Renal Denervation

Blood Pressure and Autonomic Responses to Electrical Stimulation of the Renal Arterial Nerves Before and After Ablation of the Renal Artery

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Abstract—Radiofrequency (RF) catheter ablation of the renal artery is therapeutic in patients with drug-refractory essential hypertension. This study was designed to examine the role of the renal autonomic nerves and of RF application from inside the renal artery in the regulation of blood pressure (BP). An open irrigation catheter was inserted into either the left or right renal artery in 8 dogs. RF current (17±2 watts) was delivered to one renal artery. Electrical autonomic nerve stimulation was applied to each renal artery before and after RF ablation. BP, heart rate, indices of heart rate variability, and serum catecholamines were analyzed. Before RF ablation, electrical autonomic nerve stimulation of either renal artery increased BP from 150±16/92±15 to 173±21/105±16 mm Hg. After RF ablation, BP increased similarly when the nonablated renal artery was electrically stimulated, although the rise in BP was attenuated when the ablated renal artery was stimulated. Serum catecholamines and sympathetic nerve indices of heart rate variability increased when electrical autonomic nerve stimulation was applied before RF ablation and to the nonablated renal artery after RF ablation, although it changed minimally when the ablated renal artery was stimulated, suggesting interconnectivity between afferent renal nerve stimulation and systemic sympathetic activity. Renal artery angiogram showed no apparent injury after RF ablation. In conclusion, electrical stimulation of the renal arterial autonomic nerves increases BP via an increase in central sympathetic nervous activity. This response might be used to determine the target ablation site and end point of renal artery RF ablation. (Hypertension. 2013;61:450-456.) ● Online Data Supplement

Key Words: hypertension ◼ radiofrequency ablation ◼ renal artery ◼ electrical stimulation

In 2009, Krum et al1 described a new catheter-based treatment of essential hypertension resistant to multiple antihypertensive drugs. They performed bilateral, percutaneous, catheter-based radiofrequency (RF) ablation of the renal artery in 45 patients. At 9 months after the procedure, a 24/11-mm Hg mean decrease in systemic blood pressure (BP) was measured in 20 patients. More recently, the Symplicity HTN investigators reported the treatment of 153 patients with RF catheter ablation of the renal artery at 19 medical centers.2,3 At 12 months after the procedure, BP measured in an ambulatory setting was lowered by a mean of 23/11 mm Hg in 64 patients and decreased further by 32/14 mm Hg at 24 months in 18 patients.

The putative mechanisms of RF catheter ablation of the renal artery are attributed to an attenuation of sympathetic nervous activity.1–4 However, the details of the functional effects of RF ablation of the renal artery and a marker of the optimal target site and end point of an effective RF application have not been clearly identified. To further study the role of the renal autonomic nerves and RF ablation of the renal artery in the regulation of BP, we electrically stimulated the renal arterial nerves before and after RF catheter ablation from inside the renal artery in dogs.

Methods

Animal Preparation

This study was approved by the animal studies subcommittee of our institutional review board and was in compliance with the guidelines of the National Institutes of Health for the care and use of laboratory animals. The experiments were performed in 8 intubated and artificially ventilated beagles, weighing between 10.5 and 13.0 kg, anesthetized with a 17.5-mg/kg IV bolus, followed by a maintenance dose of 3.0 to 5.0 mg/kg per hour of sodium thiopental.5

A 7F introducer was inserted into the femoral vein and advanced to the inferior vena cava for blood sampling during the experiment. A 7F quadripolar, Cool Path Duo, open irrigation catheter (St Jude Medical Inc, St. Paul, MN) was introduced via the right femoral artery and placed in the proximal right or left renal artery. The BP was continuously monitored from the left femoral artery, and fluids and drugs were administered via the median cubital vein. The limb leads of the surface electrocardiogram were monitored, and the core body temperature was kept at 37°C with a thermostatically controlled...
blanket. On completion of the experimental protocol, the animals were euthanized by electrical induction of ventricular fibrillation under deep general anesthesia.

Electrical Stimulation
To minimize the pain associated with the experiments, IV pentazocine, 1 mg/kg, was administered in addition to sodium thiopental. Electrical autonomic nerves stimulation at 20-Hz frequency, 5-ms pulse duration, and 15-mA output8-10 was applied for 60 s, 5 minutes after the drug administration and confirmation of hemodynamic stability, via the distal pair of a quadrupolar, open irrigation catheter placed in the proximal left or right renal artery (5–10 mm from the ostium of renal artery), depending on the ease of access of the target with the electrode. This was because, in our previous experience, no BP elevation was induced by electrical autonomic nerve stimulation at middle or distal portion of the renal artery (nonpublished data). Unfortunately, we did not have sufficient data to explain this observation, but distribution or density of the autonomic nerve around the renal artery may not be homogeneous in the entire length of the canine renal artery. Electrical stimulation was applied to the right renal artery first in 2 experiments and to the left renal artery in the other 6 experiments. After cessation of electrical stimulation for 10 minutes, the same protocol was applied to the contralateral renal artery. The electrical stimulation mode was the same as that used when stimulating the autonomic nerves during RF ablation procedures for atrial fibrillation.8-10 RF ablation, as described later, was then applied to the renal artery (target renal artery) on the side where electrical stimulation was applied first. The same autonomic nerves stimulation protocol was repeated 20 minutes later, first to the renal artery that was ablated, followed by the nonablated artery.

RF Catheter Ablation and Renal Angiogram
Using a 4-mm distal tip, quadrupolar, open irrigation catheter, as described earlier, RF energy was delivered for 30 seconds to a maximum of 20 watts, a temperature limit of 40°C, during irrigation at a flow of 30 mL/min, 5 times between the proximal and middle segments of the target renal artery (within 0–10 mm from the ostium of renal artery), including the portions of the electrical nerve stimulation). RF ablation was applied on the side where electrical nerve stimulation (as described earlier) had been applied first. Using a 5F angiographic catheter, renal angiograms were performed before and after RF ablation.

Blood Sampling
A blood sampling study was performed in 6 of the 8 experiments (experimental number 3–8). To minimize the effects of blood sampling on autonomic nerve activity, inferior vena cava blood was used to measure serum epinephrine and norepinephrine as follows: (1) before starting the experimental protocol (baseline); (2) immediately after cessation of electrical stimulation to each renal artery, before attempting RF ablation; (3) after RF ablation; and (4) immediately after electrical stimulation of the ablated and nonablated renal arteries. Hemoglobin and hematocrit were also counted before and at the end of the experiment protocol.

Measurements of Heart Rate Variability
In the same 6 experiments as performed for the blood sampling test (experimental number 3–8), the body surface electrocardiogram was digitized at a sampling rate of 1000 Hz by an ADX-002 analog-to-digital converter (ADT Tec Inc, Tokyo, Japan) and stored in a personal computer. The cardiac cycle length was measured using the GM-View II R-wave detection software (Signalysis Ltd, Saitama, Japan).8-10 The MemCalc method was used to calculate the high-frequency (HF; 0.15–0.40 Hz) and low-frequency (LF; 0.04–0.15 Hz) components of heart rate variability (HRV), using the MemCalc, version 2.0, software (Suwa Trust Co, Tokyo, Japan).8-10 In this study, the LF and HF components are expressed as amplitude (amp) as follows: [LF-amp = (2 • LF component)/2 and HFamp = (2 • HF component)/2] and the LF/HF ratio was calculated.

Study Protocol and Data Collection

Before RF Ablation
Electrical autonomic nerve stimulation was applied to the proximal portion of 1 renal artery (5–10 mm from the ostium of renal artery), and blood was sampled from the inferior vena cava before and immediately after stimulation. Ten minutes after cessation of the electrical autonomic nerves stimulation and after confirmation of stable hemodynamic measurements, electrical autonomic nerve stimulation was applied to the other renal artery, and blood was sampled immediately after cessation of stimulation. Bilateral renal angiograms were obtained before RF ablation.

After RF Ablation
Twenty minutes after RF ablation and after confirmation of stable hemodynamic parameter measurements, electrical autonomic nerve stimulation was repeated, beginning with the renal artery that had undergone ablation, followed by the nonablated renal artery. Under fluoroscopic guidance, electrical nerve stimulation was attempted from the same proximal portion of the renal artery before and after RF ablation. Bilateral renal angiograms were also repeated.

Statistical Analysis
The measurements are presented as mean±SD. Student t test was used to compare heart rate and BP before and after the application of electrical autonomic nerves stimulation and before and after RF ablation of the renal artery. The serum catecholamine concentrations were compared by ANOVA and Scheffe multiple-range post hoc test, where appropriate. The variables of HRV were compared using the Wilcoxon signed-rank sum test. A P value <0.05 was considered statistically significant.

Results

BP and Sinus Rate

Before RF Catheter Ablation
Systolic/diastolic BP and heart rate were 146±17/89±17 mm Hg and 119±9 bpm before versus 170±23/103±19 mm Hg (P<0.001) and 131±7 bpm (P<0.001) after electrical autonomic nerve stimulation of the target renal artery (Figures 1 and 2). The averaged changes in BP and heart rate resulting from electrical autonomic nerve stimulation of the target and nontarget renal arteries were from 150±16/92±15 to 173±21/105±16 mm Hg and from 121±8 to 133±8 bpm (P<0.001; Figures 1 and 2). The averaged changes in BP and heart rate from baseline within 4 to 6 minutes after its cessation.

After RF Ablation
Systolic/diastolic BP and heart rate were 150±20/90±16 mm Hg and 124±14 bpm before versus 152±20/92±17 mm Hg and 124±14 bpm after electrical autonomic nerve stimulation to the ablated renal artery (Figures 1 and 2). In contrast, electrical autonomic nerve stimulation to the nonablated renal
artery increased the systolic/diastolic BP from 141±17/84±19 to 171±24/103±26 mm Hg ($P=0.005$) and heart rate from 118±8 to 126±7 bpm ($P=0.014$; Figures 1 and 2).

BP and heart rate before attempting electrical autonomic nerve stimulation were similar, both before and after the RF ablation. As compared with the values before ablation, electrical autonomic nerve stimulation–induced BP augmentation was markedly attenuated by electrical autonomic nerve stimulation to the ablated renal artery. Conversely, BP after electrical autonomic nerve stimulation to the nonablated renal artery was not statistically different before or after RF ablation.

Heart Rate Variability

**Before RF Ablation**

Electrical autonomic nerve stimulation of the target renal artery increased LF-amp from 0.67±0.17 to 1.43±0.62 ms ($P=0.047$) and LF/HF ratio from 0.34±0.17 to 1.49±2.03 ($P=0.047$), whereas HF-amp changed nonsignificantly from 1.45±0.30 to 1.61±0.87 ms (Figure 3). Likewise, electrical autonomic nerve stimulation of the nontarget renal artery increased LF-amp from 0.87±0.62 to 1.59±0.56 ms ($P=0.028$) and LF/HF ratio from 0.49±0.38 to 1.15±1.40 ($P=0.047$), whereas HF-amp changed nonsignificantly from 1.33±0.47 to 2.00±1.20 ms (Figure 3).
After RF Ablation

Electrical autonomic nerve stimulation to the ablated renal artery caused changes in LF-amp from 0.71±0.37 to 0.87±0.32 ms, LF/HF ratio from 0.58±0.32 to 0.42±0.34, and HF-amp from 1.15±0.52 to 1.68±1.20 ms that were statistically non-significant. In contrast, electrical autonomic nerve stimulation to the nontarget renal artery caused significant changes in LF-amp from 0.73±0.27 to 1.92±2.45 ms (P=0.028) and LF/HF ratio from 0.62±0.50 to 1.27±0.91 (P=0.043), whereas the change in HF-amp, from 1.08±0.37 to 1.62±1.48 ms, was not significant (Figure 3).

Serum Epinephrine and Norepinephrine

The serum epinephrine and norepinephrine concentrations at baseline were 0.04±0.03 and 0.04±0.03 ng/dL, respectively. Before RF ablation, electrical autonomic nerve stimulation to the target renal artery ablation increased the epinephrine and norepinephrine concentrations to 0.51±0.20 (P=0.011) and 0.20±0.11 ng/dL (P=0.009), respectively, and autonomic nerve stimulation to the nontarget renal artery ablation to 0.57±0.33 (P=0.006) and 0.24±0.14 ng/dL (P=0.002), respectively (Figure 4).

The serum epinephrine and norepinephrine concentrations, 20 minutes after ablation of the renal artery, had returned to 0.09±0.06 and 0.05±0.02 ng/dL, respectively. Repeat electrical autonomic nerve stimulation to the ablated renal artery minimally increased serum epinephrine and norepinephrine to 0.12±0.11 and 0.07±0.04 ng/dL, respectively. In contrast, repeat electrical autonomic nerve stimulation to the nonablated renal artery increased epinephrine and norepinephrine to similar concentrations as before RF ablation of the contralateral artery (0.67±0.28 and 0.30±0.16 ng/dL, respectively; Figure 4). Hemoglobin (14.5±1.1 versus 13.3±2.6 g/dL) and hematocrit (43.2±3.2% versus 40.7±7.9%) were similar before and after completion of the experimental protocol.

RF Ablation and Renal Angiogram

RF ablation was successful and uncomplicated in all of the experiments. The mean delivered RF energy was 17±2 watts (range, 15 to 20), and mean temperature of the catheter tip was 34±2°C (range, 31°C to 40°C). Tissue impedance decreased from 157±26 before to 145±24 ohm after (P<0.001) RF ablation, suggesting that RF energy was successfully delivered and appropriately affected the renal artery. Angiograms of all of the arteries revealed no stenosis or injury to any vessel, either before or after RF ablation (Figure S3).
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renal blood flow, which, in turn, contribute to the develop-

ment and chronicity of hypertension. The kidney is also an important contributor to the regulation of central sympathetic activity. Converse et al found that muscle sympathetic nerve activity is increased in end-stage renal disease and is normalized by therapeutic nephrectomy. In rats with renal disease, Campese and Kogosov described a neurologic connection between kidney and brain. Stimulation of the afferent sympathetic nerve increases systemic sympathetic nerve activity and causes vasoconstriction via the activation of rostral ventrolateral medulla neurons in the brain. Accordingly, the attenuation of either the efferent or afferent sympathetic renal nerve theoretically contributes to lower the systemic BP. Indeed, experimental renal denervation, performed to clarify the role of these nerves in increasing the BP, decreased the BP in several models of hypertension.

**Hemodynamic Response**

Sympathetic Renal Nerve Activity and Hemodynamic Response

Both afferent and efferent sympathetic nerves are distributed around the renal artery. Renal efferent sympathetic activity participates in renin release, sodium retention, and reduced renal blood flow, which, in turn, contribute to the development and chronicity of hypertension. The kidney is also an important contributor to the regulation of central sympathetic activity. Converse et al found that muscle sympathetic nerve activity is increased in end-stage renal disease and is normalized by therapeutic nephrectomy. In rats with renal disease, Campese and Kogosov described a neurologic connection between kidney and brain. Stimulation of the afferent sympathetic nerve increases systemic sympathetic nerve activity and causes vasoconstriction via the activation of rostral ventrolateral medulla neurons in the brain. Accordingly, the attenuation of either the efferent or afferent sympathetic renal nerve theoretically contributes to lower the systemic BP. Indeed, experimental renal denervation, performed to clarify the role of these nerves in increasing the BP, decreased the BP in several models of hypertension.

**Discussion**

The main findings of this study were as follows: (1) electrical autonomic nerve stimulation of the proximal portion of renal artery increased the systemic BP; (2) the changes in serum catecholamine and HRV suggested that the increase in BP and heart rate caused by electrical autonomic nerve stimulation were attributed to an increase in systemic sympathetic nervous activity; and (3) the delivery of 17±2 watts of RF energy from the proximal to the middle potion of the renal artery attenuated the BP and heart rate responses to electrical autonomic nerve stimulation without inducing angiographic damages to the renal artery.

In this study, electrical autonomic nerve stimulation to the proximal portion of the renal artery increased the systemic BP and accelerated the heart rate, effects that are consistent with the activation of afferent sympathetic nerves around the renal artery. These effects, as well as the changes in LF/HF in the HRV analysis, were accompanied by an increase in serum epinephrine and norepinephrine. Although norepinephrine is released from the terminal portion of sympathetic nerves distributed to various tissues, epinephrine is primarily secreted by the adrenal gland. Because the efferent sympathetic nerves to the adrenal gland run from the celiac ganglia but not the renal ganglia, the increase in serum epinephrine suggests that systemic sympathetic nerve activity was increased by the electrical stimulation of afferent sympathetic nerves around the renal artery. Furthermore, the observation that RF ablation to the renal artery prevents the increase in serum epinephrine after electrical autonomic nerve stimulation suggests inter-connectivity between afferent nerve stimulation and systemic sympathetic activity.

Measurements of plasma renin activity, norepinephrine spillover, or both may help with estimating the activity of the efferent renal sympathetic nerve. However, our acute study protocol did not allow this analysis, because plasma renin activity is dynamically altered and takes longer to return to baseline, and our experimental facility was not prepared to use radioisotopes. It is, however, unlikely that the responses induced by autonomic nerve stimulation were simply caused by an augmentation of efferent sympathetic renal nervous activity alone, because we observed an increase in serum epinephrine concentrations and an increase in the sympathetic nervous indices of HRV. Measurement of serum catecholamine from multiple sites (renal arteries and veins, etc) would provide more detailed information concerning the efferent
sympathetic nerve activity by electrical autonomic nerve stimulation. However, such measurement (simultaneously performing electrical stimulation and blood sampling from the renal artery, etc) is currently technically unfeasible, and, in addition, a large amount of blood sampling from multiple sites can also subsequently alter hemodynamic status and autonomic nerve activity.

Importantly, similar responses in BP and heart rate were elicited by electrical autonomic nerve stimulation to the nonablated renal artery, although these responses were markedly attenuated when electrical autonomic nerve stimulation was applied to the ablated renal artery. These observations suggest that RF ablation from inside the renal artery attenuated the electrical autonomic nerve stimulation-induced activation of the renal sympathetic nerve. Some may argue that electrical autonomic nerve stimulation to the renal artery directly induced vasoconstriction, which increased systemic BP. However, this seems less likely, because these responses were not reproduced by electrical autonomic nerve stimulation to the ablated renal artery. The time course of BP increase by the renal arterial autonomic nerves stimulation (15–30 s after stimulation) is also consistent with the hypothesis that it was associated with an augmentation of central sympathetic activity. Previous experimental studies reported that whole body autoregulation quickly compensated for the reduction in renal perfusion, for instance, by uninephrectomy, but threshold in the amount of renal tissue that was needed to drive increased systemic sympathetic activity, and, as a consequence, hypertension was uncertain.21,22 This study, however, could not provide an answer regarding what happens in a single kidney distal to electrical stimulation that, in turn, is able to drive increased systemic sympathetic activity.

Renal Angiogram
In the clinical results of RF ablation of the renal artery for patients presenting with drug-refractory essential hypertension, BP was decreased in most patients without causing major injuries to the renal artery.1–3 Although our renal angiogram results were compatible with those clinical reports, a special catheter and ablation method was used for renal artery ablation in the hypertensive patients.1–3 The diameter and blood flow of canine and human renal arteries are different, although these factors are important to determine the effect of RF ablation.23,24 In addition, our ablation procedure was different from that used in patients with drug-refractory essential hypertension. Therefore, our result did not directly mean that the RF catheter ablation using an open irrigation catheter was safe for the treatment of clinical patients. The purpose of this study was to clarify the role of the renal autonomic system in BP regulation, not to examine whether an open irrigation catheter is applicable for ablation in human renal arteries.

Clinical Implications
We found that autonomic nervous electrical stimulation to the renal artery activates central sympathetic nervous activity. Therefore, this electrical stimulation might be used to determine the most suitable target site of RF ablation and to evaluate the effect of renal nerve RF ablation. As described above, in the current human renal artery ablations, RF current is systematically delivered without evaluating the therapeutic results during the RF ablation procedure. However, in ≈10% of patients, renal RF ablation was ineffective in decreasing the BP.1–3 Because it is unclear whether these unsuccessful outcomes were attributable to insufficient RF energy delivery to the renal artery versus the specific characteristics of the hypertension, an evaluation of the BP and heart response by electrical autonomic nerve stimulation of the renal artery might provide answers.

Study Limitations
First, we used a standard open irrigation catheter for this study, used for the treatment of arrhythmias, because we needed to apply sufficient RF energy in relatively narrow canine renal arteries. Therefore, our results do not directly apply to clinical practice. Second, the RF energy dependency of the effects on the renal sympathetic nerves was not studied. Third, the experiments were performed under general anesthesia. Autonomic nerve activity could have been altered by the anesthesia. However, the central message of this study seemed not to be affected by the use of anesthesia, because basic parameters (BP, heart rate, results of HRV, and serum catecholamine) were mostly stable during the experimental protocol, whereas electrical autonomic nerve stimulation of the target renal artery increased the systemic BP before but not after RF ablation. Fourth, effects of electrical nerve stimulation would be different from site to site in the renal artery, and this subject needs to be clarified in further studies. Fifth, we only evaluated the immediate effects of RF ablation on the renal artery. Therefore, the effects of catheter RF ablation on renal sympathetic nervous activity might be transient. Nevertheless, this study showed that renal sympathetic nervous activity was increased by electrical autonomic nerve stimulation from the renal artery and its response modulated by RF catheter ablation inside the renal artery.

Perspectives
Because RF ablation of the renal artery seems to attenuate systemic sympathetic nervous activity, its use might be extended in the future to the treatment of various cardiovascular diseases, including congestive heart failure, chronic kidney disease, metabolic syndrome, atrial fibrillation, and diabetes mellitus, all diseases where sympathetic nervous activity is considered to play a key role in their development and progression.34,25–27

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Disclosures
None.

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Blood pressure and autonomic responses to electrical stimulation of the renal arterial nerves before and after ablation of the renal artery

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Supplemental Figure 1: BP and HR responses to renal nerve electrical stimulation before (A) and after (B) renal artery nerve RF ablation. Shown are leads II and III of the surface electrocardiogram on top and BP on the bottom. Before RF application (A), electrical stimulation applied for 60 sec to the renal artery gradually increased BP. Heart rate was mildly accelerated. These responses were minimized after RF ablation (B).
Supplemental Figure 2: BP and HR responses to renal nerve electrical stimulation. Shown are leads aVR and aVL of the surface electrocardiogram on top and BP on the bottom, at baseline, and at 20 and 45 sec after the onset of electrical stimulation. Before RF electrical stimulation to the right (A) and left (B) renal arteries increased BP and accelerated HR. After RF ablation to the right renal artery, the responses to electrical stimulation of the target renal artery were attenuated (C), whereas the responses to electrical stimulation of the non-ablated (left) renal artery were unchanged (D).
Supplemental Figure 3: Renal angiograms before (A and B) and after (C and D) renal artery RF ablation. The angiograms were obtained from different experiments. Both RF ablation to the right renal artery (A and C) and RF ablation to the left renal artery (B and D) caused no angiographic injury to the renal artery.