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Research article

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 (R_1 vessels, internal radius, r_i, 50–200 μm) include narrowing of r_i, an increase in the ratio of wall thickness (w)/r_i 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 (R_2 vessels) structure is normal in all beds, except those of the kidney, where the afferent arteriolar structural changes are similar to those of the R_1 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 vasoconstrictor and two vasodilator 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 nonlinearities, 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, New South Wales, Australia) and intubated. Surgical anaesthesia was maintained with a mixture of isoflurane (Baxter Healthcare, NSW, Australia) and air via an anaesthetic vaporiser (Penlon Sigma Delta; Penlon Limited, Abingdon, UK). Prior to each surgical procedure, rabbits were administered warm 0.9% sterile saline (10 ml slow bolus i.v.; Baxter Healthcare) to prevent dehydration, the analgesic agent buprenorphine hydrochloride (0.05 mg/kg i.v.; Temgesic, Reckitt Benckiser, Berkshire, UK) and the antibiotic enrofloxacin (10 mg/kg s.c.; Ilium Enrotril, Troy Laboratories, NSW, Australia). Enrofloxacin was re-administered daily for 3 days post-surgery.

In the first operation, 5 weeks before the first experiment, a bilateral cellophane renal wrap or sham operation was performed in alternate rabbits. Bilateral cellophane renal wrap causes perinephritic 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 cellophane. The ends of the cellophane 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 S 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 cyanoacrylate 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 polypropylene 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 antagonist 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 Perivascular Flowmeter, Transonic Systems Inc.) for the measurement of renal blood flow (ml/min). The transducer and flowmeter were connected to a PowerLab 8SP (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 mg/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;

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				
	-1	-2	-3	-4	-5
Adenosine μg/kg/min	10	30	100	300	1000
Acetylcholine μg/kg/min		1	3	10	30

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

Haemaccel, Hoechst Australia Pty Ltd, Victoria, Australia). Successful ganglionic blockade was confirmed by the absence of the nasopharyngeal reflex 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.

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
From this study	$n = 8–9$	$n = 7$	
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*	
Ganglionic block	36.5 ± 1.2#	30.4 ± 0.8*	
Neurohumoral block	37.0 ± 1.6#	29.9 ± 0.9*	
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.* study, Table 2 [25] **with permission:**			
Mean arterial pressure (mmHg)	$n = 6–9$	$n = 6–9$	
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 V_1 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.

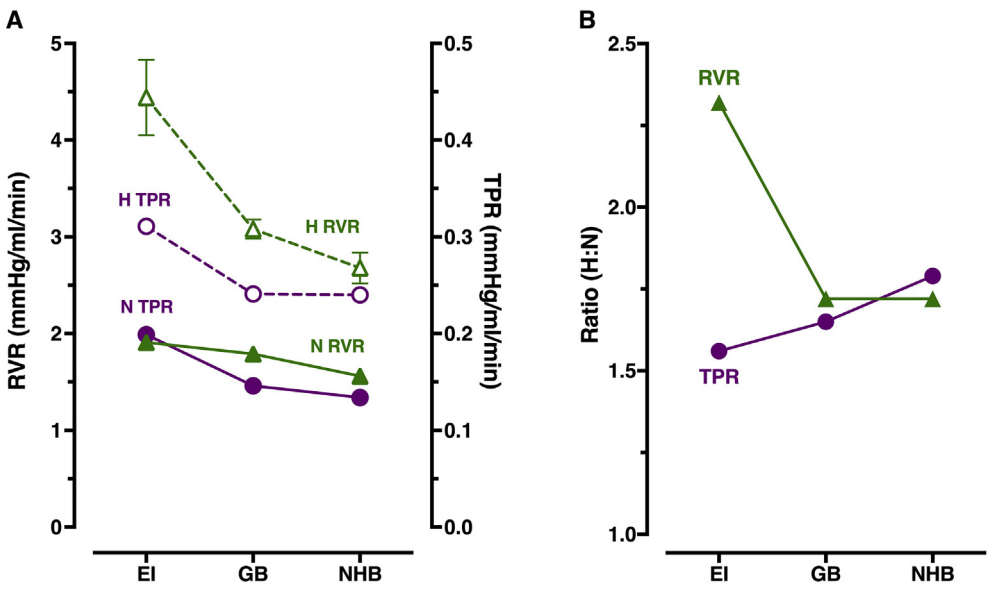

Figure 1. A. Resting renal vascular resistance (RVR; triangles) under conditions of effectors intact (EI), ganglionic block (GB) or neurohumoral block (NHB) in normotensive (N, solid symbols and lines) and hypertensive (H, open symbols and dashed lines) rabbits. For comparison, total peripheral resistance (TPR, circles; right y axis) data have been reproduced from Korner *et al.* [25]. Values are mean ± SEM (error bars not shown are contained within the symbol) from 7-9 rabbits under each condition. B. Ratios of hypertensive rabbit to normotensive rabbit (H:N) resting renal vascular resistance (RVR, triangles) and total peripheral resistance (TPR, circles) under the 3 conditions.

experimental days, to prevent excessive hypotension, the maximum i.a. dose of adenosine was 300 μg/kg/min (both normotensive and hypertensive 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 responses 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); noradrenaline bitartrate (Sigma); and vasopressin V_1 receptor antagonist des-Gly-[Phe1,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 haemodynamic 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 Student's unpaired *t* test. Within each group, circulatory variables before and after ganglionic block or neurohumoral block were compared using a Student's paired *t* test. Within each group, haemodynamic variables in rabbits with intact effectors and in rabbits during ganglionic and neurohumoral block were compared via one-way ANOVA with Dunnett's post-test for multiple comparisons; the response with effectors intact was taken as the control response. In all cases, $P < 0.05$ was taken as statistically significant.

As well as constructing dose-response curves for each individual agonist on each experimental day, extended dose-response curves were generated by combining the data from the constrictor agonists (noradrenaline and angiotensin II) and dilator agonists (adenosine and acetylcholine). Extended dose-response curves were generated using Scaled Dose units, where each unit corresponds to a half-log dose increment of a particular agonist. The allocated Scaled Dose unit at (baseline) 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 between 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 r_i from Resistance $\propto 1/r_i^4$ and again calculated the r_i 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 hypertensive rabbits, although this was only statistically significant in normotensive 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 hypertensive ($P = 0.0004$), 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

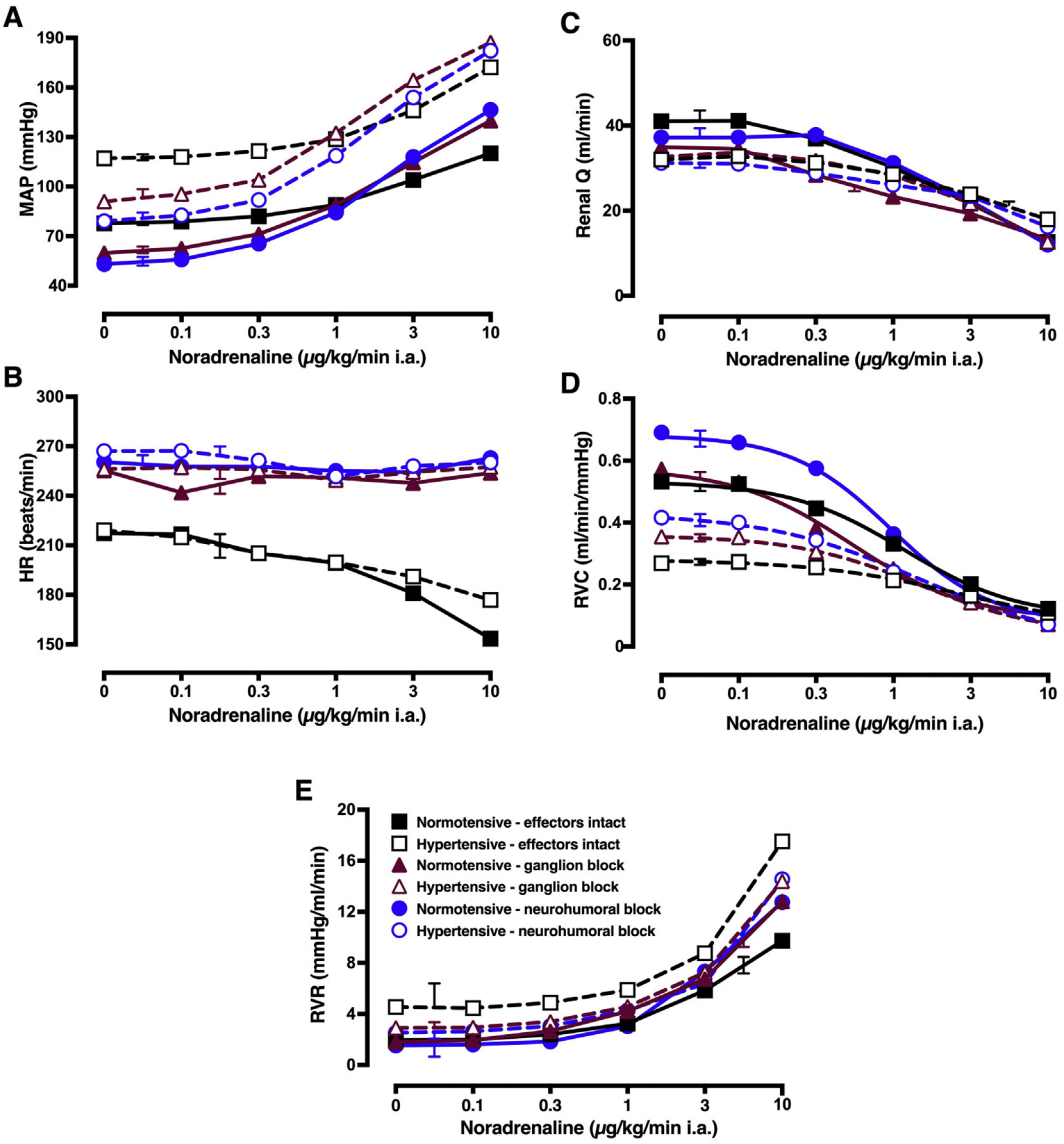

Figure 2. Noradrenaline i.a. infusion (μg/kg/min) dose-haemodynamic response curves in normotensive (n = 8–9; solid symbols and lines) and hypertensive (n = 7; open symbols and dashed lines) rabbits under three treatments (completed on separate experimental days): effectors intact (squares); ganglion block (triangles); and neurohumoral block (circles). 0, Baseline just before infusion of noradrenaline. 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.

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 during 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 (Δ -1.36 mmHg/ml/min) from the level observed with effectors intact. A greater fall was observed with neurohumoral block (Δ -1.76 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 Korner *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 (Δ -0.070 mmHg/ml/min from effectors intact), with no further change with neurohumoral block (Δ -0.071 mmHg/ml/min from effectors intact). In normotensive rabbits, the falls in resistance in each vascular bed were similar with ganglionic block 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

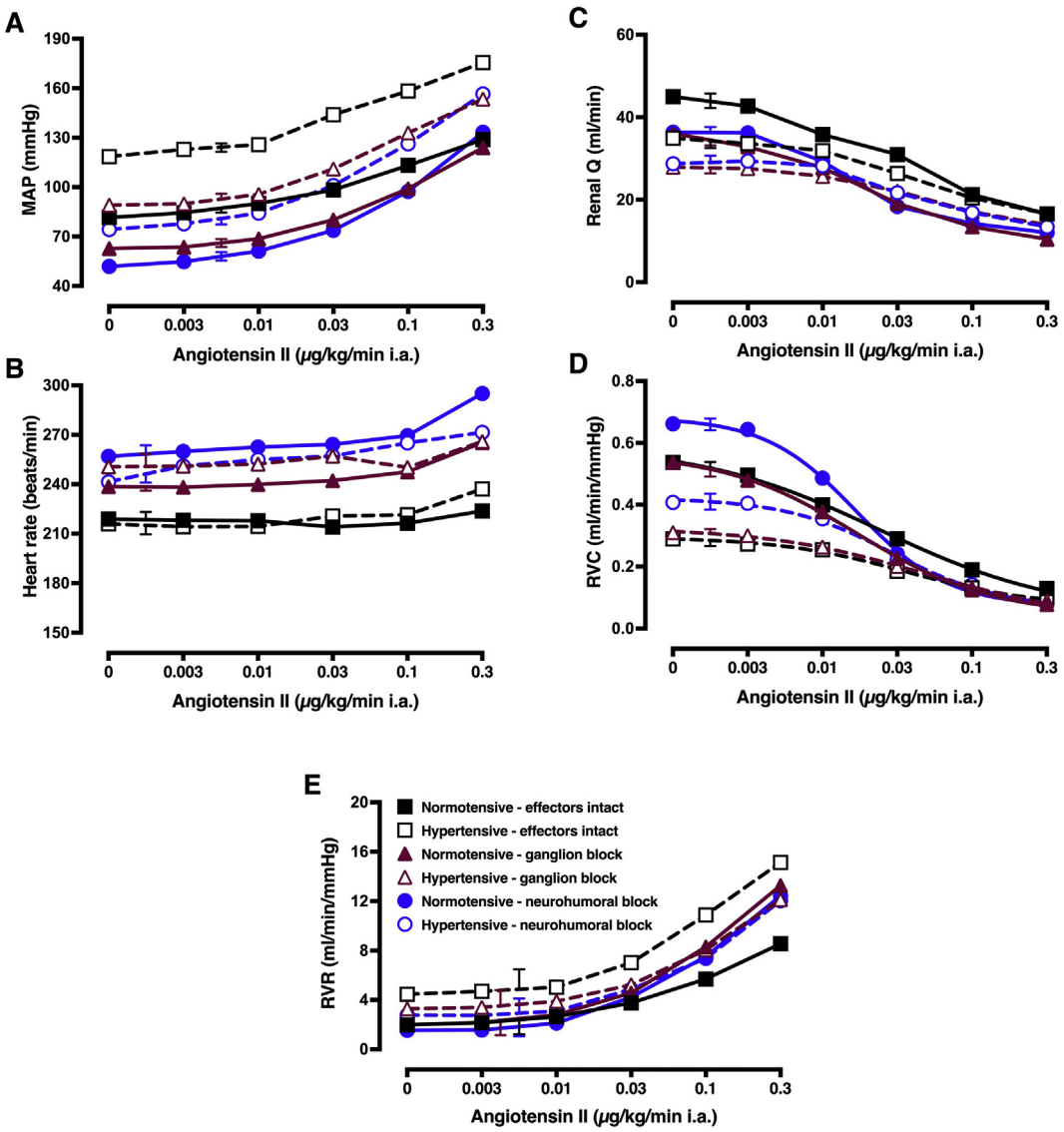

Figure 3. Angiotensin II i.a. infusion (μg/kg/min) dose-haemodynamic response curves in normotensive (n = 8–9; solid symbols and lines) and hypertensive (n = 7; open symbols and dashed lines) rabbits under three treatments (completed on separate experimental days): effectors intact (squares); ganglion block (triangles); and neurohumoral block (circles). 0, Baseline just before infusion of angiotensin II. 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.

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 α_1-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

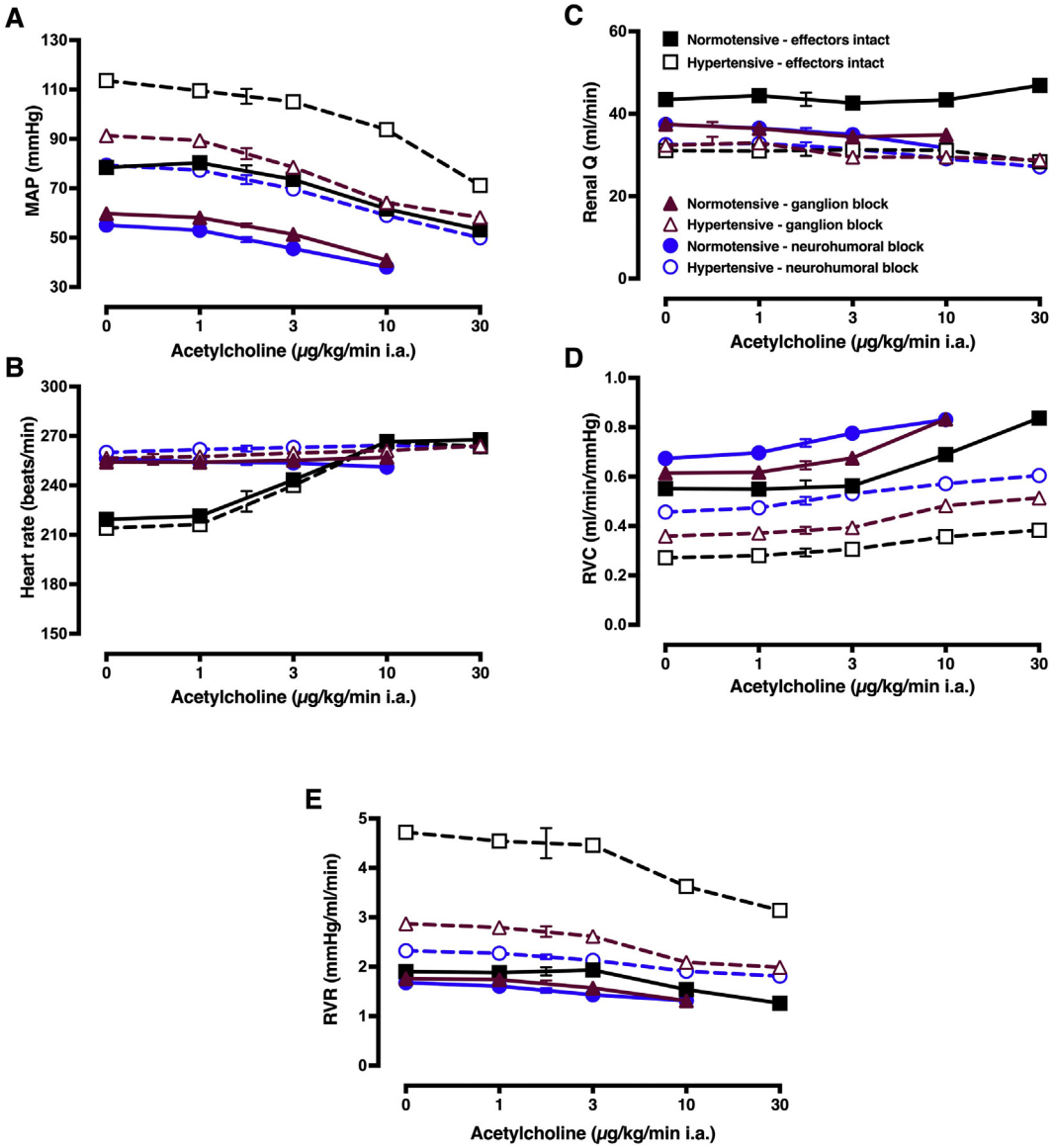

Figure 4. Acetylcholine i.a. infusion (μg/kg/min) dose-haemodynamic response curves in normotensive (n = 8–9; solid symbols and lines) and hypertensive (n = 7; open symbols and dashed lines) rabbits under three treatments (completed on separate experimental days): effectors intact (squares); ganglion block (triangles); and neurohumoral block (circles). 0, Baseline just before infusion of acetylcholine. 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.

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

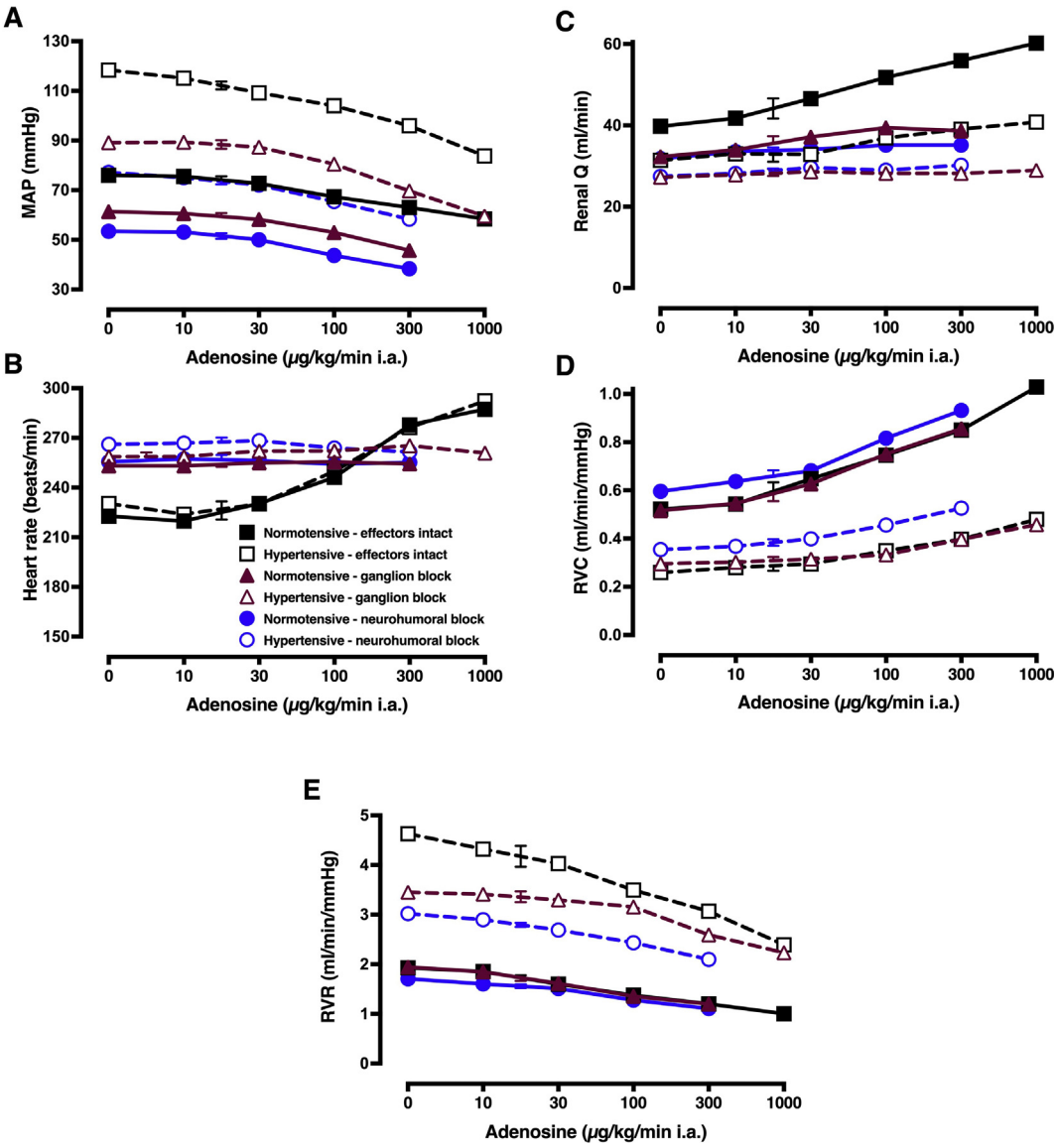

Figure 5. Adenosine i.a. infusion (μg/kg/min) dose-haemodynamic response curves in normotensive ($n = 8$–9; solid symbols and lines) and hypertensive ($n = 7$; open symbols and dashed lines) rabbits under three treatments (completed on separate experimental days): effectors intact (squares); ganglion 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.

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

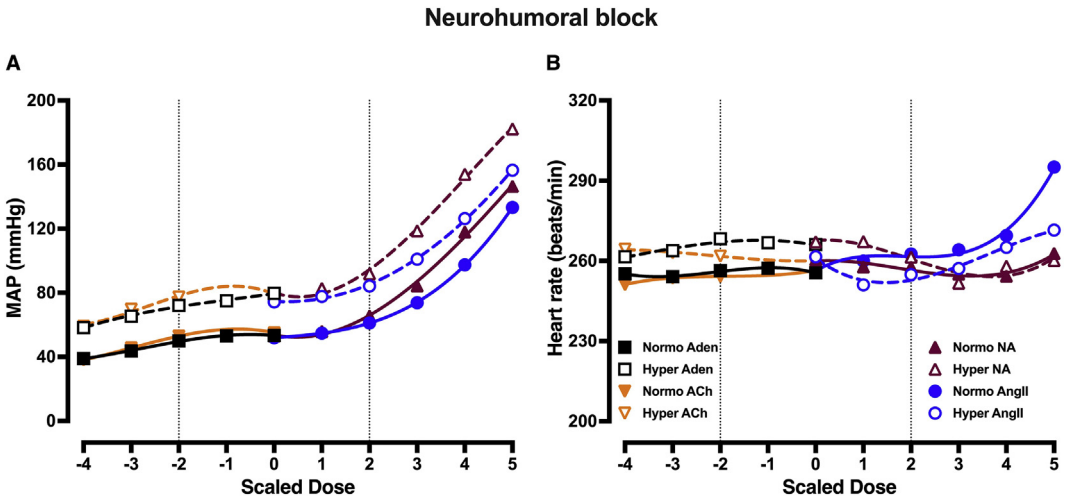

Figure 6. Average mean arterial pressure (MAP, mmHg; panel A) and average heart rate (beats/min; panel B) at rest (0 scaled dose), following i.a. infusions of acetylcholine (ACh, downward triangles) or adenosine (Aden, squares) at scaled doses -1 to -4 and following i.a. infusions of noradrenaline (NA, upward triangles) or angiotensin II (AngII, circles) at scaled doses +1 to +5. The data from the normotensive rabbits (Normo, $n = 9$) are shown by the solid symbols (solid lines) and from the hypertensive rabbits (Hyper, $n = 7$) in corresponding open symbols (dashed lines). For each agonist, 0 to -4 or 0 to +5, a 3rd order polynomial was fitted as shown by the respective lines. All measurements are from rabbits given neurohumoral block.

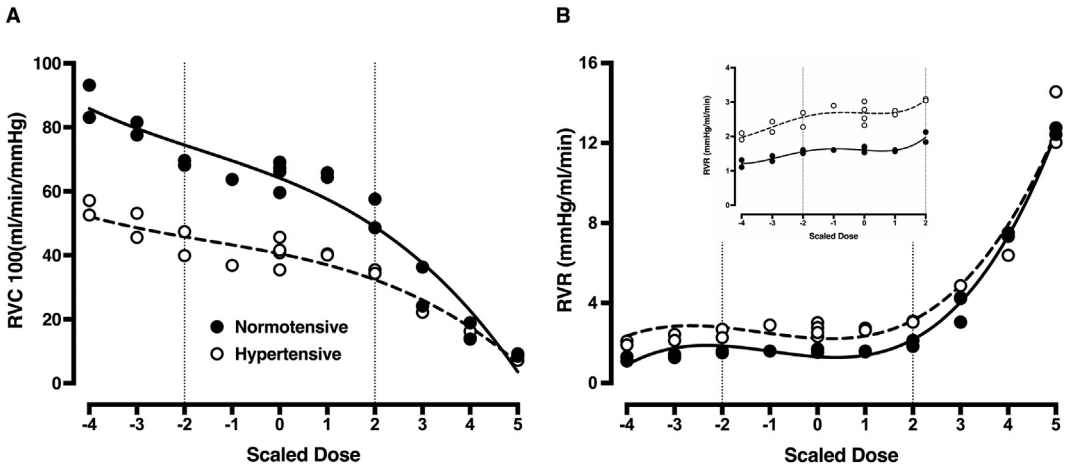

Figure 7. Combination graphs of effects of vasodilator and vasoconstrictor drug infusions on: A. renal vascular conductance (RVC); and B. renal vascular resistance (RVR). Each point is the mean of 7–9 rabbits infused i.a. with acetylcholine or adenosine at scaled doses -1 to -4 and noradrenaline or angiotensin II at scaled doses +1 to +5. The data from the normotensive rabbits ($n = 8$–9) are shown by the solid circles (solid lines) and from the hypertensive rabbits ($n = 7$) in corresponding open circles (dashed lines). In panel B, the insert graph shows data from scaled dose -4 to +2 on an enlarged y axis scale. For each rabbit group, -4 to +5, a 3rd order polynomial was fitted as shown by the respective lines. All measurements are from rabbits given neurohumoral block.

ratio 0.83–0.88 or 17-12%) in the hypertensive rabbits than in the normotensive rabbits. Secondly, r_i significantly increases from effectors intact, ganglionic block and 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

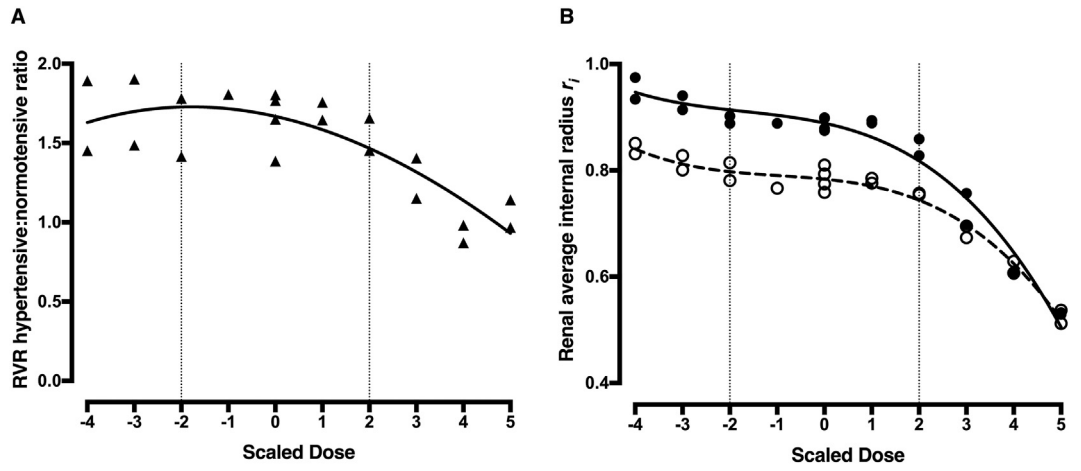

Figure 8. Combination graphs in rabbits with neurohumoral block showing (A) the hypertensive to normotensive ratio of renal vascular resistance, RVR, and (B) the renal average internal radius r_i in normotensive rabbits (solid circles and lines; $n = 8$–9) and hypertensive rabbits (open circles and dashed lines; $n = 7$) over the range of vasodilator and vasoconstrictor stimuli. Vasodilator agonists acetylcholine or adenosine were infused (i.a.) at scaled doses -1 to -4 and vasoconstrictor agonists noradrenaline or angiotensin II infused at scaled doses +1 to +5. The lines are 3rd order polynomial fits.

Table 3. Average internal radius, r_i, calculated from vascular bed resistance.

Vascular bed	Normotensive rabbits	Hypertensive rabbits	H:N
Renal vascular bed			
Effectors intact	0.856 ± 0.007	0.714 ± 0.017*	0.83
Ganglionic block	0.871 ± 0.008	0.759 ± 0.007*,#	0.87
Neurohumoral block	0.903 ± 0.009#	0.792 ± 0.011*,#	0.88
Total circulation			
Effectors intact	1.51 ± 0.04	1.33 ± 0.03*	0.88
Ganglionic block	1.63 ± 0.02#	1.42 ± 0.03*,#	0.87
Neurohumoral block	1.66 ± 0.05#	1.43 ± 0.02*,#	0.86

Values (mean ± SEM) are calculated as $r_i = \sqrt[4]{1/R}$ (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).

fell to 1.72 with ganglionic block or neurohumoral block. Interestingly, this resting value, with neurohumoral block, for total peripheral resistance was similar at a value of 1.79. The important findings from these resting RVR and TPR under the three conditions were that (i) the high level of constrictor sympathetic tone in the renal vasculature in hypertensive rabbits compared with normotensive rabbits and (ii) the lack of circulating angiotensin II and vasopressin affecting TPR in hypertensive but not normotensive rabbits. The effects of these three treatments on TPR and RVR H:N ratios illustrate the importance of obviating functional sympathetic tone and circulating angiotensin II and vasopressin when determining the effect of structural remodelling on the total and renal vasculature. Dilator and constrictor agents, while being infused locally to the renal vasculature, rapidly affected the circulation as evidenced by the changes in blood pressure and heart rate. Note that the neurohumoral block obviated the reflex bradycardia and tachycardia from scaled doses -2 to +2 (Figure 6B), but at scaled doses +3 to +5 angiotensin II and to a lesser extent noradrenaline had direct tachycardic actions at the sinoatrial node [31]. The choice of scaled doses to align the effects of two constrictor and two dilator drugs was made by allocating scaled dose -2 when MAP started to fall and +2 when MAP began to rise under neurohumoral block.

By scaled dose +3 for noradrenaline and angiotensin II, the renal vascular conductance had fallen by 50% or more in the normotensive and 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. One non-linearity from high doses of constrictor agents is elicited by functional (reversible) rarefaction with closure of a proportion of the small arterioles (R_2 vessels) and capillaries [15,32,33]. Dilator agents would also cause a non-linearity as blood pressure falls with the associated impairment of autoregulation.

Chronic hypertension will already have closed many R_2 vessels causing permanent rarefaction leaving fewer to respond to the constrictor drugs. At the highest scaled dose (+5), the renal vascular conductance (and renal vascular resistance) values were the same in hypertensive and normotensive rabbits (Figure 7A and B) suggesting that the level of total rarefaction (functional and permanent) was maximal in both groups of rabbits. Chronic hypertension may also be associated with endothelial dysfunction. However, we saw no evidence in the sensitivity (threshold dose) or the fall in MAP or RVR in hypertensive rabbits from adenosine and acetylcholine compared with normotensive rabbits.

4.2. Structual 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 r_i in chronic hypertension from vascular

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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