Renal responses to acute reflex activation of renal sympathetic nerve activity
and renal denervation in secondary hypertension

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Running title: Renal nerve activation in hypertension

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Abstract
We tested whether the responsiveness of the kidney to basal renal sympathetic nerve activity (RSNA) or hypoxia-induced reflex increases in RSNA, is enhanced in angiotensin-dependent hypertension in rabbits. Mean arterial pressure, measured in conscious rabbits, was similarly increased (+16±3 mmHg) four weeks after clipping the left (n=6) or right (n=5) renal artery, or commencing a subcutaneous angiotensin II infusion (n=9), but was not increased after sham-surgery (n=10). Under pentobarbital anesthesia, reflex increases in RSNA (51±7%) and whole body norepinephrine spillover (90±17%), and the reductions in glomerular filtration rate (-27±5%), urine flow (-43±7%), sodium excretion (-40±7%), and renal cortical perfusion (-7±3%) produced by hypoxia were similar in normotensive and hypertensive groups. Hypoxia-induced increases in renal norepinephrine spillover tended to be less in hypertensive (1.1±0.5 ng/min) than normotensive (3.7±1.2 ng/min) rabbits but basal overflow of endogenous and exogenous dihydroxyphenolglycol was greater. Renal plasma renin activity (PRA) overflow increased less in hypertensive (22±29 ng/min) than normotensive rabbits (253±88 ng/min) during hypoxia. Acute renal denervation did not alter renal hemodynamics or excretory function, but reduced renal PRA overflow. Renal vascular and excretory responses to reflex increases in RSNA induced by hypoxia are relatively normal in angiotensin-dependent hypertension, possibly due to the combined effects of reduced neural norepinephrine release and increased postjunctional reactivity. In contrast, neurally-mediated renin release is attenuated. These findings do not support the hypothesis that enhanced neural control of renal function contributes to maintenance of hypertension associated with activation of the renin/angiotensin system.

Abstract wordcount: 241

Key Words: Angiotensin II, hypertension renovascular, kidney circulation, norepinephrine, reflex, renin, sympathetic nervous system, water-electrolyte balance
Introduction

There is strong evidence that activation of the sympathetic nervous system contributes to the pathogenesis of essential hypertension (1, 11, 30). There is also evidence for a role of the sympathetic nervous system in development and maintenance of secondary hypertension. For example, depressor responses to ganglionic blockade and/or centrally acting sympatholytic agents are enhanced in hypertension induced by chronic infusion of angiotensin II in rats (27) and rabbits (5) and in two kidney, one clip (2K1C) hypertension (19). Furthermore, both muscle sympathetic nerve activity (SNA) and total body norepinephrine (NE) spillover are elevated in human renovascular hypertension (21). In hypertension induced by chronic infusion of angiotensin II, circulating angiotensin II is thought to activate SNA through actions on circumventricular organs (13). In 2K1C hypertension, an additional pro-hypertensive mechanism is thought to depend on activation of renal afferent nerves, which mediate increased SNA (49). On the other hand, renal SNA (RSNA) in conscious rabbits has been observed to be reduced during the first week of hypertension induced by chronic angiotensin II infusion (2) and 3 weeks after induction of 2K1C hypertension (18), although it had returned to control levels after 6 weeks of 2K1C hypertension (18). Moreover, renovascular hypertension is often associated with depletion of neuronal NE stores in the kidney (15). Thus, the contribution of changes in renal sympathetic nervous system function to the pathogenesis of secondary hypertension remains a matter of controversy.

A role of the renal nerves in the pathogenesis of renovascular hypertension and hypertension induced by chronic infusion of angiotensin II is supported by the observation that these forms of hypertension can be delayed or ameliorated by renal denervation (22, 48). While many studies have investigated the possibility that RSNA might be altered in these secondary forms of hypertension (see above), little attention has been paid to the possibility that the responsiveness of the kidney to RSNA is altered. This represents an important gap in our knowledge, since neural control of renal function depends both on the level of RSNA and the responsiveness of the kidney to a given level of RSNA. In turn, the responsiveness of the kidney to RSNA will depend on the level of neurotransmitter release for a given level of RSNA, the efficiency of mechanisms that remove the neurotransmitter from the biophase (eg neuronal NE re-uptake), and the responsiveness of multiple renal effector mechanisms to given levels of neurotransmitter bioavailability. We recently investigated these factors in anaesthetized rabbits (3). Electrical stimulation of the renal nerves increased renal NE spillover and the renal overflow of plasma renin activity (PRA), and reduced total renal blood flow, cortical perfusion, glomerular filtration rate, urine flow and sodium excretion. We found no evidence that these responses were enhanced in the kidney in rabbits made hypertensive by a chronic infusion of angiotensin II, or in either the clipped or non-clipped kidney
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An important caveat must be applied to our recent findings described above. Electrical stimulation of the renal nerves does not replicate the natural bursting pattern of endogenous RSNA (32). The pattern of bursts in RSNA may encode important information that influences how target organs respond to endogenous SNA (32). Furthermore, functionally specific nerves may be differentially recruited under different physiological conditions (6). Consequently, our studies using electrical stimulation of the renal nerves did not allow us to test the hypothesis that the responsiveness of renal neuroeffector mechanisms to endogenous RSNA is enhanced in 2K1C hypertension and hypertension induced by chronic infusion of angiotensin II. In the current study we tested this hypothesis by determining the effects of reflex activation of the renal nerves, and renal denervation, on kidney function in anesthetized hypertensive and normotensive rabbits from the same cohort as our previous study (3). We compared responses in the kidney of normotensive rabbits, to those of both the clipped and non-clipped kidney in 2K1C hypertension, and the kidney of rabbits with hypertension induced by chronic infusion of angiotensin II. This experimental paradigm provides the opportunity for the chronic effects of angiotensin II to be dissected from the effects of unilateral renal artery stenosis on the clipped and non-clipped kidney (3). Reflex activation of the renal nerves was induced by hypoxia, the renal effects of which in rabbits are dependent on intact renal nerves (26, 31). To identify the level of potential changes in renal sympathetic neuroeffector function we measured RSNA, renal and whole body NE kinetics, and the various renal parameters under sympathetic control. These parameters included regional renal hemodynamics, renal excretory function, and the renal overflow of PRA.

**Methods**

*Animals and experimental design*

Thirty New Zealand White rabbits (mean weight 2.82 ± 0.03 kg when they entered the study) were meal fed and provided with water *ad libitum*. All experiments were conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*, and were approved in advance by the Animal Ethics Committee of the Department of Physiology, Monash University. In the terminal experiment, performed under general anesthesia (see below), we characterized renal sympathetic neuroeffector function in normotensive rabbits, and in rabbits with
2K1C hypertension and hypertension induced by chronic infusion of angiotensin II. In the current report we present data under control conditions, during reflex activation of RSNA (hypoxia), and after renal denervation.

Baseline mean arterial pressure (MAP) was monitored in conscious rabbits via an ear artery catheter over a 1 h period and an arterial blood sample was collected for measurement of PRA (38). Rabbits were then randomized to 4 experimental groups. In three groups, a surgical procedure was performed to induce hypertension (see below). The fourth group of rabbits received sham surgery, and so served as the control group for the entire experiment. MAP and PRA were again measured 4 weeks after the surgical procedure in conscious animals. Between 4 and 6 weeks after surgery, the animals underwent a terminal experiment under pentobarbital anesthesia, for assessment of renal sympathetic neuroeffector function.

Surgical procedures to induce hypertension

Rabbits were anesthetized with halothane (Fluothane, AstraZeneca, Cheshire, UK) after induction by intravenous propofol (10 mg/kg; Sandoz, North Ryde, NSW, Australia) and endotracheal intubation. A retroperitoneal incision was made in either the left or right flank and a silver clip (gap size 0.4 - 0.6 mm) was fitted around the left (n = 6) or right (n = 5) renal artery. Because the left kidney was studied in the terminal experiment (see below), these groups allowed us to study both the clipped and non-clipped kidney in 2K1C hypertension. The sham group of animals (n = 10) underwent a similar procedure but the clip was removed immediately. For chronic infusion of angiotensin II (n = 9), a 28 day osmotic minipump (Alzet Model 2ML4, Durect Corp., Cupertino, CA) was implanted subcutaneously between the shoulder blades. The minipump was filled with angiotensin II at a concentration to deliver a dose of 20-50 ng kg\(^{-1}\)min\(^{-1}\). The dose of angiotensin II was varied between rabbits with the aim of matching the range of changes in MAP in this group to that in the groups of rabbits with 2K1C hypertension. To ensure continuous peptide delivery, the minipump was replaced, under local anesthesia (lidocaine HCl 1%, Delta West, WA, Australia), approximately 22 days later.

Terminal experiment

Rabbits were anesthetized with pentobarbital sodium (90-150 mg plus 30-50 mg/h; Sigma Chemical Company, St Louis, MO), intubated, and artificially ventilated. Extracellular fluid volume was maintained by intravenous infusion (0.18 ml kg\(^{-1}\)min\(^{-1}\)) of a 4:1 mixture of compound sodium lactate and polygeline/electrolyte solution (26). The left kidney was exposed via a flank incision and placed in a stabilized cup secured to the operating table. A catheter was placed in the iliolumbar
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vein and advanced so that its tip lay in the left renal vein, 1-2 cm from its junction with the vena cava (4) and a catheter was placed in the left ureter to facilitate collection of urine produced by the left kidney. The (left) renal nerves then were isolated and placed across a recording electrode. A needle-type laser-Doppler flow probe (26 gauge, DP4s, Moor Instruments Ltd., Millwey, Devon, UK) was advanced approximately 9 mm into the kidney (inner medulla (17)) to measure medullary laser Doppler flux (MLDF), an index of renal medullary perfusion. A standard plastic laser-Doppler flow probe (DP2b, Moor Instruments) was placed on the dorsal surface of the kidney to measure cortical laser Doppler flux (CLDF), an index of cortical perfusion. After completion of all surgical procedures [14C]-inulin (bolus dose of 10 µCi plus 45 nCi kg⁻¹ min⁻¹, Perkin Elmer Life Sciences, Boston, MA, USA) and ring labelled [3H]-NE (90 nCi kg⁻¹ min⁻¹; Perkin Elmer) were administered intravenously. The experimental protocol commenced after a 60 min equilibration period, during which ventilation rate was adjusted so that arterial blood PO₂ was 90 to 110 mmHg.

Experimental protocol

Rabbits were studied over four 20 min periods, initially during ventilation with room air, then during hypoxia (10% O₂ and 90% N₂) and again during ventilation with room air. The final experimental period was conducted after the renal nerves were sectioned. Urine produced by the left kidney was collected during the final 15 min of each period. At the mid-point of each urine collection period, samples of arterial blood (1 ml each) were taken for measurement of (i) [14C]-inulin and sodium concentrations, (ii) PRA and (iii) NE kinetics. A 0.3 ml sample of arterial blood was also taken for determination of arterial blood gas status (ABL510; Radiometer, Copenhagen, Denmark). Simultaneously, 1 ml samples were taken from the renal vein for measurement of (i) PRA and (ii) NE kinetics. Blood was replaced with that from a donor rabbit, mixed with resuspended erythrocytes from previous blood samples.

In order to calculate renal NE spillover and PRA overflow, we required an estimate of total renal blood flow (RBF). RBF was not measured directly during the experimental period, to avoid the possibility of damaging the renal nerves by placement of a perivascular flowprobe. Instead, it was estimated by calibrating CLDF to RBF measured at the end of the experimental protocol. For this, a transit-time ultrasound flow probe (type 2SB, Transonic Systems Inc, Ithaca, NY) was placed around the renal artery to allow direct measurement of RBF. The nerves were then stimulated electrically at 0.5, 1, 2, 4 and 8 Hz to allow the relationship between CLDF and RBF to be determined in each rabbit. A detailed description of the renal responses to electrical stimulation of the renal nerves is published elsewhere (3).
Recording of hemodynamic variables

Signals were processed and acquired digitally as previously described (9) to provide 2s averages of MAP (mmHg), heart rate (HR, determined from the arterial pressure pulse; beats/min), RBF (ml/min), CLDF (perfusion units), and MLDF (perfusion units). The values of CLDF (6.2 ± 0.4 units) and MLDF (15.0 ± 0.9 units), during the 60 s immediately after the rabbit was humanely killed by overdose with pentobarbital (300 mg), were subtracted from the values obtained during the experiment, before data analysis was performed. Postganglionic RSNA was processed through a low-noise differential preamplifier and amplifier combination (Baker Heart Research Institute Models 187b and 133) with bandwidth 50 Hz to 1 kHz. Amplified potentials were rectified and integrated using an integrator filter with a 20 ms time constant. RSNA was normalized relative to the initial control period (100 normalized units) and the frequency of neural bursts was also determined by counting those that exceeded a threshold set at 20% of the maximum burst amplitude.

The relationships between RBF and CLDF, in each rabbit, were determined by fitting individual lines of best fit relating CLDF to RBF during electrical stimulation of the renal nerves. Because both RBF and CLDF were dependent variables, the least products method was used for calculation of lines of best fit (28). RBF was then calculated from CLDF using the equation describing the line of best fit (RBF = (CLDF – a)/b) for each individual rabbit. The validity of this approach is evidenced by the strong correlation between RBF and CLDF ($r^2 = 0.97$; P < 0.001, Fig. 1). Across all rabbits, the 95% confidence limits of ‘a’ (x-intercept; 5.7 CLDF units) included zero (-7.3 to 18.7 units), indicating no fixed bias between RBF and CLDF. The value of ‘b’ was 10.1 CLDF units with 95% confidence limits of 9.2 and 10.9.

Analysis of blood, urine and tissue samples

Hematocrit was determined by the capillary tube method. Blood for PRA determination was collected in chilled tubes containing 2,3-dimercaptopropanol-EDTA (Sigma), centrifuged at 4 °C and 3,000 rpm for 10 minutes, and the plasma stored at -70 °C for subsequent analysis. The rate at which angiotensin I was generated per 60 min incubation period was measured via radioimmunoassay (38). Renal PRA overflow was calculated as the product of renal plasma flow (ml/min) and the arteriovenous difference in PRA (ng/ml) and so is given in units of ng/min. Urine volume was measured gravimetrically, and aliquots were stored at -20º C for subsequent analysis. [$^{14}$C]-inulin concentrations in plasma and urine were determined by liquid scintillation counting (Model LS500TA; Beckman-Coulter, Gladesville, NSW, Australia), while sodium concentrations
were determined by atomic absorption spectrophotometry (Avanta, GBC Scientific Equipment, Dandenong, Victoria, Australia). Glomerular filtration rate (GFR) was determined as the clearance of $[14C]$-inulin.

Catecholamines were extracted from plasma using alumina adsorption and quantified using HPLC with colorimetric detection as previously described (34). Whole body (total) and renal NE spillover were calculated as:

$$\text{Total NE spillover} = \text{NE clearance} \times \text{NEA}$$

where NE clearance = $[^3\text{H}]$NE infusion rate/arterial $[^3\text{H}]$NE concentration and NEA is arterial plasma NE concentration.

$$\text{Renal NE spillover} = [(\text{NERV} - \text{NEA}) + (\text{NEA} \times \text{EX}[^{3}\text{H}]\text{NE}])] \times \text{renal plasma flow}$$

where NERV is NE concentration in the renal vein and EX$[^3\text{H}]$NE is fractional extraction of $[^3\text{H}]$NE across the kidney.

Neuronal NE re-uptake in the kidney was estimated by measuring concentrations of endogenous dihydroxyphenolglycol (DHPG) and $[^3\text{H}]$DHPG overflowing into the renal venous effluent (8). DHPG is the intraneuronally produced metabolite of NE. The renal extraction of dihydroxyphenylalanine (DOPA), the precursor of NE, was also determined. DHPG overflow into the renal vein was calculated according to the following formula:

$$\text{Renal DHPG overflow} = (\text{DHPG}_{RV} - \text{DHPG}_{A}) \times \text{RBF}$$

where DHPG$_{RV}$ is the DHPG concentration in the renal vein and DHPG$_{A}$ is the arterial plasma DHPG concentration.

$[^3\text{H}]$DHPG renal production was calculated by the formula:

$$[^3\text{H}]\text{DHPG renal production} = ([^3\text{H}]\text{DHPG}_{RV} - [^3\text{H}]\text{DHPG}_{A}) \times \text{RBF}$$

where $[^3\text{H}]$DHPG$_{RV}$ and $[^3\text{H}]$DHPG$_{A}$ are the concentrations of $[^3\text{H}]$DHPG in the renal venous and arterial plasma respectively.

Renal DOPA extraction was calculated using the formula:

$$\text{Renal DOPA extraction} = (\text{DOPA}_{A} - \text{DOPA}_{RV}) \times \text{renal plasma flow}$$

where DOPA$_{A}$ and DOPA$_{RV}$ are concentrations of DOPA in the arterial and renal venous plasma respectively.

Left ventricles and kidneys were removed post-mortem and the wet weights of these tissues were expressed per kg body weight.

**Statistical Methods**

Data are expressed as mean ± SEM. Hemodynamic variables and RSNA are presented as the average over the 15 min renal clearance periods, unless otherwise stated. Arterial blood PO$_2$, NE
kinetics and PRA overflow data were derived from the blood samples collected at the mid-point of the 15 min clearance periods. Data were subjected to split-plot repeated measures analysis of variance, partitioned to test specific hypotheses (18). Two sided \( P < 0.05 \) was considered statistically significant. We tested, in a within-group fashion, whether variables had changed from their basal level by 4 weeks after surgery (\( P_{\text{basal}} \)), and whether the measured variables were altered by hypoxia (\( P_{\text{hypoxia}} \)) and renal denervation (\( P_{\text{den}} \)). We also tested, in a between-group fashion, whether baseline variables and responses to hypoxia and renal denervation differed in the specific groups of hypertensive rabbits compared to sham operated control rabbits (\( P_{\text{sham}} \)), differed in a systematic manner between all hypertensive rabbits compared to sham operated rabbits (\( P_{\text{sham vs all HT}} \)), and differed between the various groups of hypertensive rabbits (\( P_{\text{HT}} \)). Type 1 error was controlled with Bonferroni and Greenhouse-Geisser corrections (29).

**Results**

**Development of hypertension in conscious rabbits**

Before surgery, conscious MAP, HR, hematocrit and PRA were respectively 79 ± 1 mmHg, 178 ± 4 bpm, 38.3 ± 0.5% and 3.8 ± 0.4 ng/ml in the 30 rabbits we studied. These variables did not differ systematically between the four groups. Four weeks later, values in sham-operated rabbits had not altered significantly (\( P_{\text{basal}} \geq 0.05 \)). In contrast, MAP had increased by 14 ± 1 mmHg four weeks after placing a clip on the left renal artery (\( P_{\text{basal}} = 0.007 \)), 11 ± 3 mmHg four weeks after placing a clip on the right renal artery (\( P_{\text{basal}} = 0.05 \)) and 20 ± 5 mmHg after 4 weeks of angiotensin II infusion (\( P_{\text{basal}} < 0.001 \); Table 1). Arterial PRA was significantly reduced after 4 weeks of angiotensin II infusion (by 1.5 ± 0.7 ng/ml, \( P_{\text{basal}} < 0.001 \)) but was not significantly different from its basal level 4 weeks after placing a clip on the left or right renal artery (\( P_{\text{basal}} \geq 0.05 \); Table 1). Thus, by 4 weeks after surgery, PRA was markedly less in rabbits with angiotensin-induced hypertension than in sham-operated rabbits, but similar in 2K1C rabbits and sham-operated rabbits (Table 1). Hematocrit was greater in hypertensive rabbits than normotensive rabbits by 4 weeks after surgery (Table 1).

**Baseline variables in anesthetized rabbits**

The terminal experiment was conducted 4 - 6 weeks (average 4.7 ± 0.1 weeks) after surgery. Baseline values of MAP, during the 20 min control period before hypoxia, tended to be greater in the hypertensive groups than in sham-operated controls, but these apparent effects did not reach
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Renal excretory variables including GFR, urine flow and Na⁺ excretion were 58%, 90% and 90% less respectively, in the clipped kidney of the 2K1C rabbits than in sham-operated rabbits (Table 2). Arterial PRA, renal venous PRA and renal PRA overflow were significantly less in angiotensin II treated rabbits than in sham-operated rabbits (Table 2).

Renal NE extraction and renal NE spillover both tended to be less in the clipped kidney of 2K1C rabbits than in sham-operated rabbits (-22% and -79%, respectively, both \( P_{\text{Sham}} = 0.09 \)). However, whole-body (total) NE spillover was indistinguishable between the 4 groups (Table 3). The renal venous-arterial DHPG concentration gradient and renal DHPG overflow were both markedly greater in rabbits treated with angiotensin II than in sham-operated rabbits or both groups of 2K1C rabbits (Table 3). Similarly, consistent with an increased uptake and subsequent intraneuronal metabolism of NE, renal \(^{3}\text{H}\)DHPG production was markedly greater in rabbits with angiotensin-induced hypertension than in sham-operated rabbits. There was net renal DOPA extraction in all but the clipped kidney in 2K1C rabbits (Table 3).

To examine the stability of our experimental preparation, we compared parameters from the control period (before hypoxia) with those from the period of normoxia following hypoxia. None of the variables in Table 2 differed significantly between the two experimental periods of normoxia, in any group. Therefore, to increase statistical power, data from these two experimental periods were pooled for analysis of the effects of hypoxia. That is, we compared the levels of variables during hypoxia to their mean level averaged over the periods of normoxia before and after hypoxia. There were residual changes in NE kinetics after hypoxia, so levels of NE kinetic variables during hypoxia were compared with those during the initial control period only.

Effects of hypoxia

When averaged across all 30 rabbits, arterial \( PO_2 \) was 103 ± 2 mmHg during the control period of ventilation with room air, and fell to 32 ± 2 mmHg during ventilation with 10% \( O_2 \). During the subsequent recovery period of normoxia, arterial \( PO_2 \) returned to control levels (103 ± 2 mmHg). Arterial \( PO_2 \) measurements across the course of the experiment were indistinguishable in the 4 groups of rabbits, indicating that the hypoxic stimulus was similar in each group. Hypoxia did not significantly alter arterial \( PCO_2 \) or pH.

Hypoxia evoked an abrupt and sustained increase in RSNA but little change in MAP (Fig. 2). When averaged across all 4 groups, RSNA increased by 51 ± 7% (\( P_{\text{hypoxia}} < 0.001 \), Fig. 3). Renal NE spillover also increased during hypoxia (49 ± 16%; \( P_{\text{hypoxia}} = 0.009 \)) as did whole-body NE spillover
(90 ± 17%; \( P_{\text{hypoxia}} < 0.001 \)). Although the increases in RSNA and whole-body NE spillover were similar in all groups, the hypoxia-induced increase in renal NE spillover in sham-operated rabbits (3.7 ± 1.2 ng/min) was more than double that in the hypertensive groups (average of all hypertensive rabbits 1.1 ± 0.5 ng/min). However, this apparent effect did not reach statistical significance (\( P_{\text{sham vs all HT}} = 0.07 \); Fig. 3). In response to hypoxia, DHPG overflow increased in sham-operated rabbits, was reduced in rabbits with angiotensin-induced hypertension, and changed little in either the clipped or non-clipped kidney in 2K1C hypertension (Fig. 4).

The effect of hypoxia to reduce CLDF was greatest between 3 and 5 minutes after the onset of the stimulus (-16 ± 3%; \( P_{\text{hypoxia}} < 0.001 \)) but was considerably less during the 15 min clearance period that followed (-7 ± 3%) (Figs 2 and 5). MLDF was also reduced during the initial period (-13 ± 4%; \( P_{\text{hypoxia}} = 0.04 \)) but during the 15 min renal clearance period during hypoxia MLDF was on average greater (by 9 ± 4%; \( P_{\text{hypoxia}} = 0.003 \)) than during normoxia (Figs 2 and 5). Responses of CLDF and MLDF to hypoxia were indistinguishable in hypertensive compared with normotensive rabbits. Although initial reductions in CLDF appeared to be less in the clipped kidney in 2K1C hypertension than in the kidney of sham-operated rabbits, this apparent effect was not statistically significant (\( P_{\text{sham}} = 0.2 \); Figs 2 and 5).

Hypoxia was associated with reductions in GFR, urine flow and Na+ excretion (-28 ± 5%, -43 ± 7% and -40 ± 7%, respectively; \( P_{\text{hypoxia}} < 0.001 \); Fig. 6). None of these effects on renal excretory function was significantly different in hypertensive rabbits compared with sham-operated controls. PRA of arterial and renal venous blood increased during hypoxia (39-71% and 52-77% respectively; \( P_{\text{hypoxia}} < 0.001 \)), as did renal PRA overflow (\( P_{\text{hypoxia}} = 0.006 \), Fig. 7). Hypoxia induced a 253 ± 87 ng/min increase in PRA overflow in the kidney of sham-operated rabbits, but markedly smaller increases in the kidneys of hypertensive rabbits (average of all hypertensive rabbits 22.4 ± 28.7 ng/min; \( P_{\text{sham vs all HT}} = 0.01 \); Fig. 7).

**Effects of renal denervation**

Following the post-hypoxia recovery period, the left renal nerves were sectioned. This virtually abolished renal NE spillover, which averaged 0.9 ± 0.5 ng/min across all rabbits during the period after renal denervation, but did not significantly alter whole-body NE spillover. When averaged across all rabbits, renal denervation was associated with significant reductions in PRA overflow but no significant changes in renal hemodynamic and excretory variables. The reductions in both renal venous PRA and arterial PRA after renal denervation tended to be less in hypertensive compared with normotensive rabbits (\( P_{\text{sham vs all HT}} = 0.04 \) and 0.08, for venous and arterial PRA respectively),
although these effects only reached statistical significance in the case of rabbits with angiotensin-induced hypertension (Fig. 8).

**Post mortem measurements**

The average weight of the left ventricle from the hypertensive groups (1.50 ± 0.03 g/kg) was significantly greater than that of the sham-operated group (1.34 ± 0.04 g/kg; P_{sham vs all HT} < 0.001). The average weight of the kidneys was similar in sham-operated (3.34 ± 0.09 g/kg) and angiotensin II-treated (3.42 ± 0.07 g/kg) rabbits. However, in 2K1C hypertensive rabbits the clipped kidney had atrophied (2.61 ± 0.25 g/kg; P_{sham} < 0.001) and the non-clipped kidney had hypertrophied compared to sham-operated rabbits (3.99 ± 0.18 g/kg; P_{sham} = 0.001).

**Discussion**

The major findings of the current study were that hypoxia-induced increases in RSNA and reductions in renal cortical perfusion, glomerular filtration rate, urine flow, and sodium excretion, were similar in hypertensive and normotensive rabbits. Surprisingly, we also found that hypoxia-induced increases in renal NE spillover tended to be less in hypertensive rabbits than normotensive rabbits, despite similar increases in RSNA. Furthermore, basal renal DHPG overflow and [3H]-DHPG production were greater in rabbits with hypertension induced by chronic infusion of angiotensin II than in normotensive rabbits, suggestive of enhanced neuronal re-uptake and metabolism of NE. These findings suggest that hypoxia-induced increases in RSNA are relatively normal in these angiotensin-dependent forms of secondary hypertension, but that less NE may be released from post-ganglionic renal sympathetic nerves. Given that vascular and excretory responses were normal, it may be that post-junctional responsiveness is enhanced in these forms of hypertension. We also found that neurally-mediated renal renin release was markedly inhibited in hypertensive rabbits, possibly reflecting a dominance of local feedback inhibition from elevated levels of angiotensin II. Thus, our current findings indicate that the overall responsiveness of the kidney to basal levels of RSNA and reflex increases in RSNA is normal, with the exception that neurally-mediated release of renin is suppressed. Our data do not, therefore, provide support for the hypothesis that the increased contribution of the sympathetic nervous system to this form of hypertension is mediated through enhanced renal sympathetic neuroeffector function.

Our observations of the effects of hypoxia are consistent with our previously reported observations, in the same cohort of rabbits, of the effects of electrical stimulation of the renal nerves (3). Thus,
responses of total RBF, cortical perfusion, urine flow and sodium excretion to electrical stimulation of the renal nerves were not greater in hypertensive rabbits than normotensive rabbits, and increases in PRA overflow were greatly blunted in secondary hypertension. However, we did find that reductions in medullary perfusion, induced by electrical stimulation of the renal nerves, were enhanced in the hypertensive rabbits relative to the normotensive rabbits (3). The role of the renal medullary circulation in long-term control of arterial pressure is well established (33). Thus, we hypothesised that enhanced sensitivity of medullary perfusion to neurally-evoked vasoconstriction might contribute to the pathogenesis of these forms of secondary hypertension (3). However in the current study, hypoxia only induced small transient reductions in medullary perfusion, despite relatively large increases in RSNA. Thus we are unable to make any meaningful comparisons between hypertensive and normotensive rabbits. This was to be expected since previous studies have shown medullary blood flow to be relatively unresponsive to this degree of hypoxia (26). Therefore, the possibility that enhanced responsiveness of the medullary circulation to endogenous RSNA contributes to the development of 2K1C hypertension and hypertension induced by chronic infusion of angiotensin II merits further investigation.

As we have shown previously (26), hypoxia induced by ventilation with 10% O2 increased left kidney RSNA approximately 50%. This response was similar in the clipped and non-clipped kidney in 2K1C hypertension, the kidney in angiotensin-dependent hypertension, and in the kidney of normotensive rabbits. Thus, hypoxia-induced activation of RSNA appears not to be enhanced in these forms of secondary hypertension. In contrast, we previously demonstrated increased responsiveness of RSNA to air-jet stress and noise stress in rabbits with 2K1C hypertension (19). However, air-jet stress and noise stress are accompanied by changes in MAP, so responses of RSNA will depend both on the direct effects of the stimulus and the effects of altered baroreceptor input, which might differ in normotensive and hypertensive rabbits. In contrast, hypoxia did not alter MAP in our current and previous (26, 31) studies, so avoids the potentially confounding effects of changes in baroreceptor function. The systemic hemodynamic response to hypoxia reflects the balance of vasoconstriction chiefly due to reflex increases in sympathetic drive, and local vasodilator effects mediated by the direct effects of hypoxia (23). The absence of a pressor response to hypoxia in our current study, in the face of increased RSNA and both renal and whole body NE spillover, likely reflects the fact that these two opposing effects are equally balanced under the present experimental conditions. Importantly, the responses of systemic and renal hemodynamics and RSNA to hypoxia in pentobarbital anesthetized rabbits closely resemble those in conscious rabbits (26, 31). Furthermore, the renal responses to hypoxia are completely dependent on the presence of intact renal nerves. Thus, the fact that increases in RSNA of similar magnitude


were seen in the four groups of rabbits in the current study provides a basis for valid between-group comparisons of renal sympathetic neuroeffector function.

Although hypoxia increased RSNA similarly in the 4 groups of rabbits, the associated increase in renal NE spillover tended to be less in the 3 groups of hypertensive rabbits than in normotensive controls ($P_{\text{Sham vs all HT}} = 0.07$). Consistent with this finding, the renal overflow of DHPG, the deaminated, intraneuronally produced metabolite of NE, increased in response to hypoxia in normotensive rabbits but not in hypertensive rabbits. In contrast, $[^3H]-\text{DHPG}$ production (an index of neuronal re-uptake) did not change significantly during hypoxia. Thus, the blunted increase in renal NE spillover during hypoxia in hypertensive rabbits appears to be attributable to a diminished increase in NE release from renal sympathetic nerves rather than increased neuronal re-uptake. These observations accord with the finding of depletion of neuronal NE stores in the kidney in renovascular hypertension (15), although in the case of angiotensin-dependent hypertension, there is both anatomical and functional evidence of increased renal innervation (39). Blunted hypoxia-induced NE spillover appears to be specific to the kidney, since hypoxia-induced increases in total NE spillover were not significantly different in hypertensive compared with normotensive rabbits.

It also appears to be specific for reflex activation of RSNA, since we could not detect differences, between normotensive and hypertensive rabbits from the same cohort, in increases in NE spillover and DHPG overflow induced by electrical stimulation of the renal nerves (3). The mechanisms underlying the apparent attenuation in hypertensive animals, of NE release in response to hypoxia-induced increases in RSNA, remain a matter of speculation. It is unlikely to be mediated through direct actions of angiotensin II at AT$_1$-receptors, since AT$_1$-receptor activation enhances NE release from renal sympathetic nerves (45) and AT$_1$-receptor blockade attenuates increases in NE overflow induced by electrical stimulation of the renal nerves in anesthetized dogs (47).

Despite the fact that hypoxia-induced increases in renal NE spillover tended to be blunted in hypertensive rabbits, renal vascular and excretory responses to hypoxia were similar in hypertensive and normotensive rabbits. This could possibly reflect increased sensitivity of vascular and tubular elements to NE, perhaps through direct actions of angiotensin II on vascular smooth muscle cells (40), removal of the counter-regulatory actions of nitric oxide through angiotensin II-induced oxidative stress (42), or changes in intracellular signalling mechanisms. Indeed, increased sensitivity to constrictor effects of $\alpha$-adrenoceptor agonists have been observed \textit{in vivo} in renal hypertensive rabbits and rats (5, 14). However, counter to this hypothesis, angiotensin-induced hypertension in rats is not associated with enhanced vasoconstriction of isolated renal afferent arterioles (20) or arcuate arteries (39) to adrenoceptor agonists.
Hypoxia-induced increases in renal PRA overflow were markedly attenuated in hypertensive compared to normotensive rabbits. Reductions in renal venous PRA after renal denervation were also blunted in hypertensive rabbits. This latter observation is consistent with the finding that β-blockade reduces PRA less in patients with renovascular hypertension than in those with essential hypertension or in normotensive controls (44). Collectively, these observations suggest that negative feedback inhibition by angiotensin II largely over-rides neural stimulation of renin release. This likely applies in 2K1C hypertension even though arterial PRA was not significantly elevated in the rabbits we studied, since 2K1C hypertension is characterized by increased activity of the intrarenal renin/angiotensin system (35). Le Fevre et al recently demonstrated that renin release contributes to the renal vasoconstriction, antidiuresis and antinatriuresis produced by low frequency (1 Hz) electrical stimulation of the renal nerves in rabbits (24). Thus, the attenuated hypoxia-induced increase in PRA overflow we observed in hypertensive rabbits may act to attenuate the accompanying renal vasoconstriction, antidiuresis and antinatriuresis.

Differences in the levels of the various parameters we measured under baseline conditions under anesthesia, between the 4 groups of rabbits, were mostly as expected. GFR, urine flow and sodium excretion were less in the clipped kidney in 2K1C hypertensive rabbits than in the left kidney of normotensive rabbits. Renal hemodynamics and excretory function in rabbits with angiotensin-dependent hypertension were indistinguishable from normotensive rabbits. Renal PRA overflow was less in hypertensive rabbits than normotensive rabbits. These data are consistent with our observation that arterial PRA was no greater in conscious 2K1C hypertensive rabbits than normotensive rabbits, which is in turn consistent with previous observations in rabbits and other species (16). Our finding that both renal NE extraction and spillover tended to be less in the clipped kidney in 2K1C hypertension likely reflects the reduced blood flow in the clipped kidney, since renal NE spillover and clearance are blood flow dependent (10).

Basal renal DHPG overflow and [$^3$H]DHPG production were markedly greater in rabbits with hypertension induced by chronic infusion of angiotensin II than in normotensive rabbits, suggesting enhanced neuronal re-uptake and NE metabolism. This effect could be due to increased neuronal re-uptake and NE metabolism in individual neurones, and/or increased neuronal mass. It may be that both effects are involved, since angiotensin II has been shown to acutely enhance NE transporter activity and monooamine oxidase activity in cultured neurons (46), and chronic angiotensin II infusion in rats can increase sympathetic innervation density in the kidney (39). Increased basal RSNA would also be expected to enhance neuronal NE re-uptake (7), although our current experiment was unable to detect significant effects of chronic infusion of angiotensin II on basal
Renal nerve activation in hypertension

RSNA, and basal NE spillover was similar in angiotensin II-treated and sham rabbits. Nevertheless, it is tempting to speculate that basal sympathetic tone might be relatively normal in angiotensin II-induced hypertension due to the competing influences of increased RSNA and increased neuronal uptake and NE metabolism.

Comparable increases in conscious MAP were achieved 4 weeks after placing a clip on the left or right renal artery, or commencing a subcutaneous infusion of angiotensin II. We studied renal sympathetic neuroeffector function in anesthetized animals, because of the technical difficulties associated with maintenance of a renal venous catheter in conscious rabbits. As has been observed previously (41), differences in basal MAP between normotensive and hypertensive rabbits were largely lost under pentobarbital anaesthesia. This aids interpretation of our experiment by minimizing potentially confounding effects, on renal hemodynamics and excretory function, of between-group differences in renal perfusion pressure (12). On the other hand, one might expect that the relatively greater depressor effect of pentobarbital anesthesia in the groups of hypertensive rabbits compared with normotensive controls might lead to differential unloading of arterial baroreceptors and so differential effects on basal sympathetic drive. However, this seems not to be the case, since both basal total NE spillover and renal NE spillover (except for the clipped kidney in 2K1C hypertension) were similar in hypertensive and normotensive rabbits. Furthermore, because our experiment focussed on the functions of post-ganglionic sympathetic nerves and renal sympathetic neuroeffector mechanisms, effects of pentobarbital anesthesia and acute surgical trauma on central nervous system control of the circulation would be expected to have little impact on the interpretation of our findings. Importantly, increases in RSNA and reductions in renal perfusion induced by hypoxia are of a similar magnitude in conscious and pentobarbital anesthetized rabbits (26, 31). Moreover, both whole body and renal norepinephrine spillover in the anesthetized rabbits we studied were similar to values reported previously in conscious rabbits (36, 37, 43).

To avoid damaging the renal nerves by placement of a perivascular flow probe on the renal artery, we measured regional kidney blood flow by laser Doppler flowmetry. But to calculate renal NE spillover and PRA overflow, we required an estimate of total RBF. Therefore, at the end of the experiment we measured RBF by transit-time ultrasound flowmetry and induced renal vasoconstriction by graded electrical stimulation of the renal nerves. This allowed us to ‘back-calibrate’ CLDF against RBF individually in each animal. As shown in Fig. 1, and as we have shown previously (25), relationships between CLDF and RBF were linear in every animal we studied, attesting to the validity of this approach.
In conclusion, our novel approach of simultaneous assessment of RSNA, NE kinetics, and the major renal neuroeffector mechanisms, allowed us to identify the levels at which changes in renal sympathetic neuroeffector function occur in secondary hypertension. Our data suggest that renal sympathetic neuroeffector function is altered in renovascular and angiotensin II-induced hypertension in rabbits, in that NE release from sympathetic nerves, in response to increases in RSNA induced by hypoxia, is attenuated. Nevertheless, although hypoxia-induced renin release is blunted in these forms of hypertension, hypoxia-induced reductions in cortical perfusion, urine flow and sodium excretion are similar to those observed in normotensive rabbits. Thus, effector responses to hypoxia (other than renin release) are relatively normal in hypertension, despite apparently diminished neural NE release. Thus, with the caveat that we cannot exclude a role for altered neural control of medullary perfusion, our findings do not support the hypothesis that enhanced neural control of renal function contributes to maintenance of 2K1C and angiotensin-dependent hypertension.

Acknowledgements
The technical assistance of Flora Socratous and Kristy Jackson is greatly appreciated. We thank Dr Gabriela Eppel for her constructive criticisms of the manuscript.

Grants
This work was supported by grants from the National Health and Medical Research Council of Australia (317821, 143785, 182816) and the National Heart Foundation of Australia (G04M1550).
References


Figure Legends

**Fig. 1** Scatterplot of the relationships between cortical laser Doppler flux and renal blood flow during renal nerve stimulation. Each set of lines and symbols represents data from one rabbit, with separate symbols and lines for the left kidney of sham-operated (dotted line, open circles, n = 8) and angiotensin II-treated (gray lines, triangles, n = 9) rabbits, and also for the clipped (dashed line, squares, n = 6) and non-clipped (solid line, diamonds, n = 5) kidneys of rabbits with 2 kidney, 1 clip hypertension. The line of best fit (heavy black line; $r^2 = 0.97$; $P < 0.001$) and zero intercepts (dotted vertical and horizontal lines) are also shown. The 95% confidence limits of the x-intercept (5.7 units) include zero (-7.3 to 18.7 units) and the slope of the line was 10.1, with 95% confidence limits from 9.2 to 10.9.

**Fig. 2** Average responses to hypoxia in sham-operated rabbits (dotted line), rabbits receiving a chronic subcutaneous infusion of angiotensin II (gray line), and 2K1C hypertensive rabbits with the kidney with the clipped (dashed line) or non-clipped (black solid line) renal artery studied. Error bars are the standard error of the mean, indicating average within animal variance. Responses of renal sympathetic nerve activity (RSNA, normalized units), mean arterial pressure (MAP, mmHg), cortical laser Doppler flux (CLDF, units) and medullary laser Doppler flux (MLDF, units) averaged over 1 minute are shown.

**Fig. 3** Effects of hypoxia on renal sympathetic nerve activity (RSNA) and norepinephrine (NE) spillover. Data are shown for sham-operated control rabbits (open bars), rabbits receiving a chronic subcutaneous infusion of angiotensin II (AngII, gray bars), and 2 kidney, 1 clip hypertensive rabbits with the left renal artery clipped (hatched bars) or non-clipped (cross-hatched bars). Values are the mean difference ± standard error of the difference, between values observed during hypoxia relative to control values. (#) $P = 0.07$ for comparison of all hypertensive groups with sham-operated controls.

**Fig. 4** Effects of hypoxia on renal dihydroxyphenylglycol (DHPG) overflow and renal [3H]DHPG production. Values are the mean difference ± standard error of the difference, between values observed during hypoxia relative to control values. **$P_{\text{Sham}} < 0.01$** for comparison of individual hypertensive groups with sham operated controls. #$P_{\text{Sham v all HT}} < 0.05$ for comparison of all hypertensive groups with sham-operated controls. Abbreviations as for Fig. 3.

**Fig. 5** Effects of hypoxia on indices of regional kidney blood flow. Data are shown for groups as described for Fig. 3. Left panels: Values are the mean % changes ± SED between the average levels
3 - 5 minutes after the onset of hypoxia, relative to the mean level of the first control period. Right panels: Values are the mean % changes ± SED between values observed during the period 5-20 minutes after commencing hypoxia, relative to the mean levels observed across the two control periods, before and after hypoxia. CLDF = cortical laser doppler flux; MLDF = medullary laser doppler flux; other abbreviations as for Fig. 3.

**Fig. 6** Effects of hypoxia on renal excretory variables. Data are shown for groups as described for Fig. 3. Values are the mean difference ± SEM of % changes, between values observed during hypoxia, relative to the mean levels observed across the two control periods, before and after hypoxia. GFR = glomerular filtration rate; other abbreviations as for Fig. 3.

**Fig. 7** Changes (Δ) in renal plasma renin activity (PRA) overflow in response to hypoxia. Data are shown for groups as described for Fig. 3. Values are the mean difference ± SED, between values observed during hypoxia, relative to the mean levels observed across the two control periods, before and after hypoxia. * P_{sham} < 0.05 for comparison of hypertensive group with sham-operated rabbits; # P_{sham v all HT} < 0.05 for comparison of all hypertensive groups combined, with sham-operated controls. Abbreviations as for Fig. 3.

**Fig. 8** Effects of renal denervation on plasma renin activity (PRA). Data are shown for groups as described for Fig. 3. Values are the mean difference ± SED, between values observed after renal denervation, relative to the mean levels observed across the two control periods, before and after hypoxia. * P_{sham} < 0.05 for comparison of hypertensive group with sham-operated rabbits; # P_{sham v all HT} < 0.05, (#) P_{sham v all HT} = 0.08 for comparison of all hypertensive groups combined, with sham-operated controls. Abbreviations as for Fig. 3.
Table 1: Baseline levels of variables in conscious rabbits

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ang II</th>
<th>P-sham</th>
<th>2K1C clip</th>
<th>P-sham</th>
<th>2K1C non-clip</th>
<th>P-sham</th>
<th>P-sham v all HT</th>
<th>P-HT</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>80 ± 2</td>
<td>99 ± 6</td>
<td>***</td>
<td>92 ± 3</td>
<td>*</td>
<td>86 ± 3</td>
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<tr>
<td>HR (b/min)</td>
<td>162 ± 8</td>
<td>195 ± 9</td>
<td>*</td>
<td>196 ± 13</td>
<td>*</td>
<td>171 ± 4</td>
<td></td>
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<td>#</td>
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<tr>
<td>Hct (%)</td>
<td>37.3 ± 1.3</td>
<td>40.3 ± 0.9</td>
<td>40.1 ± 0.7</td>
<td>40.0 ± 1.5</td>
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<tr>
<td>Arterial PRA (ng/ml)</td>
<td>5.1 ± 1.1</td>
<td>1.6 ± 0.4</td>
<td>**</td>
<td>5.6 ± 0.9</td>
<td>4.5 ± 0.4</td>
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</tbody>
</table>

Values are mean ± SEM using between animal variance. * P-sham < 0.05, ** P-sham < 0.01, *** P-sham < 0.001 for comparison of hypertensive group with sham-operated controls; #P-sham v all HT < 0.05, ##P-sham v all HT < 0.01 for comparison of all hypertensive groups with sham-operated controls; φφP-HT < 0.01 for heterogeneity amongst hypertensive groups. MAP = mean arterial pressure; HR = heart rate; Hct = hematocrit; PRA = plasma renin activity. Rabbits with 2 kidney, 1 clip (2K1C) hypertension had the clip placed on either the left (clip) or right (non-clip) renal artery.
Table 2: Baseline levels of hemodynamic and renal variables in anesthetized rabbits prior to hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ang II</th>
<th>P&lt;sub&gt;sham&lt;/sub&gt;</th>
<th>2K1C clip P&lt;sub&gt;sham&lt;/sub&gt;</th>
<th>2K1C non-clip P&lt;sub&gt;sham&lt;/sub&gt;</th>
<th>P&lt;sub&gt;sham&lt;/sub&gt; v all HT</th>
<th>P&lt;sub&gt;HT&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>71 ± 1</td>
<td>76 ± 5</td>
<td>81 ± 3</td>
<td>74 ± 4</td>
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<tr>
<td>HR (b/min)</td>
<td>232 ± 5</td>
<td>252 ± 8</td>
<td>244 ± 9</td>
<td>244 ± 9</td>
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<tr>
<td>RSNA (bursts/s)</td>
<td>2.4 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>2.4 ± 0.3</td>
<td>2.8 ± 0.2</td>
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<tr>
<td>CLDF (units)</td>
<td>243 ± 11</td>
<td>264 ± 23</td>
<td>177 ± 32</td>
<td>275 ± 36</td>
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<tr>
<td>MLDF (units)</td>
<td>46 ± 6</td>
<td>47 ± 9</td>
<td>52 ± 12</td>
<td>55 ± 15</td>
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<tr>
<td>Hct (%)</td>
<td>35.8 ± 1.0</td>
<td>37.4 ± 1.4</td>
<td>36.3 ± 0.8</td>
<td>37.4 ± 1.3</td>
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<td>GFR (ml/min)</td>
<td>3.6 ± 0.2</td>
<td>3.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>* 4.1 ± 0.8</td>
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<tr>
<td>Urine flow (µl/min)</td>
<td>192 ± 29</td>
<td>271 ± 62</td>
<td>20 ± 4</td>
<td>* 314 ± 39</td>
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<td>FE vol (%)</td>
<td>5.7 ± 1.0</td>
<td>8.3 ± 1.5</td>
<td>4.0 ± 2.5</td>
<td>9.5 ± 2.9</td>
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<tr>
<td>FE Na⁺ (%)</td>
<td>4.9 ± 0.9</td>
<td>6.0 ± 1.1</td>
<td>3.0 ± 1.8</td>
<td>7.5 ± 2.1</td>
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<td>Na⁺ excretion (µmol/min)</td>
<td>21.3 ± 3.8</td>
<td>24.6 ± 5.7</td>
<td>2.1 ± 0.8</td>
<td>* 33.0 ± 8.4</td>
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<tr>
<td>Arterial PRA (ng/ml)</td>
<td>40.7 ± 9.2</td>
<td>12.3 ± 4.8</td>
<td>* 30.1 ± 9.6</td>
<td>21.3 ± 7.1</td>
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<tr>
<td>Renal vein PRA (ng/ml)</td>
<td>69.6 ± 16.5</td>
<td>17.5 ± 7.5</td>
<td>* 47.5 ± 14.3</td>
<td>30.1 ± 12.4</td>
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<tr>
<td>Renal PRA overflow (ng/min)</td>
<td>389.6 ± 115.3</td>
<td>96.3 ± 54.0</td>
<td>* 137.4 ± 39.2</td>
<td>109.3 ± 61.6</td>
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</table>

Values are mean ± SEM using between animal variance. *P<sub>sham</sub> < 0.05 for comparison of hypertensive group with sham-operated controls; #P<sub>sham v all HT</sub> < 0.05 for comparison of all hypertensive groups with sham-operated rabbits; ϕP<sub>HT</sub> < 0.05, ϕϕP<sub>HT</sub> < 0.01, ϕϕϕP<sub>HT</sub> < 0.001 for heterogeneity amongst hypertensive groups. MAP = mean arterial pressure; HR = heart rate; RSNA = renal sympathetic nerve activity; CLDF = renal cortical laser Doppler flux; MLDF = renal medullary laser Doppler flux; Hct = hematocrit; GFR = glomerular filtration rate; FEvol = fractional urine excretion; FE Na⁺ = fractional excretion of sodium; PRA = plasma renin activity.
### Table 3: Baseline levels of variables associated with norepinephrine release and re-uptake in anesthetized rabbits prior to hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ang II</th>
<th>Psham</th>
<th>2K1C clip</th>
<th>Psham</th>
<th>2K1C non-clip</th>
<th>Psham</th>
<th>Psham vs all HT</th>
<th>PHT</th>
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<tbody>
<tr>
<td>Arterial NE (pg/ml)</td>
<td>351.1 ± 64.2</td>
<td>414.3 ± 51.8</td>
<td>280.6 ± 65.0</td>
<td>292.7 ± 78.2</td>
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<tr>
<td>(²H)NE infusion rate (dpm/min *10⁻³)</td>
<td>284.8 ± 73.2</td>
<td>279.7 ± 89.9</td>
<td>329.9 ± 122.1</td>
<td>236.4 ± 50.8</td>
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<tr>
<td>NE clearance (ml/min)</td>
<td>172.0 ± 21.0</td>
<td>151.3 ± 27.7</td>
<td>177.2 ± 8.7</td>
<td>196.2 ± 26.1</td>
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<tr>
<td>Total NE spillover (ng/min)</td>
<td>54.5 ± 8.0</td>
<td>59.3 ± 10.5</td>
<td>51.1 ± 12.8</td>
<td>56.3 ± 18.8</td>
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<tr>
<td>Renal NE extraction (%)</td>
<td>59.3 ± 3.9</td>
<td>54.3 ± 3.9</td>
<td>46.0 ± 3.5</td>
<td>46.9 ± 5.7</td>
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<tr>
<td>Arterial DHPG/NE ratio</td>
<td>3.1 ± 0.6</td>
<td>3.0 ± 0.6</td>
<td>2.3 ± 0.4</td>
<td>2.7 ± 0.3</td>
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<tr>
<td>DHPG&lt;sub&gt;RV&lt;/sub&gt; - DHPG&lt;sub&gt;A&lt;/sub&gt; (pg/ml)</td>
<td>-270.8 ± 46.7</td>
<td>296.0 ± 121.9</td>
<td>*** -198.9 ± 79.2</td>
<td>-57.3 ± 69.9</td>
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<tr>
<td>Renal DHPG overflow (ng/min)</td>
<td>-6.1 ± 1.0</td>
<td>7.6 ± 2.7</td>
<td>*** -2.9 ± 1.2</td>
<td>-2.0 ± 1.7</td>
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<tr>
<td>(²H)DHPG production (dpm/min)</td>
<td>-2015 ± 526</td>
<td>771 ± 662</td>
<td>** -615 ± 509</td>
<td>-476 ± 634</td>
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<tr>
<td>DOPA&lt;sub&gt;RV&lt;/sub&gt; - DOPA&lt;sub&gt;A&lt;/sub&gt; (pg/ml)</td>
<td>274.6 ± 107.5</td>
<td>114.3 ± 83.5</td>
<td>-19.1 ± 146.8</td>
<td>183.9 ± 42.5</td>
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<tr>
<td>Renal DOPA extraction (ng/min)</td>
<td>3.5 ± 1.1</td>
<td>1.7 ± 1.3</td>
<td>-0.1 ± 1.3</td>
<td>3.4 ± 0.9</td>
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Values are mean ± SEM using between animal variance. ** P<sub>sham</sub> < 0.01, *** P<sub>sham</sub> < 0.001 for comparison of hypertensive group with sham-operated controls; #P<sub>sham</sub> vs all HT < 0.05, ##P<sub>sham</sub> vs all HT < 0.01 for comparison of all hypertensive groups with sham-operated controls; ¶P<sub>HT</sub> < 0.01 for heterogeneity amongst hypertensive groups. NE = norepinephrine; DHPG<sub>RV</sub> = 3,4-dihydroxyphenolglycol concentration in renal venous plasma; DHPG<sub>A</sub> = arterial plasma concentration of 3,4-dihydroxyphenolglycol; DOPA<sub>RV</sub> = dihydroxyphenylalanine concentration in renal venous plasma; DOPA<sub>A</sub> = arterial plasma concentration of dihydroxyphenylalanine.
Renal NE spillover (ng/min)

∆ RSNA (%)

0 50 100

∆ Renal NE spillover (ng/min)

0 2

∆ Total NE spillover (ng/min)

0 25 50 75

sham  AngII  clip  non-clip

(#)

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\[ \Delta \text{Renal } [{^3}\text{H}]\text{DHPG production (dpm/min} \times 10^3) \]

\[ \Delta \text{Renal DHPG overflow (ng/min)} \]