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BLOOD PRESSURE, BLOOD FLOW AND OXYGENATION IN CLIPPED KIDNEY OF CHRONIC 2K,1C RATS; EFFECTS OF TEMPOL AND ANGIOTENSIN BLOCKADE

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Abstract

Angiotensin II maintains renal cortical blood flow and renal oxygenation in the clipped kidney of early two-kidney, one clip Goldblatt hypertensive rats (2K,1C). The involvement of Ang II is believed to decline whereas oxidative stress increases during progression of 2K,1C hypertension. We investigated the hypothesis that the acute administration of drugs to inhibit reactive oxygen species (tempol) or angiotensin II type 1 receptors (candesartan) or angiotensin converting enzyme (enalaprilat), lower mean arterial pressure, and increase kidney blood flow and oxygenation in the clipped kidney of chronic 2K,1C rats in contrast to Sham controls. Twelve months after left renal artery clipping or Sham, mean arterial pressure, renal cortical blood flow and renal cortical and medullary oxygen tension were measured after acute administration of tempol followed by enalaprilat or candesartan followed by enalaprilat

The mean arterial pressure of the 2K,1C was reduced by candesartan (−9%), and more effectively by tempol (−35%). All applied treatments had similar blood pressure lowering effect in sham (average −21%). Only tempol increased cortical blood flow (+35%) and cortical and medullary oxygen tensions (+17% and +94%, respectively) in clipped kidneys of 2K,1C.

Administration of enalaprilat had no additional effect, except for a modest reduction in cortical blood flow in the clipped kidney of 2K,1C when co-administered with candesartan (−10%).

In conclusion, acute administration of tempol is more effective than candesartan in reducing the mean arterial blood pressure and improving renal blood perfusion and oxygenation in the clipped kidney of chronic 2K,1C rats.

Keywords

Goldblatt hypertension; renal oxygen tension; renal blood flow; tempol; angiotensin receptor blockers; angiotensin converting enzyme inhibitors

Introduction

A reduced renal perfusion pressure following the clipping of a renal artery increases angiotensin II (Ang II) concentrations in both kidneys.[1] There is an early development of

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Conflict of Interest/Disclosure
None.

Ang II-dependent hypertension.[2–4] Ang II acting on Ang II type 1 receptors (AT_1-Rs) results in activation of superoxide ($O_2^{\cdot -}$) production by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,[5] which is abundantly expressed in the kidney.[6] The involvement of oxidative stress in early two-kidney, one-clip (2K,1C) Goldblatt hypertensive rats has been demonstrated by prolonged administration of tempol to reduce reactive oxygen species (ROS) which reduced the mean arterial pressure (MAP), and improved the renal blood flow and glomerular filtration rate and oxygen tension (pO_2) of the clipped kidney.[7] In contrast, the administration of an AT_1-R blocker (ARB) indeed reduced the MAP both during the early[7] and the chronic phase[4] in 2K,1C hypertension, but failed to improve either the renal hemodynamics or oxygenation during the early phase.[7] Therefore, therapeutic options for correcting hypertension and renal ischemia in the chronic phase of 2K,1C renovascular hypertension are presently limited.[8] Nevertheless, prolonged Ang II infusion reduces renal tissue pO_2,[7,9,10] which is ascribed to excessive formation of ROS. Several of the conditions commonly associated with increased oxidative stress display reduced kidney pO_2, including diabetes,[11–13] lipopolysaccharide (LPS)-induced sepsis[14] and ischemia-reperfusion injury.[15] Therefore, we investigated the role of ROS in renal vasoconstriction and oxygenation in chronic 2K, 1C hypertension.

Anderson *et al* proposed that increased Ang II in the early 2K,1C model are a homeostatic modification to provide sufficient glomerular capillary pressure to sustain the glomerular filtration rate (GFR).[16] Indeed, we have reported that oxygen availability in clipped kidneys of early (3 weeks) 2K,1C is maintained by Ang II acting on Ang II type 2 receptors (AT_2-Rs) resulting in nitric oxide (NO) release.[17] This may be of importance since chronic renal hypoxia, and repeated episodes of renal ischemia, may contribute to hypertension[10] and progressive kidney disease.[18,19] However, the long-term consequences of increased levels of Ang II and subsequent oxidative stress for the function of the clipped kidney of the 2K,1C model of renovascular hypertension is currently not well understood. The role of Ang II evolves during 2K,1C hypertension.[4,20] Therefore, the present work was designed to investigate the role of ROS and/or Ang II acting on AT_1-Rs in the regulation of MAP, cortical renal blood flow and tissue oxygen availability in the clipped kidney of chronic 2K, 1C rats.

Material and Methods

These studies were performed under guidelines recommended by the National Institutes of Health and approved by the Georgetown University Animal Care and Use Committee. As described in detail previously,[7,17] young male Sprague-Dawley rats (80–100 g) were anesthetized with isoflurane (0.5–1.5%). A silver clip (0.2 mm) was placed around the left renal artery (2K,1C). Age-matched rats were used as controls (Sham). All rats received hydralazine + hydrochlorothiazide + reserpine (HHR; 30 + 10 + 0.2 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}) in the drinking water as previously described[9] in order to maximize survival of the clipped rats. The HHR treatment was discontinued 14 days prior to the acute experiments.

Twelve months after clipping, all rats were anesthetized with Inactin (100 mg $\cdot$ kg^{-1} i.p.; Sigma-Aldrich, St. Louis, MO), and an endotracheal tube was inserted for spontaneous respiration. Rats were prepared, and followed a similar protocol, as those with acute 2K,1C hypertension described in detail previously.[17] Briefly, the left femoral artery was catheterized for monitoring MAP and the left femoral vein for infusion of saline (5 ml $\cdot$ kg bw^{-1} $\cdot$ h^{-1}). The left kidney was immobilized in a plastic cup while renal cortical pO_2 and cortical blood flow (CBF) were measured with oxygen microelectrodes (Unisense, Aarhus, Denmark) and laser-Doppler needle probes (Transonic Systems Inc, Ithaca, NY) as described previously.[12,17,21] The location of each measurement was visually verified at the end of the experiments by dissecting the kidneys under a microscope. Measurements were

made before and after the administrations of candesartan (1 mg · kg bw^{-1} bolus + 1 mg · kg bw^{-1} · h^{-1}; kind gift from Astra Zeneca, Södertälje Sweden; manufacturer recommended dose for maximal inhibition of AT_1-Rs *in vivo*; Sham n=7 and 2K,1C n=8)[17] followed after 30 minutes by enalaprilat (0.3 mg · kg bw^{-1}·h^{-1}; Novaplus 1.25 mg · ml^{-1}, Baxter Healthcare Corporation, Deerfield, IL; fully effective antihypertensive dose in acute 2K,1C rats),[17], or tempol (174 μmol · kg bw^{-1} bolus + 174 μmol · kg bw^{-1} · h^{-1}; Sigma Aldrich; fully effective antihypertensive dose in spontaneously hypertensive rats; Sham n=7 and 2K, 1C n=7)[7] followed after 30 minutes by enalaprilat (0.3 mg · kg bw^{-1}·h^{-1}).

We selected this protocol, using candesartan followed by enalaprilat, for comparison with a prior series in acute 2K,1C rats[17] where an increase in blood pressure or renal vascular resistance with enalaprilat in rats pretreated with candesartan was an indication of an AT_2-Rs mediated change. Since enalaprilat reduced CBF modestly after candesartan (Fig 2), this suggested some role for AT_2-Rs in maintaining CBF even in the chronic model. Therefore, we undertook a limited study of 2K,1C rats (n=7) given the AT_2-R antagonist PD-123,319 (1 mg · kg bw^{-1} bolus + 1 mg · kg bw^{-1} · h^{-1}; Sigma Aldrich; fully effective dose in acute 2K,1C rats)[17] followed 30 minutes later by enalaprilat (0.3 mg · kg bw^{-1} bolus + 0.3 mg · kg bw^{-1} · h^{-1}) to test this hypothesis more directly.

Statistics

ANOVA was used to compare multiple data sets. When appropriate, this was followed by Dunnett's post hoc test. Two data sets within the same group were compared using Student's t-test for paired comparisons. Relative changes displayed in the figures are for visualization purpose only; statistics were calculated using the original parametric data sets (GraphPad Prism, GraphPad Software, San Diego CA). For all comparisons, $p<0.05$ was considered statistically significant. All values are expressed as mean ± SEM.

Results

A total of 54 rats were clipped and 24 rats survived until the acute experiments twelve months later, resulting in a survival ratio of 44%. Of the 24 remaining rats, one was excluded due to surgical errors and one due to having an infarcted and atrophied left kidney. All animals that underwent Sham surgery survived.

The body weights did not differ between Sham and 2K,1C rats (Table 1). Both the non-clipped and the clipped kidneys of 2K,1C rats were significantly heavier when corrected for body weight than kidneys of Sham rats.

2K,1C rats had elevated MAP, averaging 163±3 (n=22) compared to Sham 123±3 (n=14, $P<0.05$; Fig. 1 and Table 2). All acute interventions reduced the MAP modestly, but similarly, in elderly Sham rats. The elevated MAP of 2K,1C rats was reduced by candesartan. However, tempol was significantly more effective (Fig. 1). Enalaprilat did not produce a further fall in MAP in any rats administered tempol or candesartan.

The CBF was unchanged after all applied acute interventions in Sham rats. Candesartan did not change CBF significantly in 2K,1C rats (Fig. 2). However, CBF was increased in 2K,1C rats by tempol. CBF did not change further in 2K,1C rats given enalaprilat after tempol but was reduced significantly by enalaprilat following candesartan administration (Fig. 2).

The baseline renal cortical pO_2 was similar in 2K,1C and Sham rats (Table 1). Candesartan, or the combination of candesartan and enalaprilat, increased cortical pO_2 in Sham rats, whereas tempol had no effect in this group (Fig. 3). Only tempol increased the cortical pO_2 in 2K,1C rats after which there was no further change with enalaprilat (Fig. 3).

The baseline renal medullary pO_2 was reduced in 2K,1C rats compared to Sham (Table 1). Renal medullary pO_2 was increased after all the applied acute interventions in Sham, whereas again only tempol increased the medullary pO_2 in 2K,1C rats (Fig. 4). Subsequent administration of enalaprilat after tempol did not alter the medullary pO_2 further.

The 2K,1C rats administered PD-123,319 had similar body weight (495±21 g), kidney to body weight ratios (right kidney 5.01±0.62; left kidney 3.71±0.82; $P<0.05$), right to left kidney weight ratio (0.76), baseline cortical and medullary pO_2 (44±1 and 16±1 mmHg, respectively) as the other two 2K,1C groups. Administered PD-123,319 to 2K,1C rats had no effect on any of the investigated parameter. Subsequent addition of enalaprilat caused a modest reduction of MAP (Table 2).

Discussion

The main new findings from this study are that the acute administration of tempol is more effective in lowering MAP in chronic 2K,1C rats than blockade of the renin-angiotensin system with ARB or ACE inhibitor. Furthermore, only tempol increased the blood flow and the tissue pO_2 in the clipped kidney, whereas the ARB had no effect. The addition of an ACE inhibitor to rats that had received tempol or candesartan did not improve these responses.

Both the hypertension, and the intrarenal alterations in blood flow and tissue pO_2 in early 2K,1C hypertension, are highly dependent on increased Ang II action.[17] Whereas we found that the acute inhibition of AT_1-Rs or ACE reduced the blood pressure in chronic 2K,1C, the fall in BP was significantly greater following a reduction in ROS with tempol. Furthermore, only tempol effectively increased both the CBF and the tissue pO_2 in the renal cortex and medulla in the clipped kidney of chronic 2K,1C, whereas interventions directed against the renin-angiotensin system had no effect on these parameters. The altered function of the clipped kidney likely is a response to prolonged exposure to severely increased Ang II levels, and to a reduced perfusion pressure and medullary pO_2. Chronically elevated Ang II can induce self-sustaining mechanisms which maintain ROS production. Thus, prolonged Ang II can reduce the antioxidant defense systems, such as superoxide dismutase, upregulate NADPH oxidase subunits, oxidize tetrahydrobiopterin (BH_4) with subsequent uncoupling of NO synthases, stabilize the thromboxane-prostanoid (TP)-receptors and induce vascular and renal inflammation to name but a few.[10] The results from the present study show that the effects of tempol differ substantially from those that follow interruption of the renin-angiotensin system, which is consistent with the concept of a self-sustaining ROS producing system.

Tempol, whether given by acute intravenous infusion[22] or by a two week subcutaneous infusion[7] into rats with early 2K,1C renovascular hypertension reduced both the MAP and the RVR substantially. Oral administration of tempol for two[23] or five[24] weeks to rats with early 1K,1C renovascular hypertension also reduced the MAP. However, whereas we had found a reduced renal cortical pO_2 in the clipped kidney of early 2K,1C hypertensive rats, [7,17] the cortical pO_2 was maintained in the clipped kidney in the chronic model. The tissue oxygen availability is determined by the interplay between oxygen delivery and consumption. The latter is highly influenced by the tubular Na^+ transport[25] and therefore by the GFR. Although we did not measure the GFR, previous studies have established that there is a reduced GFR in the clipped kidney of chronic 2K,1C hypertensive rats that has been ascribed to a reduced renal perfusion pressure.[26] However, the absence of an atrophic stenotic kidney in this study implies that the increase in systemic pressure in these rats likely overcame the obstruction caused by the clip and that the blood perfusion distally to the mild or moderate obstruction must have been fairly well maintained. If so, it is also likely that the

GFR of the clipped kidney would have been substantially higher compared to previous reports.[26] A well maintained blood supply may also explain the near-normal pO_2 values in the kidney cortex of the clipped kidney in the present model.

In contrast to the kidney cortex, we detected a significantly reduced pO_2 in the outer medulla of the clipped kidneys of chronic 2K,1C hypertensive rats. This might contribute to progressive kidney dysfunction, as proposed in other models of hypoxia-induced kidney damage.[15,18] Therefore, it is important to elucidate the mechanisms involved and to identify potential interventions to restore the pO_2 in the medulla of the clipped kidney. The results from the present study show that tempol not only reduced the MAP, but effectively increased the blood flow and cortical and medullary pO_2 in the clipped kidney. These effects were not influenced by subsequent administration of an ACE inhibitor, which implies that tempol had corrected any effects due to ongoing Ang II generation, but the mechanism was not studied further in these experiments. It could entail a reduction by tempol of renal medullary $O_2^{\cdot -}$ with enhanced NO bioavailability which would increase blood flow and thereby oxygen delivery, and also increase the efficiency of mitochondria to produce ATP. NO reversibly inhibits mitochondrial respiration by competing for the binding site of oxygen.[27,28] This effect of NO is potentiated at the low pO_2 levels recorded in the medulla.

Chade *et al* reported that an acute intrarenal infusion of tempol into pigs fed a high fat diet and studied 12 weeks after a renal artery stenosis failed to improve the reduced levels of renal blood flow, cortical perfusion or GFR.[29] In contrast, we detected large increases in renal blood flow and cortical perfusion in rats studied at an even more prolonged stage of 2K,1C renovascular disease. It is possible that the ten-fold increase in low density lipoprotein cholesterol in the pigs fed a high fat diet, which were accompanied by extensive perivascular and tubulointestinal fibrosis and neovascularization which were not pronounced in the kidneys in our study, had limited the hemodynamic response to tempol in the hypercholesterolemic pig model.[30] Alternatively, the rather modest degree of renal artery stenosis which did not induce renal atrophy in our rat model may have provided an opportunity for a reduction in ROS to be apparent as an increase in blood flow and oxygenation.

Ang II induces NO release via AT_2-Rs within the clipped kidney in early 2K,1C and thereby sustains blood flow and pO_2 in that kidney.[17] Indeed, enalaprilat did reduce CBF modestly in the clipped kidney after candesartan, consistent with a residual role for AT_2-Rs in maintaining cortical flow, if not oxygenation in the chronic model. However, the selective AT_2-R blocker PD-123,319 had no effect on either MAP, CBF or kidney oxygenations, whereas the effects of enalaprilat persisted after blockade of the AT_2-Rs blockade. Therefore, we conclude that the involvement of AT_2-Rs is not very evident in the kidneys of rats with chronic 2K,1C. The reason for this change in the importance of the AT_2-Rs is presently unknown. In contrast to the AT_2-Rs, tempol retains its efficiency from the early to the chronic 2K,1C phase of modest hypertension in the rat.

The present study has some limitations, mainly relating to methodological difficulties. Due to the massive fibrosis resulting from the clip placed on the renal artery, it is very difficult to measure total kidney blood flow and split-kidney function in these rats. We therefore choose to use laser-Doppler methodology since the main focus of this study was to measure hemodynamic alterations occurring in response to acute drug administrations. The laser-Doppler technique is suitable for acute experiments, as described previously,[31] if care is taken to confirm each measurement location. However, the disadvantage is that the hemodynamic significance of the clip cannot be verified, as discussed further above. A second limitation was the apparently rather modest degree of renal artery stenosis engendered which in fact was accompanied by renal hypertrophy, rather than renal atrophy.

Furthermore, all animals were chronically treated with HHR throughout the course of the study to maximize survival of the clipped animals. It is also possible that only animals with only a modest renal artery restriction survived until the actuate experiments twelve months later and that this influenced the results of the present study. Although previous studies have shown that HHR indeed lowers blood pressure, this treatment has been shown to have only a marginal influence on the development of renal alterations such albuminuria, glomerolusclerosis, cytokine levels, renal blood flow response to blockade of the nitric oxide system, kidney oxygen tension and intrarenal levels of Ang II and angiotensinogen in several different models of hypertension-induced kidney damage including rats with spontaneous hypertension (SHR), 5/6 nephrectomized, deoxycorticosterone-acetate (DOCA) hypertension rats and 2K,1C.[9,32–37] These previous reports, together with the fact that the HHR therapy was discontinued 14 days before the acute experiments, indicate that this procedure should have no major influence on the results reported in the present study.

In conclusion, acute administration of tempol had superior antihypertensive efficiency and improvement of renal blood perfusion and oxygenation compared to an ARB in this model. Whether this will translate into differences in long-term renoprotection during ischemic nephropathy warrants further study.

Perspectives

Unilateral renal artery stenosis in man can lead to hypertension, renal atrophy and reduced renal function. Presently, no treatments have been found to prevent progressive kidney dysfunction in this setting. A controlled trial of renal artery angioplasty in patients with renal artery stenosis found no beneficial effects on glomerular filtration rate at one year.[38] Current pharmacologic therapy is equally unsatisfactory.[39] ACE inhibitors and ARBs are effective in reducing blood pressure, but can worsen renal insufficiency.[8] Chronic renal hypoxia has been considered an underlying cause of progressive CKD.[40] Thus, our finding that the acute administration of tempol is effective both in reducing the blood pressure and in improving the renal oxygenation in the clipped kidney at both the early[17] and the chronic phase suggests a possible role for drugs of this class in prevention or management of hypertension and renal insufficiency, at least in patients with modest degrees of renal artery stenosis. This warrants further study.

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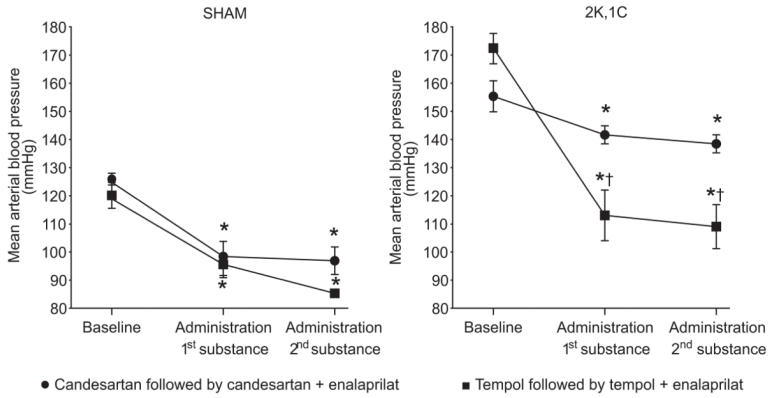

Figure 1.

Mean arterial blood pressure in anesthetized Sham and 2K,1C rats during baseline conditions and during the different acute interventions. *denotes $p<0.05$ when compared to baseline with the same group, and † denotes $p<0.05$ when compared to the candesartan, or the combined candesartan + enalaprilat treatment within the same category of animals. All values are mean ± SEM.

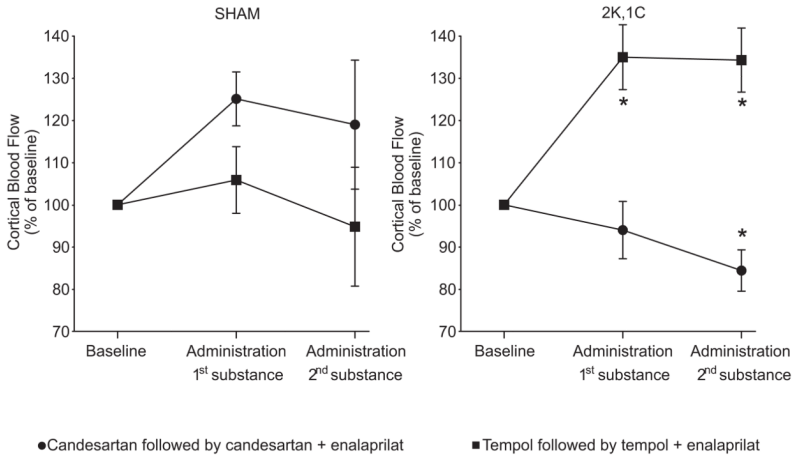

Figure 2.

Mean changes in renal cortical blood flow in Sham and 2K,1C rats during the different acute interventions. *denotes $p<0.05$ when compared to baseline with the same group, and † denotes $p<0.05$ when compared to the candesartan, or the combined candesartan + enalaprilat treatment within the same category of animals. All values are mean ± SEM.

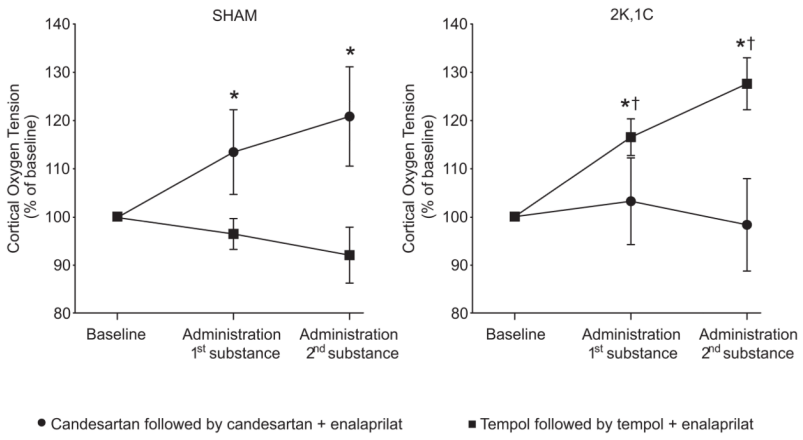

Figure 3.

Mean changes in renal cortical oxygen tension in Sham and 2K,1C rats during the different acute interventions. *denotes $p<0.05$ when compared to baseline with the same group, and † denotes $p<0.05$ when compared to the candesartan, or the combined candesartan + enalaprilat treatment within the same category of animals. All values are mean ± SEM.

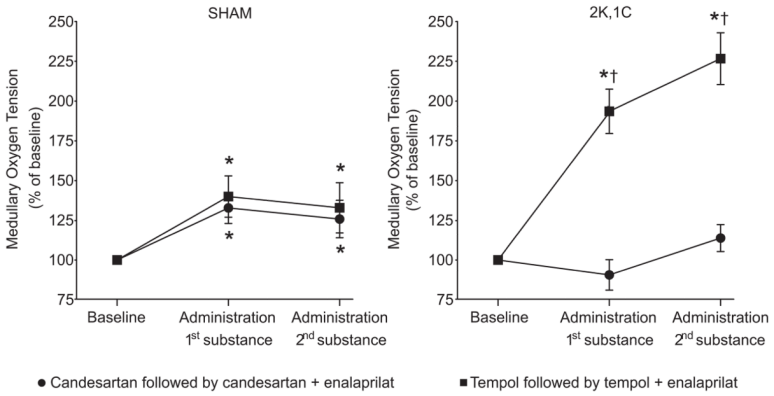

Figure 4.

Mean changes in renal medullary oxygen tension in Sham and 2K,1C rats during the different acute interventions. *denotes $p<0.05$ when compared to baseline with the same group, and † denotes $p<0.05$ when compared to the candesartan, or the combined candesartan + enalaprilat treatment within the same category of animals. All values are mean ± SEM.

Table 1

Body weights (BW), kidney weights (KW), and baseline kidney tissue pO_2 in sham or rats with a silver clip placed on the renal artery of the left kidney (2K,1C) prior to the different acute interventions.

Type	Intervention	N	BW (g)	KW/BW (x1000)		Ratio Kidney Size (Clipped/ Non-clipped)	Baseline Cortical pO_2 (mmHg)	Baseline Medullary pO_2 (mmHg)
				Non-clipped	Clipped			
Sham	Candesartan and Candesartan + enalaprilat	7	511±12	-	2.69±0.08	-	43±3	28±1
Sham	Tempol and Tempol + enalaprilat	7	492±16	-	2.65±0.09	-	47±2	28±2
2K,1C	Candesartan and Candesartan + enalaprilat	8	466±23	5.43±0.39†	3.55±0.63*	0.64±0.08	42±3	16±2*
2K,1C	Tempol and Tempol + enalaprilat	7	477±7	6.45±0.41†	4.53±0.30*	0.70±0.02	41±1	14±1*

All values are mean ± SEM.

*denotes p<0.05 when compared to corresponding Sham group, and

†denotes p<0.05 when compared to the clipped kidney within the same group.

Table 2

Response in mean arterial blood pressure (MAP), relative cortical blood flow (CBF) and kidney pO_2 to administration of the selective AT_2-R blocker PD-123,319 and the combination of PD-123,319 + enalaprilat in a separate group of 2K,1C rats.

Parameter	Baseline	After PD-123,319	After PD-123,319 + enalaprilat
MAP (mmHg)	162±5	155±5	133±5*
CBF (% of baseline)	100	101±11	88±9
Cortical pO_2 (% of baseline)	100	105±3.3	104±5
Medullary pO_2 (% of baseline)	100	96±11	91±17

All values are mean ± SEM of n=7.

* denotes $p<0.05$ when compared to baseline.