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Effects of angiotensin 1-7 on the actions of angiotensin II in the renal and mesenteric vasculature of hypertensive and streptozotocin-induced diabetic rats

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Abstract

Angiotensin 1-7, a heptapeptide derived from metabolism of either angiotensin I or angiotensin II, is a biologically active peptide of the renin–angiotensin system. The present study investigated the effect of angiotensin 1-7 on the vasopressor action of angiotensin II in the renal and mesenteric vasculature of Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR) and streptozotocin-induced diabetic rats. Angiotensin II-induced dose-dependent vasoconstrictions in the renal vasculature. The pressor response was enhanced in the SHR and reduced in the streptozotocin-diabetic rat compared to WKY rats. Angiotensin 1-7 attenuated the angiotensin II pressor responses in the renal vasculature of WKY and SHR rats. However, the ability to reduce angiotensin II response was diminished in diabetic-induced rat kidneys. The effect of angiotensin 1-7 was not inhibited by 1-[(4-(Dimethylamino)-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid ditrifluoroacetate (PD123319), an angiotensin AT2 receptor antagonist. (D-ALA7)-Angiotensin I/II (1-7) (D-ALA) (an angiotensin 1-7 receptor antagonist), indomethacin (a cyclo-oxygenase inhibitor), and Nω-Nitro-L-Arginine Methyl Ester (L-NAME)(a nitric oxide synthetase inhibitor) abolished the attenuation by angiotensin 1-7 in both WKY rats and SHR, indicating that its action is mediated by angiotensin 1-7 receptor that is either coupled to the release of prostaglandins and/or nitric oxide. The vasopressor responses to angiotensin II in mesenteric vasculature bed was also dose-dependent but smaller in magnitude compared to the renal vasculature. The responses to angiotensin II were relatively smaller in SHR but no significant difference was observed between WKY and streptozotocin-induced diabetic rats. Angiotensin 1-7 attenuated the angiotensin II pressor responses in WKY, SHR and diabetic-induced mesenteric bed. The attenuation was observed at the lower concentrations of angiotensin II in WKY and diabetic-induced rats but at higher concentrations in SHR. Similar observation as in the renal vasculature was seen with PD123319, D-ALA, and L-NAME. Indomethacin reversed the attenuation by angiotensin 1-7 only in the SHR mesenteric vascular bed. The present findings support the regulatory role of angiotensin 1-7 in the renal and mesenteric vasculature, which is differentially altered in hypertension and diabetes.

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1. Introduction

Angiotensin II is believed to be the major effector peptide of the renin–angiotensin system (Ardaillou and Chansel, 1997). However, recent evidence shows that angiotensin II is not the only active peptide of renin–angiotensin system (Kucharewicz et al., 2002). Three endopeptidases that convert angiotensins I and II directly to angiotensin 1-7 have been reported (Ferrario et al., 1991; Kucharewicz et al., 2002). Angiotensin 1-7 was found to be present in central and peripheral tissues of rats, dogs and humans and is therefore thought to be an active component of renin–angiotensin system (Ferrario et al., 1991; Santos et al., 2000). The heptapeptide has been shown to activate several subtypes of angiotensin receptors in neural, endothelial, and vascular smooth muscle cell (VSMC) preparations and to exert biological actions that are both complementary to and distinct from those of angiotensin II...
(Ferrario et al., 1991). It acts as a vasodilating agent in many vascular beds (Ferrario et al., 1997), inhibits vascular smooth muscle growth (Freeman et al., 1996), and blocks the angiotensin II-induced vasoconstriction in rat aorta (Le Tran and Forster, 1997; Loot et al., 2005), and human arteries (Roks et al., 1999). Moreover, accumulating studies demonstrated that angiotensin 1-7 stimulates the synthesis of vasodilator prostaglandins (Ferrario et al., 1991; Jaiswal et al., 1993) and nitric oxide (Broshinan, 1998), and potentiates the hypotensive action of bradykinin (Santos et al., 2001). This explains why angiotensin 1-7 exerts antihypertensive actions (Chappell et al., 1998; Ferrario, 1998), particularly in situations of increased angiotensin II activity. Angiotensin 1-7 has been demonstrated to act through its putative angiotensin 1-7 receptor or via other known angiotensin receptors. Although the angiotensin 1-7 receptor has not been cloned, its existence cannot be entirely excluded, since some of its effects could be inhibited by the angiotensin 1-7 receptor antagonist, D-ALA (Ambuhl et al., 1994; Santos et al., 2000, 2001; Vallon et al., 1998). Other studies have raised the possibility that angiotensin 1-7 may act as an endogenous antagonist of the AT1 receptor or it may modulate angiotensin II via AT1 receptor (Clark et al., 2001b; Mohan et al., 1994). With regard to these possibilities, the present study investigated the effects of angiotensin 1-7 on the angiotensin II-induced vasoconstriction in the renal and mesenteric vasculature of normal, hypertensive and experimentally-induced diabetic rats.

2. Material and methods

2.1. Animals

Male Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs) weighing 250–300 g (11–12 weeks) were obtained from the Animal House in the University of Malaya Medical Centre. Approval for the following studies was obtained from the Animal Care and Use Committee at the Laboratory Animal Center of Faculty of Medicine in the
University of Malaya and procedures were carried out according to the guidelines for ethical care of experimental animals. The animals were fed standard rat chow and tap water ad libitum.

2.2. Induction of diabetes

WKY rats were made diabetic by administration of streptozotocin 75 mg/kg intraperitoneally. Age matched controls received equal volume. Prior to injection, animal weights and blood glucose levels were recorded. Body weights and blood glucose were taken every 2 weeks until the 8-week. Animals were considered diabetic if their blood glucose concentration was >17 mM.

2.3. Isolated kidney and mesenteric vascular bed

Rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and the renal and mesenteric vascular beds were prepared as described previously (Dharmani et al., 2005). Briefly, the right kidney was exposed by midline laparotomy and the renal artery was cannulated with a catheter (PET-50) via the superior mesenteric artery. Perfusion was started in situ and the right renal vein and ureter were cut. The right kidney was excised and placed in a water-jacketed chamber maintained at 37 °C and perfused with an oxygenated Kreb’s solution by means of a peristaltic pump (Minipuls 3 Model 312, Gilson Villiers Le Bel, France) at a rate of 5 ml/min. For isolation of the mesenteric arterial bed, the remaining length of the superior mesentery artery was cannulated with a catheter. The mesentery was carefully excised from the intestine and placed in a water-jacketed chamber maintained at 37 °C and perfused with an oxygenated Kreb’s solution by means of a peristaltic pump at a rate of 5 ml/min. The composition of the Kreb’s solution was as follows (mmol/L): NaCl, 120; KCl, 4.7; CaCl₂, 2.4; MgCl₂, 1.2; NaHCO₃, 20; KH₂PO₄, 1; EDTA, 0.06; and glucose, 10).
Changes in perfusion pressure were measured by means of a pressure transducer (Model P23XL, Ohmeda Medical Devices Division Inc, USA) and recorded via a MacLab data acquisition system (AD Instruments, Australia).

2.4. Experimental protocol

After an equilibration period of 20 min, the preparation was preconstricted with phenylephrine (PE, $10^{-5}$ M) and the increase in perfusion pressure was recorded until a 5-min plateau was observed. This contractile response to phenylephrine was taken as a unity and responses to other pressor compounds were normalized against this unit. Following 1 h of perfusion, various concentrations ($10^{-13}$ – $10^{-6}$ M) of either angiotensin II or angiotensin 1-7 were used to produce a concentration response. The angiotensins were administered as a single bolus injection of 20 μl (renal) and 50 μl (mesenteric bed) into the perfusion system. The minimum time interval between successive bolus injections was 10 min or the time till the basal pressure was again recorded.

The effects of various concentrations ($10^{-15}$ – $10^{-7}$ M) of angiotensin 1-7 on the response to angiotensin II were studied with the following protocol. The preparation was first perfused with Kreb’s solution containing a concentration of angiotensin 1-7 for 30 min, prior to initiating a concentration response to angiotensin II. Each concentration of angiotensin 1-7 was studied using a different preparation. The same protocol was used to study the direct effect of indomethacin ($10^{-7}$ M), PD123319 ($10^{-5}$ M), L-NAME ($10^{-4}$ M) and D-ALA ($10^{-5}$ M) on the concentration response to angiotensin II. The effects of PD123319, indomethacin, L-NAME and D-ALA on the actions of angiotensin 1-7 on the concentration responses to angiotensin II were studied by perfusing the preparation with a effective concentration of angiotensin 1-7 and PD123319 (or indomethacin, L-NAME, D-ALA) for 30 min prior to bolus injection of angiotensin II.

2.5. Drugs

Angiotensin II, angiotensin 1-7, L-NAME and indomethacin were purchased from Sigma. D-ALA was purchased from BACHEM AC, Bubendorf, Switzerland. PD123319 was a generous gift from Parke-Davis Pharmaceutical Research, Michigan, USA.

2.6. Statistical analysis

Data are presented as mean±S.E.M. Significant difference ($p<0.05$) between means was evaluated using Student’s $t$-test when comparing two groups. When more than two groups were compared and for the comparison of the dose-response curves, data were evaluated by two-factor analysis of variance (ANOVA) followed by Bonferroni post hoc test. Results with $p<0.05$ were considered statistically significant.
3. Results

SHR rats in the experiments were age matched and have higher blood pressure than the control (173 ± 4 mm Hg vs. 124 ± 2 mm Hg). The streptozotocin-induced diabetic rats have a significantly higher blood glucose level than WKY rats (25 ± 5 mM vs. 5 ± 3 mM).

3.1. Renal vasculature

10⁻⁵ M PE caused an average increase perfusion pressure of 250 ± 20 mm Hg in SHR and 210 ± 25 mm Hg in WKY and diabetic-induced rats. The limit of detection of the system was 350 ± 50 mm Hg. Bolus injections of angiotensin 1-7 did not induce contraction in all the three groups of animals. We have reported previously that the responses to the five increasing doses of angiotensin II in SHR were significantly greater than WKY rats (p < 0.05) whilst the responses in the diabetic-induced rats were significantly lower than those in the WKY rats (Dharmani et al., 2005). No tachyphylaxis was observed with subsequent bolus addition of angiotensin II.

The contractile responses to angiotensin II were attenuated by angiotensin 1-7 (10⁻⁷ M) in both WKY and SHR rats. The attenuation was observed at the higher concentrations of angiotensin II (10⁻⁹ M–10⁻⁶ M) in SHR and from 10⁻¹⁰ M for WKY rats (Fig. 1). Angiotensin 1-7 did not affect the angiotensin II-induced vasoconstriction in streptozotocin-induced diabetic rats. The action of angiotensin 1-7 was not affected by PD123319 in WKY rats (Fig. 2A) and SHR (Fig. 3A). Indomethacin, L-NAME and D-ALA reversed the vasodepressor action of angiotensin 1-7 in WKY rats (Fig. 2) and SHR (Fig. 3).

3.2. Mesenteric vasculature

10⁻⁵ M PE caused an average increase in perfusion pressure of 100 ± 25 mm Hg in all the three groups. As reported earlier (Dharmani et al., 2005), this was much smaller than the response recorded with the renal vasculature. Angiotensin 1-7 did not induce vasopressor responses in all the three groups of animals. In contrast to responses seen in the kidney, angiotensin 1-7 attenuated the pressor responses to angiotensin II in mesenteric vascular bed of streptozotocin-induced diabetic rats. For the SHR, the attenuation was observed only at the higher concentrations of angiotensin II (10⁻⁷ M–10⁻⁶ M) (Fig. 4). PD123319 did not affect the action of angiotensin 1-7 in WKY rats, SHR and streptozotocin-induced diabetic rats (Figs. 5A, 6A and 7A respectively). L-NAME and D-ALA also reduced the attenuation by angiotensin 1-7 in all the three groups (Figs. 5, 6 and 7C). Pre-incubation with indomethacin reduced the vasodepressor actions of angiotensin 1-7 only in hypertensive animals.

4. Discussion

4.1. Renal vasculature

The present study demonstrated that angiotensin 1-7 significantly attenuated angiotensin II-induced vasoconstrictions in both the WKY rats and SHR. Stegbauer et al. (2005) and van der Wouden et al. (2006) have also demonstrated similar vasodepressor action in WKY rats with a higher concentration of the heptapeptide (10⁻⁵ M). In the SHR, the attenuation was observed at higher concentrations of angiotensin II (10⁻⁹ M–10⁻⁶ M). This action supports the reported antihypertensive actions of angiotensin 1-7 in human (Roks et al., 1999; Ueda et al., 2000). Interestingly, Mohan et al. (1994) have shown that angiotensin 1-7 attenuates the pressor response of angiotensin II in rabbit aortic rings, and in anesthetized cats. Because this effect was observed specifically with angiotensin II (and not with other vasoconstrictors) and was blocked by losartan, the authors suggested that angiotensin 1-7 modulates the effect of angiotensin II via the angiotensin AT₁ receptor. Micromolar concentration of angiotensin 1-7 caused a modest downregulation of the angiotensin AT₁ receptors in Chinese hamster ovary cells stably transfected with the angiotensin AT₁A receptor (Clark et al., 2001a) and in kidney slices from SD rats (Clark et al., 2003). Angiotensin 1-7 has also been proposed to bind to and activate angiotensin AT₁ receptor, which results in receptor internalization without coupling to G-proteins and activation of phospholipase C (Ueda et al., 2000). The downregulation and internalization of angiotensin II receptor by angiotensin 1-7 could be a possible cause of the observed reduction in pressor response of the
octapeptide in the present study. In addition, angiotensin 1-7 has been demonstrated to reduce angiotensin II-induced phosphorylation of protein kinase C-ζ and extracellular signal-regulated kinase (ERK) 1/2 (Zhu et al., 2002). The ability of angiotensin 1-7 to modulate the mechanisms of action of angiotensin II at both the receptor and cellular levels suggests that the heptapeptide plays important regulatory roles in the vascular system.

In the present study, angiotensin 1-7 significantly reduced the response to angiotensin II at a dose of $10^{-7}$ M, i.e. a concentration significantly lower than the IC$_{50}$ of the heptapeptide for the angiotensin AT$_1$ receptor (>1 μM) (Ueda et al., 2000). The finding implies that angiotensin 1-7 may act via non-AT$_1$ receptor. Similar magnitude of angiotensin 1-7 actions was observed in the WKY rats and SHR. In contrast, Kost et al. (1998) and Stegbauer et al. (2004) found the action of angiotensin 1-7 to be more potent in the SHR. Differences in periods of exposure to angiotensin 1-7 and protocol of drug administration (bolus injection) may have caused the observed differences. In the streptozotocin-induced diabetic rats, angiotensin 1-7 had no effect on the pressor response to angiotensin II. Reduced sensitivity or changes in angiotensin 1-7 receptor may have lead to the lost of its vasodepressor action.

PD123319 was without effect on the vasodepressor actions of angiotensin 1-7 in WKY rats and SHR, indicating that the angiotensin AT$_2$ angiotensin receptor was not involved. Recent studies have shown that mRNA expression of angiotensin AT$_2$ receptor was not changed in VSMCs pretreated with angiotensin 1-7 (Zhu et al., 2002). The heptapeptide has also been shown to bind to type 1 angiotensin II receptor not type 2, which is predominantly present in the rat renal cortex (Gironacci et al., 1999). Angiotensin 1-7 receptor has been postulated to exert its effect on its own functional receptors, which are distinct from angiotensin AT$_1$ and AT$_2$ receptors (Santos et al., 1994; Tallant et al., 1997).

Recently, the G-protein coupled receptor, Mas, was characterized as an endogenous angiotensin 1-7 receptor (Santos et al., 2003). Mas forms a constitutive hetero-oligomeric complex with the angiotensin AT$_1$ receptor and by so doing inhibits the action of angiotensin II (Kostenis et al., 2005). The Mas receptor has also been implicated in the reduction of blood pressure by angiotensin 1-7 (Widdop et al., 1999), and a possible reduction of Mas receptors in the kidney of streptozotocin-induced diabetic rats could be responsible for its lack of response to angiotensin 1-7. However, this suggestion requires further investigation.

Indomethacin and L-NAME reversed the action of angiotensin 1-7 in WKY rats and SHR, suggesting an involvement of prostaglandins, presumably vasodilator prostaglandins and nitric oxide in mediating the actions of the peptide. The lack of partial inhibition seen in Fig. 3B and C may suggest the possibility that the effects of prostaglandins could be mediated via nitric oxide or vice versa as either compound has been shown to upregulate the production of each other (Hsiao et al., 2007; Sugaitno et al., 2006). In agreement with results from the current study, a variety of studies clearly indicate that angiotensin 1-7 produces prostaglandins, stimulate nitric oxide, and activates a non-AT$_1$ and AT$_2$ receptor that is sensitive to [D-Ala$^7$]-angiotensin 1-7 (Jaiswal et al., 1993; Le Tran and Forster, 1997). In addition, nitric oxide has also been reported to decrease angiotensin AT$_1$ receptor mRNA levels (Ichiki et al., 1998), and angiotensin 1-7 may also modulate the effects of angiotensin II through this mechanism.

4.2. Mesentery

There are differences between the effects of angiotensin 1-7 on the renal and mesenteric vascular bed. In the mesenteric vascular bed, angiotensin 1-7 attenuated angiotensin II-induced vasoconstriction in WKY rats, SHR and streptozotocin-induced diabetic rats. The attenuation was seen at the lower concentrations of angiotensin II in WKY rats. In contrast to the kidney, angiotensin 1-7 reduced angiotensin II pressor action in streptozotocin-induced diabetic rats. Ongoing remodeling caused by activated RAS and the different roles that blood vessels play in the kidney and intestines may explain these differences. In SHR, the reduction in the pressor response by angiotensin 1-7 was seen at much higher concentrations of angiotensin II ($10^{-7}$–$10^{-6}$ M). Angiotensin II-induced vasoconstriction has been shown to be less in the SHR (Dharmani et al., 2005).

In contrast to the kidney, vasodepressor action of angiotensin 1-7 was witnessed in all the groups. In terms of the effects of angiotensin 1-7 on angiotensin II response, PD123319, D-ALA and L-NAME showed a similar pattern to those observed in the renal vasculature. Indomethacin reversed angiotensin 1-7 action in the SHR and not in WKY and streptozotocin-induced diabetic rats. This suggests that angiotensin 1-7 action is mediated via the angiotensin 1-7 receptor coupled with release of vasodilator prostaglandins and nitric oxide in SHR and only nitric oxide in WKY and streptozotocin-induced diabetic rats. Accordingly, Oliveira et al. (1999) demonstrated that angiotensin 1-7 causes both vasodilation and bradykinin potentiation in mesenteric arterioles, which was blocked by A-779, L-nitro-L-arginine methyl ester, and indomethacin, suggesting an important participation of local prostanoids and nitric oxide in the actions of angiotensin 1-7. The same authors showed that angiotensin 1-7 potentiates the bradykinin vasodilatory effect in mesenteric arterioles of SHR via release of prostanoids and endothelium derived hyperpolarization factor (EDHF) (Oliveira et al., 1999). In diabetic mesenteric bed, increased prostaglandin especially prostaglandin I$_2$ (PGI$_2$) has been demonstrated (Fuji et al., 1986, 1987). These authors have postulated that an increase in micro-circulation of PGI$_2$ may partially be protective against progression of angiopathy. As the vasodilator PGI$_2$ was already increased in diabetic mesentery, it is possible that angiotensin 1-7 does not further stimulate PGI$_2$.

The current data suggest that angiotensin 1-7 has a regulatory role in the kidney and mesenteric vasculature which includes the attenuation of the contractile effect of angiotensin II. This action is possibly modulated by angiotensin 1-7 receptor and involves a cyclo-oxygenase dependent pathway and nitric oxide release. This apparent protective effect of angiotensin 1-7 appears to be compromised in diseased state such as...
hypertension and diabetes. It is likely that angiotensin 1-7 receptors or its action is altered to accommodate the ongoing vascular remodeling.

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References


