Splanchnic sympathetic nerves in the development of mild DOCA-salt hypertension

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Kandlikar SS, Fink GD. Splanchnic sympathetic nerves in the development of mild DOCA-salt hypertension. Am J Physiol Heart Circ Physiol 301: H1965–H1973, 2011. First published September 2, 2011; doi:10.1152/ajpheart.00086.2011.—We previously reported that mild deoxycorticosterone acetate (DOCA)-salt hypertension develops in the absence of generalized sympathoexcitation. However, sympathetic nervous system activity (SNA) is regionally heterogeneous, so we began to investigate the role of sympathetic nerves to specific regions. Our first study on that possibility revealed no contribution of renal nerves to hypertension development. The splanchnic sympathetic nerves are implicated in blood pressure (BP) regulation because splanchnic denervation effectively lowers BP in human hypertension. Here we tested the hypothesis that splanchnic SNA contributes to the development of mild DOCA-salt hypertension. Splanchnic denervation was achieved by celiac ganglionectomy (CGX) in one group of rats while another group underwent sham surgery (SHAM-GX). After DOCA treatment (50 mg/kg) in rats with both kidneys intact, CGX rats exhibited a significantly attenuated increase in BP compared with SHAM-GX rats (15.6 ± 2.2 vs. 25.6 ± 2.2 mmHg, day 28 after DOCA treatment). In other rats, whole body norepinephrine (NE) spillover, measured to determine if CGX attenuated hypertension development by reducing global SNA, was not found to be different between SHAM-GX and CGX rats. In a third group, nonhepatic splanchnic NE spillover was measured as an index of splanchnic SNA, but this was not different between SHAM (non-DOCA-treated) and DOCA rats during hypertension development. In a final group, CGX effectively abolished nonhepatic splanchnic NE spillover. These data suggest that an intact splanchnic innervation is necessary for mild DOCA-salt hypertension development but not increased splanchnic SNP or NE release. Increased splanchnic vascular reactivity to NE during DOCA-salt treatment is one possible explanation.

regional sympathetic activity; celiac ganglionectomy; nonhepatic splanchnic norepinephrine spillover; whole body norepinephrine spillover; deoxycorticosterone acetate-salt

INCREASED SYMPATHETIC OUTFLOW to peripheral organs is linked to hypertension development (18). Mineralocorticoids have recently been shown to play a larger role in human hypertension than previously appreciated (21), and new evidence suggests that one mechanism is increased sympathetic nervous system activity (SNA) (36). The most commonly studied experimental model of mineralocorticoid hypertension is created by treating uninephrectomized rats with deoxycorticosterone acetate and salt (DOCA-salt rats). There is evidence both for (4, 10, 20) and against (49) increased SNA as a cause of hypertension in DOCA-salt rats. A complication of this model is that the animals become severely hypertensive and develop marked end organ damage over just several weeks’ time. In a previous study, we sought to determine the contribution of SNA to mineralocorticoid hypertension in rats using a modified DOCA-salt model (30). Animals were not subjected to uninephrectomy and were given a lower dose of DOCA. Hypertension development was more gradual, and the final increase in arterial pressure (AP) was only 20–30 mmHg. We have termed this “mild” DOCA-salt hypertension. There were two main reasons for choosing this model over the traditional DOCA-salt hypertension model. First, the magnitude of increase in blood pressure (BP) was modest and more comparable to that observed in the majority of human hypertensive subjects. Second, a smaller and slower increase in BP would be less likely to cause the end organ damage and thus allow us to more readily identify mechanisms that primarily serve as factors causing hypertension rather than those that are consequences of high BP. Our initial findings were that generalized (or global) SNA, measured using whole body norepinephrine (NE) spillover, is not increased during the development of mild DOCA-salt hypertension (30). However, in light of evidence that regionally specific changes in SNA are sufficient to produce hypertension (18, 60), we investigated the influence of renal SNA in the model using renal denervation. We concluded that fully intact renal nerves are not essential for mild DOCA-salt hypertension development, since renal denervation failed to affect the development of hypertension (31).

Previous work from our laboratory has highlighted the importance of vascular capacitance in the pathophysiology of DOCA-salt hypertension (20). This function is largely invested in the venous side of the circulation, particularly in the veins draining the splanchnic organs (20, 57). About 25% of the total blood volume is contained in the highly compliant splanchnic vasculature (35, 50). Splanchnic organs receive most of their sympathetic input from the celiac ganglion plexus (CG), formed by the celiac and superior mesenteric ganglia (26, 54). In the late 1940s and early 1950s, surgical removal of celiac ganglion plexus (celiac ganglionectomy, CGX) performed in humans proved beneficial in attenuating essential hypertension (22, 23). Those findings emphasize the importance of splanchnic SNA in human hypertension. A recent study performed in the ANG II-salt model of experimental hypertension in rats also demonstrated that CGX significantly attenuates hypertension development (35). To investigate a possible role of the splanchnic SNA in the development and maintenance of hypertension in our mild DOCA-salt model, we performed a series of experiments involving CGX and regional and whole body NE spillover measurements. We hypothesized that splanchnic SNA is increased in this model and that CGX would attenuate hypertension development and/or maintenance.
METHODS

Animals

Male Sprague Dawley rats (225–275 g) were used in the experiments. All protocols were approved by the Michigan State University Institutional Animal Care and Use Committee. After arrival, rats were acclimatized for 7 days under controlled temperature and humidity conditions with an alternate 12:12-h light-dark cycle. Rats were allowed free access to water and food.

Mild DOCA-Salt Hypertension

Under isoflurane anesthesia, a DOCA pellet (50 mg/kg) was implanted subcutaneously in one group of rats (DOCA) while the other group (SHAM) underwent sham implantation surgery with both kidneys intact. Both groups received water containing 1% NaCl and 0.2% KCl.

CGX and Radiotelemeter Implantation

Laparotomy was performed under isoflurane anesthesia by a ventral midline incision. After the abdomen was exposed, intestines were retracted to visualize the CG and soaked with warm saline gauze for the entire duration of surgery. The CG was then dissected and removed. Intestines were placed back in the abdominal cavity and lavaged with warm saline. Next, the body of a radiotelemeter was placed in the abdomen, and the attached catheter was tunneled subcutaneously to the groin. The tip of the catheter was then placed in the abdominal aorta through the femoral artery. SHAM-GX surgery was performed by visualizing the CG after laparotomy. After CGX and SHAM-GX, the incision was closed in layers.

Confirmation of Denervation

Rats were euthanized with an intraperitoneal injection of pentobarbital (100 mg/kg) at the end of the experiment. The spleen and kidneys, and sections of small intestine and liver, were harvested from each animal, frozen in liquid nitrogen, and stored at −80°C for later analysis. Tissue NE content of the samples was measured by high-performance liquid chromatography analysis with electrochemical detection. Data are reported as nanograms of NE per gram of tissue.

Catheterization

Femoral vessels. Catheters were implanted in the abdominal aorta and vena cava under 2% isoflurane anesthesia as described previously (20, 34). Rats were allowed to recover for 7 days and had free access to water and food. Catheters were flushed and refilled every day with heparinized saline (100 U/ml). Antimicrobial prophylaxis was achieved by ticarcillin-clavulanate (200 mg/kg ip) and enrofloxacin (5 mg/kg ip) administered daily for the entire duration of the experiment. Postsurgical analgesia was achieved by carprofen (5 mg/kg sc). Meloxicam (1 mg/kg po) was administered daily for three additional days after surgery.

Portal vein. For measuring nonhepatic splanchic NE spillover, an additional catheter was placed in the portal vein for sampling splanchic venous drainage. During the same surgery as for femoral catheterization, a ventral midline incision was made to expose the abdominal cavity. Intestines were retracted to the side to visualize the portal vein. A small branch going into the portal vein was carefully dissected free of connective tissue. A silicone catheter with inner diameter 0.020 in. and outer diameter 0.037 in. (Dow Corning) was placed in the portal vein through this branch such that the tip of the catheter was close to the entry of the portal vein into the liver, without obstructing portal blood flow. The catheter was then tunneled subcutaneously into the back and exteriorized at the neck along with the femoral catheters to be tethered for chronic blood sampling. The abdominal incision was closed in layers.

Hemodynamic Measurements

Whole body and regional NE spillover studies. Hemodynamic measurements were made using previously established methods in our laboratory (20, 34). Briefly, AP was measured by connecting the arterial catheter to a pressure transducer (TDX-300; Micro-Med) that senses changes in AP and relays signals to a digital pressure analyzer (BPA-400; Micro-Med) through a pressure transducer. Mean arterial pressure (MAP), systolic pressure, diastolic pressure, and heart rate (HR) were sampled at a rate of 1,000 Hz. The pressure analyzer was linked to a computer where the data were analyzed by data acquisition software (DMSI-400; Micro-Med). The pressure transducers were calibrated at the beginning of the experiment using a sphygmomanometer and balanced daily against a water column located at the level of the rat’s heart. AP and HR were measured daily for 1 h and recorded as 1-min averages.

CGX. In one study, AP and HR were measured using telemetry. A radiotelemeter (TA11PAC40; Data Sciences International) transmitted signals to an external receiver (RPC-1; Data Sciences International) that then relayed signals to a computerized data acquisition program (Dataquest ART 3.1; DSI). Hemodynamic measurements were sampled at 500 Hz, 24 h a day, with a scheduled sampling interval of 10 s every 10 min for the entire duration of the experiment. Data are reported as 24-h averages.

Whole Body NE Spillover

Whole body NE clearance and spillover were measured by an established method described previously by King et al. (34). Briefly, tracer amounts of Levo-[ring-2,5,6-3H]NE (Perkin-Elmer) were infused intravenously at 0.13 μCi·min⁻¹·kg⁻¹ at the rate of 16 μl/min for 90 min to produce a steady-state plasma concentration of [3H]NE. One milliliter of blood was collected from the arterial catheter after the infusion, and the plasma was stored at −80°C after centrifugation. Plasma NE concentration was determined by batch alumina extraction followed by separation using high-performance reversed-phase liquid chromatography with coulometric detection (ESA Biosciences). Quantification was accomplished using a modified method originally reported by Holmes et al. (25). After chromatographic analysis, the NE fraction was collected and [3H]NE was quantified by liquid scintillation counting. Whole body NE clearance and spillover were calculated using the following formulas (32):

\[
\text{Whole body NE clearance (ml/min)} = \frac{[3H]NE\text{ infusion rate (dpm/min)}}{\text{Steady state [3H]NE (dpm/ml)}}
\]

\[
\text{Whole body NE spillover (ng/min)} = \text{NE clearance (ml/min)} \times \text{plasma NE concentration (ng/ml)}
\]

Nonhepatic Splanchnic NE Spillover

Regional NE spillover was measured by a previously described method (17). Similar to the whole body NE spillover technique, tracer amounts of Levo-[ring-2,5,6-3H]NE (Perkin-Elmer) were infused intravenously at 0.13 μCi·min⁻¹·kg⁻¹ at the rate of 16 μl/min for 90 min to produce a steady-state plasma concentration of [3H]NE. One milliliter of arterial and portal venous blood was drawn simultaneously from the respective catheters, and the plasma was stored at −80°C until further analysis. Plasma NE and [3H]NE concentrations were calculated as described above. Nonhepatic splanchic NE spillover rate can be derived by measuring radiolabeled and endogenous NE concentrations in the arterial and portal venous plasma, and the portal venous plasma flow. Because of technical difficulties, we were unable to measure portal blood flow in the same rats with portal vein catheters. For this reason, we assumed constant plasma flow in DOCA and SHAM rats. This assumption is supported by several studies showing that blood flow to splanchnic organs in DOCA-salt hyper-
tensive rats is generally not different from that of SHAM animals (51, 58). The value of portal venous plasma flow was obtained from a previous study performed in our laboratory using flow probes chronically implanted around the portal vein in conscious rats (n = 4) weighing 350–375 g at the time of flow measurement (10.1 ± 0.8 ml/min, unpublished observations). Nonhepatic splanchnic NE spill-over was calculated using the following formulas:

\[
\text{Nonhepatic splanchnic NE spill-over} = \left( \frac{F_x \times NE_A}{PF} + \frac{(NE_V \times NE_A)}{PF} \right) \times PF
\]

Where, \(F_x = \left[ ^3H \right] \text{NE}_A - \left[ ^3H \right] \text{NE}_V / \left[ ^3H \right] \text{NE}_A\)

\(NE_A\) is arterial plasma NE concentration, \(NE_V\) is portal venous plasma NE concentration, \(PF\) is plasma flow in the portal vein, \(F_x\) is the fractional extraction of NE during its passage through the splanchnic bed, \(\left[ ^3H \right] \text{NE}_A\) is arterial \([H]\)NE concentration, and \(\left[ ^3H \right] \text{NE}_V\) is portal venous \([H]\)NE concentration.

Experimental Protocols

**CGX and DOCA-salt hypertension.** AP and HR recordings were started after a 7-day postsurgical recovery period. After 5 days of control recordings, rats were allowed free access to water containing 1% NaCl and 0.2% KCl for the entire duration of the experiment. After a 7-day period of salt treatment, a DOCA pellet (50 mg/kg sc) was implanted in both CGX and SHAM-GX groups, and BP was recorded for four more weeks. Twenty four-hour saline intake was also measured for 2 days at the end of the experiment before the rats were euthanized for harvesting splanchnic organs to measure tissue NE content.

**Effect of CGX on whole body NE spillover.** To study the effect of CGX on whole body NE spillover, some rats underwent CGX while others had SHAM-GX surgery. These groups were treated essentially the same as rats in the previous protocol except that they had externalized catheters instead of a radiotelemeter. After a 7-day recovery period, catheters were implanted to measure whole body NE spillover as described above. Five days after catheter implantation, control hemodynamic measurements were made for 3 days followed by a subcutaneous DOCA pellet implant in both groups. AP and HR were measured around 10:00 A.M. every day for 1 h for another 14 days. Whole body NE spillover was measured on control day 2 and days 7 and 14 following DOCA treatment.

**Nonhepatic splanchnic NE spillover.** Catheters were implanted in the femoral artery and vein and the portal vein for BP measurement, chronic infusion, and sampling purposes. After a 7-day recovery period, control hemodynamic measurements were made for 3 days. A DOCA pellet was then implanted subcutaneously to induce hypertension development. AP and HR were measured for another three weeks. Nonhepatic splanchnic NE spillover was measured on control day 2 and days 7, 14, and 21 following DOCA treatment.

**Effect of CGX on nonhepatic splanchnic NE spillover.** CGX surgery was performed on a group of rats, and catheters were implanted to measure nonhepatic splanchnic NE spillover. Rats were allowed to recover for 10 days. Hemodynamic measurements were made for 3 days following recovery. Nonhepatic splanchnic NE spillover was then measured in all rats.

Statistical Analyses

Within-group hemodynamic differences were assessed by repeated-measures ANOVA with Bonferroni’s multiple-comparisons test. Between-group hemodynamic differences were analyzed by two-way ANOVA followed by Bonferroni’s test. Plasma NE concentration, NE clearance, and NE spillover were analyzed by paired t-test. The effect of splanchnic denervation on nonhepatic splanchnic NE spillover was calculated using one-tailed t-test. A value of <0.05 was considered significant. Data are presented as means ± SE.

**RESULTS**

**Effect of CGX on DOCA-Salt Hypertension**

MAP and HR during the development of DOCA-salt hypertension in CGX and SHAM-GX rats are shown in Fig. 1. There was no difference in MAP between CGX and SHAM-GX rats during the control period. Increased salt intake alone did not change MAP significantly in either CGX or SHAM-GX groups. Upon DOCA administration, an increase in MAP was seen in both groups of rats, but this increase was attenuated significantly in CGX compared with the SHAM-GX group [15.6 ± 2.2 vs. 25.6 ± 2.2 mmHg at day 40 of the protocol (28 days after starting DOCA treatment)]. HR was significantly lower in both CGX and SHAM-GX groups during high salt intake and during DOCA treatment compared with their respective control period values, but the change in HR between the two groups was not significantly different at any time during the experiment. NE contents of splanchnic organs measured at the end of the experiment are shown in Fig. 2. NE content in the CGX group compared with the SHAM-GX group was less by 30–40% in the kidneys, 81% in liver, 67% in small intestine, and by 94% in the spleen. Consumption of saline drinking fluid, measured at the end of the experiment, was not different between the two groups (SHAM-GX 140.4 ± 22.8 and CGX 140.0 ± 29.1 ml/day).

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**Fig. 1.** Mean arterial pressure (A) and heart rate (B) during the control, high-salt, and deoxycorticosterone acetate (DOCA) treatment periods in SHAM-GX (sham operated) and celiac ganglionectomy (CGX) groups. bpm, Beats/min. *Significant difference from day 3 (control period) values. #Difference between the two groups.
Effect of CGX on Whole Body NE Spillover in DOCA-Salt Hypertension

Figure 3 shows MAP and HR during the development of mild DOCA-salt hypertension in CGX and SHAM-GX animals. During the control period, MAP was slightly lower in CGX than SHAM-GX rats, but the difference was not significant. After DOCA administration, both groups became hypertensive, but the increase in MAP was attenuated in the CGX group (Fig. 3A). However, the final absolute MAP was not statistically different between the two groups. No significant difference was observed in HR between the two groups (Fig. 3B).

During the control period, whole body plasma NE (Fig. 4A) and whole body NE spillover (Fig. 4C) were significantly higher in SHAM-GX rats compared with CGX animals but otherwise were similar in both groups on days 3, 7, and 14 of DOCA treatment. NE clearance was not different in the two groups on control day 2 or on days 3, 7, and 14 of DOCA treatment (Fig. 4B).

Nonhepatic Splanchnic NE Spillover

MAP was similar in DOCA and SHAM rats during the control period. DOCA rats became significantly hypertensive during DOCA treatment while the SHAM rats remained normotensive throughout the experimental period (Fig. 5A). There were no differences in HR between the two groups at any time during the experimental period (Fig. 5B). Arterial and portal venous plasma NE concentrations were not different at any time in DOCA vs. SHAM rats (Fig. 6). Fractional extraction of NE by the nonhepatic splanchnic organs was also similar in both groups (Fig. 7A). Hence, estimated nonhepatic splanchnic NE spillover was not different at any time in DOCA vs. SHAM rats (Fig. 7B).

Effect of CGX on Nonhepatic Splanchnic NE Spillover

After CGX, MAP was 110.1 ± 1.7 mmHg. This value was similar to the MAP measured in the SHAM group (109.9 ± 4.2 mmHg) from the previous study. Nonhepatic splanchnic NE spillover after CGX was 0.5 ± 0.53 ng·min⁻¹·kg⁻¹ (Fig. 8), a value not significantly different from zero (one-tailed “t” test). CGX also significantly attenuated NE spillover in the splanchnic region compared with values measured in SHAM rats during the control period of the previous study (2.24 ± 0.35 ng·min⁻¹·kg⁻¹; Fig. 8).

DISCUSSION

The main new findings of this study on the role of regional SNA in the development of mild DOCA-salt hypertension are: 1) splanchnic sympathetic denervation by CGX attenuates hypertension development, 2) whole body NE spillover does not reflect changes in splanchnic NE spillover and cannot be used to assess splanchnic SNA, and 3) elevated splanchnic SNA assessed by nonhepatic splanchnic NE spillover is not required for the development of hypertension.

Effect of CGX on Splanchnic Sympathetic Innervation

A decrease in splanchnic tissue NE content even 7 wk after CGX provided confirmation that denervation was achieved successfully (Fig. 2). NE content in the splanchnic organs was significantly reduced, with splenic content affected the most (by 94%). This is consistent with previous findings showing an ~85% reduction in splenic NE content (3, 37) after CGX or selective denervation of the spleen. Previous reports indicate that sympathetic postganglionic nerves show regeneration after chemical or surgical sympathectomy (24, 37). This regeneration of postganglionic neurons can reestablish neuroeffector transmission at the nerve terminal. However, regeneration does
not appear to be substantial because NE content of splanchnic organs was still very low at the end of study, and BP was attenuated throughout the experimental period in CGX rats. There was a 30–40% reduction in NE content in the kidneys. This is not surprising because 20–25% of the postganglionic neurons supplying the kidneys originate in the celiac ganglion while the majority (>80%) come from the paravertebral sympathetic chain (7, 19, 52). However, as demonstrated previously (31), fully intact renal nerves are not essential for hypertension development in this model. Thus, any effects of CGX on AP in our model are likely the result of sympathetic denervation of nonrenal splanchnic organs.

Effect of CGX on Hypertension Development

The rise in BP after DOCA administration was attenuated significantly in CGX rats compared with that of SHAM-GX rats. This suggests that an intact splanchnic sympathetic innervation is necessary for the full development of mild DOCA-salt hypertension, but additional studies were necessary to elucidate the mechanism of this effect.

Effect of CGX on Whole Body NE Spillover

The finding that splanchnic sympathectomy with CGX impairs hypertension development was surprising because, in earlier studies, we found no evidence for increased whole body SNA during the development of mild DOCA-salt hypertension. However, splanchnic SNA may not be reflected in whole body assessments of sympathetic activity. This might seem unlikely since one study reported that a large fraction (~37%) of total sympathetic outflow is directed toward splanchnic organs (2). However, NE released in the blood from the splanchnic bed must pass through the liver before reaching the systemic circulation, and the vast majority (~86%) is extracted there. Thus, nonhepatic splanchnic NE spillover is obscured when using standard methods of measuring whole body NE spillover (2). To investigate whether splanchnic denervation attenuates hypertension development by decreasing generalized sympathetic activity, we measured plasma NE levels and whole body NE spillover in CGX animals during the development of mild DOCA-salt hypertension.

During the control period, plasma NE and whole body NE spillover were in fact significantly lower in CGX vs.

Fig. 4. Plasma NE (A), whole body NE clearance (B), and NE spillover (C) in SHAM-GX and CGX on control day 2 (C) and days (D) 3, 7, and 14 after DOCA treatment. *Significant difference between the groups during the control period.

Fig. 5. Mean arterial pressure (A) and heart rate (B) during the control and DOCA treatment periods in DOCA and SHAM rats. *Significant difference from day 3 (control period) values. #Difference between the two groups.
SHAM-GX rats (Fig. 4, A and C). On the surface, this finding appears to support the conclusion that CGX causes a measurable decrease in global SNA assessed from whole body NE spillover or plasma NE levels. However, both measures were comparable in CGX and SHAM-GX rats throughout the remainder of the study. Furthermore, plasma NE and whole body NE spillover in the SHAM-GX rats during the control period in this study were markedly higher than we measured previously (30, 31). We concluded that the higher values of plasma NE and whole body NE spillover in SHAM-GX rats during the control period were most likely aberrant because of specific conditions during the blood sampling at that time. One likely possibility is that fluctuations in plasma catecholamines can occur by noise-induced stress in otherwise undisturbed rats (9). Most important for our main hypothesis was the finding of no changes in plasma NE or whole body NE spillover in CGX rats on days 3, 7, and 14 after DOCA treatment. Overall, we conclude that the effects of CGX on splanchnic SNA are not revealed by measurements of plasma NE levels or whole body NE spillover and that CGX does not attenuate hypertension development by decreasing generalized sympathetic activity.

Nonhepatic Splanchnic NE Spillover

To address the possibility that splanchnic SNA is elevated in mild DOCA-salt hypertension development without being reflected in plasma NE levels or whole body NE spillover measurements, we used the radioisotope dilution technique to estimate the rate of NE release from nonhepatic splanchnic organs (i.e., nonhepatic splanchnic NE spillover) as an index of splanchnic SNA. We also studied the effect of splanchnic denervation (via CGX) on nonhepatic splanchnic NE spillover. Sampling of portal venous blood is ideal and provides a direct estimate of the amount of NE released by nonhepatic splanchnic organs before it enters the liver. Nonhepatic splanchnic NE measurements have been reported in anesthetized human patients and in some anesthetized and conscious experimental animals (1, 2, 8, 27). This is the first study to report nonhepatic splanchnic NE spillover in conscious, undisturbed rats.

Effect of CGX on Nonhepatic Splanchnic NE Spillover

To test whether CGX attenuates splanchnic NE spillover, and to help validate the nonhepatic splanchnic NE spillover...
technical, we compared nonhepatic splanchnic NE spillover in a group of CGX and SHAM rats. We expected that CGX, by interrupting the majority of sympathetic outflow to splanchnic organs, would largely eliminate calculated spillover of NE in the splanchnic venous drainage. The value of nonhepatic splanchnic NE spillover after CGX was significantly lower (~78% reduction) than that observed in rats that did not undergo CGX (Fig. 8). Furthermore, our finding that calculated nonhepatic NE spillover was not significantly different from zero in CGX rats (Fig. 8) confirms that the technique is measuring neuronal release of NE and that most nonhepatic splanchnic innervation originates in the CG.

**Nonvascular Targets of Splanchnic SNA**

It has been postulated that sympathetic outflow to splanchnic organs is regulated in response to arterial pressure (55). Enhanced sympathetic neurotransmission to mesenteric arteries also has been found in established DOCA-salt hypertension (48). In the presence of enhanced responsiveness to NE in DOCA-treated animals, even normal levels of splanchnic SNA could produce an increase in splanchnic arterial vasoconstriction.

It has also been demonstrated that rats with established traditional DOCA-salt hypertension have an increase in venous tone mediated in part by the sympathetic nervous system (20, 57). This appears to be caused mainly by increased reactivity of mesenteric veins to released NE (57). Therefore, it is possible that sympathetically mediated venous tone also is increased in mild DOCA-salt hypertension even in the absence of higher absolute levels of splanchnic SNA. Increased venous tone would contribute to hypertension development by causing decreased venous capacitance and translocation of blood from the venous to the arterial side of the circulation.

Several investigators have reported increased release of NE from sympathetic nerve terminals in the mesenteric circulation of traditional DOCA-salt hypertensive rats (11, 48, 56). However, increased NE release should have been detectable with the spillover method but was not found in our studies. Another possibility derives from the fact that there can be a mismatch between NE synthesis and release, sympathetic firing rate and NE release, and rates of NE release and overflow (6, 15, 29, 41). This could result in normal spillover rates in the presence of increased SNA. Therefore, it is possible that nonhepatic splanchnic spillover is unchanged even when sympathetic outflow to the splanchnic bed is increased. Direct recording of splanchnic SNA would be necessary to address this possibility.

Finally, it is possible that NE might not be the primary neurotransmitter involved in sympathetic neurotransmission in the mesenteric arteries of mild DOCA-salt hypertension. Therefore, assays measuring only NE spillover in the plasma would provide insufficient information about important aspects of splanchnic sympathetic activity in the development of hypertension. It has been reported that purinergic transmission is altered in DOCA-salt hypertensive rats (13, 14, 48). Also, there is evidence suggesting the importance of neuropeptide Y (NPY) in neurotransmission in DOCA-salt hypertension (43). Measurement of portal venous NPY and/or purine levels could be used to investigate whether other neurotransmitters play a major role in sympathetic neurotransmission in hypertension development.

**Nonvascular Targets of Splanchnic SNA**

Splanchnic SNA could also contribute to the development of hypertension by mechanisms other than directly affecting splanchnic blood vessels. Even though retrograde tracer studies of the adrenal medulla have failed to demonstrate any labeling of sympathetic efferent cells within the CG (33), it is possible that some adrenal denervation might have occurred during CGX surgery. Therefore, CGX may have attenuated the release of epinephrine from adrenal gland and caused a reduction in BP. Surgical removal of the adrenal gland during established DOCA-salt hypertension decreased BP (12), and rats that were adrenalectomized and subsequently DOCA-salt treated did not develop hypertension (44). Unpublished data from our labora-
tory found no changes in circulating NE or epinephrine after CGX in rats, but we cannot rule out the possibility that CGX affected BP by altering adrenal release of catecholamines.

It is also possible that CGX affects water and salt absorption, leading to a decrease in blood volume and BP. We did not measure blood volume in CGX rats, but other studies from our laboratory have not found a consistent effect of CGX on blood volume (35). Salt and water intake measured at the end of the study were found to be similar in both groups. This is consistent with a previous finding showing that extrinsic denervation of small intestine does not alter water and electrolyte absorption (16). This is important because reduced salt intake will attenuate DOCA-salt hypertension development (45, 53), and at least one previous study showed that renal denervation (28) slowed DOCA-salt hypertension development exclusively by decreasing salt and water intake.

Effects of CGX on Visceral Afferents
CGX could alter BP by disrupting visceral sensory afferents. Activation of abdominal visceral afferents has been demonstrated to cause profound cardiovascular responses that include increases in BP, HR, and cardiac contractility (5, 38, 47). Activation of visceral afferents not only increased BP but also caused regional hemodynamic changes in the splanchnic vascular bed and a differential increase in the sympathetic outflow to the splanchnic viscera, but not to the heart and somatic tissues (46). Thus, it is possible that BP-lowering effects of CGX in hypertension development could be mediated by disruption of visceral sensory afferent nerves.

In summary, we demonstrated that selective splanchnic sympathectomy using CGX attenuates development of mild DOCA-salt hypertension and conclude that splanchnic SNA contributes to hypertension development. However, hypertension occurred in the absence of increases in splanchnic SNA as measured using nonhepatic splanchnic NE spillover. We conclude that a possible mechanism linking splanchnic SNA and hypertension is increased splanchnic vascular reactivity to NE, leading to increased vascular resistance and capacitance.

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REFERENCES
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