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Reversal of Genetic Salt-Sensitive Hypertension by Targeted Sympathetic Ablation

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Abstract—The sympathetic nervous system plays an important role in some forms of human hypertension as well as the Dahl salt-sensitive rat model of hypertension; however, the sympathetic targets involved remain unclear. To address this, we examined the role of the renal and splanchnic sympathetic nerves in Dahl hypertension by performing sham surgery (n=10) or targeted sympathetic ablation of the renal nerves (renal denervation, n=11), the splanchnic nerves (celiac ganglionectomy, n=11), or both renal and splanchnic nerves (n=11) in hypertensive Dahl rats. Mean arterial pressure increased from ≈120 mm Hg, while on a 0.1% sodium chloride diet, to ≈140 mm Hg after being fed a 4.0% sodium chloride diet for 3 weeks. At that point, rats underwent sham or targeted sympathetic ablation. Four weeks after treatment, mean arterial pressure was lower in renal denervated (150.4±10.4) and celiac ganglionectomized (147.0±6.1) rats compared with sham rats (165.0±3.7) and even lower in rats that underwent both ablations (128.4±6.6). There were no differences in heart rate or fluid balance between sham and renal denervated rats; however, rats that underwent either celiac ganglionectomy or both ablations exhibited marked tachycardia as well as sodium and water retention after treatment. These data suggest that targeted sympathetic ablation is an effective treatment for established hypertension in the Dahl rat and that the kidneys and the splanchnic vascular bed are both independently important targets of the sympathetic nervous system in this model. (***Hypertension***. 2013;61:806-811.) ● Online Data Supplement

Key Words: celiac ganglionectomy ■ Dahl S hypertension ■ renal denervation ■ sodium balance ■ water balance

Hypertension is the leading risk factor for death worldwide,[1] yet the underlying causes are poorly understood as evidenced by the staggering number of uncontrolled cases of hypertension.[2-5] Recent studies have shown that catheter-based renal nerve ablation results in a sustained reduction of arterial pressure in drug-resistant hypertensives, suggesting that targeted sympathetic ablation may be an effective treatment for hypertension that avoids the side-effects of globally acting sympatholytic drugs.[6-8]

Although the kidneys seem to be sympathetic targets in some forms of hypertension, other vascular beds, such as the splanchnic circulation, are also likely important as evidenced by the ability of splanchnic nerve stimulation to increase arterial pressure[9] and surgical splanchnic sympathectomy to attenuate drug-resistant hypertension in humans.[10,11] Additionally, we have shown that the splanchnic, but not renal nerves, contribute to the pathophysiology of angiotensin II–salt hypertension.[12] Together, these findings suggest that renal nerve ablation may be effective in some, but not all, forms of hypertension and that targeted sympathetic ablation of nonrenal vascular beds may be an effective strategy for the treatment of hypertension in some individuals.

With that in mind, the present study compared the effects of renal and splanchnic nerve ablation on arterial pressure and body fluid balance in a well-accepted and widely studied genetic model of neurogenic hypertension, the Dahl salt-sensitive (Dahl S) rat. Similar to a significant fraction of humans with essential hypertension, the Dahl S rat becomes hypertensive when fed a high-salt diet, has increased sympathetic activity,[13-18] and has increased renal and splanchnic vascular resistance.[19] Although renal nerve ablation has been consistently reported to have no effect on the development of Dahl S hypertension,[20-23] the ability of renal nerve ablation to reverse hypertension in this model has not been reported. This is an important distinction with respect to the development of new therapies, which for the foreseeable future will focus on the treatment rather than prevention of hypertension. To our knowledge, the effect of splanchnic nerve ablation on either the developmental or maintenance phase of Dahl S hypertension has not been reported.

The present study was designed to address the following questions. First, once hypertension is initiated, does renal nerve ablation decrease arterial pressure in the Dahl S rat? If so, is this response attributable to increased renal excretion of sodium and water? Second, does ablation of sympathetic nerves innervating the splanchnic vascular bed decrease arterial pressure in the Dahl S rat and, if so, how does that response compare with renal nerve ablation? Third, is combined

regional sympathectomy more effective in lowering arterial pressure than denervation of a single target?

Methods

Animals and General Procedures

Male Dahl S 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, at which time they were 64 to 70 days old and 250–340 g. The rats were allowed access to standard rat chow and distilled water ad libitum during this pre-experimental 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. Atropine sulfate (0.4 mg/kg, IP) and gentamicin sulfate (10 mg/kg, IM) were administered before surgery. For 3 days after surgery, buprenorphine (0.015 mg, SQ) was given twice per day, and the drinking water was supplemented with amoxicillin (1 mg/mL).

Experimental Protocol

The timeline for the experimental protocol is shown in Figure 1. Rats were placed on a low-salt diet (0.1% NaCl; Research Diets, New Brunswick, NJ) and instrumented with radio telemeters (model TA11PA-C40, DSI, Intl. St. Paul, MN) for monitoring of mean arterial pressure (MAP) and heart rate (HR) as previously described.[24] After a 7-day recovery period, rats were individually housed in metabolic cages (Techniplast 3701M001, Buguggiate, Italy) and allowed to acclimate for 4 days. Three days of baseline data were then collected, 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 a sham (SHAM; n=10), renal denervation (RDNX; n=11), celiac ganglionectomy (CGX; n=11), or combined renal denervation and celiac ganglionectomy (RDNX–CGX; n=11) procedure. SHAM, RDNX, and CGX procedures were performed as previously described,[25,26] and the combined RDNX–CGX was achieved by performing the RDNX and CGX in single procedure. Rats were returned to their cages and were monitored for an additional 4 weeks. On completion of the study, rats were anesthetized with isoflurane and the duodenum, liver, spleen, and both kidneys of each rat were harvested, weighed, immediately frozen with liquid nitrogen, and stored at −80°C until they were assayed for tissue norepinephrine content as previously described.[26]

Daily Measurements

The transmitter signal was monitored by a receiver (Data Sciences, model RPC-1) mounted on the side of the metabolic cage and connected to a Data Exchange Matrix (Data Sciences, Int). The arterial pressure signal was sampled at 500 samples/second for 10 seconds every 4 minutes using commercially available software (Data Sciences, Int). HR was determined from the arterial pressure 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=0.01711 mmol Na^+/g food; 4.0% NaCl=0.6844

mmol Na^+/g food). Twenty-four-hour sodium excretion was calculated by multiplying 24-hour 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-hour intake minus 24-hour excretion. Cumulative sodium and water balances were determined from sequential summation of daily balances over the duration of the protocol.

Statistical Analysis

Data were analyzed by 2-way ANOVA for repeated measures followed by the Holm–Sidak method for all post hoc comparisons (SigmaPlot version 10.0). A probability value <0.05 was considered to be statistically significant.

Results

Cardiovascular Responses to Targeted Sympathetic Ablation

As shown in Figure 2, MAP increased in all groups from ≈120 mm Hg on the final day of 0.1% NaCl diet (SHAM=120.2±2.2, RDNX=119.5±2.0, CGX=119.8±1.3, RDNX–CGX=118.3±1.8 mm Hg) to ≈140 mm Hg after 3 weeks of 4.0% NaCl (SHAM=140.1±3.1, RDNX=140.9±5.0, GCX=139.4±2.4, RDNX–CGX=138.5±3.4 mm Hg). After SHAM surgery, MAP fell transiently, most likely attributable to a transient decrease in food (and therefore sodium) intake (Figures S1 in the online-only Data Supplement), but returned to the presurgery trajectory within 7 days, reaching 165.0±3.7 mm Hg on the final day of the protocol. In contrast, MAP also fell in the RDNX group but did not rebound to the same trajectory and, by the end of the protocol, MAP was ≈15 mm Hg lower (150.4±10.4) than SHAM rats. The magnitude and time course of the MAP response to CGX were similar

Figure 2. Effect of sham surgery (SHAM), renal denervation (RDNX), celiac ganglionectomy (CGX), and combined celiac ganglionectomy and renal denervation (RDNX–CGX) on mean arterial pressure (MAP) and heart rate (HR). *P<0.05 for RDNX–CGX vs SHAM; †P<0.05 for CGX vs SHAM; ‡P<0.05 for RDNX vs SHAM.

Figure 1. Protocol timeline. CGX indicates celiac ganglionectomy; RDNX, renal denervation; RDNX–CGX, combined celiac ganglionectomy and renal denervation; and SHAM, sham surgery.

to that of RDNX rats with MAP reaching a final level of 147.0±6.1 mm Hg. Finally, RDNX–CGX resulted in a much greater decrease in MAP than RDNX or CGX alone with a final MAP of 128.4±6.6, ≈35 mm Hg lower than SHAM rats and ≈20 mm Hg lower than RDNX and CGX rats on the final day of the protocol. Moreover, the maximum pressure response was greater in RDNX–CGX than all other groups (Figure S2).

The effects of SHAM and targeted sympathetic ablation on HR are also shown in Figure 2. HR on the final day of baseline was similar in all groups (SHAM =422.5±2.7, RDNX=416.9±4.9, CGX=425.1±4.1, RDNX–CGX=420.6±4.8 bpm) and decreased to a similar level after 3 weeks of high-salt intake (SHAM=380.0±3.1, RDNX=385.2±3.0, CGX=383.6±2.8, RDNX-CGX=384.3±2.9 bpm). After treatment surgery, HR transiently increased and then gradually fell in SHAM and RDNX rats, such that HR was not statistically different between these groups throughout the protocol. In contrast, both CGX and RDNX–CGX rats exhibited a marked biphasic increase in HR after treatment. During the first peak, HR increased to 425.87±10.5 in CGX rats and 450.22±6.2 in RDNX–CGX rats and began to fall but then increased to a second peak before falling to levels slightly higher than SHAM and RDNX rats by the end of the protocol.

Sodium and Water Balance Responses to Targeted Sympathetic Ablation

Daily sodium and water intake, excretion, and balance measurements were similar between all groups during the baseline period, increased transiently when the diet was increased to 4.0% NaCl, and returned to baseline levels until the time of SHAM or targeted sympathetic ablation (Figures S1 and S3). There were no differences in these parameters between SHAM and RDNX rats at any time during the 4 weeks after surgery, with the exception of the day after surgery when urine output was higher in RDNX than in SHAM rats. Sodium and water intake, excretion, and balance decreased transiently after SHAM and RDNX but returned to preprocedure levels within 10 days.

Cumulative sodium and water balances over the duration of the protocol were calculated from the daily balance measurements in all 4 groups (Figure 3). There were no differences for cumulative sodium or water balance between RDNX and SHAM rats over the entire protocol. On the contrary, CGX rats retained sodium and water after nerve ablation, such that cumulative balances were significantly higher by the end of the protocol as compared with SHAM rats. This response was significantly attenuated by combining CGX with RDNX.

Tissue Norepinephrine Content

Tissues were collected at the end of the study to determine the extent of denervation 4 weeks postprocedure. Results of the norepinephrine assay clearly show that RDNX selectively denervated the kidneys, CGX selectively denervated the gut, and RDNX–CGX denervated both (Figure 4).

Discussion

The sympathetic nervous system plays an important role in some forms of human hypertension; however, the degree
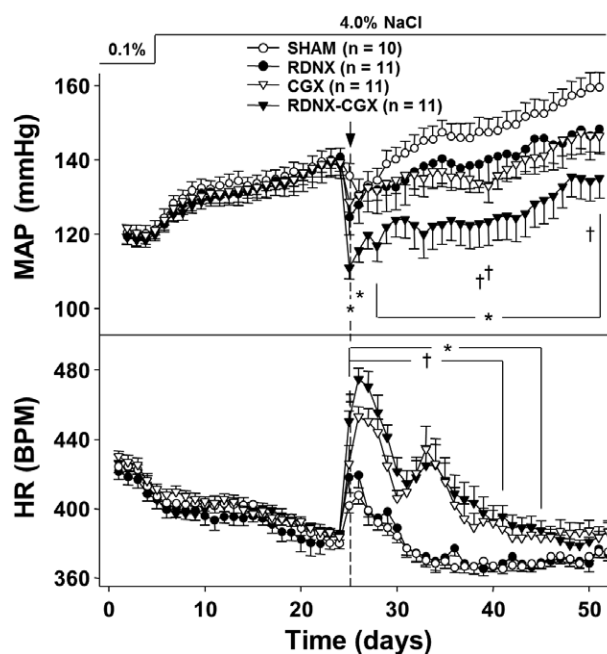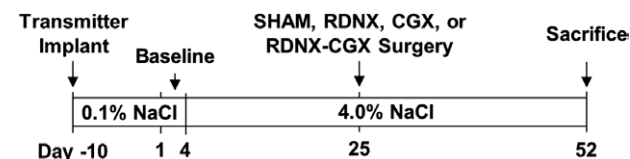

Figure 3. Effect of sham surgery (SHAM), renal denervation (RDNX), celiac ganglionectomy (CGX), and combined celiac ganglionectomy and renal denervation (RDNX–CGX) on cumulative sodium and water balance. *$P<0.05$ for RDNX–CGX vs SHAM; †$P<0.05$ for CGX vs SHAM; ‡$P<0.05$ for CGX vs RDNX–CGX.

to which sympathetic activity to the kidneys, relative to nonrenal vascular beds, contributes to the pathogenesis and maintenance of essential hypertension remains unclear.[27–29] The recent success of catheter-based renal nerve ablation to treat drug-resistant hypertension supports the concept that the kidneys are a key target of the sympathetic nervous system in hypertension, but it is still not clear whether other vascular beds are also important.

The present study was designed to address this question by measuring the cardiovascular and fluid balance responses to targeted ablation of sympathetic nerves to the kidneys and
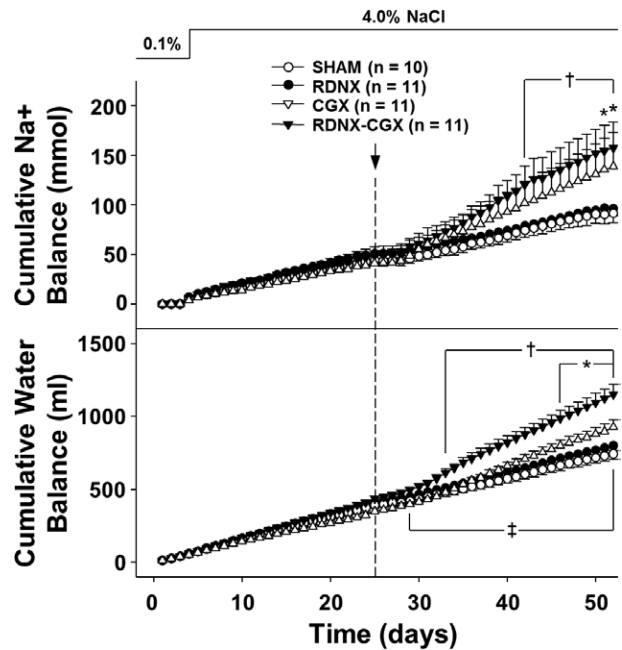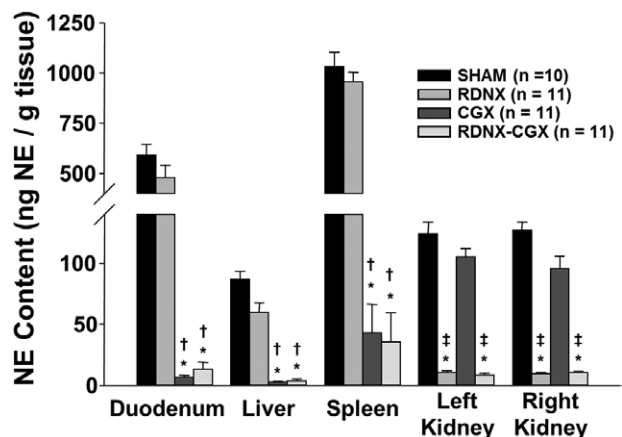

Figure 4. Effect of sham surgery (SHAM), renal denervation (RDNX), celiac ganglionectomy (CGX), and combined celiac ganglionectomy and renal denervation (RDNX–CGX) on norepinephrine (NE) content in the duodenum, liver, spleen, left kidney, and right kidney. *$P<0.05$ vs SHAM; †$P<0.05$ vs RDNX; ‡$P<0.05$ vs CGX.

the splanchnic vascular bed, separately and in combination, in Dahl S rats. The main findings of this study were RDNX decreased arterial pressure independent of changes in sodium and water balance; CGX decreased arterial pressure to a similar magnitude as RDNX, despite compensatory increases in HR and sodium and water balance; and combined RDNX and CGX induced the greatest fall in arterial pressure, suggesting that the responses to RDNX and CGX are mediated by separate mechanisms. Overall, these results suggest that targeted sympathetic ablation is an effective treatment for established hypertension in the Dahl S rat and that both the renal and splanchnic vascular beds are important sympathetic targets in this model.

Renal Denervation Partially Reverses Salt-Induced Hypertension in Dahl S Rats

RDNX has no effect on the developmental phase of Dahl S hypertension.[20–22] However, the ability of RDNX to reverse this model of hypertension has not been reported. Compared with SHAM rats, arterial pressure was ≈15 mm Hg lower in RDNX rats throughout the 4-week postprocedure period. The mechanism(s) by which RDNX attenuate(s) any form of hypertension is a subject of great debate. One explanation is that RDNX increases renal sodium and water excretion and causes a subsequent contraction of blood volume[30] by suppressing sympathetically mediated renin secretion and sodium reabsorption. However, we found no differences in daily or cumulative sodium and water balance between SHAM and RDNX rats. This is consistent with our previous reports that RDNX decreases arterial pressure in normotensive Sprague–Dawley rats, independent of sodium balance or renin release.[25,31] It is important to note that RDNX did not affect the salt sensitivity of arterial pressure in Sprague–Dawley rats[25] or Dahl S rats.

Another possibility is that RDNX results in renal vasodilation. Although this hypothesis remains to be tested in the Dahl S rat, it was recently reported that renal nerve ablation in patients with drug-resistant hypertension decreases renal vascular resistance with no change in glomerular filtration rate.[32]

A third possibility is that RDNX attenuates hypertension by ablation of centrally projecting afferent renal nerves, reducing sympathetic activity to nonrenal vascular beds. As Dahl S rats become increasingly hypertensive, renal injury worsens,[33–35] and evidence suggests that kidney disease can drive afferent renal nerve-dependent sympathetically mediated hypertension.[36,37] The progressive nature of this proposed kidney disease–dependent sympathoexcitation may explain the difference in effectiveness of RDNX to reverse, rather than prevent Dahl S hypertension. However, it is important to note that the arterial pressure response to RDNX may not require augmented afferent signaling because it occurs in normotensive Sprague–Dawley rats.[25,31]

Celiac Ganglionectomy Partially Reverses Hypertension in the Dahl S Rat: Possible Role of Blood Volume Redistribution

A novel finding of this study is that CGX decreased arterial pressure in Dahl S rats to a similar magnitude as RDNX. To our knowledge, this is the first study, in any experimental model, demonstrating the ability of CGX to reverse hypertension.

Although the mechanisms by which CGX reversed Dahl S hypertension were not explored, it is likely that, similar to our studies in the angiotensin II–salt model,[12] CGX decreased splanchnic vascular resistance and increased total vascular conductance, both of which would promote a redistribution of blood from the arterial to the highly compliant venous compartment. This hypothesis is in line with our recently published mathematical model of salt-sensitive hypertension in which the distribution of blood volume between a high compliant (ie, splanchnic) and low compliant (ie, kidney) vascular bed is determined by neural input to each vascular bed.[38] Additional experiments will be needed to test this hypothesis.

Sympathetic nerve activity was not measured in this study and, therefore, it remains to be tested whether the responses to CGX were because of decreased sympathetic nerve activity, per se. Alternative explanations include the possibility that ablation of sensory nerves in the gut may affect arterial pressure in nonsympathetically mediated ways (ie, changes in immune system function or circulating levels of vasopressin). However, we have reported in other models that CGX decreases nonhepatic splanchnic norepinephrine spillover[39] and increases total vascular capacitance.[12] These findings combined with measurements of tissue NE in the present study are most consistent with the idea that CGX reduces sympathetic input to splanchnic vasculature resulting in decreased arterial pressure. This hypothesis remains to be tested by studies of the splanchnic hemodynamic responses to CGX in the Dahl S rat.

The magnitude and time course of the arterial pressure response to CGX was nearly identical to RDNX, suggesting these procedures may act via a common pathway. However, the responses of the other variables suggest that RDNX and CGX reduced arterial pressure by separate mechanisms. Specifically, CGX resulted in marked tachycardia and sodium and water retention compared with SHAM and RDNX rats. These responses are consistent with activation of compensatory mechanisms to maintain arterial pressure after loss of sympathetic input to the splanchnic vascular bed, which may reduce effective blood volume and, therefore, cardiac output (via increased venous capacitance and reduced atrial filling) and total peripheral resistance.[40] These results are consistent with compensatory increases in both cardiac and renal sympathetic activity after CGX. The greater tachycardic response to RDNX–CGX compared with CGX suggests a baroreflex-mediated increase in cardiac sympathetic activity because the antihypertensive effect of RDNX–CGX was much greater than that of CGX. The blunted sodium and water retention after RDNX–CGX compared with CGX is consistent with the activation of renal sympathetic nerves after CGX. In addition, reduced atrial filling would be expected to inhibit the release of atrial natriuretic peptide promoting sodium retention. The extent to which these mechanisms buffer the arterial pressure response to CGX in Dahl S rats remains to be determined.

Another possibility is that the compensatory HR and fluid balance responses to CGX were secondary to volume depletion attributable to decreased sodium and water absorption from the small intestine because some rats exhibited diarrhea after CGX similar to previous studies using CGX.[41,42] Although we cannot discount this entirely, we do not feel

it was a major contributor to the responses to CGX for several reasons. First, in past studies, we have shown that CGX increases total vascular capacitance but has no effect on absolute blood volume.[12] Second, although some rats exhibited transient diarrhea (1–2 weeks), sodium retention persisted during the 2 to 3 weeks after the cessation of diarrhea. Finally, there was no correlation between final body weight (an index of nutrient absorption) and final arterial pressure in any group (Figure S4). We conclude that the compensatory responses of HR and fluid balance to CGX are secondary to a decrease in effective blood volume rather than a reduction in absolute volume. This hypothesis will be tested in future studies in which we measure the effect of CGX on mean circulatory filling pressure in Dahl S rats.

Additive Effects of Renal and Splanchnic Denervation in the Treatment of Hypertension

Further evidence that RDNX and CGX act via separate pathways is the fact that the combination of these treatments resulted in a greater response than either treatment alone. In addition, based on analysis of tissue norepinephrine content, RDNX had no effect on the duodenum, liver, or spleen, and CGX had no effect on the kidneys. Taken together, we conclude that the renal nerves and splanchnic nerves contribute to the maintenance of Dahl S hypertension independently of one another and, therefore, RDNX and CGX reduce arterial pressure via two distinct mechanisms.

It is worth noting that the compensatory increases in HR and sodium and water retention seen in CGX rats were also observed in RDNX–CGX rats. The increase in HR during the first peak tended to be greater in RDNX–CGX rats than in CGX rats, which is consistent with a baroreflex-mediated response as discussed above. Similarly, sodium retention in RDNX–CGX rats may have resulted from the greater fall in renal perfusion pressure, via the pressure-natriuresis relationship or suppression of plasma atrial natriuretic peptide as discussed above.

Perspectives

Renal nerve ablation has been shown to decrease arterial pressure in some human hypertensives; however, the lack of a method to quantitate the extent of denervation in humans makes it impossible to establish the efficacy of this treatment because failure to respond may be attributable to incomplete denervation or the fact that renal nerves do not contribute to all forms of human essential hypertension. The Dahl S rat is an excellent animal model for preclinical studies to address this issue. The response of arterial pressure to renal denervation in this model suggests that this approach can partially reverse hypertension, but sympathetic nerves to other target organs are also important. Specifically, our study suggests that targeted splanchnic nerve ablation may have an additional therapeutic effect and should be pursued as a possible stand-alone or adjunct treatment for hypertension in the future. Further studies are needed to establish the mechanisms underlying the antihypertensive effects of these interventions.

Acknowledgments

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Novelty and Significance

What Is New?

- Renal denervation (RDNX) and celiac ganglionectomy (CGX) both decrease arterial pressure in hypertensive Dahl S rats.
- CGX and RDNX–CGX, but not RDNX, cause increases in heart rate as well as sodium and water retention.
- RDNX–CGX has an additive antihypertensive effect.
- The responses to RDNX and CGX are mediated by different mechanisms.

What Is Relevant?

- RDNX decreased arterial pressure to a magnitude similar to that reported in humans.

- CGX decreased arterial pressure as has been reported in human hypertensives.

Summary

The results of this study suggest that both the renal and splanchnic nerves contribute to the maintenance of Dahl S hypertension and that targeted ablation of the splanchnic sympathetic nerves should be considered as treatment option for hypertensive patients.

ONLINE SUPPLEMENT

REVERSAL OF GENETIC SALT-SENSITIVE HYPERTENSION BY
TARGETED SYMPATHETIC ABLATION

Jason Foss[1], Gregory D. Fink[2] and John W. Osborn[1]

[1]University of Minnesota
Department of Integrative Biology and Physiology
Minneapolis, MN

[2]Michigan State University
Department of Pharmacology and Toxicology
East Lansing, MI

short title: Targeted sympathetic ablation in the Dahl S rat

Corresponding Author:

John W. Osborn, Ph.D.
University of Minnesota
Department of Integrative Biology and Physiology
Rm 6-125 Jackson Hall
Minneapolis, MN 55455
Ph: 612-624-3074
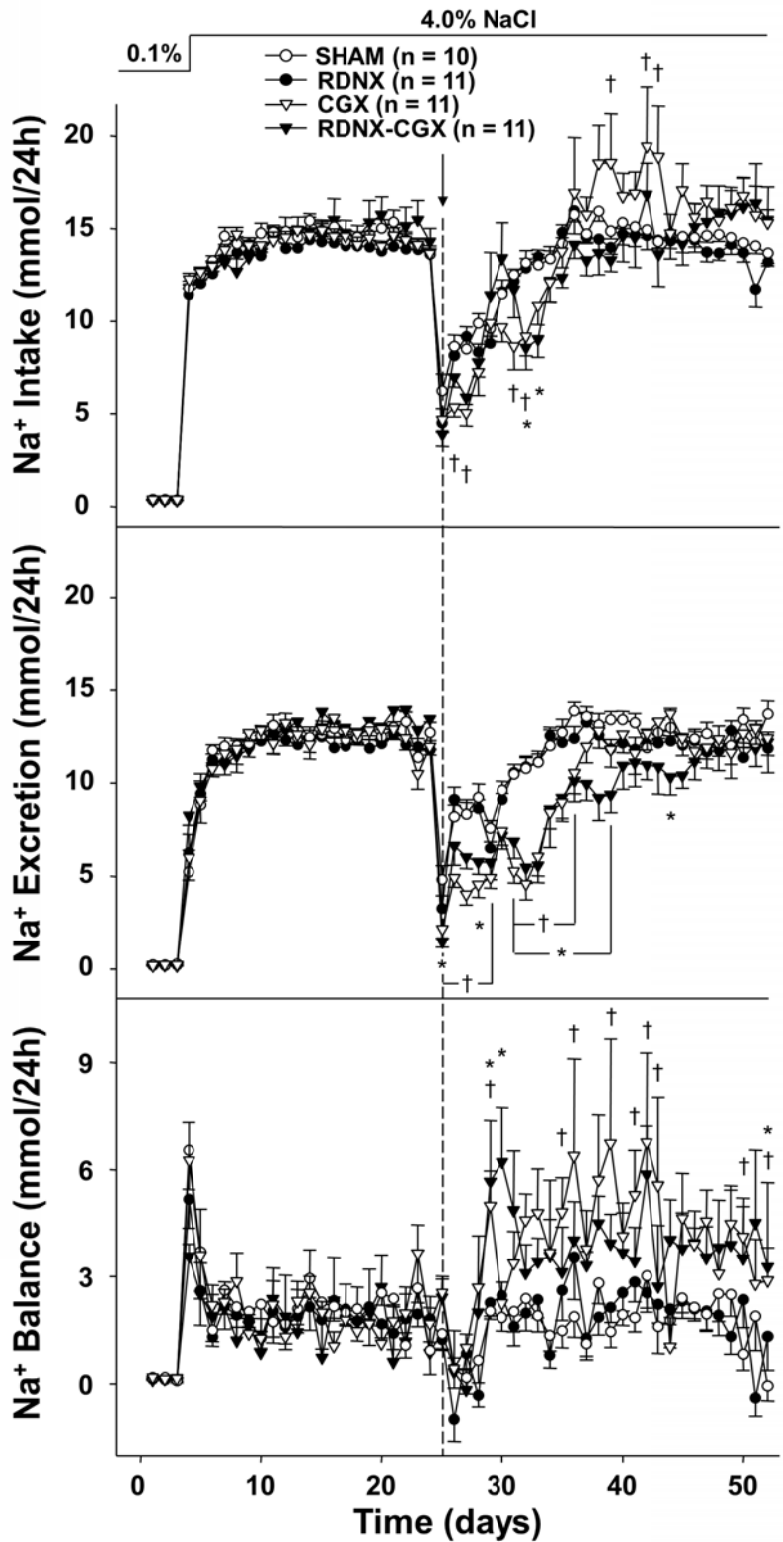Fax: 612-625-5149
Email: osbor003@umn.edu

Figure S1. Effect of SHAM, RDNX, CGX and RDNX-CGX on sodium intake, excretion and balance. * = p < 0.05 for RDNX-CGX vs. SHAM. † = p < 0.05 for CGX vs. SHAM. ‡ = p < 0.05 for RDNX vs. SHAM.
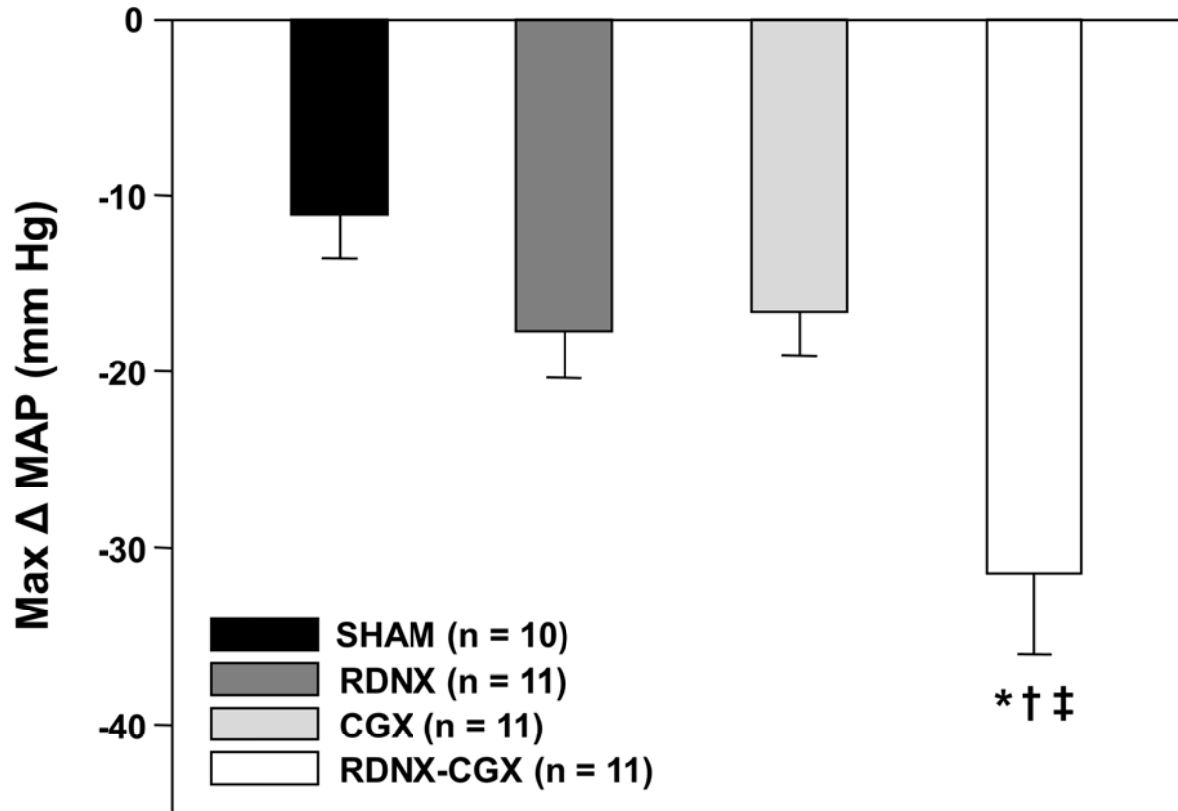
Figure S2. Maximum arterial pressure response to SHAM, RDNX, CGX and RDNX-CGX (Max ΔMAP; the lowest MAP following surgery minus MAP on the day before surgery). * = $p < 0.05$ vs. SHAM. † = $p < 0.05$ vs. RDNX. ‡ = $p < 0.05$ vs. CGX.
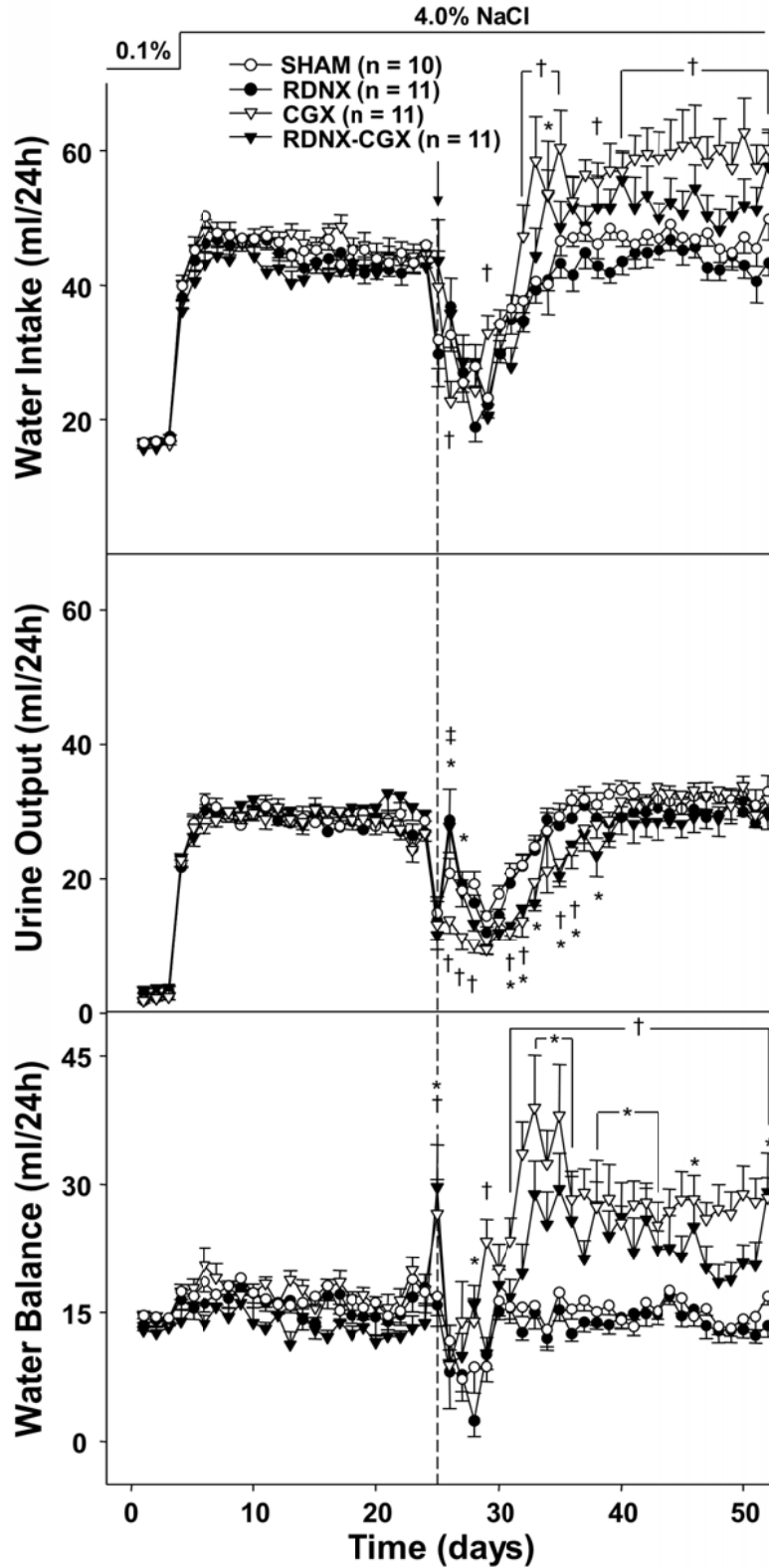
Figure S3. Effect of SHAM, RDNX, CGX and RDNX-CGX on water intake, urine output and water balance. * = p < 0.05 for RDNX-CGX vs. SHAM. † = p < 0.05 for CGX vs. SHAM. ‡ = p < 0.05 for RDNX vs. SHAM.
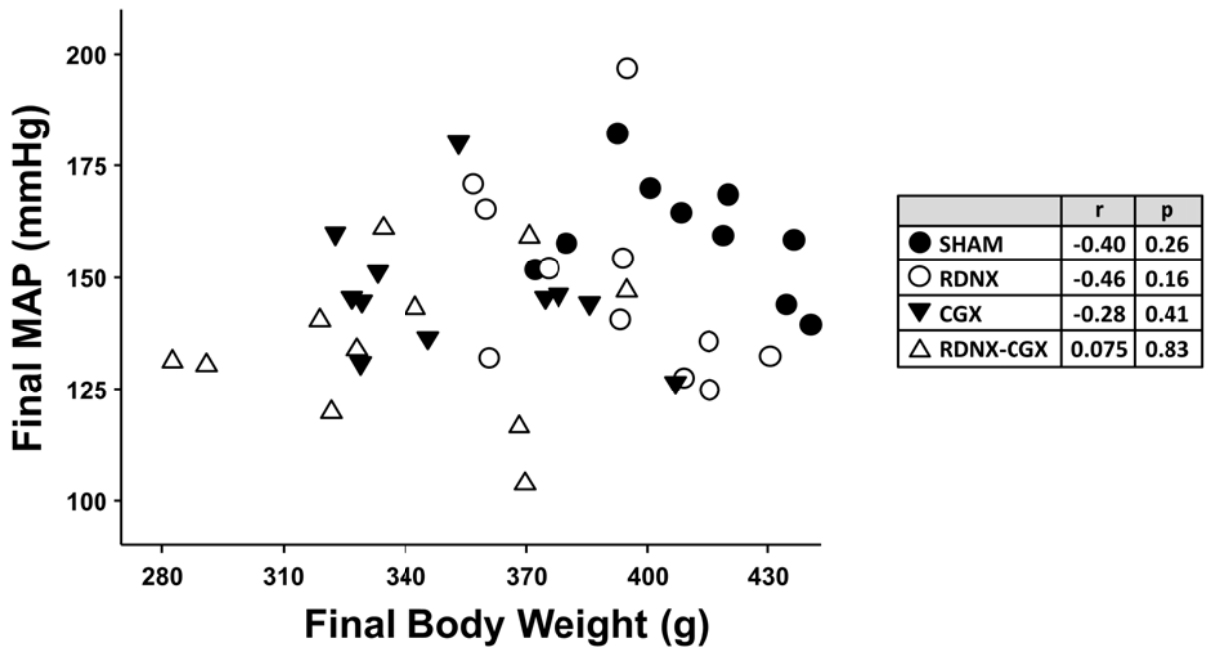
Figure S4. Final MAP vs. final body weight. No correlation exists between final MAP and final body weight in any group.