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Differential Effects of Angiotensin II on Intra-Renal Hemodynamics in Rats; Contribution of Prostanoids, NO and K^+ Channels

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ABSTRACT

Many agents are known to cause qualitative and quantitative differences in intrarenal blood flow. This study tested the hypothesis that angiotensin II (AII) evokes a differential effect on cortical (CBF) and medullary blood flow (MBF) and that AT_2 receptor mediates AII-induced increase in renal MBF by mechanisms related to nitric oxide (NO) and prostanoids. AII (100, 300 and 1000 μg/kg/min) increased mean arterial blood pressure (MABP) by 24% ± 7% ($p < 0.05$); decreased CBF by 30% ± 2% ($p < 0.05$); but increased MBF by 21% ± 8% ($p < 0.05$). Indomethacin (5 mg/kg), enhanced AII effects on MABP by 154% ± 26% ($p < 0.05$), MBF by 141% ± 46% but decreased CBF by 74% ± 54% ($p < 0.05$) indicating the involvement of dilator prostanoids in the systemic and medullary circulation but constrictor prostanoids in the cortex. N^G nitro-L-arginine (L-NNA), an inhibitor of NO synthase (100 mg/L in drinking water) enhanced AII effects on MABP (169 ± 75, $p < 0.05$) and decreased CBF (107% ± 50%, $p < 0.05$) but blunted the effects of AII on MBF (150% ± 21%, $p < 0.05$). 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ; 2 mg/kg), a guanylyl cyclase inhibitor, enhanced AII effects on MABP (118% ± 32% , $p < 0.05$) and decreased CBF(85% ± 47% , $p < 0.05$) but blunted the effects of AII on MBF (96% ± 15%, $p < 0.05$). However, glibenclamide (20 μg/kg), a K_{ATP} channel blocker, did not affect intra-renal hemodynamics elicited by AII. Blockade of AT_2 receptors with PD123319 (50 μg/kg/min) did not change basal or AII-induced changes MABP or CBF but blunted AII-induced increase in MBF by 60% ± 11% ($p < 0.05$). CGP42112 (10 μg/kg/min), an AT_2 receptor agonist, elicited a reduction in MABP and increases in CBF and MBF that were abolished or attenuated by PD123319. These findings demonstrate that AII elicited differential changes in intrarenal blood flow; an AT_1-mediated reduction in CBF but an AT_2-mediated increase in MBF. The AT_2 receptor-mediated increase in MBF involves guanylase cyclase, NO and dilator prostanoids but not K_{ATP} channels.

Keywords: Angiotensin II; Hemodynamics; Medullary Blood Flow; AT_2 Receptors; Prostanoids

1. Introduction

The intrarenal vasculature can respond to neural and a variety of humoral stimuli with vasodilatation or vasoconstriction, resulting in increased or decreased perfusion of renal tissue, respectively [1]. Such responses may have more serious functional consequences within the medulla than in the cortex. This is of major physiological and pathophysiological importance as the medulla is widely viewed as having a crucial role in maintaining body fluid homeostasis and in the control of arterial pressure [2].

The renin-angiotensin system is a coordinated hormonal cascade important to the regulation of renal sodium excretion and blood pressure. The major effector peptide, angiotensin II (AII), binds to two major receptors; AT_1 and AT_2. While the majority of AII actions are mediated via the AT_1 receptor, evidence has accumulated that the AT_2 receptor opposes the AT_1 receptor, especially by inducing vasodilation instead of vasoconstriction and may be important in the regulation of blood pressure and renal function by counterbalancing the vasoconstrictor and antinatriuretic actions of AT_1 receptors [3]. However, the roles of AT_1 and AT_2 receptors in regulating regional kidney perfusion remain unclear. In rats and rabbits, infusions of AII reduced total renal blood flow (RBF) and cortical blood flow but have a lesser effect on medullary blood flow [4,5]. AII can even increase MBF, especially when administered as a bolus [1,6]. Many studies have demonstrated that the medullary vasculature was poorly sensitive to the vasoconstrictor effects of AII

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compared with the cortical circulation [4,7,8]. A study [9] has shown that AII induced a potent vasoconstriction of isolated medullary vasa recta in Sprague-Dawley rats, a response also observed in conscious rats [10]. Conversely, other studies have shown that the systemic infusion of AII increased papillary blood flow in young Sprague-Dawley and Wistar rats [11] by increasing local medullary synthesis of vasodilator agents such as prostaglandins, nitric oxide (NO), or kinins.

Nitric oxide (NO) synthase and/or cyclooxygenase (COX) blockade can enhance AII-induced reductions in medullary blood flow (MBF) and abolish AII-induced increases in MBF, both of which are chiefly AT_1 mediated [12-14]. However, the contributions of AT_2 receptors to these effects have received little attention, even though they are expressed in vessels that might contribute to MBF control (e.g., afferent arterioles and vasa recta). In a routine experiment to address the effects of AII in the rat, we noticed a differential effect on CBF and MBF and this led us to characterize these effects. We hypothesized that AII evokes a differential effect on intrarenal hemodynamics by an AT_1-mediated cortical vasoconstriction but AT_2 receptor-mediated increase in renal medullary blood flow. To test this hypothesis, mean arterial blood pressure (MABP), MBF and CBF responses to graded doses of AII were determined in the presence of indomethacin, N^{ω}-nitro-L-arginine, ODQ (1H-[1,2,4] oxadiazolo[4,3,-a]quinoxalin-1-one), or glibenclamide. In addition, we characterized the increase in MBF using CGP42112, a highly selective AT_2 agonist, and PD123319, an AT_2 antagonist.

2. Materials and Methods

2.1. Drugs and Chemicals

N^{ω}-nitro-L-arginine (L-NNA;Sigma-Aldrich, St. Louis, MO) and indomethacin (Sigma-Aldrich, St. Louis, MO) were dissolved in 0.1 M $NaHCO_3$, and pH was adjusted to 7.0 - 7.2. Glibenclamide (Sigma-Aldrich, St. Louis, MO) and 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ; Sigma-Aldrich, St. Louis, MO) were prepared in dimethylsulfoxide (DMSO) as stock solutions of 0.1 M, from which aliquots were diluted in normal saline for intravenous administration. Angiotensin II (Sigma-Aldrich), CGP42112 (21st Century Biochemicals, USA) and PD123319 (a gift from Park Davis, USA) were dissolved in normal saline (0.9% NaCl). All agents were kept on ice during the experiments.

Female Sprague-Dawley rats (230 - 290 g body wt; Harlan Sprague Dawley, Houston, TX) were maintained on standard rat food (Purina Chow; Purina, St Louis, MO) and allowed *ad libitum* access to water and food until the beginning of the experiments. The study protocol was approved by the Animal Care and Use Committee of Texas Southern University.

2.2. Surgical Preparation

Animals were anesthetised with thiobutabarbital (Inactin), 100 mg/kg ip (Sigma-Aldrich) and placed on a heated surgical table to maintain body temperature at 37˚C. The tail vein was cannulated with a 25-gauge butterfly needle (Vacutainer, Becton and Dickson) for infusion or administration of drugs. The trachea was isolated and a polyethylene catheter (PE-250) was placed in the trachea for spontaneous ventilation. A polyethylene catheter (PE-50) was placed in the left carotid artery to monitor the blood pressure. Mean arterial blood pressure (MABP) was measured with a pressure transducer (model BLPR2, World Precision Instruments, Sarasota, FL) to a signal manifold (Transbridge, model TBM-4, World Precision Instrument, Sarasota, FL) and recorded on a data acquisition system (model DI720, DataQ Instruments, Akron, OH). The left kidney was exposed by an abdominal incision, intrarenal blood flow was measured simultaneously by laser-Doppler (LD) flowmeter (system 5000, version 1.20, Periflux, Stockholm, Sweden) via a surface probe (model PF 407) to measure CBF or an optical fiber LD probe (model PF 402) fixed to a micromanipulator and placed in the medulla (5 mm below the kidney surface) to measure MBF. CBF and MBF were recorded as perfusion units (PU).

2.3. Experimental Protocol

After surgery and placing of probes for recording regional blood flows, a 30- to 45-min equilibration period was allowed. AII was administered by an infusion pump (Model 100, SP 100i syringe pump, WPI, USA) at graded doses of 100, 300 and 1000 ng/kg/min. These graded doses were administered cumulatively. The effects on MABP, CBF and MBF were determined in the presence of indomethacin, a COX inhibitor (10 mg/kg iv; n = 6) [15], L-NNA, N^{ω}-nitro-L-arginine, a NO synthase inhibitor (100 mg/L in drinking water for 2 days; n = 6) [16], ODQ, 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one, a guanylase cyclase inhibitor (2 mg/kg iv; n = 5) [17], glibenclamide, a K_{ATP} channel blocker (20 µg/kg, iv) [18]; or their respective vehicles: 0.1 M $NaHCO_3$ for L-NNA and indomethacin, 5% DMSO for glibenclamide and ODQ and normal saline for AII. Data obtained from rats treated with 5% DMSO and 0.1 M $NaHCO_3$ were not different from those obtained from rats treated with normal saline; hence, data from both groups were pooled to represent control data for all the treatment groups.

Another set of experiments to characterize the possible mechanisms involved in AII-induced increase in MBF

was carried out. MABP, MBF and CBF responses to graded doses of AII were determined in the presence of CGP42114, an AT_2 receptor agonist and PD123319, an AT_2 receptor antagonist. Animals were anesthetized and the left carotid artery cannulated for MABP determination while intrarenal blood flow was measured simultaneously by a laser-Doppler (LD) flow meter. After recording baseline values, the effects of CGP42114 (10 µg/kg/min; n = 5) were evaluated on MABP, CBF and MBF at intervals of 5, 10, 15 and 20 min. After infusion was stopped, a 30 min interval was allowed for the values to return to baseline. PD123319, an AT_2 receptor antagonist was infused at 50 µg/kg/min [19] for 10 min before CGP42112 (10 µg/kg/min) was infused concurrently and the antagonistic effect of PD123319 was determined at intervals of 5, 10, 15 and 20 min. Doses of all agents used were those reported in the literature to produce significant desired effects.

2.4. Statistical Analysis

All data were expressed as mean ± SEM. Changes in systemic and renal hemodynamics were expressed as absolute values and changes from baseline. The effects of a particular agent were analysed using a two-way ANOVA followed by Tukey's multiple comparison test when appropriate. Statistical analysis was performed using Graph Pad Prism V. 4.01 where $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of Angiotensin II Infusion on Systemic and Renal Haemodynamics

Figure 1(a) shows a representative tracing illustrating the differential effect of AII on systemic and renal haemodynamics. AII (300 and 1000 ng/kg/min) increased mean arterial blood pressure (MABP) and medullary blood flow (MBF) while decreasing renal cortical blood flow (CBF) in a dose related manner. AII (300 and 1000 ng/kg/min) increased mean arterial blood pressure (MABP) by 24 ± 5, and 47 ± 7 mmHg; decreased renal cortical blood flow (CBF) by –202 ± 69 and –98 ± 81 perfusion units (PU), but increased medullary blood flow (MBF) by 17 ± 09 and 53 ± 14 PU respectively.

3.2. Effect of Angiotensin II Infusion on Systemic and Renal Haemodynamics in the Presence of Indomethacin, L-NNA, ODQ and Glibenclamide

Figure 2 illustrates that AII (100, 300 and 1000 ng/kg/min) dose-dependently increased MABP by 13 ± 4, 22 ± 6, and 49 ± 8 mmHg, respectively; decreased CBF by –116 ±

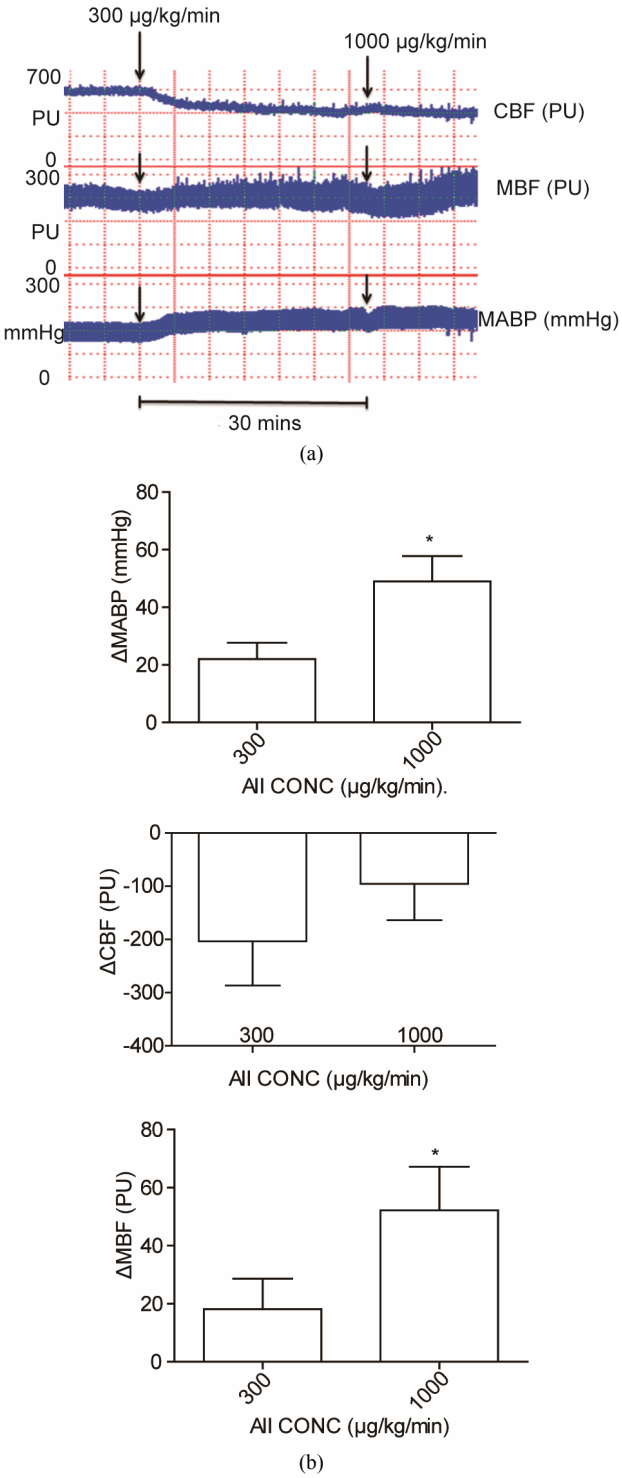


Figure 1. (a) Tracing showing the differential effects of angiotensin II (AII) on systemic (MABP) and renal hemodynamics (CBF & MBF). MABP = mean arterial blood pressure, MBF = medullary blood flow, CBF = cortical blood flow; (b) Graphical illustration showing the differential effects of angiotensin II (AII) on systemic (MABP) and renal hemodynamics (CBF & MBF). ($^*p < 0.05$ vs 300 ng/kg/min of AII).

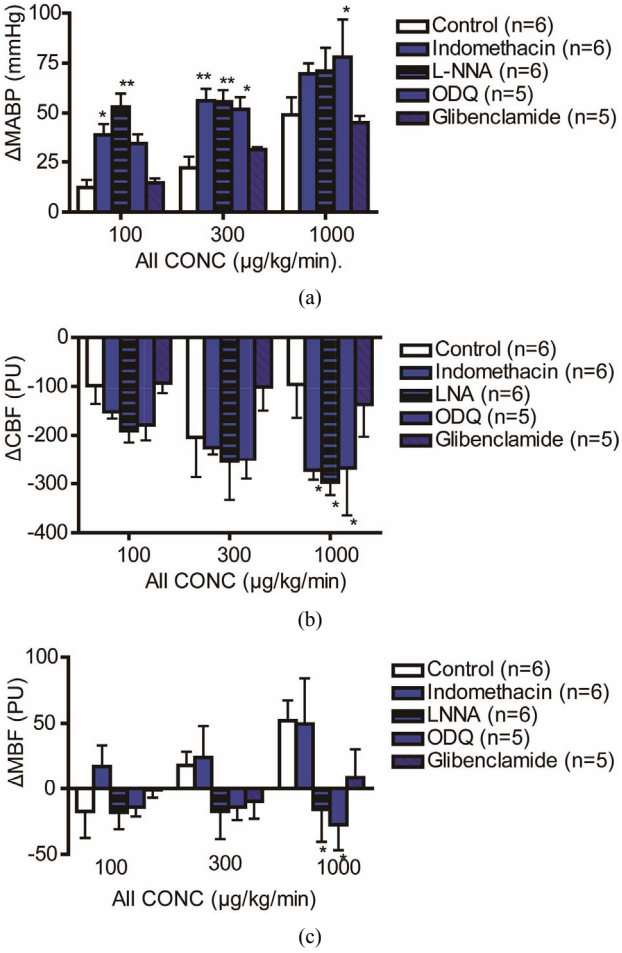


Figure 2. Effect of acute angiotensin II infusion on systemic (a) and renal (b), (c) haemodynamics in the presence of indomethacin, L-NNA, ODQ or glibenclamide ($^*p < 0.05$, $^{**}p < 0.01$ vs Control).

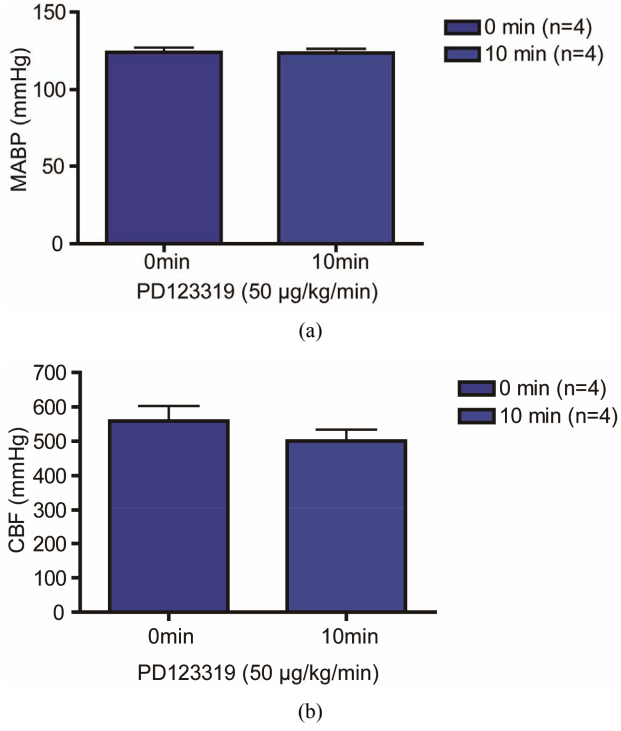
42, –204 ± 83 and –96 ± 67 perfusion units (PU), respectively but increased MBF by 18 ± 10 and 52 ± 15 PU respectively. Indomethacin (10 mg/kg) enhanced AII-induced increase in MABP by 154% ± 26% ($p < 0.05$), decreased CBF by 74% ± 54% ($p < 0.05$) and increased AII-induced increase in MBF by 141% ± 46% ($p < 0.05$) indicating that vasodilator prostaglandins may be contributing to the increase in MBF while the vasoconstrictor prostaglandins may be contributing to the increase in MABP or decrease in CBF elicited by AII. N^G nitro-L-arginine (L-NNA; 100 mg/L in drinking water for 2 days) enhanced AII-induced increase in MABP by 169 ± 75 ($p < 0.05$) and decrease in CBF by 107 ± 50 ($p < 0.05$) but blunted the effects of AII on MBF by 150 ± 21 ($p < 0.05$). ODQ, a soluble guanylyl cyclase (sGC; 2 mg/kg) inhibitor, enhanced AII-induced increase in MABP by 118% ± 32% ($p < 0.05$) and decrease in CBF by 85% ± 47% ($p < 0.05$) but blunted the effects of AII on MBF by 96% ± 15% ($p < 0.05$). This indicates that NO and activation sGC contribute to the AII-induced increase in MBF or decrease in CBF. However, in the presence of glibenclamide there were no significant changes in AII-induced increase in MABP and MBF or decrease in CBF as compared to the control, thus indicating that K^+_{ATP} channels are not involved in AII-induced increase in MBF.

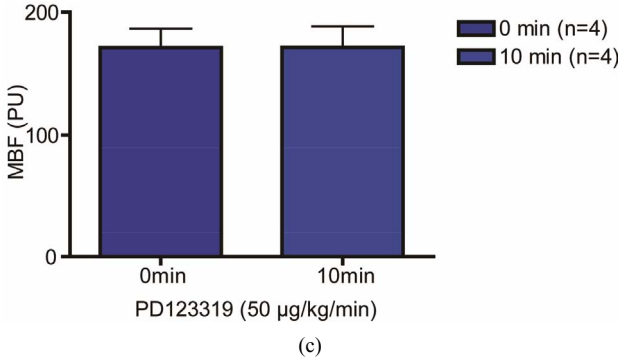
3.3. Effect of Angiotensin II Infusion on Systemic and Renal Haemodynamics as Affected by AT_2 Receptor Antagonism

The involvement of AT_2 receptors in AII-induced changes in intrarenal haemodynamics, AII (100, 300 and 1000 ng/kg/min) was infused in the presence of PD123319, an AT_2 receptor antagonist. PD123319 (50 µg/kg/min) did not change basal or AII-induced changes BP or CBF. However, PD123319 blunted AII-induced increase in MBF by 60% ± 11% ($p < 0.05$) (**Figures 3** and **4**) indicating the involvement of AT_2 receptors in AII-induced increase in MBF.

3.4. Effect of CGP42112 Alone and in Combination with PD123319 on Basal Systemic and Renal Haemodynamics

As an additional evidence to support that activation of AT_2 receptor was responsible for AII-mediated increase in MBF, the effects of CGP42112, an AT_2 receptor agonist, was tested on basal systemic and renal haemo-dynamics in the presence or absence of PD123319. **Figure 5**





(c)

Figure 3. Effect of PD123319 on basal systemic (a) and renal (b), (c) haemodynamics. 0 min represents basal values while 10 min represents values obtained after infusion of PD 123319 (50 µg/kg/min) for 10 min.

illustrates that CGP42112 decreased basal MABP, increased basal medullary perfusion and CBF in a time-dependent manner. PD123319, AT_2 receptor antagonist, attenuated CGP42112-induced decrease in MABP by 121% ± 13% ($p < 0.05$), and CGP42112 induced increase in CBF by 142% ± 3% ($p < 0.01$). CGP42112-induced increase in basal MBF was also blunted by PD123319 by 67% ± 6%. These data imply that AT_2 receptor activation accounts for the increase renal MBF by AII.

4. Discussion

There is still considerable controversy regarding the relative effects of angiotensin II on CBF and MBF in anaesthetized animals perhaps reflecting a species de pendency. Thus, studies in dogs indicated that both

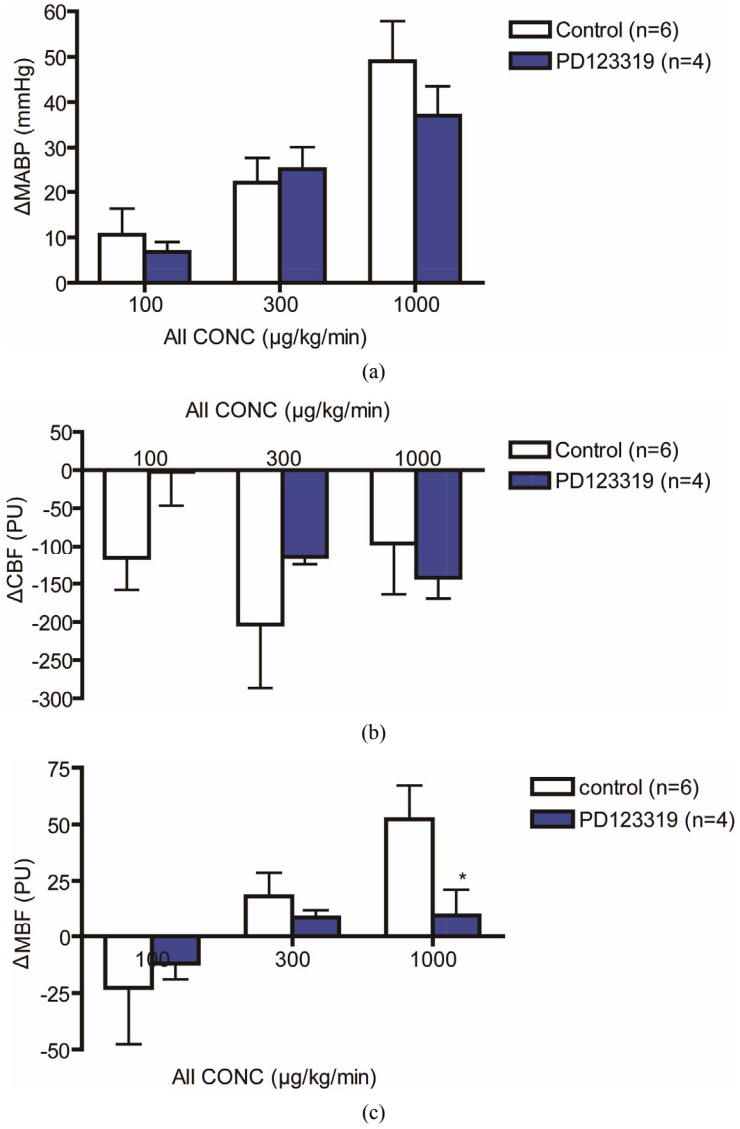


Figure 4. Effect of acute angiotensin II infusion on systemic (a) and renal (b), (c) haemodynamics in the presence of PD123319 ($^*p < 0.05$ vs control). Control animals were infused with normal saline (1 mL/h).

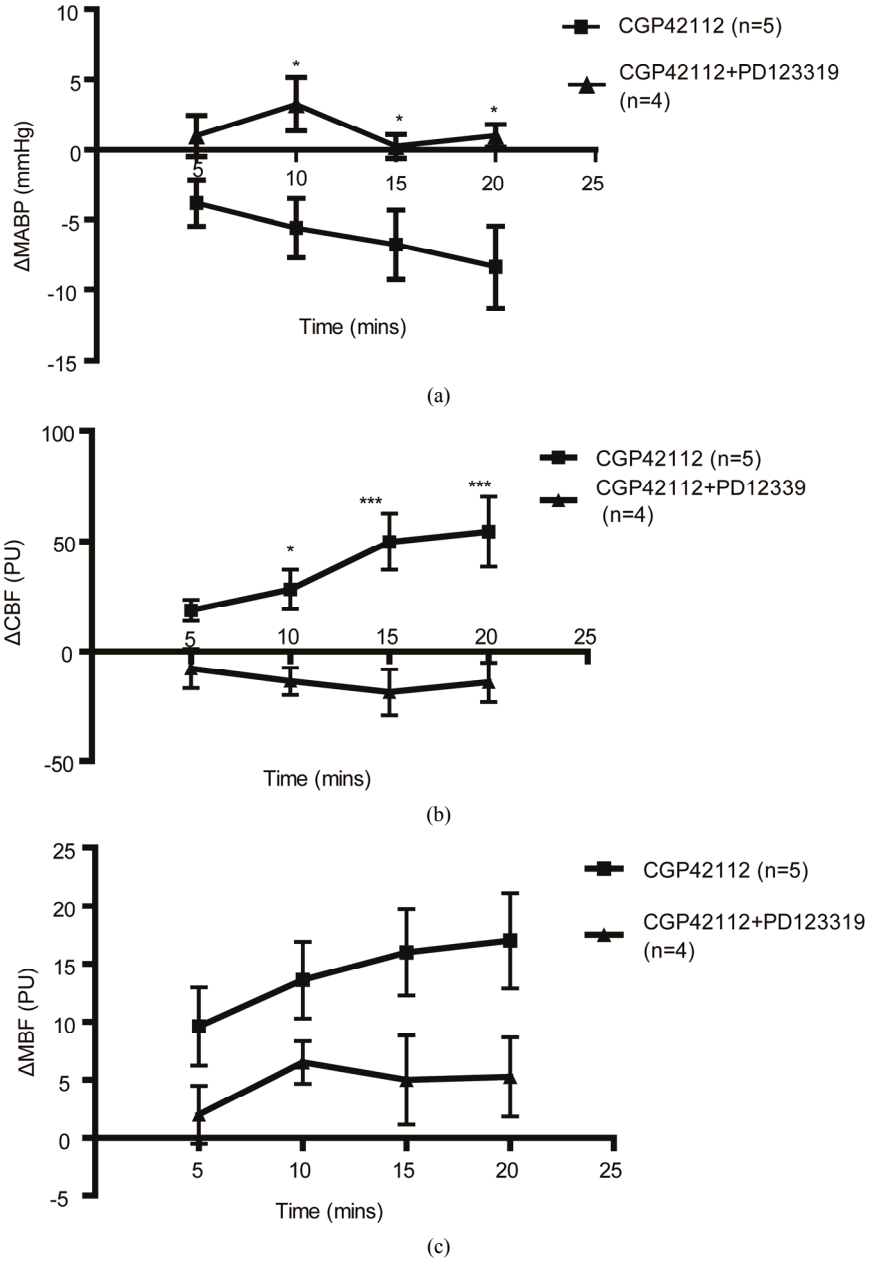


Figure 5. Effect of CGP42112 (10 µg/kg/min) alone and in combination with PD123319 (50 µg/kg/min) on basal systemic (a) renal (b), (c) haemodynamics ($^{*}p < 0.05$, $^{**}p < 0.01$ vs CGP42112 + PD123319).

exogenous and endogenous angiotensin II profoundly increase MBF, even at levels that have little impact on CBF [20,22]. In contrast, in most studies in anaesthetized rats and rabbits, intravenous or renal arterial infusion of angiotensin II significantly decreased CBF but not MBF [11,23,24].

In the present study, we tested the hypothesis that AII evokes a differential effect on intrarenal hemodynamics and that the AT_2 receptor mediates the increase in the renal medullary blood flow during acute AII infusion in rats. Infusion of AII increased MABP and MBF dose dependently with associated decrease in CBF. The pressor and renal cortical vasoconstriction is a result of the vasoconstrictive effect of AII [25] probably mediated through AT_1 receptors. Several studies have shown the paradoxical increase in MBF with AII bolus dose [1,19]. AII-induced vasodilatation, as seen in the medulla, could also have been indirect, being dependent on stimulation on the biosynthesis and/or release of vasodilator agents, such as prostaglandins, kinins or NO [26]. In order to determine which of the vasodilatory agents was involved in AII-induced increase in MBF, we examined the effect

of AII infusion in the presence of indomethacin, a COX inhibitor, L-NNA, a NO synthase inhibitor, ODQ, a sGC inhibitor or glibenclamide, a K_{ATP} channels blocker. LNNA or ODQ enhanced AII-induced increase in MABP and decrease in CBF but remarkably inhibited AII-induced increase in MBF. This is in agreement with the studies that showed that inhibition of NO synthesis prevented an increase in perfusion of the medulla after AII [14,27]. NO acts through the stimulation of sGC, with subsequent formation of cyclic GMP. These findings implicate NO involvement via sGC in the increase in MBF induced by AII. These data are in agreement with previous studies [14,27,28]. The rate of synthesis and tissue concentration of prostaglandins is much higher in the medulla compared with the cortex, and AII stimulates prostaglandin synthesis via AT_1 receptors [28]. Prostaglandin E_2 (PGE_2) is a major renal cyclooxygenase metabolite of arachidonate that modulates renal hemodynamics and salt and water excretion [29]. The maintenance of normal renal blood flow and function during physiological stress is especially dependent on endogenous prostaglandin synthesis [30] buffering the vasoconstrictor effects of AII, catecholamines, and vasopressin in the kidney thereby preserving normal renal function. Contrary to previous reports showing a tonic vasodilator influence of prostaglandins on the medullary circulation [31-33] inhibition of prostaglandins by indomethacin in this study enhanced AII-induced increase in MBF. This result suggests that vasodilator prostaglandins may be contributing to the increase in MBF while the vasoconstrictor prostaglandins may be contributing to the increase in MABP or decrease in CBF elicited by AII.

K_{ATP} channel regulation of vasoactivity in vascular beds has been documented and infusion of glibenclamide, a K_{ATP} into rats induced mesenteric, skeletal muscle, and renal vasoconstriction [34,35]. *In vivo*, K_{ATP} channel inhibition also increases resistance to blood flow in mesentery [36], renal cortex and medulla [32,37]. Previous studies demonstrated that high concentrations of AII inhibit K_{ATP} in the renal medulla [38] and infusions of K_{ATP} channel inhibitors have been shown to decrease MBF [15,32]. In the present studies, inhibition of K_{ATP} channels with glibenclamide did not significantly change AII-mediated effects on systemic and renal hemodynamics.

AII acts at two main receptor subtypes: AT_1 and AT_2 receptors. AT_1 receptors are responsible for mediating most of the known actions of AII, including vasoconstriction [39]. Moreover, a role for the AT_2 receptor in opposing the actions of AT_1 receptor stimulation has been implicated in growth and cardiovascular function [39-41]. In the kidney, infusions of AII reduce total renal blood flow (RBF) and cortical perfusion measured by laser Doppler flowmetry in rats and rabbits [41]. However, medullary perfusion is relatively insensitive to the vasoconstrictor effects of AII under most experimental conditions [1,23]. The explanation for these observations seems to be that, although AT_1-receptor activation causes vasoconstriction within vascular elements controlling MBF, it can also cause vasodilatation by release of nitric oxide and/or prostaglandins [5]. The contributions of AT_2 receptors to the control of MBF are less clear. However, recent studies in anaesthetised rabbits suggest that AT_2-receptor activation counteracts AT_1-mediated vasodilatation in the renal medulla, as the AT_2 antagonist PD123319 revealed dose-dependent increases in Medullary Laser Doppler Flux (MLDF) during renal arterial infusion of AII [19] implying an AT_2-mediated medullary vasoconstriction but AT_1-mediated vasodilation in the rabbit. This observation is at odds with the current study and contrary to the conventional view that AT_2 receptors mediate vasodilatation [42]. In our studies, blockade of AT_2 receptors with PD123319 attenuated AII-induced increase in MBF, suggesting that the increase in MBF was AT_2 receptor mediated. These data were further confirmed by infusion of CGP41112, an AT_2 receptor agonist in the absence and presence of PD123319 to determine the effect of AT_2 receptor on basal MABP, CBF and MBF. PD123319 had no detectable effect on resting systemic or renal hemodynamics. Thus, AT_2 receptors did not appear to contribute greatly to the control of resting MBF in anesthetized rats under these experimental conditions. Activation of AT_2 receptor by CGP41112 decreased basal MABP while increasing CBF and MBF. These responses were attenuated by PD123319, confirming that AT_2 receptor is not only involved in increasing MBF but also appears to be involved in the increase in CBF and decrease in MABP. These data are at odds with studies that showed that the increased medullary perfusion was AT_1 receptor-mediated [1,6,19] in rats.

In conclusion, AII evoked differential effects on intrarenal haemodynamics in the rat evoking cortical vasoconstriction but medullary vasodilation. AT_2 re-ceptor appears to mediate AII-induced increase in MBF by mechanisms involving guanylase cyclase, nitric oxide and dilator prostanoids but not K_{ATP} channels.

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