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AT_2 receptors mediate tonic renal medullary vasoconstriction in renovascular hypertension

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1 Renal medullary blood flow is relatively insensitive to angiotensin II (Ang II)-induced vasoconstriction, due partly to AT_1-mediated release of nitric oxide and/or prostaglandins. AT_2-receptor activation appears to blunt AT_1-mediated vasodilatation within the medullary circulation. This could affect long-term efficacy of antihypertensive pharmacotherapies targeting the renin/angiotensin system, particularly in Ang II-dependent forms of hypertension.

2 We tested the effects of AT_1- and AT_2-receptor blockade on basal cortical and medullary laser Doppler flux (CLDF and MLDF), and on responses to renal arterial infusion of Ang II, in rats with 2 kidney, 1 clip (2K1C) hypertension and sham-operated controls. Studies were carried out in thiobutabarbital (175 mg kg^{-1}, i.p.) anaesthetised rats, 4 weeks after clipping, or sham surgery ($n=6$ in each of eight groups).

3 Candesartan (10 μg kg^{-1} h^{-1}, intravenous (i.v.)) reduced mean arterial pressure ($\sim$17%) and increased CLDF ($\sim$24%), similarly in both sham and 2K1C rats, but did not significantly affect MLDF. PD123319 (1 mg kg^{-1} h^{-1}, i.v.) increased basal MLDF (19%) in 2K1C but not sham rats, without significantly affecting other variables.

4 In sham rats, renal arterial infusion of Ang II (1–100 ng kg^{-1} min^{-1}) dose dependently decreased CLDF (up to 44%), but did not significantly affect MLDF. These effects were markedly blunted in 2K1C rats. After PD123319, Ang II dose dependently increased MLDF (up to 38%) in sham but not 2K1C rats. Candesartan abolished all effects of Ang II, including those seen after PD123319.

5 Our data indicate that AT_1 receptors mediate medullary vasodilatation, which is opposed by AT_2-receptor activation. In 2K1C hypertension, AT_2-receptor activation tonically constricts the medullary circulation.

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Abbreviations: Ang II, angiotensin II; CLDF, cortical laser Doppler flux; DBP, diastolic blood pressure; HR, heart rate; 2K1C, 2 kidney, 1 clip; MAP, mean arterial pressure; MLDF, medullary laser Doppler flux; RAS, renin–angiotensin system; RBF, renal blood flow; RVR, renal vascular resistance; SBP, systolic blood pressure

Introduction

Angiotensin II (Ang II) is the main effector peptide of the renin–angiotensin system (RAS), acting at two main receptor subtypes: type 1 (AT_1) and type 2 (AT_2) (Carey *et al*., 2000). In rats and rabbits, infusions of Ang II reduce total renal blood flow (RBF) and cortical perfusion measured by laser Doppler flowmetry (cortical laser Doppler flux; CLDF). However, medullary perfusion is relatively insensitive to the vasoconstrictor effects of Ang II under most experimental conditions (Cupples *et al*., 1988; Parekh *et al*., 1996; Walker *et al*., 1999; Evans *et al*., 2000; 2004; Badzynska *et al*., 2002; 2003; Oliver *et al*., 2002; Rajapakse *et al*., 2002; Duke *et al*., 2003). The explanation for these observations seems to be that, although AT_1-receptor activation causes vasoconstriction within vascular elements controlling medullary blood flow, it can also cause vasodilatation. This latter effect appears to be mediated by release of nitric oxide and/or prostaglandins (Zou *et al*., 1998; Oliver *et al*., 2002; Rajapakse *et al*., 2002; Badzynska *et al*., 2003; Evans *et al*., 2004). The contributions of AT_2 receptors to the control of medullary blood flow are less clear. However, our recent observations 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 Ang II (Duke *et al*., 2003). This observation is at odds with the conventional view that AT_2 receptors mediate vasodilatation (Carey *et al*., 2000; Bautista *et al*., 2001; Widdop *et al*., 2002).

There is now strong evidence that the level of renal medullary blood flow has a major impact on urine flow and sodium excretion, and so the long-term control of blood pressure (Cowley, 1997; Cowley *et al*., 2003; Mattson, 2003; Pallone *et al*., 2003). Thus, interactions between AT_1 and AT_2 receptors in the control of medullary blood flow could have important implications for the long-term efficacy of antihypertensive therapies targeting the RAS, and for the ongoing debate on the relative merits of AT_1-receptor blockade *versus*

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Ang-converting enzyme inhibition, or their combination (Laverman *et al*., 2004). In our previous study in anaesthetised rabbits, neither candesartan nor PD123319 altered basal MLDF, suggesting that neither AT_1 nor AT_2 receptors contribute significantly to setting the basal level of medullary blood flow in normotensive animals (Duke *et al*., 2003). However, this might not be the case in hypertension, since intrarenal and/or circulating levels of Ang II are increased in most forms of hypertension, particularly that of renovascular origin (Navar *et al*., 2002). Therefore, the chief aim of the present study was to determine whether endogenous Ang II, acting at AT_1 and AT_2 receptors, contributes to the regulation of medullary blood flow in a renin-dependent form of hypertension. Therefore, we tested the effects of candesartan and PD123319, both on basal regional kidney perfusion and on responses to renal arterial administration of Ang II, in both 2 kidney, 1 clip (2K1C) hypertensive rats and sham-operated control rats. This also allowed us to confirm our findings in the rabbit, and in the most commonly used species for studies of the impact of Ang II on the renal circulation, the rat.

Methods

Animals

Male Sprague–Dawley rats ($n=48$, Biological Research Laboratories, Baker Heart Institute, Victoria, Australia) were used. Procedures were approved by the Monash University Department of Physiology Animal Ethics Committee, and accorded with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Surgery

To establish 2K1C hypertension, 4-week-old rats (100–150 g) were anaesthetised with isofluorane (1–3%, Abbott Australasia Pty Ltd, Kurnell, Australia) and a U-shaped silver clip (0.2 mm ID) was placed on the right renal artery (Li & Widdop, 1995). A control group underwent sham surgery. Tail-cuff systolic blood pressure (SBP) was measured in both groups at weekly intervals (Li & Widdop, 1995). Only 2K1C rats with SBP greater than 160 mmHg were used. At 3 to 4 weeks after this initial surgery, rats were anaesthetised (Inactin; thiobutabarbital, 175 mg kg^{-1} i.p.; Sigma, St Louis, MO, U.S.A.) and catheters were implanted into the jugular vein, and carotid and femoral arteries as described previously (Edgley *et al*., 2002). To maintain renal arterial pressure (measured from the femoral artery) at control levels during renal arterial infusion of Ang II, a clamp was placed around the aorta above the level of the renal arteries. Bovine serum albumin (2% (w v^{-1}) in 154 mM NaCl, Sigma) was infused intravenously (i.v.) throughout surgery (6 ml kg^{-1} h^{-1}) and the remainder of the experiment (1.5 ml kg^{-1} h^{-1}). The left kidney was placed in a stable cup, and a renal artery was catheterised (Edgley *et al*., 2002). Left kidney RBF was monitored by transit-time ultrasound flowmetry (type 0.7VB; Transonic Systems, Ithaca, NY, U.S.A.). For measurement of MLDF, a 26-gauge needle-type laser Doppler flowprobe (MNPIIOXP, Oxford Optronix, Oxford, U.K.) was inserted into the left kidney using a micromanipulator (Narashige, Tokyo, Japan). To allow for differences in kidney size between groups, the tip of the probe was placed $\sim$5 mm below the midregion of the lateral kidney surface in sham rats and $\sim$6 mm below the surface in 2K1C rats. We confirmed *post mortem* that this placed the tip of the probe at the margin of the inner and outer medulla. CLDF was measured with a miniature surface probe (MSP310XP, Oxford Optronix, Oxford, U.K.) placed on the dorsal surface of the kidney.

Experimental protocol

In both 2K1C rats ($n=24$) and sham rats ($n=24$), we tested the effects of (1) saline vehicle ($n=6$), (2) AT_1-receptor blockade ($n=6$), (3) AT_2-receptor blockade ($n=6$) or (4) combined AT_1-/AT_2-receptor blockade ($n=6$). Each rat then received renal arterial infusions of Ang II. Candesartan (Astra Zeneca, Switzerland) and PD123319 (synthesized as described by Cundy *et al*., 2000) were used for blockade of AT_1 and AT_2 receptors, respectively. Candesartan is a highly selective and insurmountable AT_1-receptor antagonist (Shibouta *et al*., 1993). PD123319 has 3500-fold greater affinity for AT_2 receptors relative to AT_1 receptors (Timmermans *et al*., 1991).

Effects of i.v. antagonist infusion After a 60–90 min equilibration period and a 10 min control period, rats were administered either candesartan (10 μg kg^{-1} plus 10 μg kg^{-1} h^{-1}), PD123319 (1 mg kg^{-1} plus 1 mg kg^{-1} h^{-1}), both antagonists or saline vehicle (1 ml kg^{-1} plus 1 ml kg^{-1} h^{-1} 154 mmol l^{-1} NaCl) i.v. The antagonist infusions continued for the whole experiment.

Effects of renal arterial infusion of Ang II At 30 min after commencing antagonist treatments, a series of renal arterial infusions of Ang II (0, 1, 3, 10, 30 and 100 ng kg^{-1} min^{-1}) commenced. Each dose was administered over a 15 min period, and the final dose was followed by a 15 min recovery period.

Statistical methods

Data are expressed as mean $\pm$ s.e.m. Values of $P \leqslant 0.05$ were considered statistically significant. Paired and unpaired *t*-tests, ANOVA, and where appropriate, repeated measures ANOVA (Ludbrook, 1994) were used to evaluate the effects of the various treatments.

Results

Tail-cuff SBP in conscious rats

SBP of sham rats remained constant (115 $\pm$ 2 mmHg) over the 3–4 week measurement period. In contrast, SBP of 2K1C rats rose gradually to 180 $\pm$ 6 mmHg, 3 weeks after surgery (Figure 1).

Baseline measurements in anaesthetised rats

Arterial pressure (systolic, diastolic and mean) and nonclipped (left) kidney dry weight were greater in 2K1C than sham rats (Table 1). In contrast, RBF, CLDF, MLDF and body weight were indistinguishable in the two groups. Renal vascular resistance (RVR) in the nonclipped kidney tended to be greater

in 2K1C than sham rats, but this did not reach statistical significance ($P=0.07$; Table 1). In both groups, the levels of all haemodynamic variables did not vary significantly according to the treatment that followed.

Responses to candesartan and PD123319

In sham rats, mean arterial pressure (MAP), heart rate (HR), RBF, CLDF and MLDF were not significantly affected by vehicle or PD123319 treatment. In contrast, after candesartan, MAP was decreased by 17±4%, and this was accompanied by increases in RBF (49±18%) and CLDF (22±5%). Candesartan did not significantly affect MLDF or HR in sham rats. When candesartan was coadministered with PD123319, responses of HR, MAP, RBF, CLDF and MLDF were indistinguishable from those of rats receiving candesartan alone (Figure 2).

In 2K1C rats, neither vehicle nor PD123319 treatment significantly affected MAP, HR or RBF. After candesartan, MAP of 2K1C rats was decreased by 16±3%, and this was accompanied by increased CLDF (25±10%). RBF also tended to increase (by 27±12%), but this was not statistically significant. Candesartan did not significantly affect MLDF or HR (Figure 2). In contrast to sham rats, in 2K1C rats PD123319 caused a small increase in CLDF (8±2%), although this response did not differ significantly from that in sham rats (Figure 2). Furthermore, in 2K1C rats, PD123319 increased MLDF from 550±64 to 641±57 U (19±7%), a response that was markedly different from that of sham rats when examined either at the end of the 30 min treatment period (Figure 2) or as a time course (Figure 3). Coadministration of candesartan and PD123319 resulted in responses of MAP, RBF, CLDF and MLDF that were indistinguishable from those of rats receiving candesartan alone.

Responses to infusions of Ang II

In vehicle-treated sham rats, renal arterial infusion of Ang II was accompanied by dose-dependent decreases in RBF (by 46±6% at 100 ng kg^{-1} min^{-1}) and CLDF (by 44±12% at

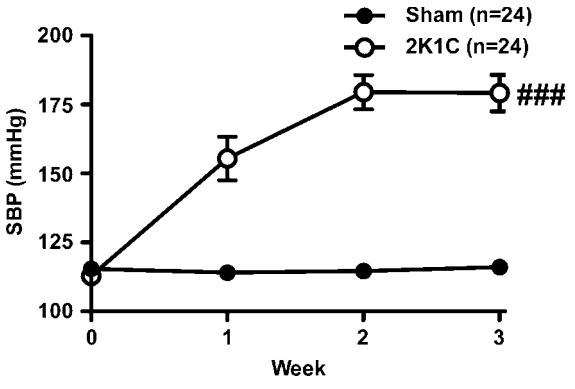

Figure 1 Tail-cuff SBP was recorded before (week 0) and at weekly intervals after surgery in 2K1C rats ($n=24$) and sham-operated rats ($n=24$). Lines and error bars represent mean±s.e.m. $^{###}P<0.001$ 2K1C *versus* sham group (analysis of variance).

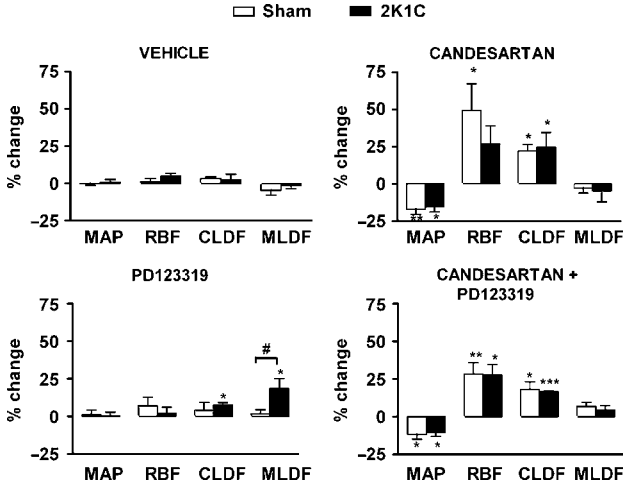

Figure 2 Effects of antagonist treatments on systemic and renal haemodynamics. Data indicate percentage differences between levels during a 10 min control period and those 20–30 min after the antagonist treatments commenced. Columns and error bars represent mean±s.e.m. ($n=6$ per group). MAP, mean arterial pressure; RBF, total renal blood flow; CLDF, cortical laser Doppler flux; MLDF, medullary laser Doppler flux. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ for change from control (paired *t*-test) and $^{\#}P<0.05$ for sham *versus* 2K1C hypertensive rats (unpaired *t*-test).

Table 1 Mean baseline levels of haemodynamic and renal variables before antagonist treatment in anaesthetised rats

Variable	*Sham-operated rats*	*2K1C rats*	P-*value*
SBP (mmHg)	125±5	155±5	<0.001
DBP (mmHg)	93±5	108±3	0.008
MAP (mmHg)	110±3	129±4	<0.001
HR (beats min^{-1})	353±8	368±8	0.2
RBF (ml min^{-1} g^{-1} dry kidney wt)	17±1	16±1	0.5
RVR (mmHg ml min^{-1} g^{-1} dry kidney wt)	7.3±0.5	8.5±0.4	0.07
CLDF (U)	1526±51	1599±39	0.3
MLDF (U)	645±47	659±42	0.8
Body weight (g)	392±9	367±12	0.1
Left kidney dry weight (g)	0.341±0.01	0.394±0.01	<0.001

Data are the mean±s.e.m. ($n=24$ per group) of levels during the 10 min period before i.v. administration of antagonist treatments. SBP, systolic blood pressure; DBP, diastolic blood pressure, MAP, mean arterial pressure; HR, heart rate; RBF, total renal blood flow; RVR, renal vascular resistance; CLDF, cortical laser Doppler flux; MLDF, medullary laser Doppler flux. *P*-values represent the outcomes of Student's unpaired *t*-test, testing whether these variables differed between the two groups. Note that analysis of variance within each group showed that none of these variables differed significantly according to the antagonist treatment that followed.

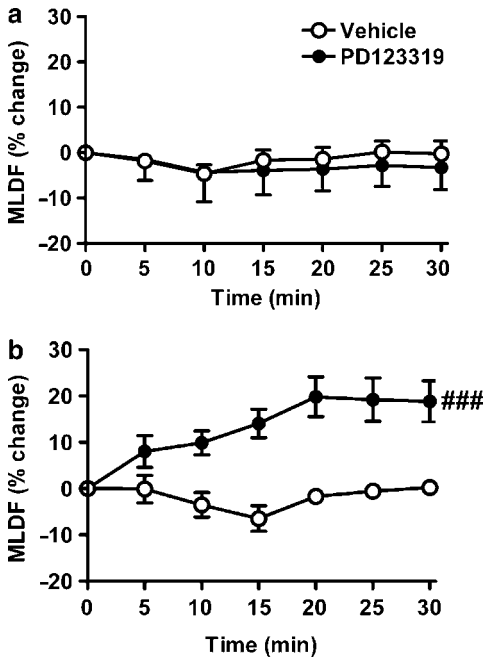

Figure 3 Time course of changes in MLDF in response to vehicle or PD123319 treatment in (a) sham and (b) 2K1C hypertensive rats. Data indicate percentage changes relative to control over 5 min intervals following commencement of antagonist infusion. Lines and error bars represent mean ± s.e.m. ($n = 6$ per group). $^{###}P < 0.001$ for main effect of PD123319 treatment from analysis of variance.

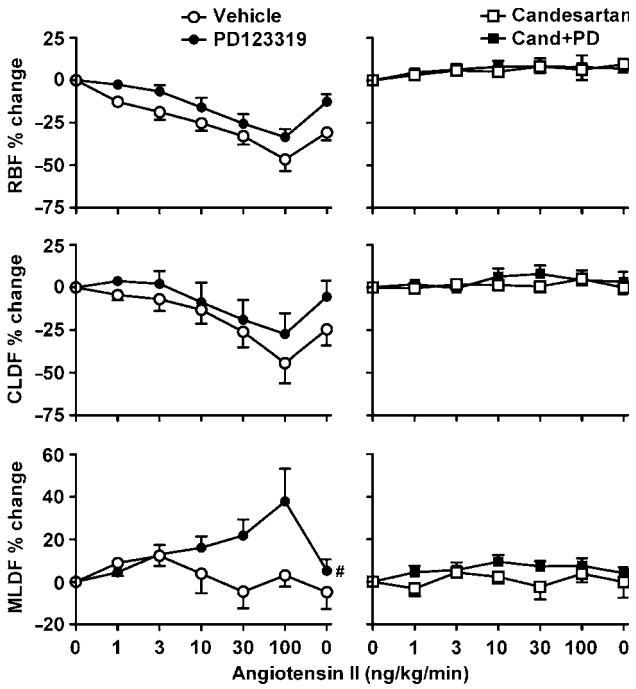

Figure 4 Responses of sham rats to renal arterial infusion of Ang II (1, 3, 10, 30 and 100 ng kg^{-1} min^{-1}, followed by a recovery period). Data indicate percentage changes, during the final 10 min of each 15 min infusion, compared with baseline levels before Ang II infusion. Lines and error bars represent mean ± s.e.m. ($n = 6$). Abbreviations are the same as for Figure 2. $^{\#}P \leq 0.05$ for main effect of PD123319 treatment from analysis of variance.

100 ng kg^{-1} min^{-1}) but not MLDF (Figure 4). These responses were virtually abolished by candesartan treatment, whether given alone or in combination with PD123319. In sham rats, PD123319 treatment had little or no effect on Ang II-induced reductions in RBF and CLDF, but uncovered a dose-dependent increase in MLDF. Thus, MLDF increased from 772 ± 84 U (control) to 808 ± 94, 874 ± 99, 883 ± 87, 932 ± 111 and 1019 ± 104 U, respectively, during infusion of 1, 3, 10, 30 and 100 ng kg^{-1} min^{-1} Ang II (Figure 4). *Post hoc* multiple comparisons, using the Ryan–Holm–Sidak procedure (Ludbrook, 1998), revealed that these changes reached statistical significance at 30 ng kg^{-1} min^{-1} ($P = 0.03$) and 100 ng kg^{-1} min^{-1} ($P < 0.001$). During the recovery period, MLDF returned to its control level (805 ± 99 U). This response was virtually abolished by candesartan treatment (Figure 4). In contrast, renal arterial infusion of Ang II in vehicle-treated 2K1C rats had little effect on RBF, CLDF and MLDF (Figure 5). In 2K1C rats, responses to Ang II were not significantly altered by PD123319 (Figure 5).

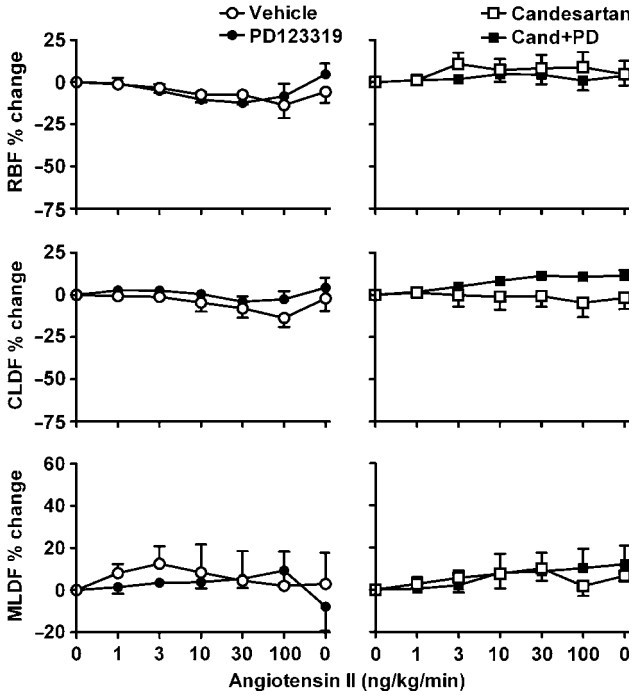

Figure 5 Responses of 2K1C hypertensive rats to renal–arterial infusion of Ang II (1, 3, 10, 30 and 100 ng kg^{-1} min^{-1}, followed by a recovery period). Abbreviations, symbols, lines and error bars are the same as for Figure 4.

Discussion

We recently obtained evidence, from studies in anaesthetised rabbits, that AT_2-receptor activation counteracts AT_1-receptor-mediated vasodilatation in the renal medulla (Duke *et al.*, 2003). The role of renal medullary blood flow in the long-term control of blood pressure is now well established, based in large part on studies showing that chronic reductions in medullary perfusion can lead to salt and water retention and hypertension (Cowley, 1997; Cowley *et al.*, 2003; Mattson, 2003). Thus, interactions between AT_1 and AT_2 receptors in the control of medullary blood flow could have important implications for antihypertensive therapies that target the RAS. These interactions could also contribute to the control

of medullary blood flow, and thus blood pressure, in renin-dependent forms of hypertension. Therefore, the chief aim of our current study was to determine the roles of endogenous Ang II in the control of medullary blood flow in 2K1C hypertension. By using a rat model for these experiments, we were also able to validate our previous observations in rabbits, in a species more commonly used for studies of regional kidney blood flow. Our key new finding was that in 2K1C rats, the AT_2-receptor antagonist PD123319 increased basal MLDF, suggesting that in this model of hypertension, AT_2-receptor activation mediates tonic vasoconstriction in the medullary circulation.

To our knowledge, this is the first study to test the effects of renal arterial administration of Ang II on regional kidney blood flow in rats. Responses to renal arterial infusion of Ang II in rats were remarkably similar to those we have observed in rabbits (Rajapakse *et al*., 2002; Duke *et al*., 2003). In sham rats, renal arterial Ang II caused dose-dependent decreases in CLDF but not MLDF, and treatment with PD123319 revealed dose-dependent increases in MLDF in response to Ang II. All of these effects were blocked by treatment with candesartan. Thus, as in the rabbit kidney, AT_2-receptor activation appears to blunt AT_1-mediated medullary vasodilatation in the rat kidney. In both species, Ang II-induced increases in MLDF (in the presence of PD123319) were dose-dependent, and occurred at doses of Ang II that produced physiologically and pharmacologically relevant reductions in total RBF (30–70%). Also, we found that neither AT_1-, AT_2-, nor combined AT_1- and AT_2-receptor blockade, altered resting MLDF in sham rats. Therefore, as in normotensive rabbits, endogenous Ang II appears to play little role in setting basal vascular tone in the medullary circulation in normotensive rats. This conclusion is consistent with the results of previous studies, showing little effect of AT_1 antagonism on medullary blood flow in rats (Ortíz *et al*., 1998; Badzynska *et al*., 2002; Sarkis *et al*., 2003). Earlier observations of increased medullary blood flow after blockade of Ang-converting enzyme likely reflect the effects of increased bradykinin bioavailability (Cupples *et al*., 1988; Mattson & Roman, 1991).

Compared to sham rats, responses of renal haemodynamics to renal arterial Ang II were greatly blunted in 2K1C rats. The most likely explanation for this is that renal vascular responses to Ang II in 2K1C rats are already near maximal, since circulating (Navar *et al*., 2001) and intrarenal levels of Ang II in the nonclipped kidney (Guan *et al*., 1992) are elevated in rats with 2K1C hypertension. We did not directly measure plasma renin activity or Ang II levels in this study, although plasma renin activity has previously been found to be elevated in 2K1C rats (15.5 ± 4.5 ng Ang I $ml^{-1} h^{-1}$) compared with sham rats (2.1 ± 0.5 ng Ang I $ml^{-1} h^{-1}$) in our laboratory (Li & Widdop, 1995). Downregulation of renal AT_{1A} receptors (but not AT_2 receptors) in the unclipped kidney of 2K1C rats (Wang *et al*., 1999) might also partly explain the blunted response to exogenous Ang II we observed. In rats, AT_1 receptors have been detected in afferent and efferent arterioles (including those of juxtamedullary glomeruli), and outer medullary descending vasa recta, as well as tubular structures associated with these vascular elements (Miyata *et al*., 1999; Wang *et al*., 1999; Helou *et al*., 2003). However, the degree to which 2K1C-hypertension downregulates expression of AT_1 receptors in each of these tissues (see Wang *et al*., 1999), and also the precise contribution of each of these tissues to the control of regional kidney blood flow (see Evans *et al*., 2004), remains to be determined.

In the present study, AT_1-receptor blockade had similar haemodynamic effects in 2K1C rats compared with sham rats. Surprisingly, few studies have examined the effects of AT_1-receptor blockade in 2K1C and sham rats simultaneously, although our results are consistent with the two previous studies of anaesthetised rats (Braam *et al*., 1995; Cervenka *et al*., 1999). Basal levels of RBF (corrected for dry kidney weight) were also similar in sham and 2K1C rats (unclipped kidney) in our study, but this does not necessarily reflect a lack of effect of RAS activation on control of renal haemodynamics. Renal arterial infusion of Ang II ($0.5\ ng\ kg^{-1}\ min^{-1}$) in conscious dogs is associated with an initial reduction in RBF, but within 48 h RBF returns to its control level (Fitzgerald *et al*., 1997). The recovery of RBF is presumably due to the impact of counter regulatory vasodilator mechanisms, since termination of the Ang II infusion, 28 days after it commenced, was associated with a marked increase in RBF that lasted for $\sim$48 h (Fitzgerald *et al*., 1997). Thus, multiple mechanisms act in concert to maintain renal circulatory homeostasis, even in the face of RAS activation.

Our observation that PD123319 treatment revealed increases in basal MLDF in 2K1C rats, which were abolished by candesartan, suggests that AT_1 receptors mediate vasodilatation in the medullary circulation in 2K1C hypertension, but that tonic AT_2-receptor-mediated vasoconstriction counterbalances this effect. These effects, on basal vascular tone in the medullary circulation, are consistent with our observation that PD123319 treatment unmasks increases in MLDF in response to exogenous Ang II in sham rats, which were also abolished by candesartan treatment in combination with PD123319. Differences between sham and 2K1C rats, in their responses to AT-receptor blockade and exogenous Ang II, are likely due to the different level of activation of circulating and intrarenal RAS in the two groups. Thus, our data indicate that in the medullary circulation, AT_2-receptor activation inhibits AT_1-mediated vasodilatation, and that in 2K1C hypertension, AT_2-receptor activation mediates tonic vasoconstriction. There is strong evidence, from both *in vitro* and *in vivo* studies, that Ang II-induced vasodilation in the medullary circulation is mediated by release of nitric oxide (Zou *et al*., 1998; Walker *et al*., 1999; Rajapakse *et al*., 2002) and/or prostaglandins (Parekh *et al*., 1996; Oliver *et al*., 2002; Badzynska *et al*., 2003). Our data regarding the effects of PD123319 therefore suggest that AT_2-receptor activation can blunt AT_1-receptor-mediated release of these vasodilator factors in the medullary circulation.

However, effects of AT_2-receptor activation on MLDF were not observed in the absence of concomitant AT_1-receptor activation, indicating that they reflect modulation of AT_1-mediated actions, rather than direct effects of activation of AT_2 receptors *per se*. For example, renal arterial infusion of Ang II did not alter MLDF (or alter RBF or CLDF) in rats or rabbits (Duke *et al*., 2003) pretreated with candesartan. Furthermore, in rabbits, renal arterial infusion of the highly selective AT_2-receptor agonist CGP42112A did not alter RBF, CLDF or MLDF (Duke *et al*., 2003). Also, PD123319 produced increases in MLDF in 2K1C rats, in which endogenous Ang II was activating AT_1 receptors near maximally (see above), but not in sham rats in which renal AT_1-receptor activation by endogenous Ang II was presum-

ably only modest. These considerations also provide a likely explanation for the lack of effect of candesartan alone on basal MLDF in 2K1C rats, since AT_1-mediated medullary vasodilatation under these conditions is normally inhibited by AT_2-receptor activation. Collectively, these observations indicate that activation of AT_2 receptors has little impact on the medullary circulation, unless AT_1 receptors are also activated.

Collectively, our results suggest that AT_2 receptors mediate vasoconstriction in the medullary circulation that counterbalances AT_1-receptor-mediated vasodilatation. AT_1-receptor-mediated vasodilatation appears to be a unique property of the medullary circulation, which relies on the close association of vascular and tubular elements within the medulla, which in turn facilitates the phenomenon of tubulovascular nitric oxide crosstalk (Dickhout *et al*., 2002). Given that AT_2-receptor activation opposes many of the actions of Ang II mediated by AT_1 receptors (Siragy & Carey, 1999; Carey *et al*., 2000), it seems logical that this is also the case for AT_1-mediated vasodilatation in the medullary circulation, even though this is at odds with the picture that has emerged in the systemic circulation, of AT_2-receptor activation opposing AT_1-mediated vasoconstriction (Carey *et al*., 2000).

Our present results suggest that this interaction between AT_1 and AT_2 receptors, in the control of medullary blood flow, has considerable impact on regulation of medullary blood flow in renovascular hypertension. It remains to be determined whether it also contributes to the regulation of medullary blood flow under other conditions associated with RAS activation (e.g. sodium depletion (Gross *et al*., 1998) and heart failure (Schrier & Abraham, 1999)). Importantly, the role of AT_2 receptors in the regulation of medullary blood flow may be enhanced under conditions of upregulation of renal AT_2 receptors (e.g. sodium depletion (Ozono *et al*., 1997) and renal failure (Bautista *et al*., 2001)). It also remains to be determined whether this mechanism contributes to the control of medullary blood flow in nonrenin-dependent models of hypertension. The implications of our present findings, for the use of AT_1-receptor antagonists in antihypertensive pharmacotherapy, also remain to be determined. However, the fact that candesartan seems not to alter basal medullary blood flow in rabbits (Duke *et al*., 2003) and rats, even when the RAS is activated (in 2K1C hypertension), does not support the notion that blockade of AT_1-mediated vasodilatation in the medullary circulation can limit the long-term antihypertensive efficacy of AT_1-receptor antagonists.

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