabsorption after stimulation of both AT_1R and non-AT_1R/non-AT_2R[18], and Mas receptor stimulation[19] appear to play a role. In addition, Ang(1-7) is a vasodilator in rabbit afferent arterioles through the release of NO after Mas receptor stimulation[20]. It also inhibits Ang II-induced vasoconstriction in isolated perfused kidneys (IPK)[5]. Blood pressure is lowered in response to Ang(1-7)[17], an effect mediated by Mas receptors[21], through the release of prostanoids[22] and NO[23] and the potentiation of bradykinin[24] and acetylcholine-induced NO release[25].

In other studies, however, Ang(1-7) was without effect or had even opposing effects on the kidney. In water-loaded rats, Ang(1-7) exerted an antidiuretic and antinatriuretic effect through Mas receptors[26]; and in hydronephrotic IPK, Ang(1-7) acted as a vasoconstrictor by stimulation of AT_1R[6]. In addition, hypertensive effects of Ang(1-7) through AT_1R stimulation have been described[27]. Differences in experimental models and protocols, such as *in vitro* versus *in vivo* studies, the use of anaesthetics, different dietary sodium intakes[28;29], water loading[26], and different Ang(1-7) doses[30] may be responsible

for these discrepancies. Because the effects of Ang(1-7) on renal vascular physiology are poorly characterised, we aim to establish the role of Ang(1-7) at several levels of the renal vasculature.

We therefore studied the effect of Ang(1-7) and its effect on the response to Ang II in isolated renal arteries, IPK *in vitro*, afferent and efferent arterioles *in vivo* under anaesthesia and on RBF in freely moving rats.

Methods

Animals

Male Wistar rats (320-350 g; n=40) were housed under standard conditions with free access to food and drinking water. Rats received a standard chow diet containing 0.3% NaCl (Hope Farms Inc., Woerden, The Netherlands). Animal experiments were approved by the institutional animal ethical committee.

Isolated small renal interlobar arteries

Kidneys (n=6) were removed under isoflurane/O_2/N_2O anaesthesia. Small renal interlobar arteries with an internal diameter of 250-300 µm were dissected and transferred to a pressurised arteriograph system. Artery segments were cannulated on glass micropipettes at both ends, secured, and perfused with Krebs solution (120.4 mM NaCl, 5.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 NaH_2PO_4, 25.0 mM $NaHCO_3$, 1.2 mM glucose, oxygenated with 5% CO_2 in O_2, pH 7.4, and 37 °C). Intraluminal pressure was set to 70 mmHg and held constant (blind sac) by a pressure servo system. The vessel chamber was transferred to the stage of an inverted light microscope with a video camera attached to a viewing tube. A video dimension analyser was used to register lumen diameter continuously.

Arteries were allowed to equilibrate for 1 h in Krebs solution. Experiments were performed in the presence of the nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (10^{-4} M, 20 min), and the ACE inhibitor, lisinopril (10^{-6} M, 15 min), to block NO-dependent relaxation and to prevent degradation of Ang(1-7) by ACE, respectively. Subsequently, dose-response curves to cumulative doses of Ang II (10^{-10} to 10^{-6} M), with and without Ang(1-7) (10^{-5} M, 10 min), were constructed by adding Ang II directly to the bath. Constriction responses are expressed as percentage of baseline lumen diameter.

Isolated perfused kidneys

Carbogenated Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4 (118 mM NaCl, 4.69 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4 \cdot 7H_20$, 25 mM $NaHCO_3$, 2.52 mM $CaCl_2 \cdot 2H_2O$), supplemented with D-glucose (1.1 g/L) was used. The IPK setup consisted of a moist temperature chamber in which the kidney was perfused through a cannula in the renal artery using a peristaltic pump. KRB was oxygenated with a mixture of 95% O_2/5% CO_2 by a capillary module, and passed through a bubble trap. Temperature was kept at 37 °C with a thermostatically controlled water bath.

Rats were anaesthetised with isoflurane/O_2/N_2O, the abdomen was opened by a mid-line incision, and the aorta (rostral from the right renal artery) and the superior mesenteric artery were ligated. Blood was removed from the kidneys by flushing them through the aorta with 10 ml of KRB at 37 °C. The

kidneys and aorta were removed *en bloc* and placed on ice. The renal arteries were cannulated, and remaining blood was removed by flushing the kidneys with KRB at 4 °C. Subsequently, the kidneys were transferred to the IPK setup and perfused with KRB at a continuous flow of 10 ml/min. Perfusion pressure was continuously monitored. After 15 minutes of equilibration, dose-response curves to Ang II were constructed by injection of 100 µl of Ang II solutions (10^{-9} to 10^{-4} M) in the buffer in close proximity to the kidney. Subsequently, Ang(1-7) was added to the buffer reservoir in a final concentration of 10^{-5} M, and after 15 minutes of equilibration, the dose-response curve of Ang II was repeated. Renal vascular resistance is expressed as percentage of maximal pressure rise (ΔP) in response to Ang II.

Intravital microscopic analysis of afferent and efferent arterioles

The experimental system consisted of a pencil-probe video microscope with a corn-shaped lens (optical magnification 3.5x) and a charge-coupled device (CCD) camera (Nihon Kohden, Tokyo, Japan), a micromanipulator, a xenon light source (LB-18 Welch Allyn, Tokyo, Japan), a monitor (KLV-17HR1, Sony, The Netherlands), a DVD recorder (RDR-GX7), and a computer for image analysis (Intel P4). The lens fitted with a 12.7 mm greyscale CCD image sensor (XC ES55L, Toshiba, Tokyo, Japan) at the focal length (200 mm) of the lens. A green filter to complement red was placed in front of a CCD image sensor to enhance the contrast on the monitor between vessels and peripheral tissue. The CCD image sensor was connected to camera module (DC700, Tokyo, Japan) and images were recorded as stacked image film (30 frames/s). The final spatial resolution of the video microscope was confirmed to be 0.86 µm with electrical magnification of 520x. The scale of the captured video image was 752x582 pixels on the display, which allowed us to monitor only one glomerulus and its region in each experimental protocol.

For the experiments, rats were briefly anaesthetised with isoflurane/O_2/N_2O to insert a cannula in the tail vein. Through this cannula, 100 µg/kg of thiobutabarbital (Inactin®, Sigma-Aldrich, Zwijndrecht, The Netherlands) was injected in two doses, and if necessary re-administered to maintain a constant level of anaesthesia. In addition, the operation regions were locally anaesthetised with lidocaine (20 mg/ml). The carotid artery and jugular vein were cannulated for measurement of arterial blood pressure and heart rate, and intravenous infusion of angiotensins, respectively. Subsequently, the abdomen was opened by mid-line incision and the left renal artery was exposed. For measuring renal blood flow, an ultrasonic flow probe (model 1RB, Transonic Systems, Ithaca, New York, USA) was placed around the left renal artery and the RBF was continuously registered with a flow meter (model T106, Transonic Systems). The blood pressure and heart rate were recorded using a pressure transducer (Edwards Lifesciences S.A., Saint-Prex, Switzerland) and amplifier (model AP641G, Nihon Kohden). The capsule of the renal cortex was removed, and a small slice of the renal surface was removed (maximum depth 0.5 mm) using a scalpel. The tip of the pencil-probe CCD video microscope was then guided to the bottom of the excision. Superficial glomeruli in which afferent and efferent arterioles could be confirmed and blood flow was not influenced by surgical insult were used in the experiment. One glomerulus per left kidney, in which the entire protocol could be completed, was monitored.

The image at each measurement point was captured as a stack of 60 frames (2 s) using an image capture board (LG-3, Scion Computer Service, Frederick, Maryland, USA) installed in the image analysis computer. Clear frames not influenced by respiration and heartbeat were selected from the 60 frames in the captured stacks and analysed. To measure afferent and efferent diameters, image software (Scion Corporation, Frederick, Maryland, USA) was used. Diameters were measured after calibration of the number of pixels with a microscope calibration glass.

Dose-response curves to Ang II were constructed by intravenous infusion of doses of 3, 10, and 30 ng/kg/min (10 min each). In other rats, responses to increasing doses of Ang II were measured, after stabilisation (10 min) on intravenous Ang(1-7) administration (300 ng/kg/min). Beside afferent and efferent arteriole diameter, mean arterial blood pressure (MAP), heart rate (HR), and RBF values were recorded at each measurement point.

Renal blood flow in freely moving rats

Rats were anaesthetised with pentobarbital (60 mg/kg), and the abdomen was opened by mid-line incision. The left renal artery was dissected and an electromagnetic flow probe (type P0.7, Skalar Medical, Delft, The Netherlands) was placed around the left renal artery. A catheter was placed in the jugular vein to allow intravenous infusion of angiotensins (in 5% glucose, 3 ml/h). Both the wire of the probe and the catheter were tunnelled subcutaneously and attached to the skull of the animal using stainless steel screws and dental cement. After recovery, the flow probe was connected to a sensor adapter (MDL 450, Skalar Medical), which was coupled to a velocity meter (MDL 401, Skalar Medical). The effect of Ang II (1, 2, 5, 10, 15, and 30 ng/kg/min) on RBF was studied. After Ang II, Ang(1-7) was administered (333 or 667 ng/kg/min). Subsequently, the RBF response to Ang II was measured in the presence of Ang(1-7).

In a second series of experiments, Ang II was infused in one dose (10 ng/kg/min). This was repeated after stabilisation on intravenous infusion of Ang(1-7) in doses of 133, 333, and 667 ng/kg/min in the same animal on the same day, which allowed each animal to serve as its own control.

Statistical analysis

Data are expressed as mean ± SEM. Dose-response curves are compared for statistical differences by general linear model analysis of variance. For other comparisons a paired-samples *t*-test was performed. Differences were considered significant at p-value less than 0.05.

Results

Isolated small renal arteries

Ang(1-7) alone had no effect on the diameter of isolated small renal arteries (data not shown). In the presence of N^G-monomethyl-L-arginine and lisinopril, Ang II dose-dependently contracted these arteries, reaching its maximum effect at 10^{-6} M with a maximum effect (E_{max}) amounting to 9.71±1.21% of the

initial baseline diameter. This response was completely blocked by additional incubation of the vessels with Ang(1-7) (10^{-5} M) in the whole concentration range (E_{max} = 3.20±0.57%, p<0.0005) (Fig. 1).

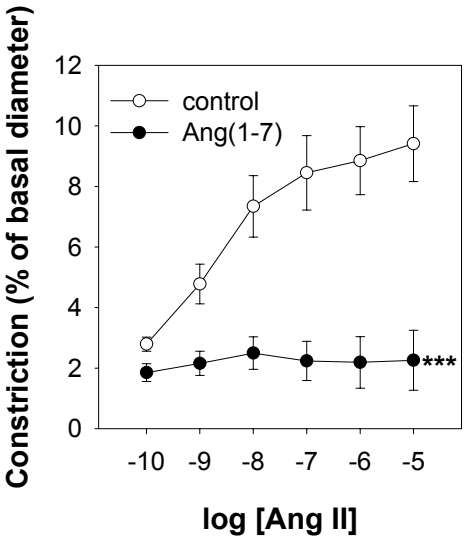

Figure 1. *Ang(1-7) inhibits the vasoconstrictor response to Ang II in isolated small interlobar renal arteries.* Ang II-induced constriction of small renal interlobar arteries (n=6) in the presence of L-NMMA and lisinopril. Ang(1-7) (10^{-5} M) completely blunted the constrictive response to Ang II (***p<0.0005).

Isolated perfused kidneys

Ang(1-7) alone did not affect renal vascular resistance (RVR) in isolated perfused kidneys (data not shown). Ang II dose-dependently increases RVR, with maximum values after injection of 10^{-5} M and reaching a half-maximal response at a negative log-concentration (pEC_{50}) of 6.69±0.19 (Fig. 2). In the presence of Ang(1-7) (10^{-5} M), the maximal response to Ang II was reduced (100±16.6 and 72.6±15.6% for control and Ang(1-7), respectively; p<0.05) and the dose-response curve of Ang II was significantly shifted rightward (pEC_{50} 6.26±0.12, p<0.05) (Fig. 2).

Intravital microscopic analysis of afferent and efferent arterioles

To extend these *in vitro* findings, we performed similar experiments in the whole rat, evaluating glomerular arteriolar diameter using intravital microscopy under anaesthesia. Ang(1-7) alone had no effect on afferent and efferent arteriolar diameter, and on the MAP and RBF (data not shown). Glomerular afferent and efferent arterioles showed a dose-dependent constriction in response to systemic Ang II infusion (3-30 ng/kg/min; Fig. 3A, B). The response to Ang II in the afferent arteriole was unaffected by Ang(1-7) infusion (Fig. 3A). In efferent arterioles, Ang(1-7) (300 ng/kg/min) diminished Ang II-induced vasoconstriction, although this difference was not statistically significant (Fig. 3B). While

infusion with Ang II alone did not significantly affect the MAP, a significant increase in MAP by Ang II was observed in the presence of Ang(1-7) ($p<0.005$). Ang II dose-response curves of the Ang II effect on MAP in the absence and presence of Ang(1-7) were still not significantly different ($p=0.509$) (Fig. 3C). In addition, Ang II strongly reduced RBF. Ang(1-7) did not attenuate the Ang II-mediated reduction in RBF (Fig. 3D).

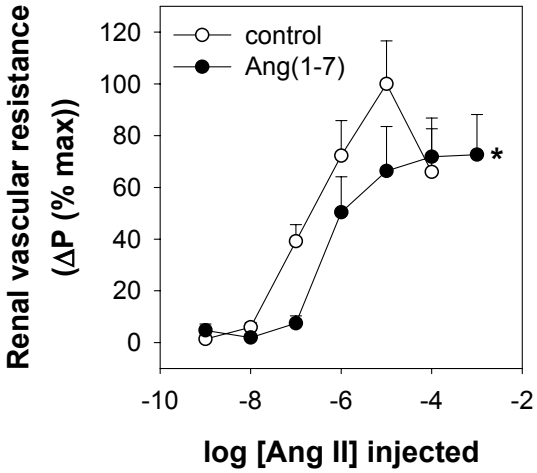

Figure 2. *Ang(1-7) inhibits the Ang II-induced increase in renal vascular resistance in isolated perfused kidneys.* Dose-dependent increase in renal vascular resistance by Ang II in isolated perfused kidney setup (n=4). Pre-incubation of the kidneys with Ang(1-7) (10^{-5} M) results in a rightward shift of the Ang II dose-response curve and depression of the maximum response to Ang II. *$p<0.05$

Renal blood flow in freely moving rats

Ang(1-7) alone had no effect on the baseline RBF (data not shown). Ang II reduced renal blood flow in freely moving rats. Ang(1-7) did not inhibit the RBF response to Ang II in doses of 333 and 667 ng/kg/min (Fig. 4A). Because of the large interindividual variability in response to Ang II, a second flow experiment was designed in which each animal served as its own control. Figure 4B shows that the renal blood flow is equally reduced by Ang II either in the absence or presence of Ang(1-7) (133, 333 and 667 ng/kg/min).

Discussion

The present study shows that Ang(1-7) alone has no effect on baseline renal vasomotor tone in any of the *in vitro* and *in vivo* experimental setups used. Second, Ang(1-7) attenuates the response to Ang II in isolated small renal arteries and IPK *in vitro*. No significant effects of Ang(1-7), however, were found on afferent and efferent arteriolar responses to Ang II in anaesthetised rats. Moreover, in freely moving rats the RBF reduction in response tot Ang II did also not change upon infusion of Ang(1-7).

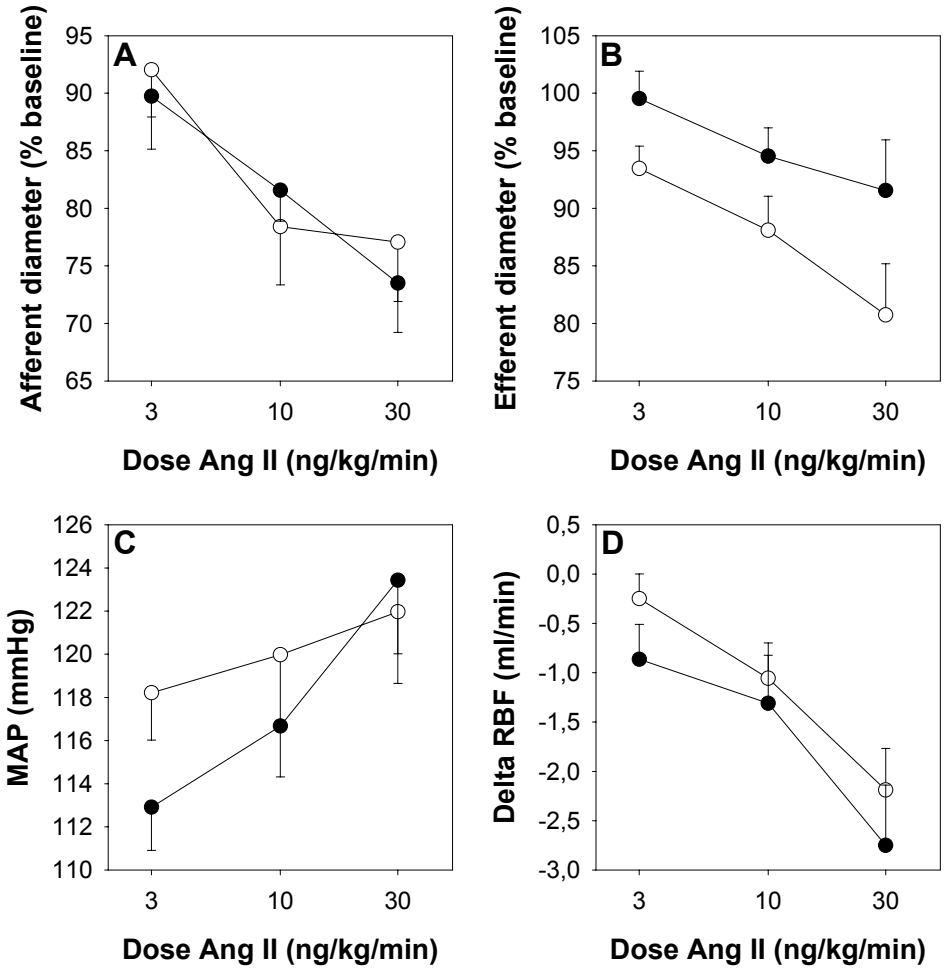

Figure 3. *Ang(1-7) does not attenuate the vasoconstrictive response to Ang II in afferent and efferent arterioles, and renal blood flow in vivo under anaesthesia.* Intravital microscopy experiment with dose-response curves for Ang II with (●) and without (○) Ang(1-7) infusion (300 ng/kg/min). Ang II dose-response curves are shown for afferent arterioles (A), efferent arterioles (B), mean arterial pressure (MAP) (C), and renal blood flow (RBF) (D). Ang II did not affect MAP, but dose-dependently reduced afferent and efferent arteriole diameter, and RBF. Ang(1-7) did not significantly affect the response of Ang II on afferent and efferent arteriole, and RBF.

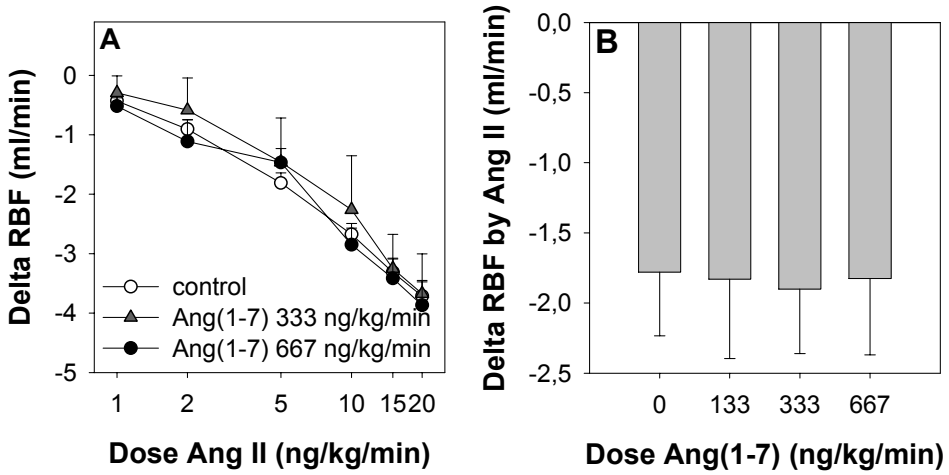

Figure 4. *Ang(1-7) does not modulate the effect of Ang II on renal blood flow in freely moving rats.* Intravenous infusion of Ang II reduced renal blood flow (RBF) (n=14). Ang(1-7) doses of 333 (n=4) and 667 ng/kg/min (n=6) could not inhibit the RBF reduction by Ang II (A). In additional experiments (n=5), Ang(1-7) in doses of 133, 333, and 667 ng/kg/min, did not antagonise the RBF response to Ang II in the same rat (B).

The finding that Ang(1-7) blocks *in vitro* constriction of renal vessels by Ang II, but has no important role in the regulation of *in vivo* RBF in normotensive rats is in agreement with other studies. Indeed, evidence for an antagonistic effect of Ang(1-7) on Ang II-induced renal vasoconstriction is achieved *in vitro*[6;20], whereas the effect of Ang II on RBF could not be blocked by Ang(1-7) *in vivo*[18].

Intriguingly, the finding on the effect of Ang(1-7) thus differs between different experimental designs. Several factors may contribute to the discrepancy between these different experimental protocols. First, the pharmacokinetics of Ang(1-7) may be important. Due to rapid degradation by ACE, Ang(1-7) has a plasma half-life of only 10 seconds in rats[31]. Despite this unfavourable pharmacokinetic property, we previously found increased plasma Ang(1-7) levels after infusion of similar doses of Ang(1-7)[14;32]. Such Ang(1-7) plasma levels, however, were considerably lower (nM range) than the concentrations used in the *in vitro* experiments (µM range). One could speculate that not plasma levels but renal tissue levels of Ang(1-7) are important for its response. Because we found no effects of Ang(1-7) *in vivo*, this would implicate that, in this study, renal levels of Ang(1-7) were insufficiently increased by intravenous infusion. Consequently, our data implicate that intravenous administration raises local concentration of Ang(1-7) insufficiently to inhibit renal ACE or antagonise AT_1R, which matches previous observations demonstrating that the effects of systemic Ang(1-7) on kidney are mediated by the Mas receptor[19;26].

A second reason for our discrepant results may be that responses were measured at different anatomical levels of the renal vasculature, each with its specific role in the regulation of renal vascular

tone. Small renal interlobar arteries are preglomerular vessels that may be contributing to the total RVR only to a limited extent. In IPK both preglomerular and postglomerular vasoconstriction determine the overall RVR; but the majority of the RVR is determined by preglomerular vasoconstriction, in particular by constriction of afferent arterioles, which contribute to 50% of preglomerular resistance[33]. Therefore, although both interlobar arteries and IPK assess (mainly) preglomerular vessels, RVR is probably more closely represented in IPK. In contrast, RBF is mainly determined by efferent vasoconstriction, although afferent arteriolar diameter also contributes to a limited extent. An Ang II-antagonising effect of Ang(1-7) in interlobar arteries and IPK is therefore not necessarily in conflict with the absence of such an effect of Ang(1-7) on RBF. Our intravital microscopy data do not, however, match the IPK experiments as an Ang II-antagonising effect of Ang(1-7) on afferent vessels was not confirmed. This could be explained if the Ang II-antagonising effect of Ang(1-7) in IPK results from effects on larger preglomerular vessels.

The different effects of Ang(1-7) in the different renal vascular beds may be the result of different levels of angiotensin receptors in the larger preglomerular arteries, afferent arterioles, and efferent arterioles. Our data may indicate that in larger preglomerular vessels either Mas receptors or AT_2 receptors are present, while they are absent in afferent and efferent arterioles. In IPK, however, the Ang II-antagonising effect of Ang(1-7) was previously shown to be mediated by an antagonistic effect on AT_1R[5]. Furthermore, there are currently no data about the exact localisation of Mas receptors within the kidney, but their presence is likely in rabbit afferent arterioles[20]. For AT_2R, it is known that they are present in larger vessels[34] and in both afferent[35] and efferent arterioles[36] of the rat. Therefore it is unlikely that the different Ang(1-7) effects are resulting from different receptor distributions in the renal vascular beds. It consequently seems more obvious that the effects of Ang(1-7) as observed *in vitro* are counterbalanced *in vivo*.

Another confounding factor in establishing the effects of Ang(1-7) may be the application of anaesthesia. Anaesthesia is known to have major influences on haemodynamics, including reduction of blood pressure and peripheral resistance. Although thiobutabarbital is an anaesthetic with minimal effects on haemodynamics[37], the glomerular filtration rate and RBF are reduced during thiobutabarbital anaesthesia, implying that renal vasomotor tone is affected by this anaesthetic[38]. In this study, therefore, the effects of Ang(1-7) were measured both under anaesthesia and in freely moving rats; however, we found no effect of Ang(1-7) on Ang II-reduced RBF even without anaesthesia, indicating that efferent Ang II-antagonising effects of Ang(1-7) are absent *in vivo* and not blunted by narcosis.

Several other factors may be contributing to the discrepant effects of Ang(1-7) *in vitro* and *in vivo*, including innervation, circulating neurohumoral substances and blood pressure regulation. Innervation may play an important role as the renal vasculature is highly innervated[39], and it is known to be important in the regulation of renal vascular tonus, in particular for afferent and efferent arterioles[40]. Consequently, the renal vasculature is less preconstricted in IPK than when it is left *in situ*, which may have major influences on the effects of Ang II and Ang(1-7). Furthermore, circulating neurohumoral substances such as NO and prostaglandins may be increased *in vivo* by Ang II alone[41], which may result in absence of an additional Ang(1-7) effects *in vivo,* as an effect of Ang(1-7) is mediated also by

NO and prostaglandins. In addition, the increase in blood pressure by Ang II *in vivo* may increase renal vascular tone and consequently mask potential effects of Ang(1-7).

Together, these data suggest that Ang(1-7) blocks Ang II mediated renal vasoconstriction *in vitro* on preglomerular vessels, but does not counterbalance Ang II in regulating renal afferent and efferent vasomotor tone *in vivo*. Detailed study on the distribution of Mas receptor in the renal vasculature is pivotal to expand our understanding of this phenomenon.

In conclusion, Ang(1-7) is antagonist of Ang II in isolated small renal arteries and IPK. Ang(1-7) does not, however, appear to have a major function in the normal physiological regulation of renal vascular constriction *in vivo*. Further studies are needed to determine the role of Ang(1-7) in renal pathophysiology.

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