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Research Article

Increased sympathetic nervous system activity has been demonstrated in established one-kidney one-clip hypertension in the rat. We have found that renal denervation in this model results in an attenuation of hypertension, unassociated with alterations in sodium or water balance or renin activity. To determine whether the depressor effect of renal denervation is associated with changes in peripheral sympathetic nervous system activity, sham operation (n = 12), renal denervation (n = 13), or unclipping (n = 13) was carried out 2 wk after the onset of one-kidney one-clip hypertension. Normotensive unine-phrectomized age- and sex-matched rats were used as controls (n = 14). Renal denervation resulted in a significant decrease in systolic blood pressure (201±7 to 151±6 mm Hg), while unclipping lowered systolic blood pressure to normotensive levels (130±6 mm Hg). 8 d after operation plasma norepinephrine and mean arterial pressure before and after ganglionic blockade with 30 mg/kg hexamethonium bromide were measured in conscious, unrestrained, resting animals, as indices of peripheral sympathetic nervous system activity. Plasma norepinephrine was significantly higher in hypertensive sham-operated rats (422±42 pg/ml) compared with normotensive controls (282±25 pg/ml) (P < 0.01). Both renal denervation and unclipping restored plasma norepinephrine to normal levels (273±22 and 294±24 pg/ml, respectively). Ganglionic blockade in hypertensive sham-operated animals resulted in a significantly greater decrease in mean arterial pressure than occurred in renal […]

Decrease in Peripheral Sympathetic Nervous System Activity following Renal Denervation or Unclipping in the One-Kidney One-Clip Goldblatt Hypertensive Rat

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ABSTRACT Increased sympathetic nervous system activity has been demonstrated in established one-kidney one-clip hypertension in the rat. We have found that renal denervation in this model results in an attenuation of hypertension, unassociated with alterations in sodium or water balance or renin activity. To determine whether the depressor effect of renal denervation is associated with changes in peripheral sympathetic nervous system activity, sham operation (n = 12), renal denervation (n = 13), or unclipping (n = 13) was carried out 2 wk after the onset of one-kidney one-clip hypertension. Normotensive uninephrectomized age- and sex-matched rats were used as controls (n = 14). Renal denervation resulted in a significant decrease in systolic blood pressure (201±7 to 151±6 mm Hg), while unclipping lowered systolic blood pressure to normotensive levels (130±6 mm Hg). 8 d after operation plasma norepinephrine and mean arterial pressure before and after ganglionic blockade with 30 mg/kg hexamethonium bromide were measured in conscious, unrestrained, resting animals, as indices of peripheral sympathetic nervous system activity. Plasma norepinephrine was significantly higher in hypertensive sham-operated rats (422±42 pg/ml) compared with normotensive controls (282±25 pg/ml) ($P < 0.01$). Both renal denervation and unclipping restored plasma norepinephrine to normal levels (273±22 and 294±24 pg/ml, respectively). Ganglionic blockade in hypertensive sham-operated animals resulted in a significantly greater decrease in mean arterial pressure than occurred in renal denervated, unclipped, or control rats. The data suggest that the depressor effect of renal denervation or unclipping in the one-kidney one-clip hypertensive rat is associated with a decrease in peripheral sympathetic nervous system activity.

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INTRODUCTION

While increased activity of the renin-angiotensin system has been implicated in the initial hypertensive response to clipping of the renal artery in a uninephrectomized rat, the renin-angiotensin system appears to play a diminishing role as hypertension becomes established in this model (1–4). Although there have been some reports to the contrary, there is increasing evidence suggesting participation of both the central and peripheral sympathetic nervous system in the maintenance of one-kidney one-clip hypertension (5–15). By 2 wk after clipping the renal artery, at a time when the blood pressure has reached a new plateau, increased plasma norepinephrine levels and increased cardiac norepinephrine turnover rates have been observed suggesting increased activity on the peripheral sympathetic nervous system (5–8). Other data implicating a role of the sympathetic nervous system in the maintenance of one-kidney one-clip hypertension are that ganglionic blockade, chemical sympathectomy, centrally or peripherally, by 6-hydroxydopamine administration, or peripheral immunosympathectomy result in lowering of blood pressure during the maintenance phase of one-kidney one-clip hypertension in the rat (9–15). Since increases in renal sympathetic efferent nerve activity facilitate the retention of sodium and result in renin release, we previously studied the effect of renal denervation on the hypertensive process in the one-kidney renal hypertensive rat at a time when increased peripheral sympathetic nervous system activity is present (16). We found that renal denervation performed 2 wk after clipping the renal artery or figure-eight wrapping the

kidney resulted in a significant attenuation of the hypertension. The depressor effect of renal denervation, however, was not mediated by alterations in sodium intake or excretion, water intake or excretion, creatinine clearance, or renin activity. These findings are consistent with recent work by others suggesting that there is an attenuation of sympathetic efferent control over renal function in the one-kidney renal hypertensive rat (17, 18).

Increased activity of the sympathetic nervous system has been shown to be a major contributor to the maintenance of hypertension in the one-kidney renal hypertensive rat. Therefore, an alternative explanation for the marked attenuation of hypertension following renal denervation could be that interruption of the renal nerves decreases peripheral sympathetic activity by some mechanism. Thus, the current study was designed to examine the hypothesis that the depressor effect of renal denervation during the established phase of hypertension in the one-kidney one-clip hypertensive rat is secondary to a decrease in peripheral sympathetic nervous system activity.

METHODS

Animal preparation. Male Sprague-Dawley rats (n = 52) obtained from Charles River Breeding Laboratories, Wilmington, Mass., were subjected to unilateral right nephrectomy at 4 wk of age. Following nephrectomy at least 14 d were allowed for compensatory renal hypertrophy to occur before a 0.40-mm silver clip (n = 38) was placed on the proximal left renal artery. Renal denervation, sham operation, or unclipping were performed 2 wk after clipping, at a time when the blood pressure had reached a plateau. The 38 animals were randomly assigned to either renal denervated, sham-operated, or unclipped groups and compared with 14 uninephrectomized nonclipped age- and sex-matched normotensive controls. Renal denervation was accomplished through a flank incision by stripping the renal artery adventitia distal to the clip and painting the renal artery with 20% phenol (wt/vol) in ethanol. Care was taken not to disturb the position of the clip during the denervation procedure. The sham operation consisted of opening and closing the flank on the side of the remaining kidney.

Throughout the study, animals were housed in a room with constant temperature (24°±1°C) and humidity (60±5%) and light from 6 a.m. to 6 p.m. Systolic blood pressures of all animals were measured using the tail-cuff method without anesthesia (Narco Bio-Systems, Inc., Houston, Tex.). Animals were weighed weekly.

Protocol. To examine the effects of renal denervation on blood pressure and peripheral sympathetic nervous system activity in one-kidney one-clip hypertension, 13 renal denervated clipped, 12 sham-operated clipped, 13 unclipped, and 14 sham-operated nonclipped rats were compared. The animals were housed in individual metabolic cages for measurement of sodium intake, urinary sodium excretion, and creatinine clearance from 1 wk before renal denervation, sham operation, or unclipping and continued until the end of the study. Plasma norepinephrine concentration and blood pressure response to ganglionic blockade were used as indices of peripheral sympathetic nervous system activity. Plasma renin activity and blood pressure response to SQ 20881 were used as indices of activity of the renin-angiotensin system. 6 d after renal denervation, sham operation, or unclipping 0.025-in. (i.d.) microline catheters were placed in the femoral artery, brought under the skin and externalized behind the animal's neck. 48 h after catheter placement, tubing was connected to the catheter and at least 0.5 h was allowed to pass before 0.5 ml of blood was sampled from conscious, unrestrained, resting animals (19). Only resting animals were sampled. All animals were sampled at the same time of the day under the same environmental conditions to avoid diurnal variation or ambient temperature influences on plasma norepinephrine (20). The blood was immediately placed on ice for measurement of plasma norepinephrine. 0.5 ml of whole blood from a donor rat was infused as volume replacement after sampling. 2 h later under the same conditions each animal's catheter was connected to a Statham P50 pressure transducer (Statham Instruments, Oxnard, Calif.). After a stable mean arterial pressure was obtained (measured using a Hewlett-Packard recorder, Hewlett-Packard Co., Palo Alto, Calif.), 30 mg/kg hexamethonium bromide was infused intraarterially and the maximum decrease in mean arterial pressure was recorded. This dose of hexamethonium bromide has been shown to interrupt sympathetic transmission controlling the cardiovascular system in the rat (21). In a subgroup of animals mean arterial pressure response to 250 μg SQ 20881 was determined using the method described above. This dose of SQ 20881 has been shown to produce >80% inhibition of the pressor response to a test dose of 100 ng of angiotensin I/kg (22). Plasma norepinephrine was measured using a modification of the radioenzymatic method of Passon and Peuler (23). Sodium concentration (milliequivalents per liter) was measured by flame photometry (model 643, Instrumentation Laboratory, Inc., Lexington, Mass.). 2 d later the animals were sacrificed by decapitation without anesthesia. Blood was collected in iced tubes containing EDTA (1 mg/ml) for determination of plasma creatinine and renin activity. Plasma renin activity was determined by radioimmunoassay of generated angiotensin I according to the method of Haber et al. (24).

Numerical results are expressed as means±1 SE. Statistical analysis of the blood pressure data was performed using analysis of variance based on a split plot in time model. Regression analysis was used to establish a linear relationship between mean arterial blood pressure and plasma norepinephrine. The test used was the test for zero slope, which in this case is exactly the same test as for zero correlation. The changes in arterial pressure with hexamethonium and SQ 20881 were compared with control using analysis of variance in conjunction with Dunnett's test (25). Changes are reported as significant if the P value was <0.05.

RESULTS

Hypertension. After clipping, the 38 rats that subsequently would undergo renal denervation, sham operation, or unclipping were observed for changes in blood pressure over 3 wk. As shown in Fig. 1 clipping the renal artery produced a rise ($P < 0.05$) in systolic blood pressure from 124±5 to 146±5 mm Hg within 5 d. The pressure continued to rise reaching a plateau of 201±7 mm Hg by day 12. Sham operation on day 14 produced no change in systolic blood pressure. In contrast, renal denervation on day 14 resulted in a

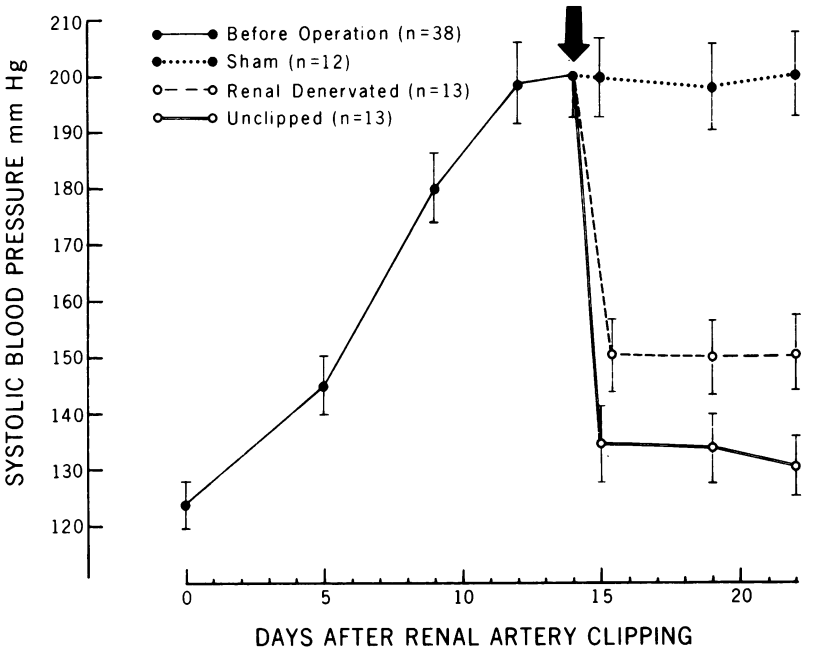

FIGURE 1 Effect of renal denervation or unclipping on one-kidney one-clip hypertension in the rat. The arrow indicates the time of renal denervation, unclipping, or sham operation.

significant sustained decrease in systolic blood pressure from 201±7 to 151±6 mm Hg ($P < 0.01$) by day 15. Unclipping the renal artery resulted in a decrease in systolic blood pressure to base-line (preclip) levels by day 15, which was significantly lower ($P < 0.05$) than the pressure of renal denervated animals.

14 control one-kidney, sham-operated, nonclipped rats were observed for 3 wk. Base-line systolic blood pressures of these animals were not significantly different from the base-line systolic blood pressures of the animals that were subsequently clipped. Over the subsequent 3 wk of observation, systolic blood pressure ranged between 122±3 and 131±4 mm Hg in this control group, representing no significant change from base line. There was no significant difference in weekly weight gain among the groups during the 3 wk of observation.

Plasma norepinephrine. 8 d after operation plasma norepinephrine was measured in conscious unrestrained, resting animals. As shown in Table I there was no significant difference in plasma norepinephrine between renal denervated animals and normotensive unclipped or control animals. In contrast, plasma norepinephrine values of sham-operated hypertensive animals were significantly ($P < 0.01$) greater than those of the renal denervated, unclipped, or control rats. There was a highly significant ($P < 0.005$) positive correlation between mean arterial pressure and the plasma norepinephrine measured in renal denervated and sham-operated rats (Fig. 2). The prediction equation relating mean arterial pressure (MAP) to plasma norepinephrine (NE) level was MAP = 73.49 + 0.2015 NE. T test for nonzero slope was significant at the $P < 0.005$ level.

Ganglionic blockade. Table I shows the mean arterial pressure before and after administration of 30 mg/kg hexamethonium bromide. Before ganglionic blockade the mean arterial pressures of sham-operated

TABLE I

*Plasma Norepinephrine and Mean Arterial Pressure before and after Ganglionic Blockade with 30 mg/kg Hexamethonium Bromide Measured 8 d after Operation in Conscious Unrestrained Resting Animals**

		Mean arterial pressure		
	NE	Pre-Hex	Post-Hex	Absolute decrease
	pg/ml		*mm Hg*	
Sham (n = 7)	422±42‡	160±6‡	78±5§	82±6‡
Denervated (n = 7)	273±22	120±5§	80±5§	40±5
Unclipped (n = 7)	294±24	105±4	75±4§	30±5
Control (n = 8)	282±25	100±4	65±4	35±4

* Values are means±SE.

‡ $P < 0.01$ and § <0.05 compared with control. NE, norepinephrine; Pre-Hex, Post-Hex, before and after hexamethonium.

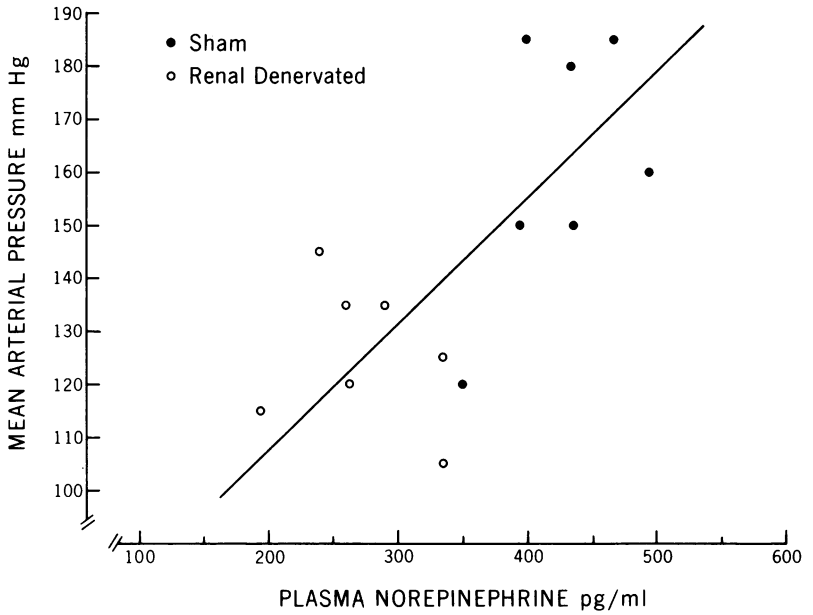

FIGURE 2 Regression analysis of the relationship between plasma norepinephrine and mean arterial pressure in sham-operated and renal-denervated one-kidney one-clip hypertensive rats. $r = 0.70$; $P < 0.005$; $n = 14$.

and renal-denervated animals were significantly greater than those of unclipped or control animals. Ganglionic blockade resulted in a significant decrease in mean arterial pressure in all groups ($P < 0.01$). The absolute decrease in mean arterial pressure (Table I) and the percent decrease in mean arterial pressure (Fig. 3) were significantly greater ($P < 0.01$) in sham-operated hypertensive animals compared with renal denervated, unclipped, or control animals. Postganglionic blockade mean arterial pressures (Table I) of control animals were significantly lower ($P < 0.05$) than those of sham-operated, renal denervated, or unclipped animals.

Response to SQ 20881. Table II shows the mean arterial pressure before and after administration of 250 μg SQ 20881. Before SQ 20881 the mean arterial pressures of sham-operated and renal-denervated animals were significantly greater than those of unclipped or control animals. In response to SQ 20881 the absolute and percent decreases in mean arterial pressure in sham-operated and renal-denervated animals were significantly greater ($P < 0.05$) than those of unclipped or control animals. Post-SQ 20881 mean arterial pressures (Table II) of sham-operated and renal-denervated animals remained significantly higher than those of unclipped and control animals.

Sodium balance and creatinine clearance. Unclipping the renal artery in six hypertensive animals resulted in an increase ($P < 0.01$) in sodium excretion during the first 24 h after the procedure (urinary sodium excretion: preoperative 1.80±0.24 vs. postoperative 2.81±0.26 meq/d). Thereafter sodium excretion in these animals returned to preoperative levels. During the 48 h before measurement of plasma norepinephrine and ganglionic blockade there was no difference in daily sodium intake (sham-operated: 2.00±0.08; renal denervated: 2.02±0.09; unclipped: 1.98±0.09; control: 2.10±0.10 meq/d) or urinary sodium excretion (sham-operated: 1.75±0.18; renal denervated: 1.83±0.25; unclipped: 1.69±0.26; control: 1.94±0.22 meq/d) between groups. There was no significant difference in creatinine clearance between renal-denervated (1.08±0.26 ml/min; $n = 12$) and sham-operated (1.21±0.19 ml/min; $n = 12$) animals. Creatinine clearances of renal-denervated and sham-operated animals were significantly lower ($P < 0.01$) that those of unclipped (1.59±0.22 ml/min; $n = 13$) or control (1.76±0.17 ml/min; $n = 14$) animals.

Plasma renin activity. Plasma renin activity of renal denervated animals (4.4±1.0 ng/ml per h; $n = 12$) was not significantly different from that of sham-operated rats (5.5±1.6 ng/ml per h; $n = 12$). Values for these groups were significantly greater ($P < 0.05$) than those of normotensive unclipped (1.6±0.6 ng/ml per h; $n = 13$) or control (1.4±0.6 ng/ml per h; $n = 14$) animals.

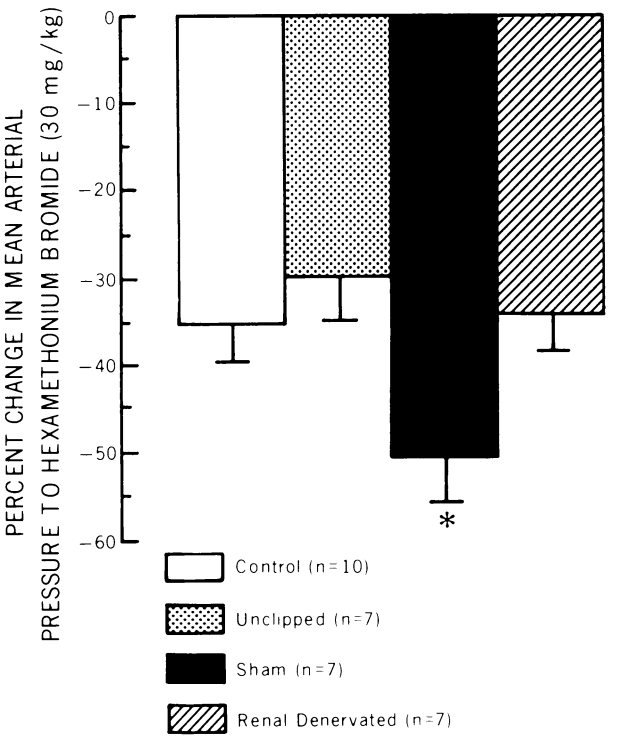

FIGURE 3 Effect of hexamethonium bromide (30 mg/kg) on mean arterial pressure 8 d after operation. The asterisk represents $P < 0.01$ comparing sham-operated, renal-denervated and unclipped animals with one-kidney normotensive age- and sex-matched controls.

DISCUSSION

There is increasing evidence that the renal nerves are important in the pathogenesis of hypertension in a number of experimental models. In both the deoxycorticosterone acetate (DOCA)-salt and spontaneously hypertensive rat renal denervation has been shown to delay the onset and slow the rate of development of hypertension (26–30). This delay in the development of hypertension in denervated animals was associated with increased urinary sodium excretion with no alteration in activity of the renin-angiotensin system (26, 30). In both of these models renal denervation did not lower blood pressure in animals with established hypertension. Renal denervation has also been shown to delay the development of one-kidney Grollman hypertension (31). In preliminary reports the authors have suggested that interruption of renal afferent connections to the anterior hypothalamus is responsible for the prevention of hypertension by renal denervation in this model (31, 32).

Our study has demonstrated: (*a*) that renal denervation during the established phase of one-kidney one-clip hypertension in the rat attenuates the severity of hypertension in this model while unclipping the renal artery normalizes the blood pressure; (*b*) that the depressor effect of renal denervation or unclipping is associated with a decrease in peripheral sympathetic nervous system activity from the increased levels present in hypertensive animals to levels comparable to those found in control normotensive uninephrectomized rats and (*c*) that plasma norepinephrine, an index of peripheral nervous system activity, is positively correlated with mean arterial pressure in sham-operated and renal-denervated groups. These observations extend our previous findings that renal denervation lowers blood pressure in this model without causing increased urinary sodium excretion or suppressed activity of the renin-angiotensin system (16). Taken together, these experiments strongly support the concept that intact renal nerves are important in the maintenance of hypertension in the one-kidney renal hypertensive rat.

The indices of peripheral sympathetic nervous system activity used in this study were plasma norepinephrine levels and the mean arterial pressure response to ganglionic blockade with hexamethonium bromide (19, 33, 34). Plasma norepinephrine in the rat is principally derived from neurotransmitter released from noradrenergic nerve endings and appears to correlate well with other indices of sympathetic function (19, 35–37). We therefore interpret the elevation of plasma norepinephrine observed in the one-kidney renal hypertensive rat as a consequence of enhanced neurotransmitter release secondary to increased sympathetic neuronal activity. Our finding of elevated norepinephrine levels in the one-kidney one-clip hypertensive rat is consistent with the observations of

TABLE II

Mean Arterial Pressure before and after Administration of 250 μg SQ 20881 Measured 9 d after Operation in Conscious Unrestrained Resting Animals

	Mean arterial pressure			
	Pre-SQ	Post-SQ	Absolute decrease	Percent change
	mm Hg			
Sham (n = 6)	155±5‡	143±5‡	12±2§	8±2%§
Denervated (n = 6)	122±5§	112±4§	10±2§	8±2%§
Unclipped (n = 6)	104±4	99±4	5±2	4±2%
Control (n = 6)	101±4	97±4	4±2	4±2%

* Values are means±SE.

‡ $P < 0.01$ and § <0.05 compared with control. Pre-SQ, Post-SQ, before and after SQ 20881.

others implicating increased sympathetic nervous system activity in the maintenance of hypertension in this model (5–15). Plasma norepinephrine levels were decreased in renal-denervated animals compared to sham-operated hypertensive rats, suggesting that renal denervation caused an attenuation in the level of peripheral sympathetic nervous system activity. The decrease in sympathetic activity following renal denervation was not related to differences in sodium balance or glomerular filtration rate (16). These are important negative findings because there is evidence to suggest that sodium may enhance the activity of the sympathetic nervous system (38). Furthermore, a markedly decreased norepinephrine excretion could result in elevated plasma levels (39, 40). The lesser absolute and percent fall in mean arterial pressure with ganglionic blockade in renal-denervated rats compared with hypertensive animals gave further evidence that the depressor effect of renal denervation in this model is associated with a decrease in peripheral sympathetic nervous system activity. Since cardiac output was not measured in the present study, we do not know whether the greater fall in mean arterial pressure from ganglionic blockade in hypertensive animals compared with renal-denervated animals indicates predominantly a decrease in peripheral vascular resistance or cardiac output. In either case, the increased response to ganglionic blockade indicates that the higher pressure in the sham-operated hypertensive animals is due, directly or indirectly, to enhanced peripheral sympathetic nervous system activity.

While enhanced peripheral sympathetic nervous system activity is important in the hypertensive process, this study confirms the work of others showing that additional factors contribute to the maintenance of early established one-kidney one-clip Goldblatt hypertension in the rat (4, 41, 42). Unclipping was accompanied by decreased activity of the renin-angiotensin system and by loss of sodium in addition to a decrease in peripheral sympathetic activity. The observation that the blood pressure of unclipped rats after hexamothonium did not fall to the levels seen in control rats suggests that vascular changes may have occurred within 2 wk after clipping (43).

The mechanism by which renal denervation or unclipping decreases peripheral sympathetic nervous system activity remains unknown. It has been suggested that the increase in peripheral sympathetic tone present in one-kidney one-clip hypertensive rats might be due to changes in central neurons, perhaps triggered by sodium retention, or an increase in circulating angiotensin II (7, 44, 45). Using this line of reasoning one could implicate either the loss of sodium and (or), decrease in renin-angiotensin system activity as possible causes of the decreased peripheral sympathetic activity seen with unclipping (4, 41, 42). However, we have found no loss of sodium or no decrease in renin-angiotensin system activity associated with renal denervation in this model as possible mechanisms for a decrease in sympathetic activity (16). Another possible explanation of our data is that renal denervation might facilitate the release of a circulating renal factor that down-regulates sympathetic nervous system activity. If this were the case, one would have to postulate that unclipping a kidney with intact renal nerves also resulted in the release of a renal factor.

A more attractive explanation for our findings is that renal denervation or unclipping in this model may decrease renal afferent nerve activity (31, 32), and thereby attenuate peripheral sympathetic tone. Consistent with this hypothesis is the increasing evidence demonstrating that afferent sympathetic signals from various organs, including the kidney, play an important role in modulating peripheral efferent sympathetic responses (31, 32, 46, 47). If clipping the renal artery in a one-kidney rat were to cause an increase in renal afferent nerve signals that triggered increased peripheral sympathetic efferent nervous system activity, then decreasing renal afferent nerves signals, whether by interrupting the renal nerves (denervation), or removing the stimulus to increased renal afferent signals (unclipping), should result in a lowering of peripheral sympathetic activity and a prompt lowering of blood pressure. Consistent with this hypothesis are our observations that clipping the renal artery resulted in increased plasma norepinephrine levels and greater responses from hexamethonium, and both renal denervation and unclipping resulted in a lowering of plasma norepinephrine concentration and a lowering of blood pressure response from ganglionic blockade to levels found in normotensive control rats. The relationship of the renal afferent nerves to the development and maintenance of hypertension in the one-kidney renal hypertensive rat merits further study.

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