Splanchnic Circulation Is a Critical Neural Target in Angiotensin II Salt Hypertension in Rats

Andrew J. King, John W. Osborn, Gregory D. Fink

Abstract—Chronic angiotensin II (Ang II) infusion, in rats fed high salt, engages the sympathetic nervous system to increase venomotor tone. The splanchnic sympathetic nervous system is the most important regulator of venous tone, indicating that splanchnic sympathetic nervous system activity may be increased in Ang II salt hypertension. We hypothesized that celiac ganglionectomy (CGx), to selectively disrupt sympathetic innervation to the splanchnic circulation, would attenuate arterial pressure (AP), and venous tone increases in Ang II salt hypertension. Rats fed 2% or 0.4% NaCl were instrumented to allow AP measurement by radiotelemetry at the same time as surgical CGx or sham operation. Ang II was delivered by minipump (150 ng/kg per minute) for 14 days. CGx reduced AP independent of salt diet during control. CGx markedly attenuated Ang II hypertension in rats on 2% NaCl but had little effect in rats fed 0.4% NaCl. To test the possibility that CGx exerted its effects via renal denervation, rats were subjected to the same protocol but received selective bilateral renal denervation. Renal denervation decreased AP during control but had no protective effect on Ang II hypertension and actually tended to exacerbate the pressor response. Finally, separate groups of rats underwent CGx or sham operation and were instrumented to allow repeated measures of mean circulatory filling pressure, an index of venous tone. In addition to attenuating Ang II salt hypertension, CGx completely prevented Ang II salt-induced increases in mean circulatory filling pressure and substantially attenuated depressor responses to acute ganglion blockade. We conclude that, in the presence of high salt, Ang II activates the splanchnic sympathetic nervous system to increase venomotor tone and AP. (Hypertension. 2007;50:547-556.)

Key Words: angiotensin II • sympathetic nervous system • splanchnic circulation • venomotor tone • renal denervation

We have shown recently, using repeated measures of mean circulatory filling pressure (MCFP) in conscious undisturbed rats, that chronic infusion of angiotensin II (Ang II), only when administered in combination with a high-salt diet, activates the sympathetic nervous system (SNS) to increase venomotor tone.¹ This increase in venomotor tone may contribute to the pathogenesis of Ang II salt hypertension by increasing central blood volume, resulting in a translocation of blood from the highly compliant venous system to the less compliant arterial circulation.¹ This redistribution of blood volume and the well-documented impairment of renal excretory function caused by Ang II would be major factors in increasing arterial pressure (AP) in this model.²–⁵

Splanchnic veins and venules account for most of the active capacitance responses in the circulation and are richly innervated by the SNS.⁶–⁸ In fact, it has been estimated that innervation to the nonhepatic splanchnic organs accounts for half of the total norepinephrine (NE) released in the entire body.⁹ Therefore, our recent observations in Ang II salt hypertension of neurogenically mediated increases in whole body venous tone would best be explained by increased SNS activity to the splanchnic circulation.¹ Indeed, this is consistent with the previously reported finding of significant increases in splanchnic nerve activity, as assessed by direct nerve recordings, in conscious rats during chronic Ang II infusion.¹⁰ Together these findings indicate the splanchnic circulation may be an important peripheral target for Ang II-mediated sympathoactivation in experimental hypertension. Vascular resistance increases in the hepatosplanchnic circulation before any other bed in humans with borderline hypertension.¹¹ Therefore, increased sympathetic activity to the splanchnic circulation may represent a common stage in the development of hypertension.

The purpose of this study was to investigate the role of sympathetic nerve activity to the splanchnic circulation in the pathogenesis of Ang II hypertension in the rat. In particular, the importance of the splanchnic SNS to increases in AP and whole body venous tone in Ang II salt hypertension was assessed. Approximately 95% of sympathetic postganglionic neurons innervating this vascular bed in the rat have their cell bodies in the celiac and superior mesenteric ganglia.¹²–¹⁴
These 2 ganglia are fused in the rat and are commonly referred to as the celiac or solar plexus. We used surgical ablation of this plexus (celiac ganglionectomy [CGx]) to investigate our hypothesis. Specifically we tested the hypothesis that CGx will attenuate increases in whole body venous tone and AP during Ang II infusion only in rats fed a high-salt diet. We previously demonstrated neurogenetically mediated increases in whole body venous tone in response to Ang II only in the setting of high dietary salt intake. Importantly, ≈70% of renal postganglionic neurons are localized to the paravertebral chain ganglia in rats and, therefore, should be spared by CGx. However, the importance of the renal nerves in various experimental models of hypertension, including Ang II hypertension, has been reported previously.

Therefore, to thoroughly assess the possibility that CGx was exerting its effects via renal denervation, separate groups of rats received selective bilateral renal denervation (RDx). The effect of CGx and RDx on chronic Ang II hypertension was initially determined by instrumenting rats with radiotelemetry transmitters to allow remote monitoring of AP. We then used repeated MCFP in conscious, undisturbed rats to investigate the effect of CGx on venomotor tone changes in chronic Ang II–induced hypertension. MCFP is the pressure measured in the vasculture immediately after cardiac arrest, after pressures in all parts of the circulation are made to equilibrate, and represents the effective driving force for venous return to the heart. The major determinants of MCFP are compliance of the venous system and blood volume, and MCFP is considered the best methodology for determination of body venous tone.

Methods

Animals

All of the protocols were approved by the Michigan State University All University Committee on Animal Use and Care. Male Sprague-Dawley rats (Charles River Laboratories), weighing 225 to 250 g at the beginning of the study, were allowed free access to either a 0.4% or 2% NaCl diet (Research Diets) and distilled water for 7 days before surgery. During this time, rats were housed 3 per cage in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. A total of 16 groups of rats were initially studied to determine the effect of CGx and RDx on Ang II hypertension. 8 of which were fed 2% NaCl, whereas the other 8 were fed a 0.4% NaCl diet. For both salt diets, the groups studied to assess the effects of CGx were as follows: CGx + Ang II (n = 8), sham + Ang II (n = 8), CGx + vehicle (n = 4), and sham + vehicle (n = 4). To assess the effect of RDx, the following groups were studied in rats consuming each diet: RDx + Ang II (n = 8), sham + Ang II (n = 8), RDx + vehicle (n = 4), and sham + vehicle (n = 4). In all of the groups, AP was measured by radiotelemetry. Four additional groups of rats (n = 7 per group) were studied to determine the effect of CGx on Ang II–mediated changes in venous tone. These rats were instrumented with exteriorized catheters, and MCFP was measured in sham and CGx rats fed either 0.4% NaCl or 2% NaCl.

Surgery

CGx, RDx, and Radiotelemetry Implantation

The first 16 groups of rats underwent CGx, bilateral RDx, or sham operation and were instrumented with a radiotelemetry transmitter (TA11PAC40, Data Sciences International) to measure AP. General anesthesia was induced in an induction chamber using 4% isoflurane in oxygen and maintained by 2% isoflurane in oxygen delivered by nose cone. All of the surgeries were performed by a ventral midline laparotomy using an aseptic technique. CGx was performed by locating the celiac plexus in between the aorta, celiac artery, and cranial mesenteric artery; dissecting it free from surrounding tissue; and removing it. Any additional nerves along these vessels in the area of the celiac ganglion were also dissected free and transected. Bilateral RDx was performed using established methods in the rat. Briefly, the renal vessels were exposed, and all of the visible nerves, fat, and connective tissue were removed. The renal vessels were then painted with 10% phenol. Sham operation was performed by exposing and visualizing the celiac plexus or renal vessels, respectively. The body of the radiotelemetry transmitter was placed in the abdominal cavity before closure of the abdomen. The catheter of the transmitter was tunneled subcutaneously to the left medial thigh region where the tip was inserted into the abdominal aorta via the femoral artery.

Antimicrobial prophylaxis and postoperative analgesia were achieved by administration of ticarcillin-clavulanate (200 mg/kg IP) and enrofloxacin (5 mg/kg IP) and buprenorphine (0.05 mg/kg SC), respectively. Rats recovered from anesthesia, under close observation, on a heating pad. Rats were then housed in individual plastic cages placed on top of a radiotelemetry receiver (RPC-1, Data Sciences International). Meloxicam (1 mg/kg PO) was administered daily for 3 days for additional analgesia.

Catheterization

The 4 additional groups of rats used to study the effect of CGx on Ang II–mediated changes in venous tone underwent CGx or sham operation as described above and were allowed to recover for 7 days. Under isoflurane anesthesia, rats were then instrumented to allow repeated measures of MCFP in conscious undisturbed animals using the methods that we have published previously. Briefly femoral arterial and venous catheters were implanted to allow measurement of AP and central venous pressure (CVP), respectively. A right atrial balloon catheter was advanced from the right jugular vein so that inflation of the balloon produced brief circulatory arrest to allow MCFP measurements. The ends of all of the catheters were tunneled subcutaneously and exited the rat between the scapulae into a stainless steel spring attached to the rat by a loosely fitted rubber jacket (Instech Solomon). Antimicrobial prophylaxis and postoperative analgesia were achieved by administration of ticarcillin-clavulanate (200 mg/kg IV) and enrofloxacin (5 mg/kg IV) and buprenorphine (0.05 mg/kg SC), respectively. The rats were then loosely tethered in individual plastic cages to allow continuous access to all of the catheters without handling or disturbing the rat. Ticarcillin-clavulanate (200 mg/kg IV) and enrofloxacin (5 mg/kg IV) were administered daily for the duration of the experiment. Vascular catheters were flushed with heparin saline each day, and the balloon catheter was briefly inflated daily to prevent adhesions to the atrial wall.

Hemodynamic Measurements

Radiotelemetry

In rats instrumented with radiotelemetry transmitters, AP and heart rate (HR) were monitored remotely using a commercially available radiotelemetry data acquisition program (Dataquest ART 3.1, Data Sciences International). Hemodynamic measurements were sampled for 10 seconds every 10 minutes for the duration of the experiment. Data are reported as 24-hour averages.

Exteriorized Catheter

In rats instrumented for MCFP measurements, hemodynamic measurements were made as described previously. Briefly, systolic, diastolic, and mean AP (MAP) and HR were recorded at the same time each morning for 20 minutes by connecting the exteriorized catheters to pressure transducers that were connected to digital pressure monitors (Digil-Med BPA-400, Micro-Med) that linked to a computerized data acquisition program (DM81–400, Micro-Med). The pressure transducers were calibrated daily against a column of water.
MCFP measurements were made according to established methods for the rat and as we have published previously. Briefly, the right atrial balloon catheter was inflated with 0.25 mL of saline for 5 seconds, resulting in a rapid fall in AP and a simultaneous rise in central venous pressure, both of which quickly plateau. MCFP was computed from arterial plateau pressure (APP) and venous plateau pressure (VPP) using the following formula:

$$ \text{MCFP} = \text{APP} + (\text{APP} - \text{VPP}) / 60 $$

Hematocrit (Hct) was measured in duplicate from an arterial blood sample. Plasma volume (PV) was estimated with the use of the 10-minute distribution volume of Evans Blue dye, and blood volume (BV) was computed with the following formula:

$$ \text{BV} = \text{PV} / [1 - \text{Hct} (0.8) / 100] $$

Experimental Protocols

Radiotelemetry

After 10 days of recovery following surgery and a 4-day control period, an Ang II or physiological saline-filled osmotic minipump (2ML2, Alzet) was implanted subcutaneously to deliver Ang II (150 ng/kg per minute) or vehicle for 14 days. This dose of Ang II, when administered subcutaneously via osmotic minipump, has been shown to increase plasma Ang II levels ~2-fold and to result in plasma concentrations within the pathophysiological range. During the entire experimental protocol, rats were allowed free access to either 0.4% NaCl or 2% NaCl diet and distilled water.

Exteriorized Catheter

Four days of recovery were allowed after catheterization followed by a 3-day control period. Ang II was then delivered subcutaneously by minipump (150 ng/kg per minute) for 14 days. MCFP was measured in duplicate before and starting 5 minutes after acute ganglion blockade with hexamethonium (30 mg/kg IV) on control day 2 and Ang II infusion on days 1, 3, 7, and 14. Each measurement of MCFP was taken 10 minutes after the previous one. Hct and PV were also measured on these days, ~6 hours after completion of the MCFP measurements. During the entire experimental protocol, rats were allowed free access to either a 0.4% NaCl or 2% NaCl diet and distilled water.

Confirmation of Denervation

On completion of the experimental period, the rats were euthanized with an intraperitoneal injection of pentobarbital (100 mg/kg). Liver, spleen, small intestine, and both kidneys were collected from each animal, immediately frozen in liquid nitrogen, and stored at −80°C for later analysis. The efficacy of CGx and bilateral RDx was assessed by measuring NE content of the tissue samples by high-performance liquid chromatography analysis with electrochemical detection. Data are reported as nanograms of NE per gram of tissue.

Statistical Analysis

The effect of CGx and RDx during the control period was assessed by Student t test comparing the control period average to the respective sham group. Between-group differences over time were assessed by a 2-way mixed-design ANOVA, and posthoc testing at each time point was performed using Bonferroni’s procedure to correct for multiple comparisons (GraphPad Prism 4). Between-group tissue NE content differences were assessed by a 1-way ANOVA and posthoc multiple comparisons with Tukey’s test. A P < 0.05 was considered significant. All of the results are presented as mean ± SE.

Results

Radiotelemetry

The effect of CGx on MAP response to chronic subcutaneous infusion of Ang II (150 ng/kg per minute) or saline vehicle in rats implanted with radiotelemetry transmitters is shown in Figure 1. During the control period, CGx rats had reduced MAP while eating 2% NaCl (sham: 102 ± 1 mm Hg; CGx: 95 ± 2 mm Hg; P < 0.05) and 0.4% NaCl diets (sham: 99 ± 1 mm Hg; CGx: 93 ± 1 mm Hg; P < 0.05). The hypotensive effect of CGx persisted for the duration of the experiment in the vehicle-infused groups on both salt diets. CGx markedly attenuated the hypertensive response to Ang II in rats on a 2% NaCl diet. Sham rats increased MAP 45 ± 6 mm Hg by day 14 of Ang II infusion, compared with only 19 ± 7 mm Hg in CGx rats (P < 0.05). However, CGx had little effect on the hypertensive response to Ang II in rats fed 0.4% NaCl. MAP increased by 14 ± 3 mm Hg in sham rats and 12 ± 5 mm Hg in CGx rats by day 14 of Ang II infusion.

The effect of RDx on MAP response to chronic subcutaneous infusion of Ang II or saline vehicle is shown in Figure 2. Similar to the effect observed with CGx, and consistent with previous reports, bilateral RDx lowered MAP in normotensive rats independent of salt diet. During the control period, MAP was lower in RDx rats fed 2% NaCl (sham: 103 ± 1 mm Hg; RDx: 97 ± 2 mm Hg; P < 0.05) and in rats fed 0.4% NaCl (sham: 101 ± 1 mm Hg; RDx: 96 ± 2 mm Hg; P < 0.05). This hypotensive effect of RDx persisted for the duration of the experiment in the vehicle-infused groups on
both salt diets. In contrast to the effect of CGx on the hypertensive response to Ang II in rats on a 2% NaCl diet, RDx had no protective effect. MAP increased in sham rats by 25/10 mm Hg compared with 31/5 mm Hg in RDx rats on day 14 of Ang II infusion. In fact, over the first 5 days of Ang II infusion, RDx seemed to exacerbate the pressor response to Ang II. Similarly, RDx had no protective effect on the Ang II-mediated increase in MAP in rats fed a 0.4% NaCl diet. Indeed, RDx tended to exacerbate the pressor response to Ang II in this group, such that MAP increased in RDx rats by 21/7 mm Hg compared with only 8/3 mm Hg in sham rats on day 14 of Ang II infusion; however, this failed to reach statistical significance (*P* > 0.05).

CGx did not affect HR during the control period in rats fed 2% NaCl (CGx: 423 ± 10 bpm; sham: 423 ± 8 bpm) or 0.4% NaCl (CGx: 411 ± 8 bpm; sham: 418 ± 6 bpm). In sham rats, HR decreased slightly but significantly (∼25 to 35 bpm) in response to Ang II infusion, and this was not affected by CGx (data not shown). RDx did not significantly affect HR in the control period in rats fed 2% NaCl (RDx: 418 ± 8 bpm; sham: 405 ± 2 bpm) or 0.4% NaCl (RDx: 408 ± 6 bpm; sham: 404 ± 6 bpm). Again, HR tended to decrease during Ang II infusion in sham rats, and RDx did not significantly affect this (data not shown). CGx did not affect body weight at the time of surgery (2% NaCl: sham: 295 ± 6 g, CGx: 286 ± 7 g; 0.4% NaCl: sham: 278 ± 5 g, CGx 278 ± 5 g), osmotic minipump implantation (2% NaCl: sham: 344 ± 10 g, CGx 344 ± 7 g; 0.4% NaCl: sham: 325 ± 10 g, CGx 318 ± 9 g), or completion of the study (2% NaCl: sham: 432 ± 8 g, CGx 426 ± 9 g).

**Exteriorized Catheters**

The effect of CGx on MAP response to chronic subcutaneous infusion of Ang II (150 ng/kg per minute) in tethered rats is shown in Figure 3. Similar to the effect seen when AP was measured by radiotelemetry, CGx caused a significant but mild (∼7 mm Hg) hypotension independent of salt intake during the control period. Consistent with the effect seen when AP was measured by radiotelemetry, CGx had little effect on chronic Ang II hypertension in rats fed 0.4% NaCl but significantly attenuated Ang II hypertension in rats fed 2% NaCl by ∼50%. Interestingly, the magnitude of the increase in AP in response to Ang II infusion was significantly greater in tethered animals compared with radiotelemetry animals by ∼25 mm Hg, although the control period AP was indistinguishable between the AP measurement methods. CGx did not affect HR during the control period in rats fed 2% NaCl (CGx: 387 ± 13 bpm; sham: 370 ± 8 bpm) or 0.4%
NaCl (CGx: 383±10 bpm; sham: 376±8 bpm) and did not affect the transient decrease in HR in response to Ang II infusion (data not shown).

Figure 4 shows MCFP and BV responses to chronic subcutaneous infusion of Ang II in sham and CGx-operated rats measured on control day 2 and Ang II infusion days 1, 3, 7, and 14. Surprisingly, in the control period, BV and MCFP were unaffected by CGx in rats fed either 0.4% or 2% NaCl. There were no statistically significant changes in BV from the control period in response to Ang II infusion in any group. Consistent with our previous study,1 MCFP was unchanged in response to Ang II infusion in sham animals fed 0.4% NaCl; however, there was a significant and marked increase in MCFP (~3 mm Hg) for the duration of Ang II infusion in sham rats fed a 2% NaCl diet. CGx had no effect on MCFP responses to Ang II in rats fed 0.4% NaCl but completely prevented MCFP increases in animals fed 2% NaCl.

The peak fall in MAP in response to acute ganglion blockade with hexamethonium (30 mg/kg IV) is shown in Figure 5 along with the fall in MCFP 5 minutes after hexamethonium administration. CGx did not alter peak depressor responses to ganglion blockade in animals fed 2% NaCl during the control period. However, quite surprisingly, CGx significantly enhanced the peak depressor response to hexamethonium in rats fed 0.4% NaCl during this control period compared with sham animals. Consistent with our previous work,1 depressor responses to ganglion blockade were not changed during Ang II infusion in sham rats fed 0.4% NaCl; CGx did not affect this. However, there were significant and marked increases in MAP depressor response to hexamethonium during Ang II infusion in rats fed a 2% NaCl diet. This increase was significant on day 1 of Ang II infusion, remained statistically significantly increased on day 3, and was further increased on days 7 and 14 of Ang II infusion. Remarkably, CGx clearly attenuated (but did not completely abolish) these increased falls in MAP in response to hexamethonium administration. Again, consistent with our previous published work,1 the fall in MCFP 5 minutes after ganglion blockade was enhanced during Ang II infusion in sham animals fed 2% NaCl but not in sham animals fed 0.4% NaCl. This increased sympathetic component contributing to the elevated MCFP in sham rats fed 2% NaCl was completely prevented by CGx.

Verification of Denervation
Tissue NE content of the abdominal organs, verifying successful CGx and bilateral RDx, is presented in Figure 6. First it should be noted that Ang II infusion alone affected tissue NE content. In sham-operated rats fed a 2% NaCl diet, Ang II infusion alone significantly (P<0.05) decreased NE content of the left and right kidney and spleen compared with vehicle by ~36%, 42%, and 30%, respectively. Small intes-
tine and liver NE was unaffected by Ang II infusion in sham rats fed 2% NaCl. In sham rats fed a 0.4% NaCl diet, Ang II infusion also tended to decreased NE content of both kidneys and spleen. However, this decrease was only statistically significant ($P < 0.05$) in the right kidney and spleen of the sham control group for CGx. Small intestine and liver NE was unaffected by Ang II infusion in sham rats fed 0.4% NaCl.

Surgical CGx and RDx caused consistent and predictable decrements in tissue NE content. In rats fed a 2% NaCl diet, CGx resulted in a significant ($P < 0.05$) reduction in NE content of the left and right kidneys, spleen, small intestine, and liver by $\approx 50\%$, $31\%$, $98\%$, $76\%$, and $95\%$, respectively. In rats fed a 0.4% NaCl diet, CGx resulted in a significant ($P < 0.05$) reduction in NE content of the left and right kidneys, spleen, small intestine, and liver by $\approx 61\%$, $60\%$, $92\%$, $64\%$, and $86\%$, respectively. In rats fed a 2% NaCl diet, RDx resulted in a significant ($P < 0.05$) reduction in NE content of the left and right kidneys by $\approx 90\%$ and $85\%$, respectively, but did not affect NE content of the spleen, small intestine, or liver. Similarly, in rats fed a 0.4% NaCl diet, RDx resulted in a significant ($P < 0.05$) reduction in NE content of the left and right kidneys by $\approx 94\%$ and $85\%$, respectively, but did not affect NE content of the spleen, small intestine, or liver.

**Figure 5.** Hemodynamic response to acute ganglion blockade with hexamethonium (30 mg/kg IV). Peak fall in MAP in sham-operated or CGx rats fed either a 2% (A) or 0.4% (B) NaCl and decline in MCFP measured 5 minutes after hexamethonium in rats fed either a 2% (C) or 0.4% (D) NaCl. *Significant difference ($P < 0.05$) control period of CGx compared with sham. #Significant difference ($P < 0.05$) between specific Ang II infusion day of CGx and sham rats.

**Discussion**

The main new finding of this study is that selective removal of sympathetic innervation to the splanchnic circulation markedly attenuated Ang II salt hypertension in the rat. This novel result was verified using 2 different techniques, radio-telemetry and exteriorized catheters, to measure AP directly. The observation that CGx attenuated the hypertensive response to Ang II only in the presence of a high-salt diet supports previous conclusions that the increment in hypertensive response to Ang II seen in rats on a high-salt diet is sympathetically driven.1-34 In contrast, bilateral RDx had no protective effect on Ang II hypertension. In fact, RDx tended to exacerbate the hypertension, especially in rats on a normal salt diet. This is consistent with previously reported baroreflex-mediated decreases in renal nerve activity in Ang II hypertension,35-38 because a sustained decrease in renal nerve activity should reduce the hypertensive response to Ang II infusion.38 Therefore, the lack of a protective effect of RDx in this model is not surprising.

CGx not only significantly attenuated Ang II salt-mediated increases in AP but also moderated enhanced AP responses to hexamethonium and completely abolished the neurogenically mediated increase in MCFP seen in Ang II salt hypertension. In the absence of a change in blood volume, increases in MCFP represent an increase in venomotor...
In the present study, blood volume was unchanged. Together, this suggests that in the presence of a high-salt diet, Ang II activates the splanchnic SNS to increase venomotor tone and AP.

To fully characterize the effect of CGx, the splanchnic organs were harvested immediately after completion of the experimental period, and tissue NE content was measured as an index of sympathetic denervation. CGx almost completely abolished NE in the spleen and liver and caused a marked reduction in small intestinal NE. These findings verify successful sympathetic denervation to the splanchnic bed. CGx also reduced renal NE content by 30% to 60%. The renal nerves have been implicated previously in the pathogenesis of various forms of experimental hypertension, including Ang II hypertension. Therefore, selective bilateral RDx was a critical control group to determine whether CGx could be exerting its effects by RDx. Because RDx did not attenuate the hypertension in response to Ang II infusion, it is clear that CGx was not exerting its antihypertensive effects via disruption of renal sympathetic innervation. Anatomically in the rat, the renal nerves are in close proximity to the celiac plexus, and the liberal use of phenol to destroy the renal nerves could inadvertently damage the celiac plexus, disrupting splanchnic sympathetic innervation. Other studies using RDx to investigate the roles of the renal nerves in rat models of hypertension did not include comprehensive evaluation of the effect of RDx on nonrenal splanchnic innervation. Therefore, it is not clear whether some of the effects attributed previously to RDx could have been the result of CGx. This is the first study to exclude an effect of bilateral RDx on tissue NE content in nonrenal splanchnic organs.

The differential effects of CGx and RDx also suggest that SNS activation in response to Ang II, in rats fed a high-salt diet, is regionally heterogeneous. Regionalized sympathetic activation has been convincingly demonstrated by direct nerve recordings in rabbits, and Esler’s group has elucidated the importance of regionalized sympathetic activation in human cardiovascular disease by using NE spillover techniques. The finding in this study that CGx markedly attenuated enhanced depressor responses to ganglion blockade during Ang II salt hypertension implies that the majority of the sympathetic activation in this model is directed toward the splanchnic bed and supports the possibility of regionalized sympathetic activation. Although our results are consistent with differential sympathetic activation, this hypothesis needs to be tested directly using the complementary methods of direct sympathetic nerve recordings and regional NE spillover measurements.

The demonstration of the importance of splanchnic SNS activity to the pathogenesis of hypertension in this study may explain the historic success of surgical thoracolumbar splanchnicectomy in prolonging survival times in patients with essential hypertension, in particular, those refractory to medical management. Interestingly, a recent study showed that poorly controlled essential hypertension in human patients was markedly improved in patients after bilateral T3

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**Figure 6.** Tissue NE content (nanograms of NE/gram tissue) in the splanchnic organs in response to CGx in rats fed 2% NaCl (A) or 0.4% NaCl diet (B) and RDx in rats fed 2% NaCl (C) or 0.4% NaCl diet (D). *Significant difference (P<0.05) compared with sham vehicle-infused rats. #Significant difference (P<0.05) compared with sham Ang II–infused rats.
endoscopic sympathetic block. Although the mechanism of the effect is unclear, it is possible that the beneficial effects are mediated through inhibition of splanchnic sympathetic nerve activity.

Another new finding in this study is that, similar to bilateral RDx, CGx chronically lowers AP independent of salt intake in normotensive rats during the control period. Surprisingly, this hypotension was not associated with a concurrent reduction in MCFP. Therefore, it seems that the hypertensive effect of CGx during the control period is not mediated through changes in whole body vascular capacitance. The fall in basal MAP could be a result of changes in the resistance bed of the splanchnic circulation. In addition, although the fall in MCFP in response to ganglion blockade was slightly less in CGx animals during the control period, it was not significantly different from sham animals. This indicates that there is nonsplanchnic neural compensation to maintain basal MCFP. However, it seems as though the compensating neural bed responsible for maintaining a normal MCFP after CGx is not activated in Ang II salt hypertension, because MCFP does not rise in these animals. The AP-lowering effects of CGx have also been reported in sheep. Transient but severe hypotension and orthostatic hypotension are also documented adverse effects of celiac plexus neurolysis for the treatment of pancreatic malignancies in humans. This emphasizes the importance of SNS innervation to the splanchnic vascular bed in the control of blood pressure under normal conditions.

Other than mild hypotension, CGx was well tolerated in this study, and no other adverse effects were observed. The metabolic effect of selective removal of splanchnic innervation in rats has previously been comprehensively evaluated. Bilateral splanchic nerve section caused no obvious behavioral signs of distress and no differences in body weight, daily food intake, abdominal fat, brown adipose tissue weight, abdominal organ weight, plasma leptin, or hypothalamic neuropeptide Y content compared with sham-operated control rats. Experimental evidence also indicates that the chronic extrinsic denervation of the small intestine does not affect net intestinal absorption of water and electrolytes. We have also shown that CGx does not affect Na^+ intake in rats fed 2% NaCl.

The exact hemodynamic mechanism by which CGx affects Ang II salt hypertension is still not completely clear and needs to be investigated further by chronic instrumentation of rats for measurements of cardiac output and total peripheral resistance determination. Neurogenically mediated increases in venous tone during Ang II salt hypertension, which are completely prevented by CGx, may contribute to the pathogenesis of hypertension in this experimental model by causing a venous-to-arterial translocation of blood volume without a change in total vascular volume. The arterial circulation in the rat is ~60 times less compliant than the venous system. A venous-to-arterial translocation of only a small volume of blood, in the presence of an impairment in renal excretory function, could significantly increase AP. This increase in AP may initiate a series of changes in the arterial system, including increased myogenic vascular tone and expression of voltage gated calcium channels, that facilitates the maintenance and progression of elevated AP by increasing TPR. CGx, which prevents the observed neurogenically mediated venoconstriction in the Ang II salt model, may prevent the venous-to-arterial translocation of blood and subsequent increase in TPR. However, in addition to removal of splanchnic venous sympathetic innervation, CGx also removes sympathetic arterial innervation, and it is therefore unclear which has the greatest hemodynamic importance. Retrograde labeling experiments have shown that the majority of neurons in the celiac ganglion supplying the vasculature dually innervates veins and arteries. In fact, only 5% of neurons solely innervate the veins, making it impossible at this time to test our hypothesis using selective venous denervation.

Another finding of this study worth noting is that the hypertensive response to chronic Ang II infusion depends on the AP measurement method, a finding consistent with our unpublished observations and those of others. Another finding of this study worth noting is that the AP measurement method, but the magnitude of the increase in AP in response to Ang II infusion was significantly greater in tethered animals. Exteriorized catheters and tethering have been proposed to introduce a stress on the animal, and from our study it seems as though the stress of tethering, although alone is not sufficient to influence AP, is acting to sensitize the animal to external stimuli, such as Ang II.

**Perspectives**

Compelling evidence indicates that SNS activation may be a common mechanism of hypertension in human essential hypertension and many experimental models. The identification of the splanchnic vascular bed as a critical target in Ang II salt hypertension greatly improves our understanding of peripheral neural mechanisms leading to hypertension. Unveiling the genomic and electrophysiological basis of Ang II salt-induced dysregulation of neurotransmission through the celiac plexus, which allows for increased sympathetic activity to the splanchnic vasculature, may identify novel therapeutic targets for the treatment of hypertension. Centrally acting sympatholytics have been demonstrated to be effective in the treatment of essential hypertension but are poorly tolerated because of their adverse effect profile. A peripheral neural target, such as the celiac plexus, may, therefore, represent a more desirable target for therapeutic intervention.

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**Disclosures**

None.


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