Differential sympathetic response to lesion-induced chronic kidney disease in rabbits

Yusuke Sata[1,2,3,4], Sandra L. Burke[1], Anna M.D. Watson[5], Jay C. Jha[5], Cindy Gueguen[1], Nina Eikelis[2,6], Kyungjoon Lim[1,7], Kristy L. Jackson[1], Gavin W. Lambert[2,6], Karin A.M. Jandeleit-Dahm[5,8], Kate M. Denton[9], Murray D. Esler[2,3,4], Markus P. Schlaich[2,10] and Geoffrey A. Head[1,11]

[1]*Neuropharmacology Laboratory, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia;* [2]*Human Neurotransmitters Laboratory, Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia;* [3]*Faculty of Medicine, Nursing and Health Sciences, Central Clinical School, Monash University, Melbourne, Victoria, Australia;* [4]*Department of Cardiology, Alfred Hospital, Melbourne, Victoria, Australia;* [5]*Department of Diabetes, Central Clinical School, Monash University, Melbourne, Victoria, Australia;* [6]*Iverson Health Innovation Research Institute and School of Health Science, Swinburne University of Technology, Hawthorn, Victoria, Australia;* [7]*Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, Victoria, Australia;* [8]*German Diabetes Centre, Leibnitz Institute for Diabetes Research at the Heinrich Heine University, Dusseldorf, Germany;* [9]*Monash Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia;* [10]*Dobney Hypertension Centre, School of Medicine, Royal Perth Hospital Unit, University of Western Australia and Departments of Cardiology and Nephrology, Royal Perth Hospital, Perth, Western Australia, Australia; and* [11]*Department of Pharmacology, Monash University, Melbourne, Victoria, Australia*

Chronic kidney disease (CKD) is associated with greater sympathetic nerve activity but it is unclear if this is a kidney-specific response or due to generalized stimulation of sympathetic nervous system activity. To determine this, we used a rabbit model of CKD in which quantitative comparisons with control rabbits could be made of kidney sympathetic nerve activity and whole-body norepinephrine spillover. Rabbits either had surgery to lesion 5/6th of the cortex of one kidney by electro-lesioning and two weeks later removal of the contralateral kidney, or sham lesioning and sham nephrectomy. After three weeks, the blood pressure was statistically significantly 20% higher in conscious rabbits with CKD compared to rabbits with a sham operation, but their heart rate was similar. Strikingly, kidney nerve activity was 37% greater than in controls, with greater burst height and frequency. Total norepinephrine spillover was statistically significantly lower by 34%, and kidney baroreflex curves were shifted to the right in rabbits with CKD. Plasma creatinine and urine output were elevated by 38% and 131%, respectively, and the glomerular filtration rate was 37% lower than in sham-operated animals (all statistically significant). Kidney gene expression of fibronectin, transforming growth factor-β, monocyte chemotactic protein1, Nox4 and Nox5 was two- to eight-fold greater in rabbits with CKD than in control rabbits. Overall, the glomerular layer lesioning model in conscious rabbits produced a moderate, stable degree of CKD characterized by elevated blood pressure and increased kidney sympathetic nerve activity. Thus, our findings, together with that of a reduction in total norepinephrine spillover, suggest that kidney denervation, rather than generalized sympatholytic treatments, may represent a preferable management for CKD associated hypertension.

Kidney International (2020) **98,** 906–917; https://doi.org/10.1016/j.kint.2020.03.039

KEYWORDS: blood pressure; chronic kidney disease; hypertension; rabbit; sympathetic nervous system

Translational Statement

A glomerular layer electro-lesioned kidney model in rabbits produces a stable moderate elevation in plasma creatinine and increased kidney profibrotic, proinflammatory, and oxidative stress markers. Blood pressure and renal sympathetic nerve activity were elevated, but global sympathetic activity was reduced. These findings suggest that targeting renal nerves with denervation may be suitable for chronic kidney disease, but treatments that target general reductions in sympathetic activity should be used with caution.

Altered sympathetic regulation has long been believed to be an important mechanism contributing to chronic kidney disease (CKD) and increased cardiovascular risk.[1,2] Early studies in edematous patients with nephrotic syndrome reported elevated plasma norepinephrine (NE) spillover.[3] Higher plasma NE concentrations are present in patients with polycystic kidney disease with and without renal failure,[4] but the pattern of sympathetic overactivity has not been established. It is assumed that renal sympathetic nerve activity (RSNA) is elevated in CKD, but this has not been established in humans. The higher SNA has generally

Correspondence: *Geoffrey A. Head, Neuropharmacology Laboratory, Baker Heart and Diabetes Institute, P.O. Box 6492, Melbourne, Victoria, 3004, Australia. E-mail: geoff.head@baker.edu.au*

Received 13 December 2019; revised 17 March 2020; accepted 26 March 2020; published online 25 April 2020

been estimated using NE kinetics[5] or by recording SNA from other vascular beds.[6,7] Hemodialysis patients and those with hypertensive CKD have elevated muscle SNA, whereas bilaterally nephrectomized patients have normal muscle SNA.[6–8] Interestingly, as renal function declined, muscle SNA increased.[2] There is certainly evidence that muscle and skin SNA are differentially regulated in end-stage renal disease,[9] and renal, cardiac, and muscle sympathetic outflow are selectively activated by different stimuli.[10,11]

Multifiber recording can be used in animal models of CKD to provide information on the pattern of SNA associated with CKD. Measurement of renal, splanchnic, and lumbar SNA in a genetic model of CKD has been performed, but no comparison between CKD and controls was possible because of the method of calibration of signals.[12] Nevertheless, reduced baroreflex sensitivity was demonstrated in the renal and splanchnic but not lumbar beds.[12] Veiga and colleagues[13] used a 5/6 nephrectomy anesthetized rat model to show that renal, splanchnic, and lumbar SNA were elevated compared with sham controls. However, this pattern may not occur in the conscious state because autonomic regulation of SNA is different under anesthesia.[14]

The aim of the current study was to determine whether there is renal-specific or generalized sympathetic activation (using direct nerve recording and whole-body NE spillover techniques, respectively) in a conscious animal model of CKD. By using conscious rabbits, RSNA could be directly measured without the confounding effects of anesthesia.[15] Importantly, RSNA could be normalized, which allows long-term comparisons to be made over time and between groups.[16,17] We used a technique of electro-lesioning 5/6 of the surface of 1 kidney and removing the contralateral 1. This produces moderate renal function impairment[18] and stable increases in plasma creatinine with no loss of body weight for up to 12 weeks.[18] In addition, we examined the effects on blood pressure (BP), autonomic reflex function, and kidney fibrosis.

RESULTS

Effects of CKD on hemodynamics, RSNA, NE kinetics, and baroreflexes

Average baseline mean arterial pressure (MAP) and heart rate (HR) before lesioning were 67 ± 1 mm Hg and 174 ± 5 beats/min, respectively (n = 10). After nephrectomy, there was an increase in MAP in the first week of +14% and by 3 weeks of +22% ($P_{baseline} < 0.001$, n = 5; Figure 1). There were no changes in HR after CKD induction or sham surgery ($P_{group} = 0.191$; Figure 1). Total RSNA at 3 weeks was 37% greater than that of sham-operated rabbits ($P_{group} = 0.006$; Figure 2). Both burst amplitude and frequency were +28% higher ($P < 0.001$ and $P = 0.029$, respectively; Figure 2). The nasopharyngeal response was similar in both groups (Figure 2).

NE spillover at this time was 34% lower in CKD rabbits than sham ($P_{group} = 0.020$), but NE clearance was similar in each group (Figure 2). Arterial NE concentration was 36% lower in CKD rabbits 3 weeks after nephrectomy than in sham-operated rabbits ($P_{group} = 0.009$; Figure 2).

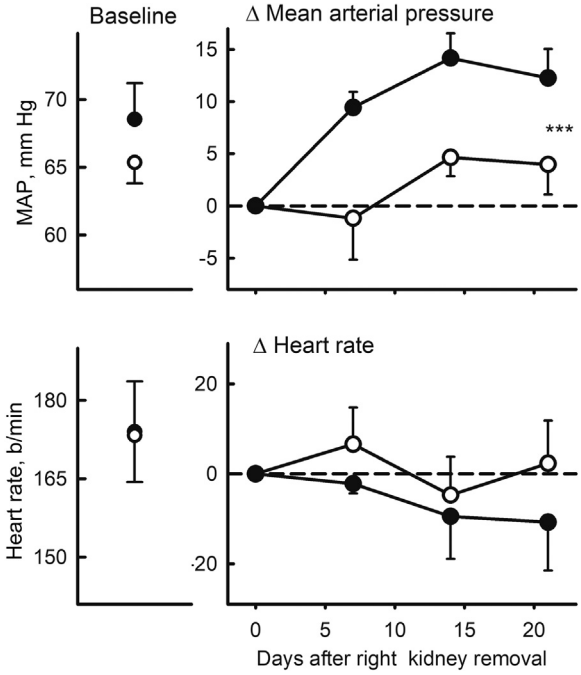

Figure 1 | Left: Baseline values of mean arterial pressure (MAP) and heart rate before lesioning (chronic kidney disease, n = 5, closed circles) or sham (n = 5, open circles) surgery. Right: Changes from baseline levels after right or sham nephrectomy in the same rabbits. Values are mean ± SEM or mean difference ± standard error of the difference, indicating between-animal variance. ***$P < 0.001$ for sham versus chronic kidney disease over 21 days.

MAP-RSNA and MAP-HR baroreflex curves were sigmoidal, with the resting value lying close to the lower plateau (Figure 3). We did not detect any differences between CKD and sham MAP-HR curve parameters, but CKD MAP-RSNA curves were shifted along the MAP axis to a higher MAP ($P_{group} = 0.005$; Table 1 and Figure 3). Baroreflex gain for both MAP-RSNA and MAP-HR curves was similar in CKD and sham rabbits (Table 1).

Effects of CKD on MAP responses to pentolinium

The response to ganglionic blocker pentolinium was determined as a measure of the sympathetic nervous system (SNS) contribution to maintaining MAP. Three or 4 weeks after nephrectomy, pentolinium lowered MAP to a greater extent in CKD than in sham rabbits (–26 ± 2 mm Hg and –19 ± 3 mm Hg, respectively; $P_{group} = 0.028$). However, the tachycardia in response to pentolinium was similar in both groups (+80 ± 13 beats/min and +75 ± 13 beats/min in CKD and sham, respectively; $P_{group} = 0.780$) as was the sympathoinhibition (–3.4 ± 0.7 normalized units in CKD and –2.7 ± 0.9 normalized units in sham, $P_{group} = 0.521$).

Effects of CKD on body weight, water and food intake, urine production, and interpolated glomerular filtration rate

Baseline body weight, water and food intake, and urine output were similar in the 2 groups (Figure 4). After

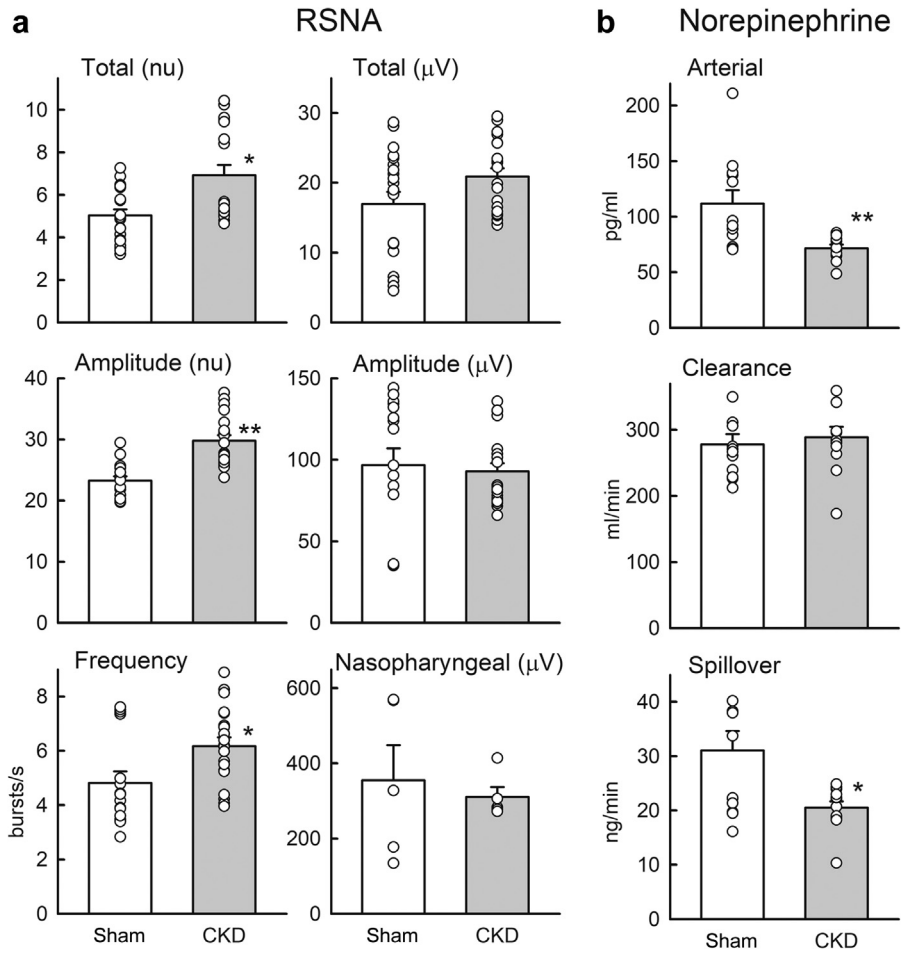

Figure 2 | (a) Renal sympathetic nerve activity (RSNA) expressed as total (upper, normalized units [nu] and μV), burst amplitude (middle, nu and μV) and burst frequency, and nasopharyngeal response (lower) 3 weeks (on day 21) after sham (open bars, n = 5) or right (chronic kidney disease [CKD], closed bars, n = 5) nephrectomy. (**b**) Norepinephrine (NE) kinetics. Arterial NE concentration (upper), NE clearance (middle), and NE spillover (lower) in sham (n = 4) and CKD (n = 4) rabbits. Data are expressed as individual points (four 15-minute averages) as well as mean ± SEM. *$P < 0.05$ and **$P < 0.01$ for sham versus CKD.

induction of CKD or sham surgery, both groups gained weight over the next 3 weeks, but this was slightly greater in the sham animals ($P_{group} < 0.001$; Figure 4). CKD rabbits also had greater water intake (+35%) and urine output (+144% by 3 weeks) than sham rabbits ($P_{group} < 0.05$), but food intake was similar in both groups (Figure 4). After 3 weeks of CKD, interpolated glomerular filtration rate (GFR) was 37% lower than in sham-operated rabbits (13 ± 1 vs 8 ± 1 ml/min, $P_{group} = 0.008$).

Effects of CKD on blood biochemistry and plasma renin activity

Plasma creatinine rapidly increased after nephrectomy, rising by 85% ($P_{group} < 0.001$) on the first day (Figure 5). Plasma urea also rose, and levels of both parameters subsided over the following week, but creatinine remained elevated throughout (+38% by day 21, $P_{group} < 0.014$ weeks 2–3; Table 2 and Figure 5). Plasma sodium and potassium levels were not altered by either nephrectomy or sham surgery. Chloride was not altered by CKD but increased over the first week in sham rabbits ($P_{group} = 0.014$; Table 2 and Figure 5). Initially, in the first 7 days after nephrectomy, hematocrit and hemoglobin levels were reduced in the sham group but not altered from baseline in the CKD animals ($P_{group} = 0.010$ and $P_{group} = 0.019$, respectively; Table 2 and Figure 5). However, by day 14 levels had returned to baseline and were not different between groups (Table 2 and Figure 5). Baseline plasma renin activity (PRA) levels were similar in each group before lesioning and averaged 3.7 ± 0.8 ng/ml/h (n = 10). We did not detect changes in PRA over 3 weeks in either group (Figure 5).

Effects of CKD on urine biochemistry, albumin, and fractional excretion of Na

Urine sodium levels over 24 hours were greater after nephrectomy in the CKD group than in the sham group ($P_{group} = 0.018$ at day 21; Table 3), but 24-hour urine albumin, creatinine, urea, chloride, and the albumin-to-creatinine ratio were not different in CKD compared with sham-CKD rabbits (Table 3). Fractional excretion of Na (FE_{Na}) was similar in each group and averaged 0.4% ± 0.1% at baseline.

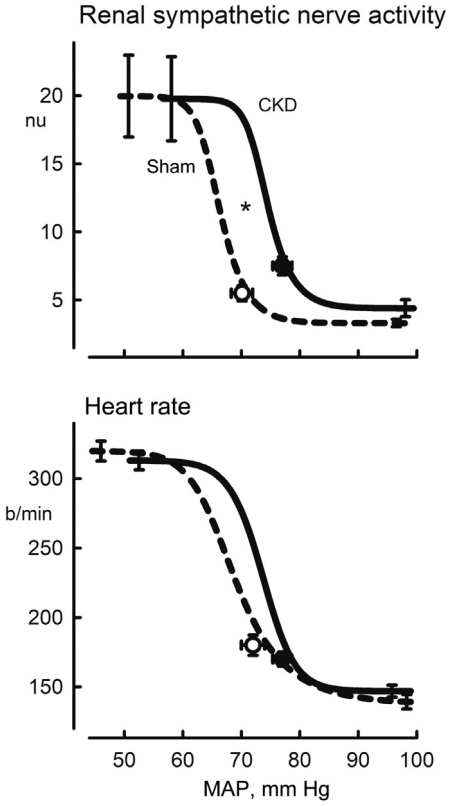

Figure 3 | Renal sympathetic nerve activity (normalized units [nu]) and heart rate baroreflex curves in sham-operated (dashed line, n = 5) and chronic kidney disease (CKD) rabbits on day 21 after nephrectomy (solid line, n = 5). Error bars are SEM indicating between-animal variance. *$P < 0.05$ for comparison of blood pressure at 50% maximum in sham and CKD rabbits. MAP, mean arterial pressure.

In the CKD group, FE_{Na} had doubled at day 21 (1.0% ± 0.1%) but did not change in the sham group ($P_{group} = 0.011$; Table 3).

Effects of CKD on left ventricular and kidney weights

Left ventricular weight, normalized to body weight, tended to be greater in CKD rabbits than in sham-operated rabbits (1.46 ± 0.06 vs 1.28 ± 0.07 g/kg, respectively; $P_{group} = 0.07$, 5 per group). The left kidney of CKD rabbits was 28% larger than the left kidney in sham-CKD rabbits at week 3 (2.7 ± 0.1 vs 2.1 ± 0.1 g/kg, $P_{group} = 0.002$, n = 5).

Effects of CKD on kidney inflammation, fibrosis, and histology

Renal mRNA of monocyte chemoattractant protein-1 was 8-fold greater, fibronectin 2.7-fold greater, and transforming growth factor (TGF)-β 1.7-fold greater in CKD rabbits than in controls ($P_{group} < 0.01$; Figure 6). Nox4 and Nox5 mRNA were 2.4- and 4.3-fold greater, respectively, in CKD rabbits than in controls ($P_{group} < 0.05$; Figure 6). However, Nox1, Nox2, and p47phox expression were similar in the 2 groups.

The lesion tracks in the renal cortex were surrounded by collagen as revealed by Masson's trichrome staining (Figure 7). When quantified, the fibrotic area was 9.8% ± 1.2% compared with 3.4% ± 1.1% in sham rabbits ($P_{group} = 0.013$; Figure 7). There were no differences in area of tubulointerstitial injury or glomerular sclerosis index between CKD and sham-treated rabbits (data not shown).

Effect of different degrees of lesioning on MAP and plasma creatinine

In a pilot study we performed 3 levels of renal lesioning, 2/6 (n = 2), 5/6 (n = 5), and 6/6 (n = 2), to determine the relationship between degree of lesioning of the surface of the left kidney and the BP or plasma creatinine concentration. There was a strong linear relationship between degree of lesioning and MAP ($r = 0.994$), but the relationship with plasma creatinine was less so ($r = 0.529$).

DISCUSSION

In the present study we found that electro-lesioning of the renal cortex and subsequent contralateral nephrectomy induced a rise in plasma creatinine levels consistent with mild CKD. There was also greater interstitial fibrosis and inflammation associated with the model, reflecting conditions observed in CKD in human subjects, with increased collagen accumulation and greater gene expression of proinflammatory and fibrotic genes such as *CCL2* (encoding monocyte chemoattractant protein-1) and TGF-β1. The hemodynamic consequence of CKD induction was an elevation in MAP and RSNA mediated by both greater burst amplitude and frequency but not HR. Surprisingly, global NE spillover in CKD rabbits was lower than in control rabbits, suggesting a marked inhibition in SNA outflow to other beds.

The ability to normalize multiunit RSNA in each individual rabbit using the nasopharyngeal reflex[16,17] was critical in being able to determine that RSNA was higher in CKD than in sham-operated rabbits. In human studies there is no standard method for normalizing burst amplitude because the voltage depends on the placement of the electrode, and thus burst frequency is used.[17] In the present study, the greater RSNA burst amplitude observed likely reflects a greater number of action potentials from activated neurons and is

Table 1 | MAP-RSNA and MAP-HR baroreflex parameters in CKD or sham-operated conscious rabbits

Baroreflex parameters	Sham CKD (n = 5)	CKD (n = 5)	P_{group}
RSNA baroreflex			
Lower plateau, nu	3.3 ± 0.3	4.4 ± 0.9	0.166
Range, nu	16.7 ± 2.7	15.4 ± 2.5	0.642
Upper plateau, nu	20 ± 2.5	19.8 ± 3.3	0.949
BP50, mm Hg	66.3 ± 2.2	74.5 ± 2.5	**0.005**
Gain, nu/mm Hg	−1.6 ± 0.4	−1.6 ± 0.1	0.910
HR baroreflex			
Lower plateau, beats/min	138 ± 5	147 ± 4	0.241
Range, beats/min	182 ± 6	166 ± 9	0.207
Upper plateau, beats/min	320 ± 7	313 ± 7	0.521
BP50, mm Hg	68.8 ± 2.2	73.6 ± 1.7	0.134
Gain, beats/min mm Hg^{-1}	−9.3 ± 1.1	−13.0 ± 2.0	0.145

BP50, blood pressure at 50% maximum; CKD, chronic kidney disease; HR, heart rate; MAP, mean arterial pressure; nu, normalized units; RSNA, renal sympathetic nerve activity.
Values are mean ± SEM, indicating between-animal variance. P_{group} for comparison of CKD vs. sham-treated rabbits, shown in bold if $P < 0.05$.

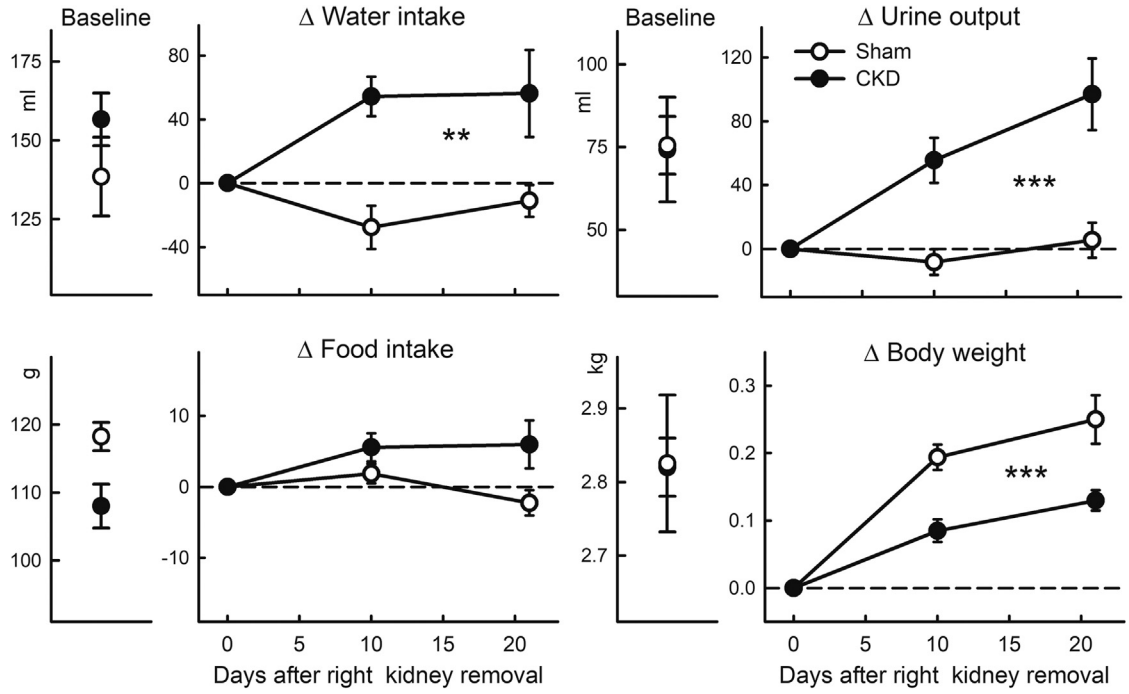

Figure 4 | Left: Water intake, urine output, food intake, and body weight measured over 24 hours at baseline before lesioning (chronic kidney disease [CKD], filled circles, n = 5) or sham (open circles, n = 4) surgery. Right: Changes from baseline levels measured over 24 hours on days 10 and 21 after right nephrectomy in the same CKD (filled circles) and sham-operated (open circles) rabbits. Values are mean ± SEM or mean difference ± standard error of the difference, indicating between-animal variance. $^{**}P < 0.01$, $^{***}P < 0.001$ for sham versus CKD over 10 to 21 days.

evidence of recruitment of previously silent fibers.[19] The greater burst frequency reflects the rhythmic generation of discharges of in-phase neurons in the central nervous system, synchronized to the cardiac cycle by the baroreceptors.[20] The increased frequency in the absence of a rise in HR suggests that the probability of a burst occurring has increased in CKD rabbits.

In conscious rabbits, renal NE spillover has been shown to closely correlate with recorded RSNA[21]; thus, the 37% increase in RSNA that we report is likely to be accompanied by a similar increase in renal NE spillover. Our results showing a one-third reduction in total NE spillover concurrent with a similar rise in RSNA in CKD rabbits suggest a marked reduction in sympathoexcitation to other beds in this model. However, the greater depressor response to pentolinium in CKD rabbits suggests an overall greater SNS support for maintaining BP. Thus, there appears to be a rebalance from partly renal to mostly RSNA supporting BP through the markedly higher SNA. This would suggest renal vascular constriction rather than an effect through increasing renin levels, because levels of PRA in both groups were similar. The mechanism may involve renal afferents that drive higher RSNA as shown by the renal injury model of kidney disease.[22] Further, our current findings are similar to the situation of angiotensin II hypertension where sympathetic activity is suppressed by a mechanism involving cardiopulmonary afferents, and this prevents neurally mediated sodium retention.[23] In CKD the stimulus from the kidney may be too strong to override the inhibition to the kidney specifically and therefore results in generalized sympathoinhibition but RSNA excitation.

Our findings are consistent with higher RSNA in anesthetized 5/6 nephrectomized rats.[13] Direct measurement of RSNA by telemetry was previously performed in a polycystic kidney disease model and showed a doubling of RSNA measured in μV compared with control rats.[24] This is a severe form of kidney disease, with BP elevated by 56% compared with the 20% increase in our rabbit lesion-induced CKD. Nevertheless, the greater depressor response to ganglionic blockade was observed in both models of CKD.[25] One difference, however, is the reduced PRA and impaired HR arterial baroreflex in the polycystic kidney disease rats,[25,26] where we saw no change in PRA. The lack of an increase in PRA in our model, also reported in 5/6 nephrectomized mice,[27] may be due to a lack of activation of the renin-angiotensin system or to depletion of the renal cortical tissue and the loss of juxtaglomerular cells by lesioning, thus altering the relationship between the renal nerves and renin release.

In patients with CKD, we could find little information about sympathetic outflow to the kidney or the contribution of whole-body SNS outflow to BP elevation. In humans, SNA is limited to measuring muscle burst rate and burst incidence,[28] which are elevated from the early stages of CKD and increase progressively with the degree of renal failure.[2,6,9] Furthermore, estimated GFR is inversely correlated to the

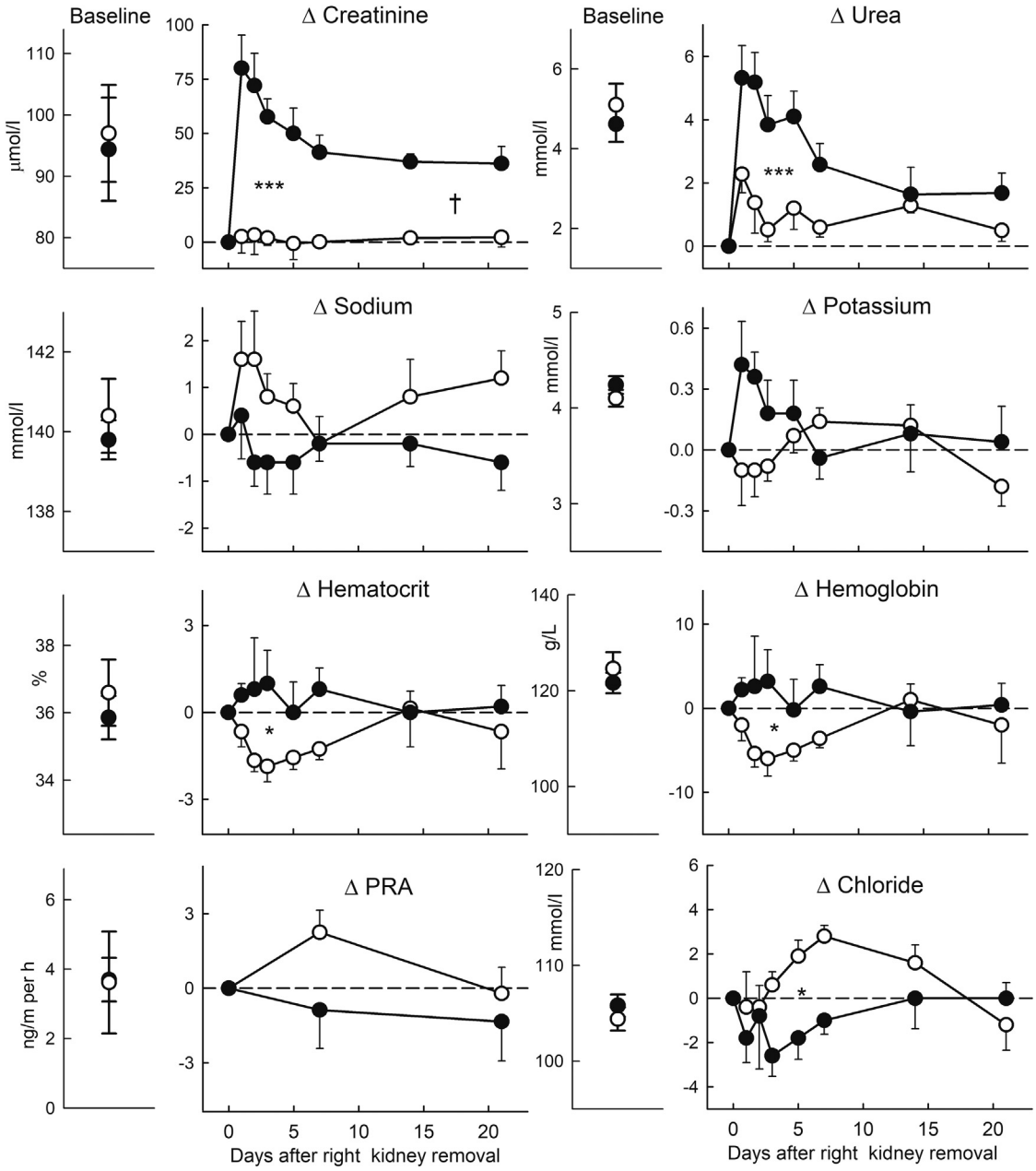

Figure 5 | Blood biochemical parameters and plasma renin activity (PRA) at baseline (before lesioning) and changes over 21 days after right nephrectomy (chronic kidney disease [CKD]) or sham surgery. Single point graphs: Baseline values before lesioning (CKD, n = 5, filled circles) or sham (n = 5, open circles) surgery. Right line graphs: Changes from baseline levels after right nephrectomy in the same CKD (filled circles) and sham (open circles) rabbits. Values are mean ± SEM or mean difference ± standard error of the difference, indicating between-animal variance. *$P < 0.05$ and ***$P < 0.001$ for sham versus CKD over first 7 days; †$P < 0.05$ for sham versus CKD over 2 to 3 weeks.

level of muscle SNA, but there was no relationship of estimated GFR with HR or plasma NE.[2] The lack of relationship of estimated GFR with HR suggests cardiac sympathetic activity may not be elevated in CKD. Further, it has been shown that skin SNA is similar in CKD patients to that observed in controls.[9,29] An explanation for this differential effect is that impairment of the baroreflex affects muscle but not skin SNA.[29] In our study, elevated RSNA was not accompanied by any change to the baroreflex except a parallel shift to the higher BP, indicating baroreceptor resetting.[15] Afferent baroreceptor resetting ensures that the steepest, most sensitive part of the curve continued to span resting BP. Thus, elevated RSNA in our study is unlikely to be caused by a dysfunctional baroreflex in the lesion model of CKD. We were also unable to detect any effect of CKD on the HR baroreflex. Lack of effect of mild CKD on HR has been interpreted as lack of cardiac sympathetic overdrive and another example of nonhomogeneous sympathetic outflow.[2] Other factors are

Table 2 | Blood parameters measured at baseline and changes from baseline after either sham or right nephrectomy in conscious rabbits

Blood parameters	Average baseline (n = 10)	Changes from baseline		Over week 1 P_{group}	Over weeks 2–3 P_{group}
		Sham CKD (n = 5)	CKD (n = 5)		
Creatinine, μmol/l	95.7 ± 5.5	1.7 ± 2	53.5 ± 4.6	**<0.001**	**0.014**
Urea, mmol/l	4.9 ± 0.3	1.1 ± 0.2	3.5 ± 0.4	**<0.001**	>0.5
Na, mmol/l	140.1 ± 0.5	0.9 ± 0.3	−0.3 ± 0.2	0.07	0.37
K, mmol/l	4.17 ± 0.07	−0.02 ± 0.04	0.17 ± 0.06	0.11	>0.5
Hematocrit, %	36.2 ± 0.6	−1.1 ± 0.3	0.5 ± 0.4	**0.010**	>0.5
Hemoglobin, g/l	123.1 ± 2	−3.3 ± 0.9	1.5 ± 1.3	**0.019**	>0.5
Glucose, mmol/l	7.1 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	>0.5	>0.5
Cl, mmol/l	105.1 ± 0.8	0.7 ± 0.4	−1.1 ± 0.5	**0.014**	>0.5
Ionized Ca, mmol/l	1.88 ± 0.02	−0.002 ± 0.01	−0.01 ± 0.016	>0.5	>0.5
Total CO_2, mmol/l	24.1 ± 0.9	−0.6 ± 0.5	0.5 ± 0.4	0.43	>0.5
Anion gap, mmol/l	16 ± 0.7	0.8 ± 0.2	0.8 ± 0.3	>0.5	>0.5

CKD, chronic kidney disease.
Values are mean ± SEM and mean change from baseline ± standard error of the difference, averaged over 3 weeks, indicating between-animal variance. P_{group} for comparison over week 1 or over weeks 2–3 of change from baseline in CKD vs. sham-treated rabbits, shown in bold if $P < 0.05$.

likely to influence HR, including possible opposing effects on the parasympathetic innervation of the heart and effects on cardiac receptor stimulation by changes in blood volume.

An important question that has not yet been adequately addressed is whether there is generalized SNS activation in human CKD or whether it is confined to the muscle bed. The only study to examine NE spillover was made in a limited number of edematous patients with nephrotic syndrome, where total plasma NE spillover was higher than in control subjects.[3] Cerasola *et al.*[4] also reported that patients with polycystic kidney disease had higher plasma NE concentrations, but this occurred irrespective of kidney impairment. In humans there are 3 lines of evidence to justify the view that SNS activity is raised in CKD and that this an important contributor to BP elevation and to adverse cardiovascular outcomes: (1) the demonstration that renal transplantation with normalization of creatinine does not alter muscle SNA if the diseased native kidneys are still *in situ*, whereas it is normalized with bilateral nephrectomy of the native kidneys; (2) the beneficial effect of catheter-based renal denervation on muscle SNA and BP in CKD[30] and end-stage renal disease[31]; and (3) the demonstration that plasma NE levels predict outcomes in end-stage renal disease.[1] Converse *et al.*[6] showed that muscle SNA in nephrectomized CKD patients was similar to control subjects, which was the first evidence that the aberrant signal leading to elevated postganglionic SNA originated in the diseased kidneys. More recently, renal denervation has become a therapeutic strategy used to treat resistant hypertension in disease states including CKD and end-stage renal disease.[30,32] Signals from mechanoreceptors,

Table 3 | Urine parameters at baseline and at days 10 and 21 after right nephrectomy or sham nephrectomy

Urine parameters	Average baseline (n = 9)	Changes from baseline				P_{group} day 10	P_{group} day 21
		Sham nephrectomy		Nephrectomy			
		Day 10 (n = 4)	Day 21	Day 10 (n = 5)	Day 21		
Total per d							
Albumin, μg/d	16 ± 3	−1.1 ± 3.9	5.5 ± 4.1	125 ± 96	46 ± 38	0.32	>0.5
Creatinine, mmol/d	0.9 ± 0.1	−0.2 ± 0.2	0.3 ± 0.3	0.3 ± 0.1	0.4 ± 0.2	0.26	>0.5
Albumin/creatinine, μg/mg/d	182 ± 42	−3.3 ± 31.9	14.4 ± 42.9	928 ± 747	188 ± 188	0.41	>0.5
Urea, mmol/d	42 ± 3	−5 ± 6.5	21.5 ± 14.4	8 ± 4.3	19.4 ± 7.2	>0.5	>0.5
Na, mmol/d	7 ± 1	−2 ± 0.4	2.5 ± 3.4	4.8 ± 0.8	10.6 ± 1.9	**0.044**	**0.018**
K, mmol/d	16 ± 2	1.4 ± 4.3	10 ± 5.5	6.4 ± 1.9	9.2 ± 1.8	>0.5	>0.5
Cl, mmol/d	13 ± 1	−1 ± 0.9	4.8 ± 5.1	6 ± 1.8	9.2 ± 1.2	0.13	0.44
Concentration							
Albumin, μg/l	261 ± 57	−54 ± 72	−26 ± 92	1021 ± 983	219 ± 230	0.46	>0.5
Creatinine, mmol/l	12.4 ± 1.4	0.8 ± 1.9	1 ± 1.3	−3.8 ± 1.9	−4.3 ± 2.2	0.27	0.18
Albumin/creatinine, μg/mg	19.7 ± 4.1	−5 ± 5.9	−3.2 ± 6.1	119 ± 102	38 ± 27	0.38	>0.5
Urea, mmol/l	690 ± 86	12 ± 197	34 ± 190	−311 ± 49	−312 ± 47	0.23	0.19
Na, mmol/l	90 ± 12	35 ± 20	25 ± 17	−6 ± 9	14 ± 20	0.26	>0.5
K, mmol/l	298 ± 67	−47 ± 117	−80 ± 111	−94 ± 33	−104 ± 24	>0.5	>0.5
Cl, mmol/l	234 ± 50	−11 ± 128	−60 ± 90	−58 ± 15	−61 ± 10	>0.5	>0.5
Urine volume, ml	67 ± 8	−14 ± 22	15 ± 21	78 ± 14	121 ± 31	0.06	**0.031**
FE_{Na}, %	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.03	0.4 ± 0.1	0.6 ± 0.1	0.32	**0.034**

FE_{Na}, fractional excretion of Na.
Values are mean ± SEM and mean change from baseline ± standard error of the difference, expressed per day and as concentration per l urine, indicating between-animal variance. P_{group} for comparison of change from baseline in chronic kidney disease vs. sham-treated rabbits, shown in bold if $P < 0.05$.

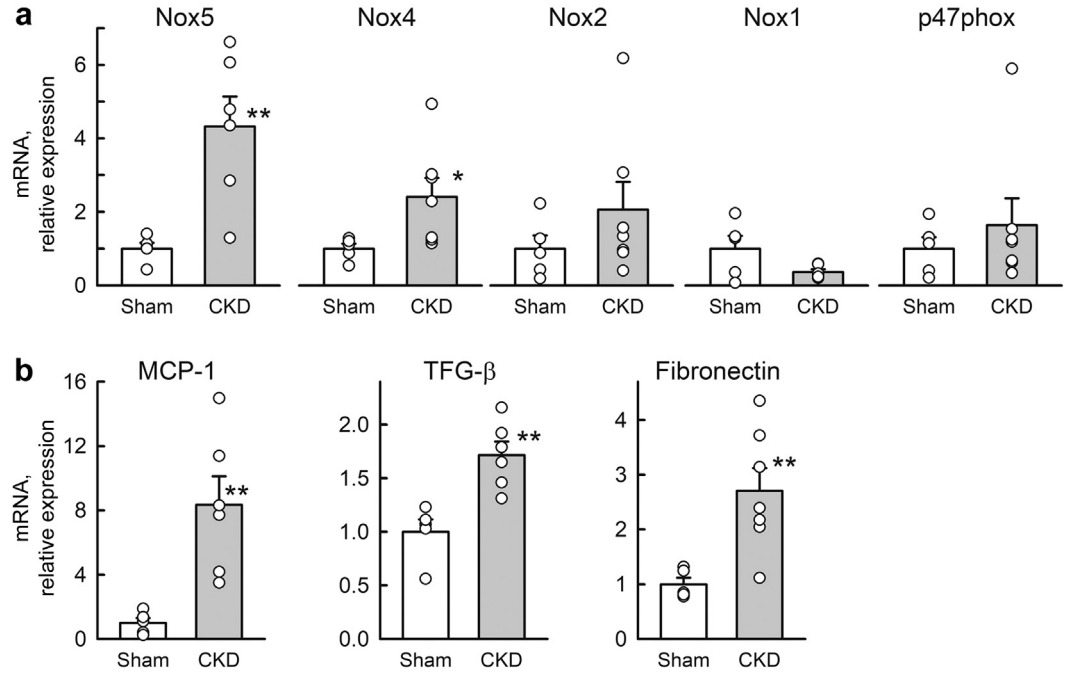

Figure 6 | Relative expression of mRNA in rabbits with chronic kidney disease (CKD; solid bars, n = 7) compared with sham-operated rabbits on day 21 (open bars, n = 5). (**a**) Relative expression of Nox isoforms Nox5, Nox4, Nox2, Nox1, and p47phox. (**b**) Relative expression of inflammatory marker monocyte chemoattractant protein-1 (MCP-1) and markers of fibrosis transforming growth factor-β (TGF-β) and fibronectin. Data are expressed as individual points as well as mean ± SEM, indicating between-animal variance. *$P < 0.05$ and **$P < 0.01$ for sham versus CKD.

responding to increased intrapelvic pressure, and chemoreceptors, activated by ischemia or uremic toxins, increase efferent RSNA by activating regions of the brain involved in modulation of sympathetic outflow.[33] Afferent nerve activity is also modified by efferent RSNA via adrenoceptors. Thus, renal denervation interrupts the reflex feedback loops and provides a way of reducing SNS activation. In light of this, renal denervation and specifically afferent denervation would provide an opportunity to determine the role of the renal sympathetic nerves in supporting arterial pressure in the CKD model described here. It is worth noting that in the current study the electrode recorded a combination of efferent and afferent SNA, although there is evidence in humans and other species that the proportion of efferent fibers is far greater than afferent fibers.[33,34]

In our model of CKD, we report greater expression of markers of fibrosis and of the inflammatory marker monocyte chemoattractant protein-1 compared with control rabbits. Greater abundance of Nox4 and Nox5 mRNA in CKD is consistent with studies of human disease states and animal models of renal inflammation.[35] Reduced nicotinamide adenine dinucleotide phosphate oxidase isoforms, in particular Nox4, which is highly expressed in the proximal tubule, mediate oxidative stress and inflammation in experimental CKD.[36] Recent studies have suggested a pathogenic role for the Nox5 isoform, which is not present in rodents. Nox5 is expressed in proximal tubules and glomeruli and is upregulated in human diabetic nephropathy.[35,37] Indeed, tubulointerstitial fibrosis is evident in many forms of progressive renal impairment irrespective of their origin.[38] In the present study, evidence of greater collagen (as shown by Masson's trichrome staining) and greater gene expression for fibronectin and TGF-β, a key cytokine in the development of tubulointerstitial fibrosis that also promotes synthesis of fibronectin, is consistent with the development of tissue fibrosis.[39]

Although most experimental studies use rodents,[40] the model of glomerular layer lesioning plus nephrectomy remains one of the few models appropriate for rabbits.[18] Advantages of this rabbit model include the moderate degree of CKD that is stable by 2 to 3 weeks after nephrectomy, shown by sustained elevation of plasma creatinine. The rabbits remain in good health with body weight increasing. Normally urine output is half the water intake (49%) to account for other avenues of water loss due to respiration and via the gastrointestinal tract. In CKD rabbits the urine output increases to 80% of the fluid intake. Thus, assuming nonurine water loss is approximately the same, there is a 50-ml/day insufficient water intake to maintain fluid balance, suggesting a reduced volume. The higher RSNA via innervation of tubules is likely preventing sodium loss and limiting urine excretion.[41] The higher urine production is now believed to be initiated early in the process of renal failure, not as a consequence of uremia.[2,42] Renal function as calculated by interpolated GFR in CKD rabbits was only one-third less than that of controls and is remarkable because the kidney had

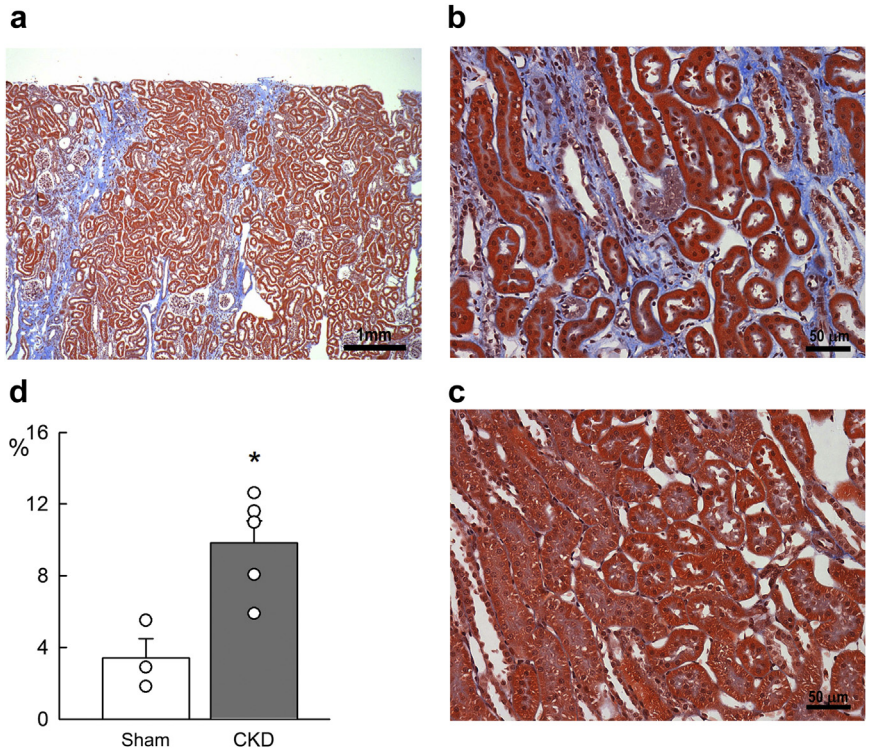

Figure 7 | (a) Masson's trichrome staining of renal cortex of a chronic kidney disease (CKD) rabbit on day 21, showing fibrotic tracks made by lesions (blue staining; bar = 1 mm). (**b,c**) Masson's trichrome staining of renal cortex in (**b**) 1 CKD and (**c**) 1 sham-operated rabbit (bars = 50 μm). (**d**) Effect of CKD on percentage of collagen (blue stain) accumulation in the renal cortex, which was greater in CKD than in sham kidneys. Data are expressed as individual points as well as mean ± SEM, indicating between-animal variance in sham (white, n = 4) and CKD (gray, n = 5) rabbits. *$P < 0.05$ for CKD versus sham. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

lesions over much of its surface. We noted that the damage done by the lesioning needle followed discrete pathways and did not induce mesangial expansion. Thus, the importance of measuring RSNA versus muscle SNA in CKD cannot be underestimated because of the multifaceted role of RSNA in promotion of renin secretion, sodium retention, water reabsorption, and vasomotor tone.

A further consideration with our control rabbits is that they have 2 kidneys, whereas our CKD model has only 1. Although this is common practice, renorenal reflexes are

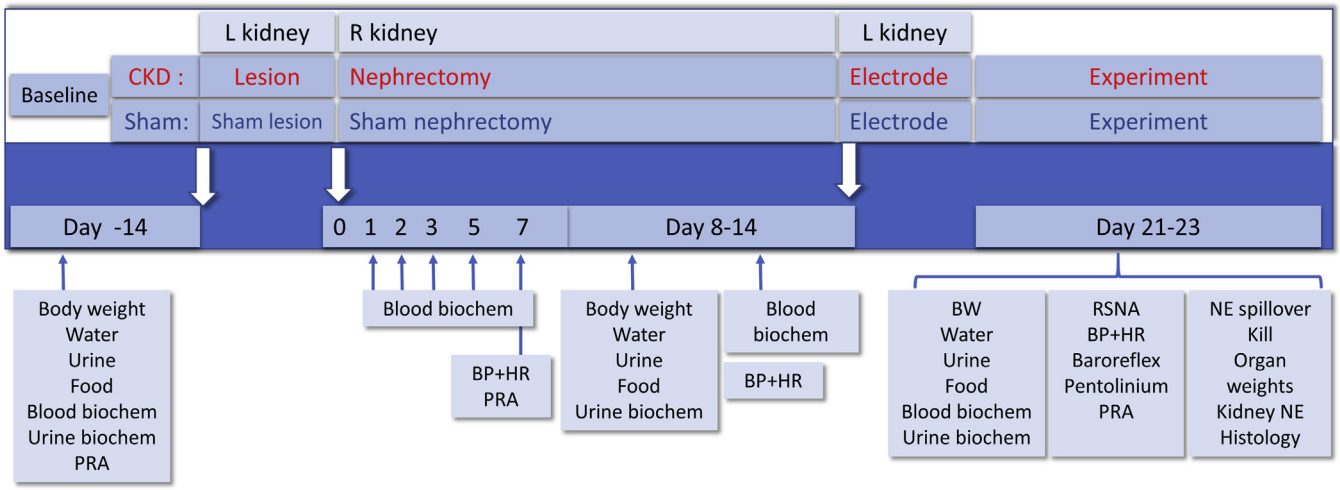

Figure 8 | Timeline of measurement (shown by blue arrows) and surgery days (shown by white arrows). Biochem, biochemistry; BP, blood pressure; BW, body weight; CKD, chronic kidney disease; HR, heart rate; NE, norepinephrine; PRA, plasma renin activity; RSNA, renal sympathetic nerve activity.

absent in the CKD rabbit but present in the controls. Although a further uninephrectomized control might be desirable, there still would be differences because of compensatory hypertrophy in the intact kidney that may impact remodeling and tissue growth. Moreover, comparing FE_{Na} in the CKD rabbits with the 2-kidney controls having twice as many glomeruli may well result in the magnitude of the responses obtained being confounded. An additional limitation of the current study is that we only included male rabbits, but we point out that unlike rodents, rabbits are induced ovulators and do not have a fixed estrous cycle.[43]

In conclusion, the lesion-induced model of CKD consistently produced stable but moderate CKD and allowed us to measure BP and RSNA in conscious rabbits. Both BP and RSNA were elevated, but global SNA, determined by whole-body NE spillover, was lower than in controls. The glomerular layer lesioning and nephrectomy model of CKD was characterized by augmented markers of oxidative stress, inflammation, and fibrosis that occurred within 5 weeks of lesioning, all contributing to renal dysfunction. We propose that RSNA was increased predominantly via changes induced by the damaged kidney. Interrupting the signal from renal afferent nerves may prove to be a means of controlling hypertension and elevated RSNA associated with CKD.

MATERIALS AND METHODS

Animals

Experiments were conducted in 29 male New Zealand White rabbits (initial body weight, 2.7–3.6 kg) and were approved by the Alfred Medical Research Education Precinct Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for Scientific Use of Animals.

Experimental procedures and protocol

The surgical and experimental protocol spanned a period of 5 weeks (Figure 8). During the first experiment in conscious rabbits, before any surgery, baseline MAP and HR were recorded over 1 hour, always beginning at approximately 10 AM, via an indwelling ear artery catheter inserted under local anesthetic and connected to a pressure transducer (Statham P23DG; Hato Rey, Puerto Rico). At the same time, arterial blood samples were taken for measurement of PRA and other blood parameters (i-Stat system, Chem8$^+$ cartridge; Abbott, Melbourne, VIC, Australia).

Rabbits underwent operations under isoflurane anesthesia (3% in oxygen; Abbott Australasia, Botany, NSW, Australia) after induction with propofol (10 mg kg^{-1}; Fresenius Kabi, Pymble, NSW, Australia). Carprofen (Rimadyl; Pfizer, West Ryde, NSW, Australia) was given before and 24 hours after surgery for analgesia and anti-inflammation. The left kidney was exposed via a retroperitoneal incision and placed on a stainless steel plate. The kidney was lesioned in a 1-mm grid pattern over 5/6 of the surface with a 9-mA cathodal current (2-second duration; depth, 2 mm) using a lesion maker (Grass Instruments, model DCLM5A; Quincy, MA). To validate the choice of 5/6 surface lesioning, an additional 4 rabbits underwent a pilot study in which 2/6 of the kidney surface or 6/6 was lesioned. Sham surgery involved exposure of the kidney, but it was not lifted onto the plate and there was no lesioning (Figure 8).

After 2 weeks of recovery, contralateral renal nephrectomy (n = 18) or sham nephrectomy (n = 11) was performed and blood samples taken over the next 3 weeks (Figure 8). MAP and HR were measured in 1 subset of conscious rabbits on days 7 and 14 (n = 10). Water intake, urine output, and food consumption were measured in 9 rabbits housed in metabolic cages over 2 days, at baseline, and at days 10 and 21 after nephrectomy, and a further 10 rabbits were used for polymerase chain reaction and biochemical analysis (Figure 8). Every rabbit with a lesioned renal surface plus contralateral nephrectomy produced elevated plasma creatinine (i.e., success rate was 100%).

All rabbits then underwent surgery to implant a stainless steel recording electrode on the left renal nerve[15] (Figure 8). The renal nerves, usually protected by the tissue between the renal artery and vein, were accessed in both CKD and sham rabbits without disturbing the kidney. Thus, any scarring caused by the previous exposure had no discernable impact on the implantation of the electrode, with 100% success 1 week later.

After 1-week recovery from electrode surgery, RSNA as well as MAP and HR were measured in conscious rabbits (21 days after nephrectomy). The central ear artery was catheterized for arterial pressure and a lateral ear vein for i.v. injection with 24G catheters (BD Insyte, Singapore). The RSNA signal was recorded as described previously[16] (see Supplementary Methods), and burst amplitude and frequency were calculated.[44] Resting parameters were recorded for 1 hour, and then RSNA and HR baroreflexes were derived in duplicate from slow ramp rises and falls in MAP by i.v. infusions of 0.5 mg/ml phenylephrine and 1 mg/ml sodium nitroprusside, respectively.[16] The response to ganglionic blockade was assessed by i.v. injection of pentolinium tartrate (6 mg/kg; Apin Chemicals, Abingdon, UK) (Figure 8).

A 2-day recovery was allowed before total NE spillover was measured in conscious rabbits. This is sufficient time for recovery of MAP, HR, and RSNA.[45] Blood samples were taken before and at 40, 50, and 60 minutes during i.v. infusion of ring-labeled [^{3}H]NE (110 nCi/kg per min; Perkin Elmer, Boston, MA).[46] At the end of the experiment, rabbits was killed and kidney and heart removed.

Data analysis

BP and RSNA were digitized at 1000 Hz, and total RSNA and amplitude were scaled to 100 normalized units by the maximum nasopharyngeal response evoked by smoke.[16] Resting levels were measured over 60 minutes. Baroreflex MAP-RSNA and MAP-HR curves were fitted to a 5-parameter logistic function.[47]

Measurement of PRA, urine albumin, NE spillover, GFR, FE_{Na}, and mRNA

PRA was determined by radioimmunoassay,[48] and urine albumin was measured using an ELISA kit (ab108793; Abcam, Melbourne, VIC, Australia) according to the manufacturer's instructions. NE was determined in plasma[49] and whole-body NE spillover to plasma calculated[46] (see Supplementary Methods for details).

GFR was interpolated from the relationship between creatinine and GFR measured directly in a cohort of 20 rabbits using [^{14}C] inulin (28 nCi/kg per min; Perkin Elmer) clearance.[50] FE_{Na} was calculated as $FE_{Na} = Urine_{Na} \times Plasma_{creatinine}/PlasmaNa \times Urine_{creatinine} \times 100$.

An RNA isolation kit (Z3105; Promega Australia, Alexandria, NSW, Australia/Madison, WI) was used for RNA extraction, and complementary DNA synthesis was performed[51] (see Supplementary Methods for details).

Histopathology

Assessment of kidney histology was made with periodic acid–Schiff or Masson's trichrome, and the percentage of cortical area from 20 photomicrographs per kidney (in RBG) was assessed using Image-Pro Analyzer (Media Cybernetics, Rockville, MD, version 7.0).[52] Area of tubulointerstitial injury was made blinded using a grading system according to the severity of the tubular and interstitial damage (17–20 images per section).[53] Glomerular injury was estimated using the glomerular sclerosis index.[54]

Statistical analysis

Values are expressed as mean ± SEM or mean difference ± standard error of the difference. Data were analyzed by split-plot repeated-measures (mixed model) analysis of variance (ANOVA). The main contrasts were the within-group effect of treatment compared with baseline ($P_{baseline}$) and between sham and CKD groups (P_{group}). One-way ANOVA was used for comparisons taken at a single time point. Type I error was controlled using Bonferroni adjustment to the probability threshold and Greenhouse-Geisser correction to reduce inflated residual degrees of freedom.[55] A probability of $P < 0.05$ was considered significant.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The study was supported by the Japan Heart Foundation/Bayer Yakuhin Research Grant Abroad and Grant-in-Aid for Scientific Research (25.5473) promoted by the Ministry of Education, Culture, Sports, Science and Technology of Japan, Postdoctoral Fellowship (101325) from National Heart Foundation of Australia, and Emerging Leader Fellowship from the Baker Heart and Diabetes Institute (YS); National Health and Medical Research Council of Australia (NHMRC) project grant 1046594 and project grant to AMDW (GNT1079645); NHMRC Early Career Fellowship to JCJ (APP1126169); NHMRC Postdoctoral Fellowships to KL (APP 1053928) and KLJ (APP1091688); NHMRC Senior Research Fellowship to KAMJ-D (APP 1059124); and NHMRC Principal Research Fellowship to GAH (APP367631), KMD (APP1136813), and MPS (APP1080404). GWL and NE were supported by Swinburne Research, Swinburne University of Technology. The study was supported in part by the Victorian Government's Operational Infrastructure Support Program.

We thank Ms. Lisa van Rensch, Ms. Sarah Phillips, and Ms. Aozhi Dai for their excellent technical contribution to the study and Ms. Josepha Pete and Dr. Carlos Rosado for designing the primers.

AUTHOR CONTRIBUTIONS

Experiments were conducted in the Neuropharmacology Laboratory, Baker Heart and Diabetes Institute and Department of Diabetes, Monash University, Melbourne, Victoria, Australia. GAH, MPS, KMD, and SLB conceived of and designed the research; YS, SLB, JCJ, and AMDW performed surgery and experiments; SLB, AMDW, JCJ, KL, KLJ, and CG analyzed data; YS, GAH, SLB, AMDW, and GWL interpreted results of experiments; YS, SLB, CG, and GAH prepared figures; YS, SLB, AMDW, and GAH drafted the manuscript; and all authors edited, revised, and approved final version of manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Materials and Methods. Measurement of RSNA, NE spillover, and of mRNA.

REFERENCES

1. Zoccali C, Mallamaci F, Parlongo S, et al. Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. *Circulation*. 2002;105:1354–1359.
2. Grassi G, Quarti-Trevano F, Seravalle G, et al. Early sympathetic activation in the initial clinical stages of chronic renal failure. *Hypertension*. 2011;57:846–851.
3. Rahman SN, Abraham WT, Van Putten VJ, et al. Increased norepinephrine secretion in patients with the nephrotic syndrome and normal glomerular filtration rates: evidence for primary sympathetic activation. *Am J Nephrol*. 1993;13:266–270.
4. Cerasola G, Vecchi M, Mule G, et al. Sympathetic activity and blood pressure pattern in autosomal dominant polycystic kidney disease hypertensives. *Am J Nephrol*. 1998;18:391–398.
5. Grassi G, Esler M. How to assess sympathetic activity in humans. *J Hypertens*. 1999;17:719–734.
6. Converse RL Jr, Jacobsen TN, Toto RD, et al. Sympathetic overactivity in patients with chronic renal failure. *N Engl J Med*. 1992;327:1912–1918.
7. Hausberg M, Kosch M, Harmelink P, et al. Sympathetic nerve activity in end-stage renal disease. *Circulation*. 2002;106:1974–1979.
8. Ligtenberg G, Blankestijn PJ, Oey PL, et al. Reduction of sympathetic hyperactivity by enalapril in patients with chronic renal failure [see comments]. *N Engl J Med*. 1999;340:1321–1328.
9. Park J, Campese VM, Nobakht N, et al. Differential distribution of muscle and skin sympathetic nerve activity in patients with end-stage renal disease. *J Appl Physiol*. 2008;105:1873–1876.
10. Dean C, Seagard JL, Hopp FA, et al. Differential control of sympathetic activity to kidney and skeletal muscle by ventral medullary neurons. *J Auton Nerv Syst*. 1992;37:1–10.
11. Okada Y, Ninomiya I. Different cardiac and renal inhibitory and excitatory areas in rabbit hypothalamus. *Am J Physiol*. 1983;244:H832–H838.
12. Yao Y, Hildreth CM, Farnham MM, et al. The effect of losartan on differential reflex control of sympathetic nerve activity in chronic kidney disease. *J Hypertens*. 2015;33:1249–1260.
13. Veiga GL, Nishi EE, Estrela HF, et al. Total renal denervation reduces sympathoexcitation to different target organs in a model of chronic kidney disease. *Auton Neurosci*. 2017;204:81–87.
14. Mayorov DN, Burke SL, Head GA. Relative importance of rostral ventrolateral medulla in sympathoinhibitory action of rilmenidine in conscious and anesthetized rabbits. *J Cardiovasc Pharmacol*. 2001;37:252–261.
15. Dorward PK, Riedel W, Burke SL, et al. The renal sympathetic baroreflex in the rabbit. Arterial and cardiac baroreceptor influences, resetting, and effect of anesthesia. *Circ Res*. 1985;57:618–633.
16. Burke SL, Head GA. Method for in vivo calibration of renal sympathetic nerve activity in rabbits. *J Neurosci Methods*. 2003;127:63–74.
17. Burke SL, Lambert E, Head GA. New approaches to quantifying sympathetic nerve activity. *Curr Hypertens Rep*. 2011;13:249–257.
18. Gotloib L, Crassweller P, Rodella H, et al. Experimental model for studies of continuous peritoneal dialysis in uremic rabbits. *Nephron*. 1982;31:254–259.
19. Ninomiya I, Malpas SC, Matsukawa K, et al. The amplitude of synchronized cardiac sympathetic nerve activity reflects the number of activated pre- and postganglionic fibers in anesthetized cats. *J Auton Nerv Syst*. 1993;45:139–147.
20. Malpas S, Ninomiya I. Fundamental rhythm of renal sympathetic nerve activity in anesthetized cats. *J Auton Nerv Syst*. 1992;37:11–18.
21. Noshiro T, Saigusa T, Way D, et al. Norepinephrine spillover faithfully reflects renal sympathetic nerve activity in conscious rabbits. *Am J Physiol Renal Physiol*. 1991;261:F44–F50.
22. Campese VM. A new model of neurogenic hypertension caused by renal injury: pathophysiology and therapeutic implications. *Clin Exp Nephrol*. 2003;7:167–171.
23. Lohmeier TE, Lohmeier JR, Haque A, et al. Baroreflexes prevent neurally induced sodium retention in angiotensin hypertension. *Am J Physiol Regul Integr Comp Physiol*. 2000;279:R1437–R1448.
24. Salman IM, Sarma Kandukuri D, Harrison JL, et al. Direct conscious telemetry recordings demonstrate increased renal sympathetic nerve activity in rats with chronic kidney disease. *Front Physiol*. 2015;6:218.
25. Phillips JK, Hopwood D, Loxley RA, et al. Temporal relationship between renal cyst development, hypertension and cardiac hypertrophy in a new

rat model of autosomal recessive polycystic kidney disease. *Kidney Blood Pressure Res*. 2007;30:129–144.
26. Harrison JL, Hildreth CM, Callahan SM, et al. Cardiovascular autonomic dysfunction in a novel rodent model of polycystic kidney disease. *Auton Neurosci*. 2010;152:60–66.
27. Salas SP, Giacaman A, Vio CP. Pregnant rats with 5/6 nephrectomy have normal volume expansion despite lower renin and kallikrein. *Hypertension*. 2003;42:744–748.
28. Hart E, Head G, Carter J, et al. Recording sympathetic nerve activity in conscious humans and other mammals: guidelines and the road to standardization. *Am J Physiol Heart Circ Physiol*. 2017;312:H1031–H1051.
29. Grassi G, Seravalle G, Arenare F, et al. Behaviour of regional adrenergic outflow in mild-to-moderate renal failure. *J Hypertens*. 2009;27:562–566.
30. Hering D, Mahfoud F, Walton AS, et al. Renal denervation in moderate to severe CKD. *J Am Soc Nephrol*. 2012;23:1250–1257.
31. Schlaich MP, Bart B, Hering D, et al. Feasibility of catheter-based renal nerve ablation and effects on sympathetic nerve activity and blood pressure in patients with end-stage renal disease. *Int J Cardiol*. 2013;168:2214–2220.
32. Zelniker TA, Wiviott SD, Raz I, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet*. 2019;393:31–39.
33. DiBona GF, Kopp UC. Neural control of renal function. *Physiol Rev*. 1997;77:75–197.
34. Sakakura K, Ladich E, Cheng Q, et al. Anatomic assessment of sympathetic peri-arterial renal nerves in man. *J Am Coll Cardiol*. 2014;64:635–643.
35. Holterman CE, Thibodeau JF, Towaij C, et al. Nephropathy and elevated BP in mice with podocyte-specific NADPH oxidase 5 expression. *J Am Soc Nephrol*. 2014;25:784–797.
36. Duni A, Liakopoulos V, Rapsomanikis KP, et al. Chronic kidney disease and disproportionally increased cardiovascular damage: does oxidative stress explain the burden? *Oxid Med Cell Longev*. 2017;2017:9036450.
37. Jha JC, Banal C, Okabe J, et al. NADPH oxidase Nox5 accelerates renal injury in diabetic nephropathy. *Diabetes*. 2017;66:2691–2703.
38. Nath KA. Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis*. 1992;20:1–17.
39. Toda N, Mukoyama M, Yanagita M, et al. CTGF in kidney fibrosis and glomerulonephritis. *Inflamm Regen*. 2018;38:14.
40. Gagnon RF, Gallimore B. Characterization of a mouse model of chronic uremia. *Urol Res*. 1988;16:119–126.
41. DiBona GF. Functionally specific renal sympathetic nerve fibers: role in cardiovascular regulation. *Am J Hypertens*. 2001;14:163S–170S.
42. Schlaich MP, Socratous F, Hennebry S, et al. Sympathetic activation in chronic renal failure. *J Am Soc Nephrol*. 2009;20:933–939.
43. Dal Bosco A, Rebollar PG, Boiti C, et al. Ovulation induction in rabbit does: current knowledge and perspectives. *Anim Reprod Sci*. 2011;129:106–117.
44. Lim K, Burke S, Moretti J-L, et al. Differential activation of renal sympathetic burst amplitude and frequency during hypoxia and stress in angiotensin hypertension. *Exp Physiol*. 2015;100:1132–1144.
45. Moretti J-L, Burke SL, Davern PJ, et al. Renal sympathetic activation from long-term low-dose angiotensin II infusion in rabbits. *J Hypertens*. 2012;30:551–560.
46. Esler M, Jennings G, Korner P, et al. Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension*. 1988;11:3–20.
47. Ricketts JH, Head GA. A five parameter logistic equation for investigating asymmetry of curvature in baroreflex studies. *Am J Physiol Regul Integr Comp Physiol*. 1999;277:R441–R454.
48. Oliver JR, Korner PI, Woods RL, et al. Reflex release of vasopressin and renin in haemorrhage is enhanced by autonomic blockade. *Am J Physiol Heart Circ Physiol*. 1990;258:H221–H228.
49. Lambert GW, Jonsdottir IH. Influence of voluntary exercise on hypothalamic norepinephrine. *J Appl Physiol*. 1998;85:962–966.
50. Burke SL, Evans RG, Head GA. Effects of chronic sympatho-inhibition on renal excretory function in renovascular hypertension. *J Hypertens*. 2011;29:945–952.
51. Tikellis C, Xuereb L, Casley D, et al. Calcitonin receptor isoforms expressed in the developing rat kidney. *Kidney Int*. 2003;63:416–426.
52. Jha JC, Thallas-Bonke V, Banal C, et al. Podocyte-specific Nox4 deletion affords renoprotection in a mouse model of diabetic nephropathy. *Diabetologia*. 2016;59:379–389.
53. Calkin AC, Giunti S, Jandeleit-Dahm KA, et al. PPAR-alpha and -gamma agonists attenuate diabetic kidney disease in the apolipoprotein E knockout mouse. *Nephrol Dial Transplant*. 2006;21:2399–2405.
54. Watson AM, Li J, Schumacher C, et al. The endothelin receptor antagonist avosentan ameliorates nephropathy and atherosclerosis in diabetic apolipoprotein E knockout mice. *Diabetologia*. 2010;53:192–203.
55. Ludbrook J. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc Res*. 1994;28:303–311.