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Angiotensin-(1–7)-dependent vasorelaxation of the renal artery exhibits unique angiotensin and bradykinin receptor selectivity

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ABSTRACT

Angiotensin-(1–7) [Ang-(1–7)] exhibits blood pressure lowering actions, inhibits cell growth, and reduces tissue inflammation and fibrosis which may functionally antagonize an activated Ang II-AT_1 receptor axis. Since the vascular actions of Ang-(1–7) and the associated receptor/signaling pathways vary in different vascular beds, the current study established the vasorelaxant properties of the heptapeptide in the renal artery of male Wistar male rats. Ang-(1–7) produced an endothelium-dependent vasodilator relaxation of isolated renal artery segments pre-contracted by a sub-maximal concentration of phenylephrine (PE) (3×10^{-7} M). Ang-(1–7) induced vasodilation of the rat renal artery with an ED_{50} of 3 ± 1 nM and a maximal response of $42 \pm 5\%$ (N = 10). The two antagonists (10^{-5} M each) for the AT_7/Mas receptor (MasR) [D-Pro^7]-Ang-(1–7) and [D-Ala^7]-Ang-(1–7) significantly reduced the maximal response to $12 \pm 1\%$ and $18 \pm 3\%$, respectively. Surprisingly, the AT_2R receptor antagonist PD123319, the AT_1R antagonist losartan and B_2R antagonist HOE140 (10^{-6} M each) also significantly reduced Ang-(1–7)-induced relaxation to $12 \pm 2\%$, $22 \pm 3\%$ and $14 \pm 7\%$, respectively. Removal of the endothelium or addition of the soluble guanylate cyclase (sGC) inhibitor ODQ (10^{-5} M) essentially abolished the vasorelaxant response to Ang-(1–7) ($10 \pm 4\%$ and $10 \pm 2\%$, $P < 0.05$). Finally, the NOS inhibitor LNAME (10^{-4} M) reduced the response to $13 \pm 2\%$ ($p < 0.05$), but the cyclooxygenase inhibitor indomethacin failed to block the Ang-(1–7) response. We conclude that Ang-(1–7) exhibits potent vasorelaxant actions in the isolated renal artery that are dependent on an intact endothelium and the apparent stimulation of a NO-sGC pathway. Moreover, Ang-(1–7)-dependent vasorelaxation was sensitive to antagonists against the AT_7/Mas, AT_1, AT_2 and B_2 receptor subtypes.

1. Introduction

Angiotensin-(1–7) [Ang-(1–7)] is an alternative peptide product of the renin-angiotensin-aldosterone system (RAAS) present in the circulation and various tissues including the kidney, brain and heart [8,28]. In contrast to Ang II, Ang-(1–7) exhibits blood pressure lowering actions and beneficial effects on end organ damage such as reduced fibrosis and inflammation [7,13,29]. Ang-(1–7) is formed from either Ang I by several endopeptidases (neprilysin, thimet oligopeptidase) or from Ang II by carboxypeptidases that include angiotensin-converting enzyme 2 (ACE2) and prolyl carboxypeptidase (9). Similar to bradykinin, angiotensin converting enzyme 1 (ACE) metabolizes Ang-(1–7) resulting in the formation of Ang-(1–5) and the dipeptide His-Leu [9]. Indeed, the administration of ACE inhibitors contributes to increased circulating levels of Ang-(1–7) that likely reflect both the reduced metabolism of the peptide and the processing of Ang I by neprilysin instead of ACE [7]. The cardiovascular actions of Ang-(1–7) are mediated predominantly through the G protein-coupled receptor Mas and involve the release of vasodilatory prostaglandins and formation of nitric oxide (NO) [28]. Moreover, chronic blockade of the Ang-(1–7) receptor with the selective antagonist [D-Ala^7]-Ang-(1–7) (A779) partially reverses the cardiovascular effects of either ACE inhibitors or AT_1 receptor antagonists [12]. Importantly, these data support the concept that at least part of the therapeutic effects of RAS blockade may be mediated through activation of the Ang-(1–7)-AT_7/Mas receptor axis.

Within the kidney, the beneficial effects of Ang-(1–7) include both hemodynamic and tubular actions [12]. Acute infusion of Ang-(1–7) generally increases glomerular filtration (GFR) and augments renal sodium excretion. In contrast to Ang II, the increase in GFR by Ang-(1–7) likely reflects the vasorelaxant properties of the peptide.

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In higher species including dog and sheep, administration of Ang-(1–7) increases renal blood flow [15,32]. In this regard, the vascular response to Ang-(1–7) was absent in adult male sheep exposed to glucocorticoids during fetal development and the lack of a renal Ang-(1–7) response may contribute to the sustained elevation in blood pressure in this ovine model of fetal programming [12]. In pre-constricted afferent arterioles of the rabbit kidney, Ang-(1–7) induces vasodilation that is dependent on the release of NO [24]. The vasorelaxant effects of Ang-(1–7) within the kidney are consistent with the actions of the peptide on other vascular beds and the ability of the peptide to stimulate NO [6,14,26,37]. However, there is relatively little data on the vascular actions of Ang-(1–7) on isolated renal vessels. Therefore, the present study characterized the vascular responses of Ang-(1–7) in the renal artery of the normotensive Wistar rat, the major conduit vessel of the kidney, as well as the influence of various angiotensin receptor antagonists and signaling inhibitors to better define the Ang-(1–7) response in the renal vessel.

2. Materials and methods

2.1. Renal artery preparation

Male Wistar rats (age 12–15 weeks) were euthanized by decapitation under light ether anesthesia. The renal artery was isolated carefully and transferred into a Petri dish containing oxygenated Krebs' solution. The vessel was cut into ring segments of about 5 mm. The ring segments were mounted in baths containing 50 ml Krebs-Henseleit (KH) solution at pH 7.4. The composition of KH-solution is as follows (mM): NaCl (118.3), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1.2), $NaHCO_3$ (25), KH_2PO_4 (1.2) and glucose (11.2). The tissue bath solution was maintained at 37°C and was aerated with 95% O_2/5%CO_2. Changes in vascular reactivity of the renal arteries were recorded by measurement of changes in the isometric tension to vasoactive agonists using computerized Automatic organ bath LSI Letica Scientific Instruments (Powerlab/8sp ADIinsturments, Panlab, Spain). A pre-tension of 0.5 g was applied and the preparations were allowed to stabilize (45 min) until a stable baseline tone was obtained. The integrity of the endothelial layer was tested before every experiment by testing the vasodilator response to carbachol (10^{-7} M). All studies involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication number 85–23, Revised in 1985) as approved by Kuwait University Research Administration.

2.2. Ang-(1–7) responsiveness

Following the period of equilibration, the isolated renal artery segments were pre-contracted by a sub-maximal concentration of PE (3×10^{-7} M) and a cumulative concentration response curve for Ang-(1–7) (10^{-12} to 3×10^{-6} M) was established. The response to any given concentration was left to stabilize before adding the next drug concentration. The relaxant responses were expressed as the percent reduction (%) of the tension induced by PE. Sigmoidal dose-response curves were established by nonlinear regression of the log dose of Ang-(1–7) and the percentage (%) of the vasorelaxation response (3 parameter) using a least squares fit for each experiment with GraphPad Prism 6.0 (GraphPad Software, San Diego, CA). Based on the constructed dose-response curves, the ED_{50} (concentration to reduce tone by 50%) was determined for Ang-(1–7) alone. The maximal vasorelaxation response (%) was determined for Ang-(1–7) alone or in the presence of various receptor antagonists or enzyme inhibitors.

2.3. Effect of angiotensin-receptor blockers

The effect of pre-incubation of the vessels with [sarcosine^1, threonine^8]-Ang II (Sarthran, 10^{-5} M); losartan (10^{-6} M); PD123319 (10^{-6} M); [D-Alanine^7]-Ang-(1–7) (DALA, 10^{-5} M); [D-Proline^7]-Ang-(1–7) (DPRO, 10^{-5} M) on the vasodilator response to Ang-(1–7) was established. The vasodilator effect to Ang-(1–7) was determined by pre-contracting the tissues with PE (3×10^{-7} M) added to the organ baths. After obtaining a steady level of contraction, the relaxant effect to Ang-(1–7) from 10^{-12} to 3×10^{-6} M was assessed. The tissues were then incubated for 20 min in KH solution containing the antagonists and the vasodilator effect to Ang-(1–7) was then re-assessed. The effect of the different antagonists was investigated using different tissue segments.

2.4. Effect of inhibition of nitric oxide synthase, soluble guanylate cyclase and cyclooxygenase blockade

The effect of nitro-L-arginine methyl ester (LNAME) (10^{-4} M), an inhibitor of nitric oxide synthase, indomethacin (10^{-6} M), a combination of LNAME (10^{-4} M) and indomethacin (10^{-6} M) or 1H-[1,2,4] oxadiazolo-quinoxalin (ODQ) (10^{-5} M), an inhibitor of soluble guanylate cyclase, on the vasodilator response induced by Ang-(1–7) was also established in the renal artery preparation. Note that the dose response range for Ang-(1–7) for these agents was 10^{-12} to 10^{-6} M. We also assessed the effect of endothelium removal on the Ang-(1–7) response (10^{-12} to 3×10^{-6} M). Denuded vessels were prepared by gentle rubbing of the endothelial layer which was confirmed by loss of the vasodilator response to carbachol (10^{-7} M). The vasodilator effect to Ang-(1–7) was determined by pre-contracting the tissues with PE (3×10^{-7} M) added to the organ baths. The contribution of the bradykinin pathway was addressed by addition of the bradykinin B_2 receptor antagonist HOE140 (5×10^{-8} M) and combined HOE and LNAME (10^{-4} M and 3×10^{-8} M, respectively) on Ang-(1–7)-induced vasorelaxation.

2.5. Effect of potassium channel inhibitors

The effects on Ang-(1–7) dependent vasorelaxation of the potassium (K^+) channel blockers glibenclamide (10^{-5} M) to inhibit ATP-sensitive K^+ channels and iberiotoxin (5×10^{-8} M) to block calcium-activated K^+ (BK) channels were also investigated in this study.

2.6. AT_2 receptor binding

The assessment of the various angiotensin antagonists on AT_2 receptor binding using the non-selective radioligand ^{125}I-[Sarcosine^1, Threonine^8]-Ang II (Sarthran) or ^{125}I-Ang-(1–7) was performed in isolated membranes from the rat renal artery and the pancreatic AR42J cell line (ATTC, Manassas, VA) as previously described [11]. In brief, 50 μg of the cell membrane preparation was incubated with 0.2 nM of the radioligand with or without the antagonists in 20 mM HEPES buffer pH 7.4 containing 125 mM NaCl, 5 mM $MgCl_2$ and 2.5 mM EGTA, 0.2% BSA and a cocktail of inhibitors [22]. The binding reaction was terminated by addition of ice cold buffer and centrifugation in a high-speed microfuge at 20,000 xg. The resultant pellet was washed, centrifuged again and the pellet counted in a γ-counter. The ^{125}I-labeled peptides were generated by chloramine T and purified by HPLC [11]. Competition curves were constructed in Prism 6 by non-linear regression using a one-site fit to determine the IC_{50} values [11].