Estimation of the vascular resistance amplifier in the renal vascular bed in conscious hypertensive rabbits: comparison with the total peripheral vasculature

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ABSTRACT

Objectives: The vascular amplifier in hypertension is a result of structural changes in resistance arteries. We estimated the vascular amplifier hypertensive:normotensive (H:N) ratio in the renal bed compared with the total peripheral bed in conscious rabbits during infusion of vasoconstrictor and vasodilator stimuli.

Methods: Rabbits were subjected to bilateral renal cellophane wrap or sham operation. A perivascular ultrasonic flow probe was implanted on the left renal artery to measure renal blood flow. A catheter was inserted into the thoracic aorta for agonist administration. Blood pressure, heart rate and renal blood flow were measured on three separate days in conscious rabbits with intact effectors, ganglionic block or neurohumoral block. Dose-response curves were constructed to intra-arterial infusion of noradrenaline, angiotensin II, adenosine and acetylcholine.

Results: Resting renal vascular resistance in hypertensive rabbits was markedly decreased by ganglionic block and further by neurohumoral block. With effectors intact, ganglionic block or neurohumoral block, the H:N ratio for renal vascular resistance was 2.32, 1.72 or 1.72, respectively. The ratio was generally maintained during the infusion of constrictor and dilator drugs although distortions occurred at higher concentrations of constrictor or dilator drugs.

Conclusions: Estimation of the renal resistance amplifier in renal wrap hypertension with neurohumoral block accords with our earlier estimates of the total peripheral resistance amplifier (1.79). This vascular resistance amplifier is consistent with a decrease in internal radius through structural remodelling in the renal vascular bed as is reflected in the total arterial circulation in hypertension.

1. Introduction

In most types of chronic hypertension structural changes in the large resistance vessels (R2 vessels, internal radius, r2, 50–200 μm) include narrowing of r2, an increase in the ratio of wall thickness (w)/r2 and often a decrease in wall distensibility [1, 2, 3, 4, 5, 6, 7]. The first two increase the effect of a given constrictor or dilator stimulus on vascular resistance, whilst less wall distensibility moderates this increase [1,8,9]. The net effect is enhancement of the vascular resistance responses in chronic hypertension, which is often referred to as the vascular amplifier. In the smaller arterioles (R3 vessels) structure is normal in all beds, except those of the kidney, where the afferent arteriolar structural changes are similar to those of the R1 vessels of other beds [10, 11, 12, 13]. Many believe that the vascular amplifier contributes significantly to the elevation of blood pressure [1, 2, 8, 9, 14, 15, 16, 17, 18, 19, 20, 21]. However, this has been challenged by other in vivo studies where enhanced vascular resistance responsiveness was not observed (e.g. [22, 23, 24]).

In our earlier paper on total peripheral resistance (TPR) responsiveness in conscious rabbits with renal cellophane wrap (Page) hypertension [21], the data from individual log dose–response curves to constrictor and dilator drugs were combined into extended scaled dose (ScD)–TPR and –total peripheral conductance (TPC) curves, in which major non-linearities are easier to detect [25]. One non-linearity was elicited by high doses of constrictor agonists and was due to functional (reversible) rarefaction. A second non-linearity occurred at high doses of dilator drugs and was due to impaired autoregulation associated with falls in blood pressure. However, there remained a substantial dose range between these non-linearities over which TPR responsiveness to constrictor

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and dilator drugs was enhanced in hypertensive animals, in accord with theoretical properties of a structural amplifier [8,9,14]. Polynomial regression equations were used to characterize the extended ScD–TPR curves under various conditions of autonomic and pressor hormone function. This involved measuring the ratio of TPR in hypertensive and normotensive rabbits (H:N ratio) at frequent intervals between the above non-linearities and taking the mean over this segment as the measure of the amplifier's magnitude. H:N was similar for different agonists, suggesting that the enhancement was non-specific, due to the structural changes. In Page hypertension the ratio was 1.79 during neurohumoral blockade where, in addition to ganglionic block, the effects of two major pressor hormones, angiotensin II and arginine vasopressin were also blocked [25]. The ratio was significantly greater than 1.00, hence the conclusion that the structural amplifier made a large contribution to the elevation of TPR and blood pressure in Page hypertension.

Our research question was to ask whether the magnitude of the structural amplifier is the same in different beds [26,27]. Specifically, our purpose here was to compare renal amplifier estimates with our TPR amplifier estimates in rabbits with Page hypertension. We infused two vasodilator and two vasoconstrictor agents intra-arterially via the thoracic aorta and assessed the haemodynamic responses in conscious rabbits with neural and humoral effectors intact and in rabbits subjected to ganglionic and neurohumoral blockade. We found that over a limited range of dilator and constrictor stimuli, to avoid non-linearities, the H:N ratios for renal vascular resistance and total peripheral resistance were similar at 1.72 and 1.79, respectively, in the presence of neurohumoral block. This finding suggests that the resistance amplifier is mainly structural in nature, stimulus nonspecific and aligns with a general adaptation in vascular beds in Page hypertension.

2. Materials and methods

This study was approved by the Animal Ethics Committee of the University of Melbourne and performed in accordance with the Australian code for the care and use of animals for scientific purposes (8th edition, 2013, National Health and Medical Research Council, Canberra). New Zealand White rabbits (2.4–2.8 kg) of either sex were used (Nanowie, Small Animal Production Unit, Bellbrae, Victoria, Australia). Rabbits were housed in pairs in floor pens in the Biomedical Science Animal Facility under constant climatic conditions (21 °C, 12 h light/dark cycle) and provided with water and food ad libitum.

2.1. Surgical procedures

Two preliminary surgical operations were performed before the day of the first experiment. For each operation, rabbits were anaesthetised with intravenous propofol (10 g/kg; Diprivan, AstraZeneca, North Ryde, Australia) to prevent dehydration, the analgesic agent buprenorphine were administered warm 0.9% sterile saline (10 ml slow bolus i.v.; Baxter Healthcare) and provided with water and food ad libitum. For the first experiment, 5 weeks before the first experiment, a bilateral ccelophene renal wrap or sham operation was performed in alternate rabbits. Bilateral ccelophene renal wrap causes perinephric hypertension over 4–5 weeks in rabbits [20] and was performed as previously described [28]. Briefly, kidneys were accessed via flank incisions, mobilised from surrounding tissue and wrapped in sterile ccelophene. The ends of the ccelophene were gathered at the hilum and held in place by loosely-tied sutures. In the sham (normotensive) group, kidneys remained undisturbed. In the same operation, rabbits were implanted with a perivascular ultrasonic flow probe (sized for vessel of 2 mm o.d., MC2PSB Precision 5 flow probe with back exit; Transonic Systems Inc., Ithaca, USA) around the left renal artery for the measurement of renal blood flow. The flow probe connector plug was tunnelled subcutaneously and buried at the nape for future retrieval.

In the second operation, at least 2 days before the first experiment, a polyvinyl catheter (o.d. 1.7 mm; i.d. 1.2 mm) was inserted into the left carotid artery and passed retrogradely to lie freely in the thoracic aorta for the subsequent intra-arterial infusion of vasoactive agonist drugs. The catheter was filled with heparin (1000 units/ml; Pfizer, NY, USA) to prevent clotting, secured in position with a Dacron patch and cymocrylate glue (Vetbond, 3M, North Ryde, NSW, Australia) and the distal tip heat-sealed. The carotid artery blood flow was unobstructed and the artery remained patent. The catheter and the previously implanted renal blood flow probe plug were exteriorised and protected from damage by a custom-made rabbit denim jacket that allowed full range of movement.

2.2. Experimental day protocols

Each rabbit underwent 3 experimental days: (i) effectors intact; (ii) ganglionic block; and (iii) neurohumoral block. Two to four days separated each experimental day. For the duration of each experiment, rabbits sat alert and undisturbed in a polystyrene box (without head restraint). On each day, minor surgical procedures were performed under local anaesthesia (50/50% v/v mix of 1% w/v ropivacaine and 1% w/v lignocaine; Naropin and Xylocaine, respectively, Astra, NSW, Australia). Catheters were placed in the central ear artery for the measurement of arterial pressure and in the marginal ear vein for the infusion of antagonistic drugs. The intra-thoracic aortic catheter was retrieved from the rabbit jacket for the infusion of agonist drugs.

The central ear artery catheter was connected to a pressure transducer (Argon Medical Devices Inc., Texas, USA) for the measurement of arterial pressure (mmHg). The flowprobe connector was retrieved from the rabbit jacket and connected to a flowmeter (TS420 Transit Time Peripheral Flowmeter, Transonic Systems Inc.) for the measurement of renal blood flow (ml/min). The transducer and flowmeter were connected to a PowerLab BSP (AD Instruments Pty Ltd, Bella Vista, NSW, Australia) via a bridge amplifier (Quad Bridge Amplifier, AD Instruments) for data collection. Heart rate (HR; beats/min) and renal vascular conductance (RVC; ml/min/mmHg) were continuously computed by Chart v 5.5.6 (AD Instruments); renal vascular resistance (RVR; mmHg/ml/min) was calculated as the reciprocal of RVC values. Haemodynamic parameters were allowed to stabilise over a period of 30 min prior to the generation of agonist dose-response curves (reflexes intact day) or, on the second and third experimental day, to the ganglionic or neurohumoral block regimen, followed by the generation of agonist dose-response curves.

At the end of each experimental day, the ear catheters were removed, the thoracic aortic catheter re-sealed and, together with the flow probe plug, placed securely in the rabbit jacket; the rabbit was then returned to its home floor pen. For the second experimental day, pharmacological inhibition of autonomic ganglionic transmission was achieved with mecamylamine (4 mg/kg i.v. bolus followed by 2.5 mg/kg/h at 10 ml/h i.v. infusion), a previously optimised protocol [20]. For the third experimental day, neurohumoral block was achieved with i.v. administration of: (i) vasopressin V1 receptor antagonist, des-Gly–[Phe1,D-Tyr(Et)2,Lys6, Arg8]–vasopressin (1 μg/kg bolus and 0.1 μg/kg/h infusion); (ii) angiotensin-converting enzyme inhibitor enalaprilat (1 mg/kg bolus and 1.5 mg/kg/h infusion); and (iii) mecamylamine (4 μg/kg slow bolus and 2.5 mg/kg/h infusion) at 10 ml/h (Terufusion Syringe Pump, Terumo Corporation, Japan). This protocol has been used to successfully elicit and maintain neurohumoral blockade in rabbits over several hours [21]. All rabbits receiving mecamylamine were administered a warm 10% polygeline/electrolyte solution, a plasma volume expander that prevented precipitous falls in blood pressure (10 ml i.v. slow bolus;
Successful ganglionic blockade was confirmed by the absence of the nasopharyngeal reflux activated upon exposure to cigarette smoke [29,30]. Haemodynamic parameters were allowed to stabilise for 40 min before the generation of agonist dose-response curves.

### Table 1. Agonist doses and respective scaled doses used to construct combined scaled dose-response curves in the renal vascular bed of normotensive and hypertensive rabbits.

| Constrictor drugs – Scaled Dose | 1 | 2 | 3 | 4 | 5 |
|--------------------------------|---|---|---|---|---|
| Angiotensin II μg/kg/min        | 0.003 | 0.01 | 0.03 | 0.1 | 0.3 |
| Noradrenaline μg/kg/min         | 0.1 | 0.3 | 1 | 3 | 10 |

| Dilator drugs – Scaled Dose |
|-----------------------------|
| Adenosine μg/kg/min          | -1 | -2 | -3 | -4 | -5 |
| Acetylcholine μg/kg/min      | 10 | 30 | 100 | 300 | 1000 |

Scaled doses are positive for constrictor drugs and negative for dilator drugs.

### 2.3. Agonist dose-response curves

Agonist dose-response curves were constructed, in the following order, to angiotensin II (0.001–1.0 μg/kg/min i.a.), adenosine (10–1000 μg/kg/min i.a.), noradrenaline (0.1–10 μg/kg/min i.a.) and acetylcholine (1–30 μg/kg/min i.a.). On the ganglionic block and neurohumoral block

### Table 2. Resting haemodynamic variables in sham-operated (normotensive, N) and renal cellophane-wrapped (hypertensive, H) conscious rabbits.

| Variable                               | Normotensive rabbits | Hypertensive rabbits | H:N |
|----------------------------------------|----------------------|----------------------|-----|
| Mean arterial pressure (mmHg)          |                      |                      |     |
| Effectors intact                       | 78 ± 1               | 115 ± 2*             | 1.47|
| Ganglionic block                       | 60 ± 1*              | 90 ± 2*              | 1.50|
| Neurohumoral block                     | 53 ± 1*              | 77 ± 3*              | 1.45|
| Heart rate (beats/min)                 |                      |                      |     |
| Effectors intact                       | 221 ± 6              | 216 ± 13             |     |
| Ganglionic block                       | 247 ± 5*             | 255 ± 12             |     |
| Neurohumoral block                     | 259 ± 13*            | 240 ± 15             |     |
| Renal blood flow (ml/min)              |                      |                      |     |
| Effectors intact                       | 43.0 ± 1.5           | 33.0 ± 3.1*          | 2.32|
| Ganglionic block                       | 36.5 ± 1.2*          | 30.4 ± 0.8*          | 1.72|
| Neurohumoral block                     | 37.0 ± 1.6*          | 29.9 ± 0.9*          | 1.72|
| Renal vascular resistance (mmHg/ml/min)|                      |                      |     |
| Effectors intact                       | 1.91 ± 0.07          | 4.44 ± 0.39*         | 2.32|
| Ganglionic block                       | 1.79 ± 0.07          | 3.08 ± 0.10*         | 1.72|
| Neurohumoral block                     | 1.56 ± 0.07*         | 2.68 ± 0.16*         | 1.72|

From Korner et al., Table 2 [25] with permission:

| Variable                               | Normotensive rabbits | Hypertensive rabbits | H:N |
|----------------------------------------|                      |                      |     |
| Mean arterial pressure (mmHg)          |                      |                      |     |
| Effectors intact                       | 69 ± 1               | 111 ± 3*             | 1.61|
| Ganglionic block                       | 53 ± 1*              | 95 ± 2*              | 1.79|
| Neurohumoral block                     | 52 ± 3*              | 93 ± 3*              | 1.79|
| Heart rate (beats/min)                 |                      |                      |     |
| Effectors intact                       | 217 ± 9              | 210 ± 4              |     |
| Ganglionic block                       | 280 ± 16             | 283 ± 12*            |     |
| Neurohumoral block                     | 288 ± 20*            | 284 ± 13*            |     |
| Cardiac output (ml/min)                |                      |                      |     |
| Effectors intact                       | 367 ± 12             | 368 ± 10             |     |
| Ganglionic block                       | 392 ± 10             | 415 ± 14*            |     |
| Neurohumoral block                     | 411 ± 10*            | 403 ± 10*            |     |
| Total peripheral resistance (mmHg/ml/min) |                    |                      |     |
| Effectors intact                       | 0.199 ± 0.004        | 0.311 ± 0.006*       | 1.56|
| Ganglionic block                       | 0.146 ± 0.002*       | 0.241 ± 0.003*       | 1.65|
| Neurohumoral block                     | 0.134 ± 0.004*       | 0.240 ± 0.005*       | 1.79|

Each value is the average of the resting values obtained before performing each agonist dose-response curve; with the four curves per rabbit on a particular day, each mean ± SEM is based on 28–36 observations. Ganglionic block was achieved with mecamylamine; neurohumoral block was achieved with concomitant vasopressin V1 antagonism, angiotensin-converting enzyme inhibition and mecamylamine (see Methods for details). H:N, hypertensive:normotensive ratio. *P < 0.05 compared to corresponding normotensive group value (Student’s unpaired t test); and **P < 0.05 compared with corresponding effectors intact values within group (one-way ANOVA with Dunnett’s post-test for multiple comparisons). n, number of rabbits.
experimental days, to prevent excessive hypotension, the maximum i.a.
dose of adenosine was 300 μg/kg/min (both normotensive and hyper-
tensive rabbit groups) and in the normotensive group acetylcholine was
limited to 10 μg/kg/min. Each agonist dose was infused into the thoracic
aorta at a variable rate (0.003–3.0 ml/min) until haemodynamic re-
sponses plateaued. NaCl (0.9%) given over these rates has no effect on
regional haemodynamics [20]. Doses of agonists have been previously
optimised [20]. At least 50 min separated the infusion of each different
agonist to allow all haemodynamic parameters to return to baseline.

2.4. Drugs

Drugs and suppliers were as follows: acetylcholine bromide (Sigma, St.
Louis, MO, USA); adenosine (Sigma), angiotensin II amide; arginine
vasopressin (AusPep, Parkville, Victoria, Australia); enalaprilat (gift from
Merck, Rahway, New Jersey, USA); mecamylamine (Sigma); noradrena-
line bitartrate (Sigma); and vasopressin V1 receptor antagonist des-Gly-
[Phel1,D-Tyr(Et)2,Lys6,Arg8]-vasopressin (Bachem, Bubendorf,
Switzerland). All drugs used for in vivo assessment were prepared using
sterile 0.9% sodium chloride solution. Angiotensin II was stored as a stock
solution at -20 °C until required. All other drugs were made fresh daily.

2.5. Statistical analysis

Values are presented in text and tables as mean ± SEM. The haem-
odynamic variables assessed were mean arterial pressure (MAP), heart rate
(HR), renal blood flow, renal vascular conductance (RVC; renal blood
flow/MAP) and renal vascular resistance (RVR; MAP/renal blood flow).
On each experimental day, haemodynamic variables at rest were
compared between groups (hypertensive vs. normotensive) using Stu-
dent’s unpaired t test. Within each group, haemodynamic variables in
rest (x = 0) was 0, while the Scaled Dose units for constrictor and dilator
drugs were +1 to +5 and -1 to -5, respectively, as shown in Table 1.
Extended dose-response curves were examined with 3rd order polynomial
regression equations using Prism 8 (GraphPad Software, La Jolla, CA,
USA), which gave the best fit [25]. Using the mean values obtained at each
Scaled Dose unit along the extended dose-response curve, the ratio be-
tween the renal vascular conductance (or resistance) of hypertensive and
normotensive rabbits was determined over the dose range of interest.
In addition, we calculated the average rj from Resistance x 1/rj² and again
calculated the rj ratio for hypertensive:normotensive responses.

3. Results

3.1. Baseline haemodynamic variables with neurohumoral effectors intact

With effectors intact, mean arterial pressure (MAP) was 37 mmHg
greater in hypertensive than normotensive rabbits (P < 0.0001), while
heart rate (HR) was comparable (Table 2). Renal blood flow and vascular
conductance (RVC) were substantially lower in hypertensive than in
normotensive rabbits (P = 0.0024 & P < 0.0001, respectively), with a
hypertensive (H) to normotensive (N) H:N ratio for RVC of 0.52 (or 2.32
in terms of renal vascular resistance, RVR).

3.2. Effect of ganglionic block on baseline haemodynamic variables

During ganglionic block, both normotensive and hypertensive rabbits
had lower MAP compared with effectors intact (P < 0.0001), but higher
than with neurohumoral block. HR tended to be higher during ganglionic
block compared with effectors intact in both normotensive and hyper-
tensive rabbits, although this was only statistically significant in normoten-
sive rabbits (P = 0.045). Renal blood flow fell significantly in hypertensive (P
= 0.0002) and normotensive (P = 0.0032) rabbits. RVR decreased (RVC
increased) with ganglionic block compared with effectors intact in hyper-
tensive (P = 0.0004), but not normotensive rabbits (Table 2).

3.3. Effect of neurohumoral block on baseline haemodynamic variables

During neurohumoral block, both normotensive and hypertensive
rabbits again had lower MAP (P < 0.0001) and normotensive rabbits had

![Graphical representation](image-url)
higher HR compared to when effectors were intact ($P = 0.0107$); the elevation in HR was not significant in hypertensive rabbits ($P = 0.24$; Table 2). While the fall in resting MAP with neurohumoral block was more marked in hypertensive than in normotensive rabbits (-38 vs. -25 mmHg in hypertensive vs. normotensive rabbits, respectively), MAP remained much higher in hypertensive than normotensive rabbits across both experimental days ($P < 0.0001$).

Compared with effectors intact, RVC was elevated in normotensive ($P < 0.0001$) and hypertensive ($P = 0.0008$) rabbits during neurohumoral block (Table 2). RVC remained significantly lower (and RVR significantly higher) in hypertensive than normotensive rabbits across neurohumoral block ($P < 0.0001$); the H:N ratio for RVC was 0.60 (1.72 for RVR).

### 3.4. Comparison of resting vascular resistance

With ganglionic block, the resting renal vascular resistance in the hypertensive rabbits fell steeply ($-1.36 \text{ mmHg/ml/min}$) from the level observed with effectors intact. A greater fall was observed with neurohumoral block ($-1.76 \text{ mmHg/ml/min}$ from effectors intact value) indicating that in the effector intact circulation there was a strong additional constrictor effect in the renal bed from angiotensin II and potentially vasopressin (Figure 1A). The total circulation (total peripheral resistance, TPR) data have been reproduced from Körner et al. [25] and are added for comparison with the renal resting vascular resistance (Figure 1A; Table 2). Here, in the hypertensive rabbits, a major fall in TPR was observed with ganglionic block ($-0.070 \text{ mmHg/ml/min}$ from effectors intact), with no further change with neurohumoral block ($-0.071 \text{ mmHg/ml/min}$ from effectors intact). In normotensive rabbits, the falls in resistance in each vascular bed were similar with ganglionic or with neurohumoral block (Figure 1A).

In general, the H:N ratio of resting RVR or TPR values in hypertensive rabbits and normotensive rabbits in all 3 settings of effectors intact, ganglionic block and neurohumoral block fell between 1.5-1.8 (Figure 1B and Table 2), except for RVR in rabbits with intact effectors where the ratio was higher at 2.3. Interestingly, the TPR H:N ratio increased from 1.56 with effectors intact to 1.65 and 1.79 with ganglionic block and.
neurohumoral block, respectively, while the RVR H:N ratios decreased with ganglionic block and neurohumoral block (Figure 1B).

3.5. Vasoconstrictor agonist dose-response curves

The predominantly α1-adrenoceptor agonist noradrenaline at doses >0.1 μg/kg/min i.a. increased MAP and RVR, with decreased RVC, in normotensive and hypertensive rabbits with effectors intact (Figure 2). In both rabbit groups, HR slowed significantly at higher doses (>1 μg/kg/min; Figure 2B). In hypertensive rabbits with intact effectors, RVC was significantly lower at rest (x = 0) than in normotensive rabbits, however it fell to equivalent maximum responses with noradrenaline 10 μg/kg/min i.a. (Figure 2D). Similar responses were also observed with noradrenaline administration during ganglionic or neurohumoral block, albeit from generally raised baseline values of RVC in each group. Ganglionic or neurohumoral block inhibited any significant (reflex) bradycardia, even with noradrenaline 10 μg/kg/min (Figure 2B).

With i.a. infusion of angiotensin II, qualitatively similar differences were observed in the dose-haemodynamic response curves in normotensive and hypertensive rabbits as seen with noradrenaline, indicating that these responses were not agonist-specific (Figure 3). The exception was the effect of angiotensin II infusion on HR where there was no reflex bradycardia in the effectors intact groups, despite marked increases in MAP. In the presence of ganglionic or neurohumoral block, there was a larger tachycardia with higher doses of angiotensin II (particularly in the normotensive group) suggestive of direct positive chronotropic effects of the agonist (Figure 3B).

3.6. Vasodilator agonist dose-response curves

Both adenosine and acetylcholine caused dose-dependent decreases in MAP. In rabbits with intact effectors, the maximum decrease in pressure induced by acetylcholine tended to be greater in hypertensive (-48 ± 5 mmHg) than normotensive (-34 ± 3 mmHg) rabbits, though this was
not statistically significant (P = 0.083; Figure 4A). Adenosine elicited a significantly larger decrease in MAP in hypertensive (-35 ± 3 mmHg) than normotensive (-18 ± 3 mmHg; P < 0.0001; Figure 5A) rabbits. Both dilator agents also elicited a reflex tachycardia in response to the decrease in pressure. Acetylcholine elicited a comparable peak increase in HR of 44 ± 11 and 52 ± 9 beats/min in hypertensive and normotensive rabbits, respectively (Figure 4B); the magnitude of the tachycardia during adenosine infusion was also similar between both rabbit groups (61 ± 11 vs. 65 ± 10 beats/min; Figure 5B). Reflex tachycardia was not observed in either hypertensive or normotensive rabbits during ganglionic or neurohumoral block in response to adenosine or acetylcholine. In the presence of ganglionic or neurohumoral block, to avoid dangerous falls in MAP, the highest dose of acetylcholine (30 μg/kg/min i.a.) or adenosine (1000 μg/kg/min i.a.) was not given to the normotensive rabbits; with neurohumoral block, the highest dose of adenosine was also not administered to the hypertensive rabbits.

In both rabbit groups, acetylcholine infusion caused a dose-dependent fall in RVR (Figure 4E) and increase in RVC (Figure 4D). With intact effectors, the falls in RVR appeared to be more marked in the hypertensive rabbits than in their normotensive counterparts, however the values were not statistically different. In the presence of ganglionic or neurohumoral block, the vasodilator effects of acetylcholine were comparable (Figure 4).

With intact effectors, the graded infusion of adenosine caused an increase in RVC and thus decrease in RVR of both hypertensive (P = 0.008) and normotensive rabbits (P < 0.0001) (Figure 5D-E). In the presence of ganglionic or neurohumoral block, the vasodilator effects of adenosine were similar in each rabbit group, albeit from lower respective baseline haemodynamic values.

### 3.7. Extended scaled dose–haemodynamic curves

The “full” range of MAP values from maximum vasodilatation to maximum vasoconstriction can be graphed using scaled doses (minus scale) for adenosine and acetylcholine, zero being baseline (no infusion) and scaled doses (plus scale) for the vasoconstrictor agonists...
noradrenaline and angiotensin II (Table 1). In the presence of neurohumoral block, the MAP full range was 39–146 mmHg for the normotensive group and 58–182 mmHg for the hypertensive group (Figure 6A). HR did not change during the dilator infusions or with noradrenaline to any significant extent, despite the large changes in MAP, consistent with effective neurohumoral block. The one surprise was the tachycardia with the highest dose of angiotensin II due to direct agonist action on the sinoatrial node that was more prominent in the normotensive rabbits than in the hypertensive rabbits (Figure 6B).

Extended scaled dose–RVC and –RVR curves with fitted polynomial lines are shown in Figure 7. In general, over a –2 to +2 scaled dose range (dotted vertical lines in Figure 7), the polynomial fitted line was generally flat for RVR in both hypertensive and normotensive animals at the level of baseline (zero) without stimulus (agonist) infusion. In each rabbit group, comparison of RVR values from –2 to +2 doses with respective baseline (0 scaled dose) values showed no significant difference (P = 0.34 and 0.68 in normotensive and hypertensive groups, respectively; one way ANOVA with Dunnett’s post hoc test; Figure 7B inset). Further, there was a clear separation of the normotensive from the hypertensive values over the scaled dose range of –2 to +2. Above this –2 to +2 scaled dose range, the separation of hypertensive and normotensive values generally disappears for RVC (Figure 7A) and for RVR (Figure 7B), with non-linearities in the intact haemodynamics of conscious rabbits at very high pressures.

Further analysis of the RVR ratio of hypertensive:normotensive (H:N) with neurohumoral block showed again the consistency of the vascular amplifier in the renal bed between scaled doses –2 to +2 (Figure 8A); the average value for RVR H:N was 1.65 ± 0.05. Consistent with these values of the vascular resistance amplifier in hypertensive:normotensive rabbits, the average internal radius (r_i) again showed a decreased r_i over the scaled dose range –2 to +2 (Figure 8B) in the hypertensive rabbits compared with the normotensive rabbits. Table 3 shows the resting baseline r_i estimations for both renal and total peripheral vascular beds under the conditions of effectors intact, ganglionic and neurohumoral block. Although the scale is different between the average r_i for the renal bed and the total vasculature, the r_i is always significantly lower (H:N

Figure 5. Adenosine i.a. infusion (μg/kg/min) dose-haemodynamic response curves in normotensive (n = 8–9; solid symbols and lines) and hypertensive (n = 7; open symbols and dashed lines) rabbits under three treatments (completed on separate experimental days): effectors intact (squares); ganglion block (triangles); and neurohumoral block (circles). 0, Baseline just before infusion of adenosine. Error bars are average SEM from repeated measures ANOVA (see Methods). Haemodynamic variables shown are: A. mean arterial pressure, MAP; B. heart rate; C. renal blood flow, Q; D. renal vascular conductance (renal Q/MAP), RVC; and E. renal vascular resistance (MAP/renal Q), RVR.
ratio 0.83–0.88 or 17–12%) in the hypertensive rabbits than in the normotensive rabbits. Secondly, \( r_n \) significantly increases from effectors intact, ganglionic block or finally neurohumoral block for both the renal and total vasculature (Table 3).

4. Discussion

Our estimation of the renal vascular amplifier in Page hypertension in conscious rabbits (1.72) is almost identical to our estimate of the total vascular amplifier of 1.79, previously published [25]. Our experimental approach was similar in that instrumented rabbits were infused with constrictor or dilator agonists in close upstream proximity to the renal bed, as previously done for the total vasculature through the left atrial appendage or lower abdominal aortic infusion for the hindquarter bed [20]. We were keen to estimate the role of the autonomic nervous system and humoral factors in the renal bed that would influence the estimation of resting vascular resistance and interfere with responses to dilator or constrictor agonists. Thus, three experimental days with effectors intact, ganglionic block or neurohumoral block were conducted.

As previously demonstrated for total vascular resistance, we estimated renal vascular resistance and derived the internal vessel radius \( r_i \) across the resting (baseline) values and either side as the renal bed was constricted or dilated with angiotensin II, noradrenaline, acetylcholine or adenosine. Combining these dose-response curves from these stimuli by creating a "scaled dose" metameter allowed the inspection of the resultant "full" dose-response curve from maximum dilatation to maximum constriction.

4.1. Resting renal vascular resistance and effects of ganglionic and neurohumoral block

First, the resting renal vasculature appeared to be under considerable functional constrictor tone when effectors were intact especially in the hypertensive rabbits (Figure 1A). The ratio H:N for RVR was 2.3 which
vascular conductance had fallen by 50% or more in the normotensive and neurohumoral block. Lesser extent noradrenaline had direct tachycardic actions at the sino- 
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when MAP started to fall and constrictor and two dilator drugs was made by allocating scaled dose -2 to -2 (Figure 6B), but at scaled doses -3 to +5 angiotensin II and to a 1 angiotensin II and vasopressin when TPR and RVR H:N ratios illustrate the importance of obviating functional non-linearities outside this range. One non-linearity from high doses of constrictor agents is elicited by functional (reversible) rarefaction with closure of a proportion of the small arterioles (R2 vessels) and capillaries [15,32,33]. Dilator agents would also cause a non-linearity as blood pressure falls with the associated impairment of autoregulation.

Values (mean ± SEM) are calculated as ri = √1/R (arbitrary units) from individual rabbit values for the renal bed from renal vascular resistance and for the total circulation from total peripheral resistance data shown in Table 2. H:N, hypertensive:normotensive ratio. *P < 0.05 compared to corresponding normotensive group value (Student’s unpaired t test); and #P < 0.05 compared with corresponding effectors intact values within group (one-way ANOVA with Dunnett’s post-test for multiple comparisons).

4.2. Structural amplifier

The renal vascular resistance at baseline (calculated as the average of the baselines at 0 scaled dose just before each of the 4 agonist infusions in each respective group) under neurohumoral block was 2.69 ± 0.17 mmHg/ml/min (hypertensive group) and 1.61 ± 0.08 mmHg/ml/min (normotensive group), giving a H:N ratio of 1.67 (Figure 7B). Taking data from scaled dose -2 to +2 excluding the 0 scaled dose gave group values of hypertensive 2.77 ± 0.13 and normotensive 1.70 ± 0.06, i.e. a H:N ratio of 1.63.

H:N ratios calculated from the resting data during neurohumoral block provide a reasonable one-point assay of the renal vascular amplifier without requiring local drug infusions as this estimate was not significantly different from the estimate from scaled doses -2 to +2 (excluding baseline; P > 0.05 in each group).

In conclusion, the estimate of the renal vascular resistance amplifier is 1.7; very similar to that of the total peripheral circulation. This estimate under experimental conditions of neurohumoral block and constrained dilator and constrictor local drug infusions to limit non-linearities caused by rarefaction or hypotension-limited autoregulation is consistent with a structural decrease in ri in chronic hypertension from vascular hypertensive neurohumoral block-treated rabbits. For the dilator agonists under neurohumoral block, there were sharp falls in MAP at acetylcholine scaled dose -3 (3 μg/kg/min i.a.) and adenosine -3 (100 μg/kg/min i.a.) in the normotensive rabbits. Therefore the prudent choice of scaled dose -2 to +2 allowed estimations of the vascular amplifier without distorting the estimate from non-linearities outside this range.

Figure 8. Combination graphs in rabbits with neurohumoral block showing (A) the hypertensive to normotensive ratio of renal vascular resistance, RVR, and (B) the renal average internal radius ri from indi- vidual rabbit values for the renal bed from renal vascular resistance and for the total circulation from total peripheral resistance data shown in Table 2. H:N, hypertensive:normotensive ratio. *P < 0.05 compared to corresponding normotensive group value (Student’s unpaired t test); and #P < 0.05 compared with corresponding effectors intact values within group (one-way ANOVA with Dunnett’s post-test for multiple comparisons).
remodelling. This finding suggests that this is a general structural adaptation in vascular beds in Page hypertension.

**Declarations**

**Author contribution statement**

Makhala M. KHAMMY: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

James A. ANGUS, Christine E. WRIGHT: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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**Competing interest statement**

The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

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