Impaired Renorenal Reflexes in Two-Kidney, One Clip Hypertensive Rats

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In normotensive rats, stimulation of renal mechanoreceptors by an increase in ureteral pressure results in a contralateral inhibitory renorenal reflex response with contralateral natriuresis. Similar effects are produced by stimulation of renal chemoreceptors by renal pelvic perfusion with 0.9 M NaCl. However, in spontaneously hypertensive rats the renorenal reflex responses to renal mechanoreceptor and chemoreceptor stimulation are impaired. The present study was performed to examine whether the renorenal reflexes were altered in two-kidney, one clip hypertensive rats, a model of hypertension in which it has been suggested that the afferent renal nerves contribute to the enhanced peripheral sympathetic nervous activity. A 0.2 mm silver clip was placed around one renal artery 4 weeks before the study. At the time of study, mean arterial pressure was 156±4 mm Hg. Renal mechanoreceptor and chemoreceptor stimulation of either the nonclipped or clipped kidney failed to affect ipsilateral afferent renal nerve activity, contralateral efferent renal nerve activity, and contralateral urine flow rate and urinary sodium excretion. Renal denervation of the nonclipped kidney increased ipsilateral urinary sodium excretion from 0.65±0.13 to 1.50±0.42 μmol/min/g and decreased contralateral urinary sodium excretion from 0.18±0.03 to 0.13±0.03 μmol/min/g (p<0.05). Thus, denervation of the nonclipped kidney resulted in a similar contralateral excitatory renorenal reflex response as in normotensive rats. However, denervation of the clipped kidney increased both ipsilateral and contralateral urinary sodium excretion, from 1.29±0.33 to 2.09±0.59 μmol/min/g (p<0.01), respectively. Taken together these data suggest that the lack of inhibitory renorenal reflexes from the clipped kidney may enhance efferent sympathetic nervous activity and thereby contribute to the hypertension in two-kidney, one clip hypertensive rats. (Hypertension 1989;14:445-452)
adult SHR treated with captopril from 3 weeks of age to prevent the development of hypertension. An impairment of the renorenal reflexes such as that shown in adult hypertensive SHR would enhance ERNA, favor sodium and water retention, and thus potentially contribute to hypertension.

The two-kidney, one clip hypertensive rat is another model of hypertension in which peripheral sympathetic activity is enhanced. In contrast to SHR, in which the delay in the development of hypertension after renal denervation is associated with increased water and sodium excretion, renal denervation of the clipped kidney significantly lowers mean arterial pressure in the absence of an increase in daily urinary sodium excretion. Denervation of the nonclipped kidney is without effect on arterial pressure. The depressor effect of denervation of the clipped kidney is associated with a decrease in peripheral sympathetic nervous system activity. Similarly, in one-kidney, one clip hypertensive rats, denervation of the clipped kidney or ipsilateral dorsal rhizotomy (T1–L2) lowers arterial pressure. The fall in arterial pressure is associated with a fall in hypothalamic norepinephrine content and plasma norepinephrine concentration. Taken together, these findings suggest that afferent renal nerves contribute to the hypertension in renal vascular hypertensive rats by enhancing peripheral sympathetic nervous activity.

Thus, in contrast to normotensive Sprague-Dawley rats in which unilateral renal denervation results in an increase in contralateral ERNA, denervation of the clipped kidney decreases peripheral sympathetic nervous system activity. Therefore, the present study was undertaken to examine whether the inhibitory renorenal reflexes present in normotensive Sprague-Dawley rats were altered in two-kidney, one clip hypertensive Sprague-Dawley rats. Renal MR and CR stimulation were performed on the clipped kidney, which was exposed to normal renal perfusion pressure, and the nonclipped kidney, which was exposed to the elevated renal perfusion pressure. In addition, the effects of unilateral renal denervation of either the clipped or nonclipped kidney on contralateral urinary sodium excretion were examined.

Materials and Methods

The study was performed in male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Indiana). The rats (195±1 g) were anesthetized with a high frequency cutoff at 3,000 Hz and a low voltage integrator (Beckman 9873B). Assessment of renal nerve activity was done by its pulse-synchronous rhythmicity and its abolition by load-
Renal Denervation

In 22 of the 53 rats used in the study renal denervation of either left or right kidney was performed by sectioning all visible renal nerves and by stripping the renal artery and painting it with 10% phenol in absolute alcohol. This technique results in complete renal denervation as shown by total abolition of the renal vasoconstrictor response to electrical renal nerve stimulation of the ipsilateral lumbar sympathetic chain and a reduction of the renal tissue norepinephrine content to 3.1% of that observed in sham–renal-denervated rats.13

Experimental Procedure

Approximately 1.5 hours were allowed to elapse between the end of surgery and start of the experiment. The study was divided into seven groups. In groups 1–4, two 20-minute experimental periods were performed during which renal MRs and CRs were stimulated at random order. Each of the experimental periods was preceded by a 20-minute control period and followed by a 20-minute recovery period. In groups 1 and 2, renal MR and CR stimulation was performed on the nonclipped kidney. Ipsilateral ARNA was recorded in six of nine rats in group 1, and contralateral ERNA in six of eight rats in group 2. In groups 3 and 4, renal MR and CR stimulation was performed on the clipped kidney. Ipsilateral ARNA was recorded in group 3 (n=8) and contralateral ERNA in group 4 (n=6). In groups 5 and 6, the experiment started with two 20-minute control periods. Then denervation was performed in either the nonclipped kidney, group 5 (n=6), or the clipped kidney, group 6 (n=11). Two 20-minute post–denervation periods were started 20 minutes later. In group 7 (n=5), denervation of either the left or right kidney was performed in Sprague-Dawley rats that had not been previously exposed to renal artery clipping. The experimental protocol was identical to that in groups 5 and 6. Group 7 served as a control group for groups 5 and 6. Thus, in groups 5–7 no renal MR or CR stimulation was performed.

Urine was collected in 20-minute periods, and an arterial blood sample, 200 μl, was taken at the end of each period. Blood was replaced with an equal amount of saline. In groups 1–4, renal nerve activity was measured and averaged over each 20-minute control, experimental, and recovery period.

Analytical Procedure

Plasma and urine concentrations were determined with a flame photometer (model 143, Instrumentation Laboratories, Lexington, Massachusetts). Plasma and urine samples were analyzed for inulin by an anthrone method.14 Urinary clearance of inulin was used for measurement of glomerular filtration rate. Values of glomerular filtration rate, urine flow rate, and urinary sodium excretion are expressed per gram kidney weight.

Statistical Analysis

In groups 1–4, the effects of renal MR and CR stimulation on systemic hemodynamics and renal function were evaluated by comparison of the experimental value with the average of the control and recovery values. In groups 5–7, the average of the two postdenervation values was compared with the average of the two predenervation values. Friedman two-way analysis of variance, short-cut analysis of variance, Wilcoxon matched-pairs, signed-rank test, and Mann-Whitney U test was used.15,16 A significance level of 5% was chosen. Data in text and figures are expressed as mean±SEM.

Results

Renal Mechanoreceptor and Chemoreceptor Stimulation

The kidney weight of the clipped kidney was significantly less than that of the contralateral nonclipped kidney, 1.36±0.07 vs. 2.29±0.08 g or 0.0040±0.0002 vs. 0.0069±0.0003 g/g body wt (p<0.01). Basal glomerular filtration rate, urine flow rate, and urinary sodium excretion from the clipped kidney were also significantly less than those from the contralateral nonclipped kidney: glomerular filtration rate, 0.30±0.05 vs. 0.69±0.6 ml/min/g; urine flow rate, 2.10±0.37 vs. 11.02±1.12 μl/min/g; and urinary sodium excretion, 0.29±0.11 vs. 1.67±0.23 μmol/min/g (p<0.01). Basal mean arterial pressure and heart rate were 159±5 mm Hg and 354±8 beats/min, respectively. Microscopic examination of the kidneys revealed ischemic structural damage in the clipped kidney. The nonclipped kidney was hypertrophied but otherwise normal.

Groups 1 and 2

Because basal values of systemic hemodynamics and renal function and the responses to renal MR and CR stimulation were similar, whether ipsilateral ARNA (group 1) or contralateral ERNA (group 2) was measured, the data have been pooled. The results are shown in Figure 1. Renal MR and CR stimulation of the nonclipped kidney failed to affect ipsilateral ARNA and contralateral ERNA. Contralateral urine flow rate and urinary sodium excretion...
increased slightly \( p<0.01 \) during renal MR stimulation but did not return toward control values during the recovery period and remained unchanged during renal CR stimulation. Mean arterial pressure, heart rate, and contralateral glomerular filtration rate remained unchanged throughout the experiment.

At the end of the experiment, the capability of the afferent and efferent renal nerves to respond to other stimuli, such as an intravenous injection of air or an intravenous injection of a pressor dose of norepinephrine, was tested. Ipsilateral ARNA increased from \( 1.4\pm0.9 \) to \( 4.1\pm2.2 \) resets/min \( (p<0.05, \ n=6) \) in response to an intravenous injection of air. Contralateral ERNA decreased from \( 2.0\pm0.9 \) to \( 0.6\pm0.1 \) resets/min \( (p<0.05) \) in response to an intravenous injection of norepinephrine, 2 \( \mu \)g.

Groups 3 and 4
Because basal values of systemic hemodynamics and renal function and the responses to renal MR and CR stimulation were similar, whether ipsilateral ARNA (group 3) or contralateral ERNA (group 4) was measured, the data have been pooled. The results are shown in Figure 2. Renal MR and CR stimulation of the clipped kidney did not affect ipsilateral ARNA, contralateral ERNA, or contralateral glomerular filtration rate, urine flow rate, and urinary sodium excretion. Similarly, mean arterial pressure and heart rate remained unchanged throughout the experiment.

At the end of the experiment an intravenous injection of air increased ipsilateral ARNA from \( 1.8\pm0.4 \) to \( 5.6\pm1.1 \) resets/min \( (p<0.01, \ n=8) \), and an intravenous injection of norepinephrine, 2 \( \mu \)g, decreased contralateral ERNA from \( 2.2\pm0.6 \) to \( 0.8\pm0.3 \) resets/min \( (p<0.05, \ n=6) \).

Renal Denervation
The kidney weight of the clipped kidney was significantly less than that of the contralateral nonclipped kidney, \( 1.09\pm0.12 \) vs. \( 2.05\pm0.13 \) g or \( 0.0033\pm0.0003 \) vs. \( 0.0064\pm0.0004 \) g/g body wt \( (p<0.01, \ n=17) \). There was no difference between...
Denervation of either the left or right kidney increased ipsilateral urinary sodium excretion by 0.58 ± 0.23 μmol/min/g and decreased contralateral urinary sodium excretion by 0.70 ± 0.29 μmol/min/g (p < 0.01), as shown in Figure 3. Ipsilateral urine flow rate increased from 14.5 ± 4.5 to 17.5 ± 3.9 μl/min/g (p < 0.05). The decrease in contralateral urine flow rate from 15.1 ± 3.6 to 10.1 ± 2.4 μl/min/g did not reach statistical significance. Ipsilateral glomerular filtration rate, 0.86 ± 0.06 ml/min/g, and contralateral glomerular filtration rate, 0.93 ± 0.07 ml/min/g were slightly decreased after ipsilateral renal denervation. Mean arterial pressure and heart rate decreased after renal denervation from 121 ± 5 to 110 ± 6 mm Hg and 374 ± 18 to 300 ± 11 beats/min, respectively (p < 0.05).

Discussion

There is considerable evidence for enhanced activity of the sympathetic nervous system contributing to the development and maintenance of hypertension in the two-kidney, one clip hypertensive rats. Although increased activity of the renin-angiotensin system has been implicated in the initial hypertensive response to clipping the renal artery in two-kidney, one clip hypertensive rats, the role of the renin-angiotensin system as the main or sole mechanism appears to diminish during the chronic phase of hypertension. Rather, there is evidence to suggest that central and peripheral neurogenic mechanisms interact in the development and maintenance of hypertension. Injection of angiotensin II antagonists intracerebroventricularly lowers arterial pressure at doses that have no effect when injected systemically in rats made hypertensive by occlusion of the aorta between the two renal arteries. Renal denervation of the clipped kidney in two-kidney, one clip hypertensive rats lowers arterial pressure and plasma norepinephrine concentrations. Furthermore, oral administration of clonidine, a centrally acting α2-adrenergic receptor agonist, lowers arterial pressure and plasma renin activity in two-kidney, one clip hypertensive rats. Taken together these data suggest that hypertension in two-kidney, one clip hypertensive rats may be mediated through angiotensin-dependent central neurogenic mechanisms that are influenced by the afferent renal nerves.

The results of the present study demonstrate that renal MR and CR stimulation of either the clipped or nonclipped kidney fails to affect ipsilateral ARNA, contralateral ERNA, and contralateral urine flow rate and urinary sodium excretion in two-kidney, one clip hypertensive rats. Whereas renal denervation of the nonclipped kidney results in a decrease
Renal MR and CR stimulation of the nonclipped or clipped kidney failed to affect ipsilateral ARNA, contralateral ERNA, and contralateral renal excretory function in two-kidney, one clip hypertensive Sprague-Dawley rats. It is unlikely that the slight increases in contralateral urine flow rate and sodium excretion observed during renal MR stimulation of the nonclipped kidney are related to stimulation of renal MR since contralateral urine flow rate and sodium excretion remained unchanged during the recovery period when ureteral pressure was returned to zero. The present findings are in sharp contrast to our previous studies in normotensive nonclipped Sprague-Dawley rats in which renal MR and CR stimulation of similar magnitude as that applied in the present study elicited a contralateral inhibitory renorenal reflex response with contralateral diuresis and natriuresis. 1 In the present study, ipsilateral ARNA increased and contralateral ERNA decreased in response to intravenous injection of air and norepinephrine, respectively, demonstrating that the lack of an effect of renal MR and CR stimulation was specific for the stimuli applied. It may be argued that the impairment of the renorenal reflexes may be related to the decreased renal catecholamine content and decreased responsiveness to neural stimuli observed in renal vascular hypertensive animals. 23 However, the evidence concerning decreased renal responsiveness to neural stimuli derives from studies on the renal vasculature. The data from the present study, which show no difference in the percent increase in ipsilateral urinary sodium excretion produced by unilateral renal denervation between the control rats and the two-kidney, one clip hypertensive rats do not support the view that the impairment of the renorenal reflexes is related to decreased renal responsiveness to changes in renal nerve activity. Rather, it may be proposed that the impaired renorenal reflexes are related to structural changes in the clipped and nonclipped kidney related to ischemia and elevated renal perfusion pressure, respectively. An increased wall-to-lumen ratio in the interlobular arteries and thickening of the intima in arcuate and interlobular arteries have been shown in the nonclipped kidney in two-kidney, one clip hypertensive rats. 24 Furthermore, the findings in the present study are similar to our previous findings in untreated adult SHR, 5 which showed that the impairment of the renorenal reflex responses to renal MR and CR stimulation in SHR was related to the elevated blood pressure. 6

Renal denervation of either kidney in the normotensive control group resulted in an increase in ipsilateral urinary sodium excretion and a decrease in contralateral urinary sodium excretion. The magnitudes of the increase in ipsilateral urinary sodium excretion and decrease in contralateral urinary sodium excretion after unilateral renal denervation were similar, resulting in no change in total urinary sodium excretion from both kidneys. These findings are in agreement with previous studies in volume-expanded normotensive rats that showed that the decrease in contralateral urinary sodium excretion after unilateral renal denervation was associated with an increase in contralateral ERNA. 3,4 Renal denervation of the nonclipped kidney in the two-kidney, one clip hypertensive rats showed a similar response to that observed in normotensive rats (i.e., an increase in ipsilateral urinary sodium excretion and fall in contralateral urinary sodium excretion). Mean arterial pressure and heart rate were decreased after unilateral renal denervation in both groups. Because the magnitude of the reduction of mean arterial pressure and heart rate was greater in the normotensive control rats (group 7) than in the nonclipped kidney renovascular hypertensive rats (group 5), it is not likely that the decreases in these parameters are necessarily related to the presence of renal vascular hypertension. In the two-kidney, one clip hypertensive rats, the fall in contralateral urinary sodium excretion after unilateral renal denervation of the nonclipped kidney was significantly smaller than the increase in ipsilateral urinary sodium excretion, resulting in a net total loss of excreted sodium excretion, resulting in a net total loss of excreted sodium excretion.
sodium. Although this may be related to a central abnormality of the reflex control of ERNA, it is more likely related to the low basal value of urinary sodium excretion from the contralateral clipped kidney. The presence of a renorenal reflex response to unilateral renal denervation of the nonclipped kidney in the two-kidney, one clip hypertensive rats would suggest that the impairment of the renorenal reflex responses to renal MR and CR stimulation is localized to the nonclipped kidney and not due to an impairment in the more central or efferent portions of the renorenal reflex arc. Of interest in this context is the study by Protasoni et al, which showed the presence of a renorenal reflex response to unilateral renal denervation in adult hypertensive SHR.

In contrast to the contralateral antinatriuretic response to denervation of the nonclipped kidney, acute unilateral renal denervation of the clipped kidney resulted in an increase in both ipsilateral and contralateral urinary sodium excretion. The increase in contralateral urinary sodium excretion was associated with a fall in directly measured contralateral ERNA. Previously, Katholi et al showed that chronic denervation of the clipped kidney 7 weeks after clamping in two-kidney, one clip hypertensive rats lowered plasma norepinephrine concentration to control levels and decreased peripheral sympathetic nervous activity to levels present in normotensive rats as judged by the depressor response to the ganglionic blocker, hexamethonium. Our acute unilateral renal denervation increases urinary sodium excretion from both kidneys without changing mean arterial pressure, whereas Katholi et al observed a fall in mean arterial pressure 2–3 days after unilateral renal denervation, which probably accounted for the transient fall in urinary sodium excretion. The differences in results may be explained by the differences in the experimental design of the two studies. In the present study, it is not likely that the natriuretic response to denervation was solely related to anesthesia since denervation natriuresis has also been shown in volume-expanded conscious rats in which renal nerve activity is low.

There is considerable evidence for a role of the afferent renal nerves in the maintenance of hypertension in the one-kidney, one clip hypertensive rats. Arterial pressure is reduced by renal denervation as well as by ipsilateral dorsal rhizotomy. Furthermore, the fall in arterial pressure is associated with a fall in hypothalamic norepinephrine content and plasma concentration of norepinephrine. Several studies have focused on the stimulus to afferent renal nerve activity in the clipped kidney. Microscopic examination of the clipped kidney revealed ischemic structural damage in the present study. Renal ischemia with decreased renal blood flow increases the release of adenosine by proximal tubular cells and urinary adenosine concentration is significantly higher in one-kidney, one clip hypertensive rats compared with control rats. Studies in dogs and rats have shown that administration of adenosine intrarenally results in increases in ipsilateral ARNA, contralateral ERNA, plasma norepinephrine concentration, and mean arterial pressure, which are abolished by ipsilateral renal denervation. Furthermore, intrarenal administration of adenosine deaminase reduces mean arterial pressure in one-kidney, one clip hypertensive rats. Taken together, these studies suggest that the increased urinary adenosine concentration derived from ischemic kidneys activates afferent renal nerves, which in turn results in increased peripheral (renal) sympathetic nerve activity.

In summary, the results from the present study show that renal MR and CR stimulation of the nonclipped or clipped kidney failed to elicit a renorenal reflex response in two-kidney, one clip hypertensive rats. The data suggest that the impairment of the renorenal reflexes in the nonclipped kidney is related to the elevated renal perfusion pressure, whereas the impairment of renorenal reflexes in the clipped kidney is related to ischemic structural damage. Since the nature of the inhibitory renorenal reflexes in normotensive rats is that of decreased ERNA and a diuresis and natriuresis, an impairment of the inhibitory renorenal reflexes would result in a lesser decrease or possibly an increase in ERNA leading to renal water and sodium retention. Furthermore, the data show that denervation of the clipped kidney results in an increase in both ipsilateral and contralateral urinary sodium excretion. These findings, together with those of Katholi et al, suggest that the contralateral natriuretic response to ipsilateral renal denervation is the result of a decrease in contralateral ERNA. The lack of inhibitory renorenal reflexes from the clipped kidney supports the hypothesis that the afferent renal nerves from the clipped kidney contribute to the hypertension in two-kidney, one clip hypertensive rats by enhancing efferent (renal) sympathetic nervous activity.

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