Differential Contribution of Afferent and Central Pathways to the Development of Baroreflex Dysfunction in Chronic Kidney Disease

Ibrahim M. Salman, Cara M. Hildreth, Omar Z. Ameer, Jacqueline K. Phillips

Abstract—The effects of chronic kidney disease on baroreflex control of renal sympathetic nerve activity (RSNA) and deficits in afferent and central components of the baroreflex were studied in juvenile and adult male Lewis Polycystic Kidney (LPK) and control Lewis rats under anesthesia (n=35). Blood pressure (BP), heart rate (HR), aortic depressor nerve activity (ADNA), and RSNA were determined after pharmacological manipulation of BP. Responses to ADN stimulation (4.0 V, 2.0 ms, 1–24 Hz) were determined, and the aortic arch was collected for histomorphometry. In juvenile LPK versus age-matched Lewis rats, gain of RSNA (~1.5±0.2 versus ~2.8±0.2/mm Hg; P<0.05) and ADNA (2.5±0.3 versus 5.0±0.6/mm Hg; P<0.05), but not HR barocurves, were reduced. BP, HR, and RSNA responses to ADN stimulation were normal or enhanced in juvenile LPK. In adult LPK versus age-matched Lewis, the gain and range of RSNA (gain: −1.2±0.1 versus −2.2±0.2%/mm Hg, range: 62±8 versus 98±7%) and HR (gain: −0.7±0.1 versus −3.5±0.7 bpm/mm Hg, range: 44±8 versus 111±19 bpm) barocurves were reduced (P<0.05). The gain and range of the ADNA barocurves were also reduced in adult LPK versus Lewis [1.5±0.4 versus 5.2±1.1 (%/mm Hg) and 133±35 versus 365±61 (%) P<0.05] and correlated with aortic arch vascular remodeling. BP, HR, and RSNA responses to ADN stimulation were significantly reduced in adult LPK. Our data demonstrate a deficit in the afferent component of the baroreflex that precedes the development of impaired central regulation of RSNA and HR in chronic kidney disease, and that progressive impairment of both components is associated with marked dysfunction of the baroreflex pathway. (Hypertension. 2014;63:804-810.) • Online Data Supplement

Key Words: baroreflex • heart rate • hypertension • renal insufficiency, chronic • sympathetic nervous system

Autonomic dysfunction is a major complication of chronic kidney disease (CKD)\(^3\) and is likely a key contributor to the high incidence of cardiovascular mortality in this patient population. In addition to sympathetic overdrive, evidenced by increased sympathetic nerve activity (SNA)\(^3\)–\(^5\) and plasma noradrenaline levels,\(^6\) baroreflex control of heart rate (HR) is impaired.\(^7\)–\(^8\) Impaired baroreflex control of HR is directly correlated with the severity of CKD\(^9\) and is an independent risk factor for sudden cardiac death in people with CKD.\(^10\) Whether or not baroreflex control of SNA is impaired in CKD is unclear, with mixed reports of normal\(^11\) and impaired\(^12\) responses. Moreover, the mechanisms underlying baroreceptor dysfunction in CKD are unknown and could relate to an inability for the baroreceptor afferents, including the aortic depresor nerve (ADN) and carotid sinus nerve,\(^13\) to sense changes in blood pressure (BP), influenced by factors such as altered vascular distensibility and mechanotransduction at the receptor level. Alternatively, central relay nuclei such as the nucleus tractus solitarius, nucleus ambiguous, or ventrolateral medullary sites may fail to produce sufficient change in vagal or sympathetic outflow, or the heart and vasculature may inadequately respond to these autonomic inputs.

Previously, we demonstrated that the Lewis Polycystic Kidney (LPK) rat, an animal model of autosomal recessive cystic kidney disease arising from a mutation in the Nek8 gene,\(^14\) develops impaired baroreflex control of HR between 13 and 12 weeks of age.\(^15\) In the present study, we wished to identify whether a temporal impairment in baroreflex control of renal SNA (RSNA) also occurs in the LPK, and at what point within the baroreflex arc dysfunction occurs. Therefore, we compared the functionality of the afferent and central components of the baroreflex in LPK and control Lewis rats, at 7–8 weeks of age, when the HR reflex is intact, and 12–13 weeks of age, when the HR reflex is impaired,\(^15\) and renal function has deteriorated.\(^16\) Because progressive remodeling occurs along the thoracic aorta in the LPK between 6 and 12 weeks of age,\(^17\) we hypothesized that similar vascular remodeling would occur along the aortic arch, a site of origin of baroreceptors, and be associated with reduced functionality of the afferent component of the baroreflex.

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From the Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia.
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Correspondence to Jacqueline K. Phillips, Australian School of Advanced Medicine, 2 Technology Place, Macquarie University, Sydney, NSW 2109, Australia. E-mail jacqueline.phillips@mq.edu.au
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Methods
A detailed description of all experimental methods can be found in the Online Supplement.

Results
Baseline Data
Baseline levels of mean arterial pressure (MAP), systolic blood pressure (SBP), pulse pressure (PP), HR, and RSNA were elevated in the LPK compared with age-matched controls (Table S1 in the online-only Data Supplement). An age-related increase in PP was observed in the LPK. No difference in aortic depressor nerve activity (ADNA) was observed between LPK and age-matched controls but there was an age-related increase in the LPK, despite no concomitant increase in HR. Urinary protein:creatinine ratio was elevated in both juvenile and adult LPK versus age-matched controls and further elevated in adult versus juvenile LPK (Table S1).

Baroreceptor Reflex Control of HR, RSNA, and ADNA
Reflex HR, RSNA, and ADNA responses to pharmacologically evoked increases and decreases in BP after administration of phenylephrine and sodium nitroprusside, respectively, are illustrated in Figure 1. Representative curves showing the sigmoidal fit of the MAP-HR, MAP-RSNA, and MAP-ADNA relationship in adult Lewis and LPK rats are shown in Figure S1, and group data in Figure 2.

Baroreflex Control of HR
In both juvenile and adult LPK, there was a rightward shift in the HR baroreflex function curve compared with age-matched controls (Figure 2A), as indicated by an increase in the MAP_{50} (Table 1). In the juvenile LPK, the curve was shifted upward as demonstrated by an increase in the upper plateau of the curve. However, both the lower plateau and the range of the curve were not significantly different between the juvenile LPK and Lewis (Tables 1 and S2). In the adult LPK, the upper plateau of the curve did not differ compared with age-matched controls. However, the lower plateau was higher and, therefore, there was a reduction in the range of the curve in the adult LPK compared with adult Lewis (Tables 1 and S2). The gain of the reflex was comparable in the juvenile LPK and Lewis but was reduced in the adult LPK versus Lewis (Table 1). Consequently, there was an age-related reduction in both the range and the gain of the HR baroreflex in the LPK (Table 1).

Baroreflex Control of RSNA
RSNA baroreflex function curves were shifted to the right in both the juvenile and adult LPK (Figure 2B). Accordingly, MAP_{50}, MAP threshold (MAP_{th}), and MAP saturation (MAP_{sat}) were higher in the LPK versus age-matched Lewis (Tables 1 and S2). In juvenile LPK, there was no difference in the upper and lower plateau, and therefore range, of the reflex compared with juvenile Lewis (Tables 1 and S2). In adult LPK, the upper plateau was comparable; however, the lower plateau and therefore RSNA at MAP_{sat} were higher versus Lewis and juvenile

Figure 1. Representative raw data traces, illustrating responses of aortic depressor nerve activity (ADNA), renal sympathetic nerve activity (RSNA), and heart rate (HR) to evoked changes in arterial pressure (AP) from an (A–C) adult (12–13 weeks old) Lewis and (D–E) adult Lewis Polycystic Kidney (LPK) rats. Bursts of ADNA can be seen in association with each pulse of AP in both Lewis and LPK. In response to phenylephrine (PE, 10–50 \( \mu \)g/kg), RSNA is silenced and HR reduced in the Lewis (B); however, in the LPK (E), reflex sympathoinhibition and bradycardia are reduced. Significant reductions in ADNA and reflex tachycardia are observed when AP is reduced by sodium nitroprusside (SNP, 50–70 \( \mu \)g/kg; C and F). bpm indicates beats per minute.
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Because baseline RSNA was elevated in the LPK, baroreflex function curves were also generated using microvolt RSNA (Figure S2). Accordingly, there was an increase of the upper plateau of the reflex and RSNA at MAP_{up} and MAP_{sat} in the juvenile LPK versus age-matched Lewis (Table S3). Compared to age-matched Lewis, the lower plateau was shifted upward in adult LPK, and this was associated with markedly higher measures of RSNA at MAP_{sat}. The gain and range of the reflex expressed in microvolts was not different between the LPK and Lewis at any age; however, both parameters declined with age in the LPK.

**Baroreflex Control of ADNA**

Administration of sodium nitroprusside markedly reduced ADNA, whereas phenylephrine resulted in an increase in ADNA (Figure 1).

Baroreflex control of ADNA was shifted to the right in both the juvenile and adult LPK (Figure 2C). Accordingly, MAP_{50} and MAP_{sat} were higher in the LPK versus Lewis (Tables 1 and S2). The range of the curves was comparable between the juvenile LPK and Lewis; however, in the adult LPK, the upper plateau, range, and ADNA at MAP_{sat} were reduced relative to Lewis and an age-related decrease in the range was evident. The gain of the reflex was also reduced in both juvenile and adult LPK versus Lewis controls, and this reduction tended to be greater (P=0.065) in adult versus juvenile LPK (Table 1).

**Central Component of Baroreflex Arc**

Electric stimulation of the ADN between 1 and 24 Hz reduced RSNA, HR, and MAP in all groups (Figure 3). In juvenile LPK, the reflex sympathoinhibition was comparable with Lewis controls, consistent with results showing the relationship between ADNA and RSNA was also not different at this age (P=0.13; Figure S3). Reflex bradycardic and depressor responses were however enhanced in juvenile LPK (Figure 3).

In adult LPK, reflex responses were reduced compared with Lewis controls and juvenile LPK. This was most noticeable at the higher frequencies.

Sympathoinhibitory responses to ADN stimulation were also analyzed as microvolt data (Figure S4). Results were comparable with those described in terms of percentage change, with no difference between juvenile LPK and Lewis controls, but reduced RSNA reflex responses in the adult LPK, and an age-related reduction evident.

**Histomorphometry of the Aortic Arch**

Vascular remodeling along the aortic arch was evident in both the juvenile and adult LPK (Figure S5; Table S4). Notably, at both ages, the aortic arch was characterized by an increase in medial wall thickness, reduction in elastin content, elastin-to-collagen ratio, and an increase in the number of elastin lamellae frictions and collagen density in the tunica media. Aortic medial calcium deposition was markedly elevated in adult LPK. These parameters progressively changed with age, indicating an age-related increase in vascular hypertrophy and arteriosclerotic remodeling along the aortic arch.

There was a significant negative correlation between the gain of the ADNA baroreflex function curves and medial wall thickness, number of elastin lamellae frictions, collagen density and nucleus cross-sectional area, and a significant positive correlation with the total elastin density and elastin-to-collagen ratio (Table S5; Figure 4). The range of the ADNA baroreceptor function curve was negatively correlated with aortic medial wall thickness, number of elastin lamellae frictions, and total calcium density, whereas a positive association was seen with total elastin density and elastin-to-collagen ratio (Table S5; Figure 4).
The major goal of this study was to identify whether any temporal change in baroreflex control of HR and RSNA in CKD is associated with a deficit in the afferent or central component of the baroreflex circuit. The important new findings are the following: (1) there is a temporal decline in baroreflex control of RSNA in the LPK; (2) in juvenile LPK, a deficit in the afferent component of the baroreflex is
the primary deficit; (3) in adult LPK, further reduced afferent function and a decrease in central processing are associated with markedly impaired RSNA and HR baroreflexes; (4) a decline in the functionality of the afferent component of the reflex is correlated with vascular remodeling of the aortic arch. Together this shows that in CKD, full expression of baroreflex dysfunction is dependent on both impaired afferent signaling and abnormal central processing.

Afferent Signaling
An early deficit in the afferent component of the baroreflex was observed in the LPK evidenced by a reduction in the gain of the ADNA baroreflex function curves in the juvenile LPK. This impairment worsened with age in the LPK, with the adult animals exhibiting a marked reduction in the range of the reflex. This indicates that in the adult LPK, in response to changes in BP, the ADN does not respond as fast or as effectively in comparison with either juvenile LPK or age-matched Lewis. Dysfunctional baroreceptor afferent function has been previously shown in the spontaneously hypertensive rat and Dahl salt-sensitive hypertensive rat. To our knowledge, this is the first report of impaired baroreceptor afferent function in CKD. Hypertension and increased SNA are known to cause vascular hypertrophy and loss of vessel structure, leading to the loss of aortic receptor function. Previously, we demonstrated vascular remodeling in the thoracic aorta of the LPK and an associated functional increase in pulse wave velocity, indicating aortic stiffness. In CKD patients, impaired baroreflex function directly correlates with a reduction in arterial distensibility, as evidenced by increased pulse wave velocity. In patients with polycystic kidney disease, this has been further demonstrated to be apparent before the onset of hypertension or reduced renal function. Here, we demonstrate that vascular remodeling occurs along the aortic arch, the site of aortic baroreceptor afferents, correlating with a decline in baroreceptor afferent function. These findings strongly support the hypothesis that in the LPK, hypertrophy and a reduction in elastic properties reduce aortic wall distensibility and hence impair the ability of the aortic baroreceptors to effectively transduce changes in BP.

Central Processing
Altered afferent baroreceptor function preceded any decline in the functionality of the central component of the baroreceptor reflex in the LPK. In juvenile LPK, the central component of the baroreceptor reflex was intact, and greater reductions in HR could be evoked by ADN stimulation when compared with juvenile Lewis. This enhancement may indicate a compensatory mechanism, such that in response to a reduction in afferent input, the HR baroreceptor reflex is able to buffer changes in BP. In adulthood, the LPK demonstrated reduced reflex responses to stimulation of the ADN, indicating a decline in the central component of the reflex. The impairment in central processing seems independent of the afferent fiber type because the reflex responses to both low (<10 Hz) and high (>10 Hz) frequency stimulation were reduced in the LPK, indicating that both A- and C- fiber input are impaired. Altered central processing of the baroreceptor reflex is a feature of other models of hypertension, including the spontaneously hypertensive rat, renal wrap hypertensive rats, and obese Zucker rats, and although the exact location of the deficit cannot be elucidated from the present study, it is plausible that key medullary nuclei, such as the rostral ventrolateral medulla, responsible for generating changes in SNA in

Figure 4. Correlation between key histomorphometric variables examined in the aortic arch and afferent baroreceptor function in the juvenile (7–8 weeks old) and adult (12–13 weeks old) Lewis and Lewis Polycystic Kidney (LPK) rats. A, Elastin density vs aortic depressor nerve activity (ADNA) gain. B, Collagen density vs ADNA gain. C, Aortic media thickness vs ADNA range. D, Number of elastin lamellae fractures vs ADNA range. n=21.
accordance with the baroreflex, are abnormal in the LPK, as seen in other hypertensive rodent models.27,28

Baroreflex Function

Previously we demonstrated that, under conscious conditions, the LPK develops a temporal decline in the sensitivity of the HR baroreflex between 10 and 12 weeks of age.15 In the present study, we replicate and extend on this finding under anesthesia by showing that there is also a temporal decline in the range of the HR baroreflex in the LPK. Here, we further show that there is a temporal decline in baroreflex control of RSNA in CKD, which is associated with an increase in resting RSNA. This renal sympathetic overactivity was already evident in juvenile LPK but, interestingly, despite a decline in renal function during the same time-frame, RSNA did not further increase. This suggests that, analogous to the human condition, RSNA is increased early in the disease-course and may contribute to the further deterioration in renal function.29

In the juvenile LPK, the RSNA baroreflex gain (%) was reduced; however, the range (μV) of the reflex was comparable. This suggests that at this age, the baroreflex is capable of producing a full range of RSNA change, albeit at a slower rate. The decreased gain of the RSNA baroreflex is potentially contributed to by the decreased responsiveness of the ADN that we describe. In contrast, in adult LPK, the gain and range of the RSNA (%) baroreceptor function curves were impaired in comparison with both adult Lewis and juvenile LPK. We think that this change reflects a temporal decline in baroreflex control of RSNA, rendering the reflex impaired in adulthood because (1) there was no further increase in RSNA in the adult LPK that could potentially bias this data; (2) the ability to produce reflex inhibition of RSNA in response to ADN stimulation was reduced when RSNA was expressed in both normalized and absolute units; and (3) there was an age-related reduction in the gain and range of the RSNA (μV) baroreceptor curves in the LPK that was not observed in the Lewis rats. The deficit in baroreflex control of RSNA in the adult LPK observed in this study contrasts with previous findings that baroreflex control of splanchnic sympathetic outflow, while unable to maximally suppress nerve activity, is comparable in the LPK and Lewis in terms of the sensitivity of the reflex.7 Our finding of reduced baroreflex control of RSNA suggests that the reflex control of sympathetic outflow may be differentially regulated and impaired in CKD.

In conclusion, our findings indicate that in the juvenile LPK, a deficit in baroreceptor afferent function is compensated for by the central component of the baroreflex, and consequently HR baroreflex function is preserved and only a minimal impairment in baroreflex control of RSNA is observed. In the adult LPK, however, there is a loss of function in the central component of the baroreflex that, together with a reduction in afferent baroreflex function, results in markedly impaired baroreflex control of both HR and RSNA.

Perspectives and Significance

Cardiovascular autonomic dysfunction is a major cause of morbidity and mortality in patients with CKD1;2 however, the critical underlying mechanisms are not fully understood. Herein, we provide direct evidence of sympathetic overactivity, compounded with impaired baroreflex control of HR and SNA, thus emphasizing the complexity of this pathological condition. The study further highlights key pathways within the baroreflex arc that explain the mechanisms involved and could potentially be targeted for future therapeutics. We suggest that early interventional measures to treat CKD that reduce SNA, lower BP, and limit vascular remodeling may well serve to ameliorate autonomic dysfunction and therefore reduce overall cardiovascular risk in these patients.

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Disclosures

None.

References


Novelty and Significance

What Is New?

• This is the first comprehensive investigation to demonstrate mechanisms underlying dysfunctional baroreflex control of heart rate and sympathetic nerve activity (SNA) in chronic kidney disease, and changes associated with disease progression.

What Is Relevant?

• The kidney plays a key role in high blood pressure, with a vicious circle whereby hypertension in turn can further damage the kidney. An increase in SNA and impaired autonomic reflex responses is increasingly being recognized as a major cardiovascular risk factor for patients with chronic kidney disease. By identifying dysfunction within the baroreflex arc, future therapeutic targeting to reduce mortality accompanying chronic kidney disease may be developed.

What Is Relevant? What Is New?

Summary

In the Lewis Polycystic Kidney model of chronic kidney disease, SNA is elevated early in the course of renal dysfunction, whereas reflex control of heart rate and SNA becomes progressively impaired. Our novel data indicate that there is a deficit in the afferent component (aortic depressor nerve) of the baroreflex that precedes the development of impaired central regulation of RSNA and heart rate, and that the progressive impairment of both components is associated with a marked dysfunction of the baroreflex pathway. We also demonstrate that the decline in aortic depressor nerve function correlates with structural remodeling of the aortic arch.
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Differential Contribution of Afferent and Central Pathways to the Development of Baroreflex Dysfunction in Chronic Kidney Disease

Online Supplement

Ibrahim M. Salman, Cara M. Hildreth, Omar Z. Ameer, Jacqueline K. Phillips
Methods

Animals

Male juvenile (7–8 weeks old) and adult (12–13 weeks old) Lewis Polycystic Kidney (LPK; total \( n = 32 \)) and Lewis (total \( n = 31 \)) were sourced from the Animal Resource Centre, Murdoch, Western Australia, Australia. All experiments were approved by the Animal Ethics Committee of Macquarie University and carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

The LPK model is a result of a mutation in \( \text{Nek8} \),\(^1\) which in humans is responsible for nephronophthisis (NPHP)\(^2\)\(^3\). Multiple NPHP genes have been identified that encode for the nephrocystin protein family and overall they constitute a group of autosomal-recessive cystic kidney disorders that are the leading genetic cause of end-stage renal disease in children and young adults.\(^3\) In the juvenile cystic kidney (jck) mouse\(^4\) and LPK rat\(^5\), the \( \text{Nek8} \) mutation leads to a phenotypic presentation of cystic kidney disease that resembles human autosomal recessive polycystic kidney disease.

Anaesthesia and Surgical Procedures

For in-vivo studies, animals (\( n = 35 \) total) were anaesthetised with ethyl carbamate (urethane, 1.3 g/kg i.p., Sigma Aldrich, Australia). Depth of anaesthesia was confirmed by absence of reflex response to tactile (corneal stroking) and noxious (hindpaw pinch) stimuli. Supplemental doses of urethane (10–20 mg i.v.) were administered as required. Immediately following induction of anaesthesia, rectal temperature was monitored and maintained at 37ºC using a thermostatically controlled heating blanket and infrared heating source, and supplemental oxygen provided. A tracheostomy was performed and if required, the animal was artificially ventilated with oxygen enriched room air and ventilation adjusted to maintain pH at 7.40 ± 0.05 and pCO\(_2\) at 40 ± 5 mmHg.

The right jugular vein and both femoral veins were cannulated for the administration of fluids (Ringer’s solution, 5 ml/kg/hr) and drugs respectively. The carotid artery was cannulated and connected to a pressure transducer for the recording of blood pressure (BP), which was sampled at 250 Hz and acquired using a CED 1401 plus (Cambridge Electronic Designs Ltd, Cambridge, UK) and Spike 2 (v7, Cambridge Electronic Designs Ltd., Cambridge, UK). Heart rate (HR) was derived online from the BP signal.

The left aortic depressor nerve (ADN) was isolated as it joined the cervical vagus nerve near the superior laryngeal nerve. Isolation of the ADN was confirmed using the following criteria: 1. audio confirmation that the nerve had a bursting discharge pattern; 2. pulse synchronous discharge of nerve activity with the systolic phase of the cardiac cycle; and 3. an increase and a decrease in activity following administration of phenylephrine (PE) and sodium nitroprusside (SNP), respectively.

The left renal nerve was isolated retroperitoneally. Both the renal nerve and ADN were dissected by the same investigator, maintained in paraffin oil and recorded whole using bipolar silver wire recording electrodes, amplified, band-pass filtered (10–1000Hz, CWE Inc., Ardmore, PA, USA) and sampled at 5 kHz using a CED 1401 plus and Spike2, using the same bioamplifier calibrated to a pre-set 50 \( \mu \)V setting.
Experimental Protocols

Following stabilisation of baseline parameters, rats underwent one or both of Protocols I and II (detailed below) to assess the different components of the baroreflex arc. Baroreflex control of HR and sympathetic outflow were examined by measuring changes in HR and RSNA in response to changes in MAP. The afferent component of the baroreflex arc was assessed by determining changes in ADNA in response to changes in MAP. The central component of the baroreflex arc was tested by measuring the changes in HR and RSNA to direct stimulation of the ADN and also by correlating changes in ADNA and RSNA in response to alterations in MAP.

Protocol I: Assessment of the relationship between BP and aortic depressor nerve activity (ADNA), renal sympathetic nerve activity (RSNA) and HR.

Blood pressure was manipulated using sequential bolus injections of PE (10–50 µg/kg i.v.) and SNP (50–70 µg/kg i.v.), in order to increase mean arterial pressure (MAP) to 200-250 mmHg maximum and reduce it down to 50 mmHg, respectively. This was repeated at least 3 times. Changes in ADNA, RSNA and HR were recorded in response to each drug administration and all variables were allowed to return to baseline levels prior to subsequent drug administration. Where possible the relationship between MAP and ADNA was assessed simultaneously to the relationship between MAP and RSNA. In animals that subsequently underwent Protocol II (see below), the ADN was cut distal to the recording electrode and background ADNA recorded. Animals that did not undergo Protocol II were euthanased with an overdose of sodium pentobarbital (60 mg/kg i.v.) and background ADNA recorded.

Protocol II: Assessment of the RSNA, HR and BP response to ADN stimulation.

The ADN was cut and the proximal end stimulated sequentially using a 5-second train (4.0V, 2.0-ms pulses) of 1, 2, 4, 8, 16 and 24 Hz separated by a period of 3–5 minutes, as described previously.6-8 Peak RSNA, HR and BP responses were observed within the first two seconds of ADN stimulation and were sustained until the stimulation was ceased. Responses were continuously recorded.

At the end of the experiment, the renal nerve was cut proximally to the recording electrode and background RSNA recorded. The animal was then either euthanased with an overdose of 60 mg/kg sodium pentobarbital i.v. or transcardially perfused with heparinised 0.9% saline followed by 4% formalin in saline and the aortic arch removed and stained with Shikata’s orcein, Martius Scarlet Blue (MSB) or Von Kossa for histomorphometry.

Urinary protein:creatinine ratio

A separate cohort of age-matched animals (n = 25 total) were individually held in metabolic cages for at least 4 hours to collect urine samples. Urine was then centrifuged at 3000 rpm for 5 min and stored at -20 °C until further assayed for urinary protein: creatinine ratio (each g/L) using an IDEXX VetLab analyser (IDEXX Laboratories Pty Ltd., NSW, Australia).
Data analysis

All data was analysed offline using Spike 2 software, GraphPad Prism (GraphPad Prism software v6 Inc., La Jolla, CA, USA) and/or Axiovision software (AxioVs40 v4.8.2.0, Carl Zeiss Microimaging, Gottingen, Germany).

(i) Baseline data

Mean arterial pressure, systolic BP (SBP), diastolic BP (DBP) and HR were derived from the arterial pressure waveform. Both RSNA and ADNA waveforms were full-wave rectified, a 1-second smoothing constant applied and the level of activity following nerve transection or euthanasia subtracted.

Baseline measurements of MAP, SBP, DBP, HR, RSNA and ADNA were taken over a 30s period immediately prior to commencement of the experimental protocol.

In order to confirm the integrity of the RSNA signal and eliminate renal afferent nerve activity as a confounding variable, a pilot study using adult LPK (n=3) was undertaken where RSNA recordings were assessed before and after ganglionic blockade [hexamethonium (20 mg/kg i.v.)] and then compared to levels after euthanasia. RSNA was significantly reduced after ganglionic blockade (6.4 ± 0.8 vs. 2.2 ± 0.2 µV, P = 0.003) and did not change any further after euthanasia (2.3 ± 0.3 µV, P > 0.999 vs. after hexamethonium). P values are the outcomes of one-way ANOVA and Bonferroni post hoc analysis.

(ii) Baroreceptor afferent, central and reflex function curves

Baroreceptor reflex regulation of ADNA, RSNA and HR were examined by comparing changes in ADNA, RSNA and HR, in response to pharmacologically-evoked increases and decreases in BP using PE and SNP, respectively (Figure 1). In animals where simultaneous recordings of ADNA and RSNA were made, indirect assessment of the central component of the baroreceptor reflex was evaluated by comparing RSNA (efferent output) with ADNA (afferent input) over the pharmacologically-evoked increase and decrease in BP achieved using PE and SNP.

Both RSNA and ADNA were normalised, setting a 30s period immediately prior to PE and SNP administration as 100% and the level of background nerve activity as 0%. The baseline levels of RSNA, ADNA and HR prior to administration of PE or SNP were compared to ensure that resting levels of nerve activity and HR did not differ between drug administrations; however, due to a range in the time intervals between PE and SNP administration, nerve activity immediately prior to each drug administration was used to normalise to 100%.

The relationship between the active phase of MAP change (from resting level of blood pressure through to the peak blood pressure change induced by PE or SNP) and induced responses in HR (BPM), RSNA (%) and µV or ADNA (%) were fitted to a four-parameter sigmoid logistic function curve (GraphPad Prism software) using the following equation:

\[ y = \frac{A_1}{1 + \exp \left[ A_2 \left( MAP - A_3 \right) \right]} + A_4 \]

where \( A_1 \) is the y axis range of the curve, \( A_2 \) is the gain coefficient, \( A_3 \) is midpoint of the curve and \( A_4 \) is the lower plateau. Curves with a \( R^2 \) value less than 0.9 were not included in the data set (see Figure S1 for example curves showing goodness of fit). In order to account for the time delay between a change in BP and the reflex change in HR, the relationship
between an increase and a decrease in BP and the concomitant change in HR was shifted by 10 beats with respect to their corresponding R-R interval as described previously. From each individual non-linear regression curve generated, the following parameters were obtained: range of the curve (A_1), the gain coefficient (A_2), midpoint of the curve (A_3) and lower plateau (A_4). Using these parameters, the range of the reflex (i.e. HR range, RSNA range and ADNA range), the gain of the reflex, MAP_{50}, MAP threshold (MAP_{th}), and MAP saturation (MAP_{sat}), MAP operating range and HR, RSNA and ADNA values at MAP_{th} and MAP_{sat} were calculated as described previously. Each individual parameter was then averaged to create a mean value for each group of animals (juvenile LPK, juvenile Lewis, adult LPK and adult Lewis).

Using the same equation described above, the relationship between ADNA and RSNA was fitted to a sigmoid regression curve as described previously. Curves with a R^2 value less than 0.85 were not included in the data set.

Each individual non-linear regression curve was averaged to obtain single logistic function curve per group. The MAP and HR, RSNA or ADNA relationships were then plotted over a fixed range of 50–200 mmHg in the Lewis and 50–250 mmHg in the LPK, reflecting the maximal PE and SNP-induced changes in MAP evoked in these two strains. The ADNA-RSNA curves were plotted over the range of ADNA evoked in response to these same blood pressure ranges in each strain.

(iii) ADN stimulation

Reflex responses to ADN stimulation were determined by measuring peak changes in RSNA (% and µV), HR (beats per minute: bpm) and MAP (mmHg) relative to an immediate 30-s baseline prior to the application of each electrical stimulus.

(iv) Histomorphometry

After perfusion with fixative, the aortic arch from each animal was excised, cleaned of adherent fat and connective tissue, and stored in 70% ethanol. The aortic arch was then dehydrated and fixed, as described previously, embedded in paraffin and transverse sections (5 µm) cut using a microtome (Leica Microm HH325, Germany), and mounted onto glass slides. Sections were stained for Shikata’s orcein, MSB, and Von Kossa. Photos were captured using a video camera mounted on a microscope (Carl Zeiss Microimaging, Gottingen, Germany) and processed with Zeiss Axiovision. Images were corrected for brightness and contrast only and analysis performed using Image J (v1.47d, National Institute of Health, USA). Evaluation of aortic thickness, elastin lamellae fracture points, calcium density and average size of calcium deposits was performed on the whole aortic segment (captured at 10x magnification) for each animal. All other histomorphometric parameters were calculated from an average of 4 fields equally distributed around the circumference of the aorta from each segment of the aortic arch (captured at 20 x magnifications). Wall thickness was measured on at least eight separate aortic arch regions for each animal and averaged. Specific analysis for each stain was as follows:

**Shikata’s orcein:** Analogue images were digitized and separated into 3 coloured images (red, blue and green). Subsequent image processing was performed on the red image only, indicating the elastin component of the aortic arch. The images were then binarized to extract relative measures of elastin in the field examined.

The number of elastin lamellae fracture points was normalised using the following equation:

\[
\text{number of fracture points} \div \text{average of inner and outer circumference} \times \text{number of lamellae}
\]
Martius Scarlet Blue (MSB): The MSB stained images were analysed by setting a threshold, which allowed for visualisation and quantification of the blue colour, indicating the total collagen density. All images were then binarized to extract relative measures of collagen and the smooth muscle cell nuclei in the studied field. Nuclei were detected by setting a minimum threshold on the binarized image and the total number of nuclei summated.

Von Kossa: The total calcium density and average size of calcium deposit in the media on Von Kossa-stained sections was quantified by setting a minimum threshold on the binarized image to visualise the black calcium deposits in the aortic media. All binarized images were visually inspected by two investigators and compared with the original image to ensure that all parameters were detected accurately.

(v) Correlation analysis

The gain and range of the MAP-ADNA function curves were correlated with the aortic arch histomorphometrical indices detailed above including medial wall thickness and measures of elastin, collagen and calcium content. Correlation analysis was performed using a Pearson correlation followed by stepwise linear regression.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software. A two-way ANOVA with Bonferroni correction was used to identify differences between groups (strain and age). A Brown-Forsythe test was used to determine if there were any differences in the variance, and if so, the data was log-transformed before statistical analysis. Significance was defined as \( P \leq 0.05 \).
Supplementary References


Table S1. Baseline parameters in Lewis and Lewis Polycystic Kidney (LPK) rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Juvenile</th>
<th>Adult</th>
<th>Strain</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lewis</td>
<td>LPK (6)</td>
<td>Lewis (6)</td>
<td>LPK (5)</td>
</tr>
<tr>
<td>BW (g)</td>
<td>214 ± 11</td>
<td>167 ± 6*</td>
<td>361 ± 8†</td>
<td>239 ± 7†</td>
</tr>
<tr>
<td>UPC</td>
<td>0.04 ± 0.01</td>
<td>0.94 ± 0.25*</td>
<td>0.05 ± 0.004</td>
<td>1.90 ± 0.3†</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>86 ± 3</td>
<td>108 ± 4*</td>
<td>85 ± 2</td>
<td>111 ± 6*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>116 ± 5</td>
<td>165 ± 4*</td>
<td>123 ± 5</td>
<td>179 ± 6*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>65 ± 3</td>
<td>76 ± 4*</td>
<td>64 ± 2</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>51 ± 4</td>
<td>89 ± 6*</td>
<td>59 ± 6</td>
<td>111 ± 6†</td>
</tr>
<tr>
<td>HR (BPM)</td>
<td>363 ± 11</td>
<td>439 ± 14*</td>
<td>350 ± 16</td>
<td>402 ± 13*</td>
</tr>
<tr>
<td>RSNA (µV)</td>
<td>3.6 ± 0.7</td>
<td>7.3 ± 1.5*</td>
<td>2.7 ± 0.4</td>
<td>5.3 ± 0.8*</td>
</tr>
<tr>
<td>ADNA (µV)</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>1.2 ± 0.3†</td>
</tr>
</tbody>
</table>

BW, body weight; UPC, urinary protein (g/L) to creatinine (g/L) ratio, MAP, mean arterial pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; and ADNA, aortic depressor nerve activity.

Results are expressed as mean ± SEM.

* P<0.05 vs. age-matched Lewis.
† P<0.05 vs. strain-matched juvenile rat.

(n) values denoted in subscript and represent the minimum number in each group.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Juvenile</th>
<th>Adult</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lewis</td>
<td>LPK</td>
<td>Lewis</td>
</tr>
<tr>
<td>HR barocurve (n)</td>
<td></td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>HR upper plateau (BPM)</td>
<td></td>
<td>370 ± 10</td>
<td>463 ± 8*</td>
<td>383 ± 17</td>
</tr>
<tr>
<td>HR lower plateau (BPM)</td>
<td></td>
<td>283 ± 24</td>
<td>331 ± 21</td>
<td>272 ± 21</td>
</tr>
<tr>
<td>MAPthr (mmHg)</td>
<td></td>
<td>102 ± 6</td>
<td>135 ± 8*</td>
<td>101 ± 5</td>
</tr>
<tr>
<td>HR at MAPthr (BPM)</td>
<td></td>
<td>352 ± 12</td>
<td>435 ± 7*</td>
<td>359 ± 16</td>
</tr>
<tr>
<td>RSNA barocurve (n)</td>
<td></td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>RSNA upper plateau (%)</td>
<td></td>
<td>110 ± 3</td>
<td>108 ± 4</td>
<td>111 ± 5</td>
</tr>
<tr>
<td>RSNA lower plateau (%)</td>
<td></td>
<td>9 ± 2</td>
<td>12 ± 6</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>MAPsat (mmHg)</td>
<td></td>
<td>109 ± 4</td>
<td>136 ± 6*</td>
<td>106 ± 7</td>
</tr>
<tr>
<td>RSNA at MAPsat (%)</td>
<td></td>
<td>89 ± 3</td>
<td>88 ± 3</td>
<td>90 ± 4</td>
</tr>
<tr>
<td>ADNA barocurve (n)</td>
<td></td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>ADNA upper plateau (%)</td>
<td></td>
<td>346 ± 26</td>
<td>324 ± 26</td>
<td>406 ± 60</td>
</tr>
<tr>
<td>ADNA lower plateau (%)</td>
<td></td>
<td>48 ± 15</td>
<td>37 ± 8</td>
<td>40 ± 5</td>
</tr>
<tr>
<td>MAPthr (mmHg)</td>
<td></td>
<td>103 ± 3</td>
<td>116 ± 7</td>
<td>93 ± 2</td>
</tr>
<tr>
<td>ADNA at MAPthr (%)</td>
<td></td>
<td>111 ± 11</td>
<td>98 ± 5</td>
<td>117 ± 12</td>
</tr>
<tr>
<td>MAPsat (mmHg)</td>
<td></td>
<td>143 ± 3</td>
<td>195 ± 13*</td>
<td>145 ± 6</td>
</tr>
<tr>
<td>ADNA at MAPsat (%)</td>
<td></td>
<td>283 ± 21</td>
<td>263 ± 19</td>
<td>328 ± 47</td>
</tr>
<tr>
<td>MAP operating range (mmHg)</td>
<td></td>
<td>40 ± 4</td>
<td>80 ± 13*</td>
<td>52 ± 6</td>
</tr>
</tbody>
</table>

MAPthr, threshold mean arterial pressure to trigger a change in HR, RSNA or ADNA; and MAPsat, saturation mean arterial pressure at which there is no further change in HR, RSNA or ADNA.

Results are expressed as mean ± SEM.

* P<0.05 vs. age-matched Lewis.
† P<0.05 vs. strain-matched juvenile rat.

(n) = number in each group.
Table S3. Parameters describing the relationship between mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) expressed in microvolts (µV) in the juvenile and adult Lewis and Lewis Polycystic Kidney (LPK) rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Juvenile</th>
<th>Adult</th>
<th>P value</th>
<th>Strain</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lewis (8)</td>
<td>LPK (9)</td>
<td>Lewis (9)</td>
<td>LPK (7)</td>
<td></td>
</tr>
<tr>
<td>Upper plateau (µV)</td>
<td></td>
<td>4.5 ± 0.7</td>
<td>8.6 ± 1.7*</td>
<td>3.3 ± 0.6</td>
<td>5.0 ± 0.7</td>
<td>0.0143</td>
</tr>
<tr>
<td>Lower plateau (µV)</td>
<td></td>
<td>0.4 ± 0.1</td>
<td>1.4 ± 0.6</td>
<td>0.4 ± 0.1</td>
<td>1.8 ± 0.5*</td>
<td>0.0050</td>
</tr>
<tr>
<td>Range (µV)</td>
<td></td>
<td>4.1 ± 0.7</td>
<td>7.2 ± 1.5</td>
<td>2.9 ± 0.6</td>
<td>3.1 ± 0.6†</td>
<td>0.0900</td>
</tr>
<tr>
<td>Gain (µV/mmHg)</td>
<td></td>
<td>0.019</td>
<td>0.024</td>
<td>0.013</td>
<td>0.010†</td>
<td>0.7118</td>
</tr>
<tr>
<td>MAP50 (mmHg)</td>
<td></td>
<td>121 ± 3</td>
<td>157 ± 5*</td>
<td>125 ± 4</td>
<td>167 ± 11*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MAPthr (mmHg)</td>
<td></td>
<td>110 ± 4</td>
<td>135 ± 6*</td>
<td>107 ± 6</td>
<td>149 ± 12*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RSNA at MAP thr (µV)</td>
<td></td>
<td>3.6 ± 0.6</td>
<td>7.1 ± 1.5*</td>
<td>2.7 ± 0.5</td>
<td>4.3 ± 0.6</td>
<td>0.0102</td>
</tr>
<tr>
<td>MAPsat (mmHg)</td>
<td></td>
<td>133 ± 3</td>
<td>180 ± 6*</td>
<td>143 ± 2</td>
<td>186 ± 11*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RSNA at MAP sat (µV)</td>
<td></td>
<td>1.2 ± 0.2</td>
<td>2.9 ± 0.8*</td>
<td>1.0 ± 0.2</td>
<td>2.5 ± 0.5</td>
<td>0.0029</td>
</tr>
<tr>
<td>MAP operating range (mmHg)</td>
<td></td>
<td>24 ± 3</td>
<td>45 ± 6*</td>
<td>36 ± 6</td>
<td>37 ± 4</td>
<td>0.0305</td>
</tr>
</tbody>
</table>

MAP50, mean arterial pressure at the midpoint of the curve; MAPthr, threshold mean arterial pressure to trigger a change in RSNA; and MAPsat, saturation mean arterial pressure at which there is no further change in RSNA.

Results are expressed as mean ± SEM.

* P<0.05 vs. age-matched Lewis.
† P<0.05 vs. strain-matched juvenile rat.

(n) values denoted in subscript and represent the minimum number in each group.
**Table S4. Differentiating histomorphometric variables in the aortic arch of Lewis and Lewis Polycystic Kidney (LPK) rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Juvenile</th>
<th>Adult</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lewis (6)</td>
<td>LPK (7)</td>
<td>Lewis (5)</td>
<td>LPK (6)</td>
</tr>
<tr>
<td>Medial thickness (µm)</td>
<td>131 ± 4</td>
<td>166 ± 3*</td>
<td>145 ± 8</td>
<td>222 ± 5*</td>
</tr>
<tr>
<td>Number of elastin lamellae</td>
<td>12 ± 0.3</td>
<td>11 ± 0.5</td>
<td>11 ± 0.5</td>
<td>13 ± 1.1</td>
</tr>
<tr>
<td>Total elastin density (%)</td>
<td>74 ± 2</td>
<td>61 ± 1*</td>
<td>68 ± 2</td>
<td>44 ± 3*</td>
</tr>
<tr>
<td>Lamellae elastin density (%)</td>
<td>48± 1</td>
<td>43 ± 1</td>
<td>44 ± 2</td>
<td>34 ± 2*</td>
</tr>
<tr>
<td>Interlamellae elastin density (%)</td>
<td>25 ± 2</td>
<td>17 ± 0.9*</td>
<td>24 ± 2</td>
<td>10 ± 1*</td>
</tr>
<tr>
<td>Lamellae-to-interlamellae elastin ratio</td>
<td>2.0 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>3.8 ± 0.4*</td>
</tr>
<tr>
<td>Thickness of elastin lamellae (µm)</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3.2 ± 0.1*</td>
</tr>
<tr>
<td>Elastin lamellae spacing (µm)</td>
<td>7.6 ± 0.2</td>
<td>9.9 ± 0.4</td>
<td>9.3 ± 0.3</td>
<td>14.6±1.2*</td>
</tr>
<tr>
<td>Number of elastin lamellae fractures</td>
<td>0.02 ±</td>
<td>0.05 ±</td>
<td>0.02 ±</td>
<td>0.08 ±</td>
</tr>
<tr>
<td>Total collagen density (%)</td>
<td>21 ± 1.6</td>
<td>29 ± 0.5*</td>
<td>23 ± 1.4</td>
<td>34 ± 1.9*</td>
</tr>
<tr>
<td>Elastin-to-collagen ratio</td>
<td>3.7 ± 0.4</td>
<td>2.1 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>1.3 ± 0.1*</td>
</tr>
<tr>
<td>Nuclear density (%)</td>
<td>4.8 ± 0.2</td>
<td>5.8 ± 0.3</td>
<td>3.7 ± 0.4</td>
<td>5.0 ± 0.1*</td>
</tr>
<tr>
<td>Nucleus CSA (µm²)</td>
<td>9.6 ± 0.5</td>
<td>13.7±1.0*</td>
<td>9.4 ± 1.1</td>
<td>14.2±0.7*</td>
</tr>
<tr>
<td>Number of nuclei per µm²</td>
<td>0.10 ±</td>
<td>0.08 ±</td>
<td>0.12 ±</td>
<td>0.07 ±</td>
</tr>
<tr>
<td>Calcium density (%)</td>
<td>0.7 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.2</td>
<td>5.1 ± 1.9*</td>
</tr>
<tr>
<td>Average calcium deposit (µm²)</td>
<td>5.7 ± 0.7</td>
<td>4.0 ± 0.7*</td>
<td>6.4 ± 0.5</td>
<td>8.4 ± 1.7*</td>
</tr>
</tbody>
</table>

CSA: cross sectional area.
Results are expressed as mean ± SEM.
* P<0.05 vs. age-matched Lewis.
† P<0.05 vs. strain-matched juvenile rat.
(n) values denoted in subscript and represent the minimum number in each group.
Table S5. Pearson’s correlation coefficients (r) for aortic depressor nerve activity (ADNA) baroreflex function curve parameters relative to vascular structure in juvenile and adult Lewis and Lewis Polycystic Kidney (LPK) rats

<table>
<thead>
<tr>
<th>Morphometric parameter</th>
<th>MAP-ADNA function curve parameter Gain (21)</th>
<th>Range (21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial thickness (µm)</td>
<td>-0.71 (P=0.0005)</td>
<td>-0.58 (P=0.0072)</td>
</tr>
<tr>
<td>Total elastin density (%)</td>
<td>0.76 (P&lt;0.0001)</td>
<td>0.68 (P=0.0006)</td>
</tr>
<tr>
<td>Number of elastin lamellae fractures (fracture/lamellae/mm)</td>
<td>-0.74 (P=0.0001)</td>
<td>-0.69 (P=0.0005)</td>
</tr>
<tr>
<td>Total collagen density (%)</td>
<td>-0.49 (P=0.0201)</td>
<td>ns</td>
</tr>
<tr>
<td>Elastin-to-collagen ratio</td>
<td>0.56 (P=0.0062)</td>
<td>0.44 (P=0.0383)</td>
</tr>
<tr>
<td>Nucleus CSA (µm²)</td>
<td>-0.58 (P=0.0049)</td>
<td>ns</td>
</tr>
<tr>
<td>Calcium density (%)</td>
<td>ns</td>
<td>-0.50 (P=0.0260)</td>
</tr>
</tbody>
</table>

Correlation analysis of the relationship between the gain/range of ADNA baroreflex function and aortic arch vascular remodelling. Mean arterial pressure: MAP, cross-sectional area: CSA, ns: no significance. (n) = number in each group. Only those parameters for which there was a significant correlation are reported.
Figure S1. Representative raw data curves from individual animals showing the sigmoidal relationship between mean arterial pressure (MAP) and heart rate (HR; A-B), renal sympathetic nerve activity (RSNA; C-D) and aortic depressor nerve activity (ADNA; E-F) in adult (12-13 weeks old) Lewis (left panels) and Lewis Polycystic Kidney rats (right panels).
Figure S2. Logistic function curves illustrating the relationship between mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) expressed in microvolts (µV) in juvenile (7-8 weeks old) and adult (12-13 weeks old) Lewis and Lewis Polycystic Kidney (LPK) rats. Results are expressed as mean ± SEM. n values are as detailed in Table S3. ($R^2 = 0.94$ ± 0.01 all groups).
Figure S3. Logistic function curves illustrating the relationship between aortic depressor nerve activity (ADNA) and renal sympathetic nerve activity (RSNA) in juvenile (7-8 weeks old) and adult (12-13 weeks old) Lewis and Lewis Polycystic Kidney (LPK) rats. Results are expressed as mean ± SEM. n/group juvenile Lewis = 5, juvenile LPK = 4, adult Lewis = 4 and adult LPK = 7. (R^2 = 0.94 ± 0.03 all groups).
Figure S4. Effect of the left aortic depressor nerve stimulation on reflex responses in renal sympathetic nerve activity (RSNA) expressed in microvolts (µV) in juvenile (7-8 weeks old; A) and adult (12-13 weeks old; B) Lewis and Lewis Polycystic Kidney (LPK) rats. Results are expressed as mean ± SEM. "P<0.05 vs. age-matched Lewis and "P<0.05 vs. strain-matched juvenile rat. "P<0.05, overall two-way ANOVA strain effect within indicated age group. n/group: juvenile Lewis = 5, juvenile LPK = 5, adult Lewis = 6 and adult LPK = 7.
Figure S5. Representative histological sections of the aortic arch in adult (12–13 weeks old) Lewis (left panels) and Lewis Polycystic Kidney rats (right panels), stained with Shikata's orcein (panels A, B) showing the elastin component in red, Martius Scarlet Blue (MSB; panels C, D) showing the collagen component in blue and the nuclei in black and Von Kossa (panels E, F) showing calcification in black. Block arrow in B shows an example of elastic lamellae fracture (point of lamellae discontinuation). Line arrows in F indicate areas of medial calcification. Scale bar in panel F = 50 µm for all panels.