Renal Denervation Does Not Abolish Sustained Baroreflex-Mediated Reductions in Arterial Pressure

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Abstract—Recent studies indicate that suppression of renal sympathetic nerve activity and attendant increments in renal excretory function are sustained baroreflex-mediated responses in hypertensive animals. Given the central role of the kidneys in long-term regulation of arterial pressure, we hypothesized that the chronic blood pressure–lowering effects of the baroreflex are critically dependent on intact renal innervation. This hypothesis was tested in 6 dogs by bilaterally activating the carotid baroreflex electrically for 7 days before and after bilateral renal denervation. Before renal denervation, control values for mean arterial pressure and plasma norepinephrine concentration were 95±2 mm Hg and 96±12 pg/mL, respectively. During day 1 of baroreflex activation, mean arterial pressure decreased 13±1 mm Hg, and there was modest sodium retention. Daily sodium balance was subsequently restored, but reductions in mean arterial pressure were sustained throughout the 7 days of baroreflex activation. Activation of the baroreflex was associated with sustained decreases in plasma norepinephrine concentration (~50%) and plasma renin activity (30% to 40%). All of the values returned to control levels during a 7-day recovery period. Two weeks after renal denervation, control values for mean arterial pressure, plasma norepinephrine concentration, plasma renin activity, and sodium excretion were comparable to those measured when the renal nerves were intact. Moreover, after renal denervation, all of the responses to activation of the baroreflex were similar to those observed before renal denervation. These findings demonstrate that the presence of the renal nerves is not an obligate requirement for achieving long-term reductions in arterial pressure during prolonged activation of the baroreflex. (Hypertension. 2007;49:373-379.)

Key Words: baroreflex ■ arterial pressure ■ renal nerves ■ sympathetic nervous system ■ norepinephrine ■ renin–angiotensin system ■ sodium excretion

Novel experimental approaches in chronically instrumented animals have recently provided greater insight into the role of baroreflexes in long-term control of arterial pressure.1–10 These studies, conducted over several weeks, indicate that baroreflex resetting is incomplete in experimental models of hypertension. They also indicate that during chronic increases in arterial pressure, there is sustained baroreflex-mediated suppression of renal sympathetic nerve activity and attendant increments in renal excretory function, responses expected to attenuate the severity of hypertension. However, the quantitative importance of renal sympathoinhibition in mediating the chronic blood pressure–lowering effects of sustained baroreflex activation remains unclear.

We have recently developed methodology that is ideal for evaluating the time dependency and underlying mechanisms of the blood pressure–lowering effects of the baroreflex.5,10 To activate the carotid baroreflex, an externally adjustable pulse generator is used to electrically stimulate electrodes chronically implanted around both carotid sinuses of dogs. With this methodology, one can achieve sustained and controllable reductions in mean arterial pressure (MAP) that are associated with distinct reductions in circulating levels of norepinephrine (NE), indicating inhibition of sympathetic activity.

Because increments in renal excretory function seem to play a critical role in promoting sustained reductions in arterial pressure and are mediated, at least in part, by suppression of renal sympathetic nerve activity, the renal nerves provide a logical link between changes in central sympathetic output and renal function that lead to a lowering of blood pressure during prolonged baroreflex activation.8,11 Thus, the primary goal of this study was to test the hypothesis that long-term reductions in MAP during prolonged baroreflex activation are critically dependent on intact renal innervation. To this end, the carotid baroreflex was electrically activated in the same dogs both
before and after bilateral renal denervation. We reasoned that if the renal nerves are critical mediators of the long-term blood pressure–lowering effects of the baroreflex, then activation of the baroreflex after renal denervation would not produce sustained decreases in arterial pressure. To our surprise, bilateral renal denervation had no impact on the normal blood pressure response to prolonged baroreflex activation.

Methods

Animal Preparation

All of the procedures were performed in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee. Surgical procedures were performed under isoflurane anesthesia (1.5 to 2.0%) after premedication with acepromazine (0.15 mg/kg) and induction with thiopental (10 mg/kg). Six male dogs weighing 22 to 25 kg were used in this study. The procedures for renal denervation, implantation of vascular catheters, implantation of stimulating electrodes around each carotid sinus, and the connection of electrode lead bodies to a pulse generator have been described previously.1,6,12 Also, as described previously, the dogs were maintained in metabolic cages.1,6 The electrodes and the pulse generator were provided by CVRx, Inc.

Experimental Protocol

During a 3-week postoperative period and throughout the study, the dogs were given free access to water and maintained on a fixed daily diet of two 15.5-oz cans of prescription heart diet (Hill’s Pet Products) supplemented with 5 mL of vitamin syrup. Two cans of heart diet provide ≈5 mmol of sodium and ≈55 mmol of potassium. The dogs received a continuous intravenous infusion of isotonic saline at a rate of 350 mL/day providing a total daily sodium intake of ≈60 mmol.1,6 Water consumption was monitored daily, and 24-hour urine samples were collected at 11:00 AM each day at the time of feeding.

After achieving steady-state conditions at the end of the third postoperative week, control measurements were made. The carotid baroreflex was then electrically activated for 7 days using stimulation parameters described previously.6 This was followed by a 7-day recovery period. Subsequently, the dogs underwent bilateral renal denervation, and after a 2-week recovery period, the above experimental protocol was repeated.

Analytical Methods

The plasma levels of hormones and NE and the renal cortical content of NE were measured by radioimmunoassay and high-performance liquid chromatography with electrochemical detection (Agilent 1100), respectively, as described previously.1,6,12 Standard techniques were used to measure hematocrit and the plasma concentrations of sodium, potassium, and protein.6

Arterial pressure and heart rate were monitored continuously, 24 h/day, from an arterial catheter.1 The daily hemodynamic values presented were averaged from the 20-hour period extending from 11:30 PM to 7:30 AM. The hours excluded from the 24-hour analysis included the time required for flushing catheters, calibrating pressure transducers, feeding, and cleaning cages.

Statistical Analysis

Results are expressed as mean±SE. A repeated-measures ANOVA followed by the Dunnett multiple comparisons test was used to compare experimental and recovery responses to control. Single comparisons between conditions (before and after renal denervation) were made with the Student’s paired t test. Statistical significance was considered to be P<0.05.

Results

Arterial Pressure and Heart Rate

Figure 1 illustrates the changes in MAP and heart rate in response to prolonged baroreflex activation both before and after renal denervation. When the kidneys were innervated, control values for MAP and heart rate were 95±2 mm Hg and 65±3 bpm, respectively. During the initial 20 minutes of baroreflex activation, MAP decreased ≈15 to 20 mm Hg.
and for day 1, MAP was reduced by $13 \pm 1$ mm Hg. This reduction in MAP persisted throughout the entire 7 days of baroreflex activation, and on day 7, MAP was $16 \pm 2$ mm Hg below control levels. Heart rate decreased in parallel with MAP, and on day 7 of baroreflex activation, heart rate was reduced by $12 \pm 3$ bpm. When baroreflex activation was discontinued, both MAP and heart rate returned toward control values; however, 2 to 3 days were required for complete recovery. A representative 20-hour plot of MAP during the control period and on day 7 of baroreflex activation is presented in Figure 2.

After renal denervation, basal values for MAP and heart rate were $91 \pm 3$ mm Hg and $71 \pm 3$ bpm; these values were not significantly different from those measured before renal denervation. Moreover, and most importantly, the long-term reductions in MAP ($13 \pm 1$ mm Hg) and heart rate (10$\pm$2 bpm) in response to baroreflex activation were comparable to those achieved before renal denervation.

**Urinary Electrolyte Excretion**

When the kidneys were innervated, the excretion rates of sodium and potassium were 57$\pm$3 and 44$\pm$2 mmol/day, respectively, reflecting the intake of these electrolytes (Figure 3). As reported previously,$^{6,10}$ during the first 24 hours of baroreflex activation, there was modest retention of sodium ($\approx 15$ mmol) before daily sodium balance was reestablished in subsequent days. This retained sodium was excreted on day 1 of the recovery period. There were no significant changes in potassium excretion. Comparable changes in urinary electrolyte excretion occurred in response to baroreflex activation after renal denervation.

**Neurohormonal Profile**

In the presence of the renal nerves, there was an $\approx 50\%$ decrease in plasma NE concentration from control levels ($96 \pm 12$ pg/mL) throughout the entire 7 days of baroreflex activation (Figure 4). In addition, despite the marked fall in MAP, PRA (control $0.55 \pm 0.05$ ng of angiotensin I per milliliter per hour) did not increase during sustained activation of the baroreflex (Figure 4). In fact, throughout the entire 7 days of baroreflex activation, PRA was reduced 30% to 40%, achieving statistical significance on days 1 and 7. Both NE and PRA returned to control levels within 24 hours of cessation of baroreflex activation.

After renal denervation, control values for plasma NE concentration ($100 \pm 7$ pg/mL) were comparable to those present before renal denervation (Figure 4). Furthermore, the suppression of plasma NE concentration during baroreflex activation was modest retention of sodium ($\approx 15$ mmol) before daily sodium balance was reestablished in subsequent days. This retained sodium was excreted on day 1 of the recovery period. There were no significant changes in potassium excretion. Comparable changes in urinary electrolyte excretion occurred in response to baroreflex activation after renal denervation.

**Figure 2.** A 20-hour plot of MAP in 1 dog during the control period and on day 7 of prolonged baroreflex activation.

**Figure 3.** Effects of prolonged baroreflex activation on the daily excretion of sodium and potassium before and after bilateral renal denervation. Values are mean$\pm$SEM ($n=6$). $^*P<0.05$ vs before baroreflex activation.
activation was similar in the presence and absence of the renal nerves. Control levels for PRA were lower in 5 of 6 dogs after renal denervation compared with the intact state, but for the group as a whole (PRA = 0.27 ± 0.09 ng of angiotensin I per milliliter per hour), the difference fell short of statistical significance (Figure 4). After renal denervation, PRA did increase several-fold during baroreflex activation in the dog with the greatest fall in MAP; but in remaining dogs, PRA was unchanged. Consequently, after renal denervation, changes in PRA during baroreflex activation were not statistically significant. During baroreflex activation, values for PRA were statistically equivalent before and after renal denervation.

Control plasma levels for aldosterone and cortisol were 2.7 ± 0.3 ng/dL and 1.4 ± 0.3 µg/dL, respectively, before renal denervation. The corresponding values after renal denervation (2.1 ± 0.2 ng/dL and 1.5 ± 0.3 µg/dL) were not significantly different from the above. As in previous studies, in neither group was there significant changes in the plasma levels of these steroids during baroreflex activation. Because of an insufficient amount of plasma, plasma cortisol concentration was measured in only 3 of the 6 dogs.

**Hematocrit and Plasma Concentrations of Electrolytes and Protein**

Before renal denervation, changes in hematocrit and in the plasma concentrations of electrolytes and protein in response to prolonged baroreflex activation were similar to those reported previously with the exception that there was no increase in plasma potassium concentration. In association with the modest retention of sodium (Figure 3), there were small (5% to 10%), but nevertheless significant, reductions in both hematocrit (control = 0.39 ± 0.03 g/dL) and plasma protein (control = 6.1 ± 0.2 g/dL) concentration during baroreflex activation. As reported previously, these changes in hematocrit and plasma protein concentration were achieved by day 1 of baroreflex activation. There were no significant changes in plasma sodium concentration (control = 150 ± 1 mmol/L) or plasma potassium concentration (control = 4.5 ± 0.2 mmol/L) during prolonged activation of the baroreflex. Values for hematocrit and the plasma concentrations of electrolytes and protein after renal denervation were not significantly different from the innervated state during either the control period or baroreflex activation.

**Renal Tissue Levels of NE**

In dogs with intact renal innervation, levels of NE in the renal cortex were 987 ± 80 ng/g tissue. In the denervated dogs of the present study, tissue levels of NE were reduced markedly to 10 ± 5 ng/g renal cortex. Thus, the ~100-fold difference in renal tissue NE content between innervated and denervated kidneys indicates the completeness of renal denervation.

**Discussion**

Long-term regulation of arterial pressure is closely linked to volume homeostasis through the renal body fluid feedback mechanism. According to this concept, if sodium intake is constant, long-term changes in arterial pressure are not possible unless there is a shift in the pressure natriuresis mechanism. The current study tested the hypothesis that inhibition of renal sympathetic nerve activity is the critical signal emanating from the central nervous system that increases pressure natriuresis and leads to a sustained fall in arterial pressure during prolonged baroreflex activation. This hypothesis was based on recent findings in chronically instrumented animals indicating that resetting of the arterial baroreflex is incomplete in hypertension and that chronic activation of the baroreflex has sustained effects to inhibit renal sympathetic nerve activity and promote sodium excretion. Contrary to our hypothesis, the major finding of this study is that the presence of the renal nerves is not essential for achieving long-term reductions in arterial pressure during prolonged activation of the baroreflex.
As expected from previous studies, prolonged activation of the baroreflex produced sustained decreases in sympathetic activity (plasma NE concentration) and arterial pressure. In addition, before renal denervation, these responses were associated with a fall in PRA, presumably reflecting the effects of reflex-induced renal sympathoinhibition on renin release. Although baroreflex-mediated suppression of renin secretion, a dependence that is manifested at reduced renal perfusion pressures, could not possibly account for the failure of PRA to increase significantly in response to reductions in arterial pressure during baroreflex activation in denervated kidneys. After renal denervation, the inability of reduced arterial pressure to elicit significant increases in PRA during baroreflex activation may relate to the obligatory role of the renal nerves in maintaining normal rates of renin synthesis and secretion, a dependence that is manifested at reduced renal perfusion pressures. The functional significance of suppression of pressure-dependent renin release, whether induced by reflex inhibition of renal sympathetic nerve activity or a result of chronic renal denervation, should be emphasized, because increases in circulating levels of angiotensin II attenuate the chronic blood pressure–lowering effects of the baroreflex.

Because recent studies suggest that the renal nerves play a role in mediating the long-term effects of the baroreflex on arterial pressure, how then does prolonged baroreflex activation lead to chronic reductions in arterial pressure after renal denervation? The arterial baroreflex has a well-established role in the acute regulation of arterial pressure by its actions on peripheral resistance, vascular capacity (a determinant of mean circulating filling pressure and, thus, venous return), and cardiac function. No doubt the above reflex-induced responses were dominant in mediating the initial sodium retention and blood pressure–lowering effects of baroreflex activation. However, according to the concept of the renal body-fluid mechanism for long-term control of arterial pressure, these autonomic responses, even if sustained chronically, would not be expected to produce a persistent fall in arterial pressure unless they were associated with a simultaneous effect to enhance the pressure natriuresis mechanism, because otherwise the kidneys would retain fluid until arterial pressure returned to control levels. Strong experimental support for this notion comes from a study in dogs in which the renal perfusion pressure to 1 kidney was servocontrolled at a reduction in pressure comparable to that achieved in the present study. Throughout the duration of this study (12 days), there was a sustained reduction in sodium excretion in the kidney with the reduced renal perfusion pressure and an increase in sodium excretion in the high-pressure contralateral kidney. Because both kidneys were exposed to the same neurohumoral influences, this study supports a premise that has been difficult to test experimentally and one that is critical to the renal body fluid feedback mechanism: that renal perfusion pressure is an important long-term controller of sodium excretion. However, not all of the investigators share this point of view. Investigators from Odense and Berlin have argued that the pressure natriuresis mechanism is not always operative during modest changes in arterial pressure. In regard to the present study, if this interpretation were correct, and the concomitant neurohumoral responses did not enhance pressure natriuresis, this would indicate that renal excretory function adapts to changes in arterial pressure and that reductions in either cardiac output or peripheral resistance could be causal mechanisms that account for long-term reductions in arterial pressure during prolonged baroreflex activation. Additional long-term studies are needed to provide greater insight into this controversy.

Studies in dogs with unilateral renal denervation and surgical division of the urinary bladder into hemibladders to allow separate 24-hour urine collection from denervated and innervated kidneys indicate that excretory function is impaired in chronically denervated kidneys. Reduced renal excretory function in denervated kidneys may result from the diminished actions of several natriuretic agents, including NO and endothelin. Another possibility is that this relative impairment in sodium excretion in denervated kidneys may reflect renal denervation supersensitivity, which may have diminished functional significance during prolonged activation of the baroreflex.

Because renal denervation is a method commonly used to investigate the role of the renal nerves in the control of renal function, several studies have addressed the issue of renal denervation supersensitivity, an increased renal response to circulating NE that has the potential to mask the functional effects of renal denervation. As discussed previously, early studies in which the possibility of renal denervation supersensitivity was originally proposed preclude an understanding of the true functional significance of this potential confounder. More recent studies in animals with an innervated and denervated kidney provide strong evidence that chronically denervated kidneys do not have either exaggerated renal vascular or sodium excretory responses to either physiological or pathophysiological increments in circulating levels of NE. It must be emphasized, however, that because of expediency, virtually all of the studies relating to the topic of renal denervation supersensitivity have investigated renal responses to increases, but not decreases, in adrenergic stimulation. Consequently, it is not known whether denervated kidneys are supersensitive to normal circulating levels of NE. If so, this may be one factor that accounts for the impaired excretion of sodium in chronically denervated kidneys. Moreover, if denervated kidneys are supersensitive to normal circulating levels of NE, then the pronounced reductions in plasma levels of NE in the present study during baroreflex activation could lead to increases in renal excretory function of sufficient magnitude to account for the observed decreases in arterial pressure. This possibility merits further investigation and is readily testable using the current technology while clamping circulating levels of NE at control levels during prolonged activation of the baroreflex.

Atrial natriuretic peptide is another potential mediator of the blood pressure–lowering effects of prolonged baroreflex activation. Our preliminary studies indicate that prolonged baroreflex activation leads to an ~2-fold increase in the plasma concentration of atrial natriuretic peptide (control = 18.8 ± 5.8 pg/mL; n = 4) presumably because of a small increase in atrial pressure. Modest fluid
retention and subtle autonomic effects on cardiac function may account for increased atrial pressure during baroreflex activation. By mimicking the functional effects of sustained baroreflex activation by chronic α- and β-adrenergic blockade, a computer model of the circulation from the Department of Physiology and Biophysics, University of Mississippi Medical Center (Quantitative Circulatory Physiology, http://physiology.umc.edu/themodelingworkshop/index.html) indicates that increased secretion of atrial natriuretic peptide may account for ∼50% of the fall in arterial pressure during prolonged activation of the baroreflex. It should be emphasized, however, that the quantitative significance of this prediction has not been formally tested in experimental studies. Nonetheless, if atrial natriuretic peptide does contribute to the blood pressure–lowering effects of prolonged baroreflex activation, its effects to enhance pressure natriuresis and, therefore, to reduce arterial pressure may be more substantial in denervated than innervated kidneys, because renal nerve activity opposes the natriuretic effects of the peptide.14,27,28

In summary, this study clearly demonstrates that the renal nerves are not an obligate requirement for achieving long-term reductions in arterial pressure during prolonged activation of the baroreflex. The failure of bilateral renal denervation to attenuate the blood pressure–lowering effects of prolonged baroreflex activation is incongruous with the emerging evidence in experimental models of hypertension linking increased renal excretory function to sustained baroreflex activation via the renal nerves. Thus, the mechanisms that mediate the long-term blood pressure–lowering effects of the baroreflex remain unclear, and the possibility that the renal nerves may serve as an important link for baroreceptor-induced suppression of central output through which long-term reductions in arterial pressure are achieved must be corroborated by additional experimental studies.

Perspectives
Technical barriers persist that limit an understanding of the role of the baroreflex in long-term control of arterial pressure. The issue of whether baroreflex-mediated inhibition of renal sympathetic nerve activity truly plays an important role in attenuating chronic hypertension will require additional integrative studies using multiple technologies to assess sympathetic outflow to the kidneys and neurally induced changes in renal excretory function. Studies using the current technology for chronically activating the baroreflex have demonstrated impressive sustained reductions in arterial pressure in normotensive and hypertensive dogs.6,10,29 In so doing, they have also provided considerable insight into the efferent mechanism mediating the long-term blood pressure–lowering effects of the baroreflex. Further mechanistic insight is now especially important, because feasibility trials are now underway to evaluate the efficacy of this technology in lowering arterial pressure in patients with severe refractory hypertension that is resistant to drug therapy.30,31

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T.E.L. and E.D.I. received consultant fees and are on the scientific advisory board at CVRx Inc. M.A.R. is employed by CVRx Inc, and R.S.K. is president and chief operating officer of CVRx Inc.

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