Differential role of afferent and efferent renal nerves in the maintenance of early- and late-phase Dahl S hypertension

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Foss JD, Fink GD, Osborn JW. Differential role of afferent and efferent renal nerves in the maintenance of early- and late-phase Dahl S hypertension. Am J Physiol Regul Integr Comp Physiol 310: R262–R267, 2016. First published December 9, 2015; doi:10.1152/ajpregu.00408.2015.—Clinical data suggest that renal denervation (RDNX) may be an effective treatment for human hypertension; however, it is unclear whether this therapeutic effect is due to ablation of afferent or efferent renal nerves. We have previously shown that RDNX lowers arterial pressure in hypertensive Dahl salt-sensitive (S) rats to a similar degree observed in clinical trials. In addition, we have recently developed a method for selective ablation of afferent renal nerves (renal-CAP). In the present study, we tested the hypothesis that the antihypertensive effect of RDNX in the Dahl S rat is due to ablation of afferent renal nerves by comparing the effect of complete RDNX to renal-CAP during two phases of hypertension in the Dahl S rat. In the early phase, rats underwent treatment after 3 wk of high-NaCl feeding when mean arterial pressure (MAP) was ~140 mmHg. In the late phase, rats underwent treatment after 9 wk of high NaCl feeding, when MAP was ~170 mmHg. RDNX reduced MAP ~10 mmHg compared with sham surgery in both the early and late phase, whereas renal-CAP had no antihypertensive effect. These results suggest that, in the Dahl S rat, the antihypertensive effect of RDNX is not dependent on pretreatment arterial pressure, nor is it due to ablation of afferent renal nerves.

Dahl salt-sensitive rat; afferent renal nerves; efferent renal nerves; Dahl salt-sensitive rat

DESPITE THE STAGGERING WORLDWIDE prevalence of hypertension and its significant association with death and disability, little progress has been made in developing more effective treatments for the disease (16, 20). However, recent technological advances have led to the development of catheter-based ablation of renal nerves (renal denervation, RDNX) as a possible treatment for drug-resistant hypertension in humans. The majority of clinical data that have been published using this technique have demonstrated a significant antihypertensive effect of RDNX, although a number of studies have failed to show any effect (4). This discrepancy has highlighted the need for further investigations into the mechanisms underlying the effects of RDNX.

One mechanistic question regarding this treatment is whether RDNX lowers arterial pressure (AP) secondary to ablation of afferent or efferent renal nerves. Because efferent renal nerves influence renin release, tubular sodium reabsorption, and renal vascular resistance, it has been proposed that the antihypertensive effect of RDNX may result from ablation of efferent renal nerves (5). Indeed, data from one clinical study suggest that RDNX reduces renal vascular resistance, as indicated by a reduction in renal resistive index (17). However, one study has found no correlation between the blood pressure lowering effect of RDNX and sodium excretion (21), and data regarding the effect of RDNX on plasma renin concentration are limited and mixed (6, 27).

An alternative possibility is that the antihypertensive response to RDNX is due to ablation of sympathoexcitatory afferent renal nerves, resulting in decreased nonrenal sympathetic nerve activity (SNA). This hypothesis is supported by clinical studies demonstrating reductions in muscle SNA, plasma glucose, cardiac arrhythmias, and events of sleep apnea following RDNX (10, 23, 28). Although these studies suggest that there are physiological consequences of afferent renal nerve ablation, it is still unclear whether ablation of afferent renal nerves is responsible for the decrease in AP following RDNX. It should also be noted, that at least one study has failed to show reductions in SNA following RDNX (2).

We have recently established the Dahl salt-sensitive (DS) rat as an animal model of hypertension in which RDNX lowers AP to a degree similar to that reported in humans (7). This commonly used model is widely accepted as a good model of human hypertension, as it displays many comorbidities commonly observed in hypertensive patients, such as renal pathology, impaired glucose metabolism, and overactivity of the sympathetic nervous system (30). Although we have shown that RDNX decreases AP in DS rats, it is unknown whether this response is due to ablation of afferent or efferent renal nerves.

To address this question, we recently developed and validated a novel method for selective ablation of afferent renal nerves in the rat, termed renal-CAP (8). In the present study, we tested the hypothesis that the antihypertensive response to RDNX in the DS rat is due to ablation of afferent renal nerves specifically by comparing the antihypertensive effect of complete RDNX to that of renal-CAP. Moreover, because pretreatment AP has been suggested to be a predictor of the antihypertensive effect of RDNX (24), we tested this hypothesis in both the early phase (after 3 wk of high NaCl feeding when hypertension is moderate) and the late phase (after 9 wk of high NaCl feeding when hypertension is more severe) of hypertension.

MATERIALS AND METHODS

Animals and General Procedures

A total of 56 male DS rats were purchased from Charles River Laboratories (Wilmington, MA) and housed in pairs in a temperature- and light-controlled room until the beginning of the study. Rats were allowed access to standard rat chow and distilled water ad libitum during this preexperimental period. All procedures were approved by...
the University of Minnesota Animal Care and Use Committee and were conducted in accordance with the institutional and National Institutes of Health guidelines. For all surgeries, rats were anesthetized with 2.0% isoflurane (Phoenix Pharmaceutical, St. Joseph, MO). Atropine sulfate (0.4 mg/kg ip; West-Ward Pharmaceuticals, Eatontown, NJ), gentamicin sulfate (10 mg/kg im; Hospira, Lake Forest, IL) and ketoprofen (5 mg/kg sc; Fort Dodge Animal Health, Overland Park, KS) were administered prior to surgery. For three days following surgery, ketoprofen (2.5 mg/kg sc) was given once per day, and the drinking water was supplemented with amoxicillin (1 mg/ml; Sandoz International, Holzkirchen, Germany).

Treatment Surgeries

Renal denervation. Complete denervation of the kidneys was performed as previously described (8). Briefly, the left renal nerves were exposed through a midline abdominal incision. All visible renal nerve fibers were sectioned, and the renal artery and vein were exposed with a small piece of gauze soaked in 10% phenol in ethanol. The area was then dried, and the procedure was repeated on the contralateral side.

Renal-CAP treatment. Selective ablation of afferent renal nerves was performed as previously described (7). Briefly, a midline abdominal incision was made, and the left renal artery and vein were exposed through a small hole in the peritoneal membrane. The fat surrounding the renal artery and vein was then dissected away, and parafilm was placed under the vessels. A small piece of gauze soaked in a capsaicin solution (33 mM in 5% ethanol, 5% Tween 80 and 90% normal saline) was wrapped around the renal artery and vein for 15 min. The gauze and parafilm were removed, the area was dried, and the procedure was repeated on the contralateral side.

Sham surgery. The left and right renal nerves were visualized through a midline abdominal incision without physical disruption of the area.

Experimental Protocol

Early-phase protocol. Sixty-seven to seventy-four-day-old rats were placed on a low-salt diet (0.1% NaCl; Research Diets, New Brunswick, NJ) and instrumented with radio telemeters (model TAI1-PA-C40; Data Sciences International (DSI), St. Paul, MN) for monitoring of mean arterial pressure (MAP) and heart rate (HR), as previously described (25). After a 7-day recovery period, rats were individually housed in metabolic cages (Techniplast 3701M001; Buggiate, Italy) and were allowed to acclimate for 4 days. Three days of baseline data were then collected (see below for details), and rats were placed on a high-salt diet (4.0% NaCl; Research Diets) for the remainder of the protocol. After 21 days of high-salt intake, rats were anesthetized with isoflurane and, via a midline approach, subjected to sham surgery (Sham; n = 10), renal denervation (RDNX; n = 10), or selective ablation of afferent renal nerves (renal-CAP; n = 10), as described above. Rats were returned to their cages and monitored for an additional 4 wk.

Late-phase protocol. Sixty-two to sixty-four-day-old rats were placed on a low-salt diet (0.4% NaCl; Research Diets), and after 49–50 days, they were instrumented with radio telemeters (DSI) for monitoring of MAP and HR. After a 6–7-day recovery period, rats were individually housed in metabolic cages (Techniplast) and allowed to acclimate for 4 days. Three days of baseline data were then collected. After 9 wk total of a high-salt diet, rats were anesthetized with isoflurane and, via a midline approach, subjected to a sham surgery (Sham; n = 8), renal denervation (RDNX; n = 9), or selective ablation of afferent renal nerves (renal-CAP; n = 9), as described above. Rats were returned to their cages and monitored for an additional 2 wk.

At the end of the study, rats were anesthetized and exsanguinated, and the kidneys were removed immediately after death. The renal artery, renal vein, ureter, and capsule were removed, and the kidneys were placed in a cold normal saline bath for further dissection. Renal parenchymal samples were taken from the poles and lateral portion of the kidney, and they were flash frozen in liquid nitrogen. The renal pelvis was then carefully dissected from the remaining portion of kidney and flash frozen in liquid nitrogen. All frozen samples were stored at −80°C until being assayed.

The renal parenchymal samples were assayed for norepinephrine (NE) using HPLC, as previously described (15). The detection limit of this assay is 100 pg/g with an intra-assay coefficient of variation (CV) of 4.4% and interassay CV of 6.7%. The isolated renal pelvic samples were assayed for calcitonin gene-related peptide (CGRP) using a commercially available ELISA kit (Cayman Chemicals, Ann Arbor, MI; item no. 589001). Tissues were homogenized in 1 M acetic acid, and CGRP was extracted using C18 Sep-columns (Peninsula Laboratories, San Carlos, CA; item no. Y-1000). To eliminate any interassay variance, all pelvic samples were run on a single 96-well ELISA plate.

Daily Measurements

The transmitter signal was monitored by a receiver (Data Sciences, model no. RPC-1) mounted on the side of the metabolic cage and connected to a Data Exchange Matrix (DSI). The AP signal was sampled at 500 samples/s for 10 s every 4 min using commercially available software (DSI). HR was determined from the AP profile using the same software. Twenty-four-hour averages of MAP and HR were determined and plotted for each day of the study.

Twenty-four-hour food intake, water intake, and urine output were measured gravimetrically. Twenty-four-hour sodium intake was calculated by multiplying food intake (grams) and sodium content of the diet (0.1% NaCl = 0.0171 mmol Na+/g food; 4.0% NaCl = 0.6844 mmol Na+/g food). Twenty-four-hour sodium excretion was calculated by multiplying 24-h urine output (ml) and urinary sodium concentration (mmol Na+/ml), which was measured using an ion-specific electrode (NOVA-5+ electrolyte analyzer, Nova Biomedical, Waltham, MA). Twenty-four-hour sodium and water balances were calculated as 24-h intake minus 24-h excretion. Cumulative sodium and water balance were then calculated by sequential summation of daily balances beginning on the day of treatment surgery.

Statistical Analysis

MAP, HR, and cumulative Na+/H2O balance data were analyzed by 2-way ANOVA for repeated measures followed by the Bonferroni method for post hoc comparisons using GraphPad Prism v6 (GraphPad Software, San Diego, CA). Baseline MAP and HR, as well as CGRP and NE data, were analyzed by one-way ANOVA. A P value less than 0.05 was considered to be statistically significant.

RESULTS

Early-Phase Protocol

As shown in Table 1, early-phase pretreatment MAP (after 3 wk of high-NaCl feeding) was similar in all groups (~140 mmHg). Figure 1 shows the change in MAP from the day before treatment (ΔMAP). Following treatment, MAP dropped transiently in all three groups and then continued to rise throughout the protocol. This drop was significantly greater on the day of surgery in RDNX (~16 ± 3 mmHg, P < 0.05) compared with Sham (~3 ± 2 mmHg) and renal-CAP (~5 ± 2 mmHg), and on the final day of the protocol, MAP had risen significantly less in RDNX (11 ± 2 mmHg, P < 0.05) than Sham (23 ± 2 mmHg) and renal-CAP (22 ± 3 mmHg).

As shown in Table 1, early-phase pretreatment HR was similar in all groups (~400 bpm). Figure 1 shows the change in HR from the day before treatment (ΔHR). In all three groups, HR transiently increased following treatment and then
fell slightly below baseline. Although there were no differences between Sham and renal-CAP throughout the protocol, ΔHR on day 2 after surgery was greater in RDNX (30 ± 5 BPM) compared with Sham (21 ± 4 BPM, P < 0.05) and renal-CAP (19 ± 8 BPM, P < 0.05). Additionally, HR fell more toward the end of the protocol in RDNX, and ΔHR was significantly lower in RDNX than Sham on days 22 (−27 ± 8 vs. −7 ± 5 BPM, P < 0.05) and 28 (−24 ± 8 vs. −6 ± 4 BPM, P < 0.05).

As shown in Fig. 2, there were no differences in cumulative sodium or water balance between groups at any time following treatment.

Late-Phase Protocol

As shown in Table 1, late-phase pretreatment MAP (after 9 wk of high NaCl feeding) was similar in all groups (~170 mmHg) and nearly 30 mmHg higher than rats fed a high-NaCl diet for 3 wk. MAP dropped transiently in all three groups following treatment and then continued to rise throughout the remainder of the protocol (Fig. 3). While the initial drop was similar in all groups (~25 mmHg), MAP increased less in RDNX, such that ΔMAP was lower in RDNX than Sham and renal-CAP throughout much of the protocol. On the final day, ΔMAP was significantly lower in RDNX (4 ± 1 mmHg) than Sham (12 ± 4 mmHg, P < 0.05) and renal-CAP (13 ± 1 mmHg, P < 0.05).

As shown in Table 1, late-phase pretreatment HR was similar in all groups and similar to the pretreatment HR in the early-phase protocol (~400 bpm). As shown in Fig. 3, HR initially increased in RDNX (25 ± 8 bpm), decreased in Sham (−15 ± 13 bpm), and was largely unchanged in renal-CAP (5 ± 8 bpm). HR in all groups then returned roughly to baseline levels for the remainder of the protocol, and there were no differences between groups except on day 9 when RDNX (−13 ± 4 bpm) was significantly less that of Sham (3 ± 5 bpm, P < 0.05).

As with the early-phase protocol, there were no differences in cumulative water balance between groups (Fig. 4). However, cumulative sodium balance trended slightly lower in RDNX and renal-CAP rats compared with Sham, but these differences were only significant between RDNX (58.1 ± 4.7 mmol) and Sham (68.3 ± 6.0 mmol, P < 0.05) on the final day of the protocol.

Renal NE and CGRP Content

To confirm the denervation procedures, we measured renal tissue content of the afferent nerve marker, CGRP, and the efferent nerve marker, NE (Fig. 5). In the early-phase protocol, both RDNX and renal-CAP resulted in significant reductions in

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Mean arterial pressure (MAP) and heart rate (HR) on the day before treatment surgery in Sham, renal denervation (RDNX), and renal-CAP (selective ablation of afferent renal nerves) rats in both the early-phase and late-phase protocols. There were no statistical differences in MAP or HR between groups in either protocol.
Renal Denervation, but not Selective Ablation of Afferent Renal Nerves, Attenuates DS Hypertension

To test whether the antihypertensive effect of RDNX in the DS rat is due to ablation of afferent renal nerves, we compared the effect of RDNX and renal-CAP on MAP in both the early and late phase of DS hypertension. In both protocols, RDNX...
lowered MAP —10 mmHg compared with Sham. Importantly, selective ablation of afferent renal nerves by renal-CAP had no effect on MAP compared with sham surgery in either protocol. These data suggest that the antihypertensive effect of RDNX in the DS rat is not due to ablation of afferent renal nerves. This is in contrast to other animal models of hypertension to which afferent renal nerves appear to contribute. Specifically, we have shown that selective ablation of afferent renal nerves attenuates the development of DOCA-salt hypertension to a similar extent as RDNX (8). Moreover, others have implicated afferent renal nerves in the pathogenesis of hypertension in various other animal models (3, 29). Still others have suggested that, under conditions of dietary sodium loading, hypertension may result from impaired afferent renal nerve activity (14, 26).

The reason for this discrepancy is presently unknown; however, many of the preclinical models in which afferent renal nerves appear to play a role involve severe renal pathology, such as reduced renal mass, renal hypoxia, and even renal necrosis. In the case of the DOCA-salt model used in this study, renal mass is reduced by unilateral nephrectomy, which results in compensatory renal hypertrophy of the remaining kidney. This is followed by chronic administration of a salt-retaining hormone and excessive salt intake induced by replacing drinking water with a saline solution. This results in a profound inflammatory state in the kidney that includes marked leukocyte infiltration and increased tissue levels of inflammatory cytokines such as TNF-α, IFNγ, and IL-6 (1, 22). In contrast, although some renal inflammation has been reported in the Dahl S rat, it involves moderate infiltration of T lymphocytes into the kidneys with no increase in either IFNγ or IL-6 (9). Since numerous cytokines have been shown to chronically modulate the activity of visceral afferent nerve function (19), the difference in the renal inflammatory profile may explain why ablation of afferent afferent nerves attenuates hypertension in DOCA-salt model, but not Dahl S model, of hypertension. Our laboratory is currently investigating in greater detail the mechanisms linking renal inflammation to modulation of afferent renal nerves in various preclinical models of hypertension.

**Antihypertensive Effect of RDNX Is Similar in Both the Early and Late Phase of DS Hypertension**

Because pretreatment AP has been suggested to be a predictor of the antihypertensive effect of RDNX (24), we subjected DS rats to RDNX and renal-CAP in both the early phase (after 3 wk of high NaCl feeding), when MAP was ~140 mmHg, and in the late phase (after 9 wk of high NaCl feeding), when MAP was ~170 mmHg. Interestingly, the antihypertensive effect of RDNX was nearly identical in both the early and late phase of DS hypertension, suggesting that, unlike in humans with resistant hypertension, pretreatment MAP may not always be a good predictor of response to RDNX (24).

**Antihypertensive Effect of RDNX Is not Due to Sodium and Water Unloading**

While total assessment of the efferent renal nerve-specific responses to RDNX (i.e., plasma renin concentration and renal vascular resistance) was outside of the scope of this study, we did measure whole body cumulative sodium and water balance. These measurements revealed that the antihypertensive effect of RDNX did not correspond with a reduction in sodium or water balance, thus demonstrating that a reduction in MAP can occur in the absence of an enhanced natriuresis and diuresis. This finding is consistent with our earlier report on the effect of RDNX in DS rats (7). Combined with our findings that afferent renal nerves do not play a role in this model, these data suggest that the antihypertensive effect of RDNX in the DS rat is likely due to reduced activity of the renin angiotensin system, a reduction in renal vascular resistance, or another effect of efferent renal nerve ablation. Further investigation will be needed to test these possibilities.

**Renal Nerves May Play a Minor Role in the Regulation of Heart Rate**

Our previous work has suggested that renal nerves may play a minor role in the long-term regulation of heart rate. Specifically, renal-CAP resulted in a blunting of the bradycardic response to dietary sodium loading in normotensive Sprague-Dawley rats (8). Alternatively, the bradycardic response to DOCA-salt hypertension was actually enhanced in RDNX rats compared with Sham rats, despite the antihypertensive effect of RDNX (12). Interestingly, there was a slightly enhanced bradycardia in the early phase of DS hypertension in this study. While the effect was minor and only significant on a few days, these results are consistent with some minor role of the renal nerves in heart rate regulation. The mechanisms underlying this effect are unclear but may involve changes in baroreceptor reflex sensitivity, possibly secondary to decreased circulating ANG II following RDNX. This recurring phenomenon merits further investigation to uncover the mechanisms involved, as it may have important implications for the possible effects—both therapeutic and detrimental—of RDNX on the heart.

Although RDNX appears to be a promising treatment option for drug-resistant hypertension, the mechanisms underlying this therapeutic effect remain unclear. To further improve this treatment, it is important to determine whether the antihypertensive effect is due to ablation of afferent or efferent renal nerves. To this end, we have shown that the antihypertensive effect of RDNX in the DS rat is not due to ablation of afferent renal nerves. This is in contrast to our previous study in the DOCA-salt model in which renal-CAP blunted the development of hypertension. These results are critically important because they suggest that the mechanisms underlying the antihypertensive effect of RDNX will likely vary from patient to patient. Therefore, it may be ideal to develop selective ablation techniques, which could be used to target either subset of renal nerves to improve efficacy and reduce side effects. Furthermore, it may be possible to develop clinical tests to identify patients that would benefit from such selective ablations. Further animal studies will be critical in developing such tests.

**Limitations**

We recently validated the renal-CAP method as a novel technique to target afferent renal nerves while leaving efferent renal nerves intact (8). This was demonstrated by the observation that renal-CAP markedly reduces CGRP but has no effect on renal NE content. However, in the present study, we observed a modest reduction in renal NE content in the early-phase renal-CAP group, which raises the possibility of an...
off-target effect of this treatment. We feel this was not likely due to the capsaicin itself since sympathetic fibers lack receptors for capsaicin. However, it is possible that some efferent fibers were disrupted when the fat was dissected away from the renal vessels prior to renal-CAP treatment. Although there was a small decrease in renal NE content in the kidneys of these rats, the decrease appeared to be insufficient to have a physiological effect, as there was no effect on MAP or HR compared with Sham.

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DISCLOSURES

J. W. Osborn was a paid consultant for Medtronic Cardiovascular, Inc., at the time this study was conducted. No other conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: J.D.F. and J.W.O. conception and design of research; J.D.F. performed experiments; J.D.F. analyzed data; J.D.F., G.D.F., and J.W.O. approved final version of manuscript.

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