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Time-dependent changes in autonomic control of splanchnic vascular resistance and heart rate in ANG II-salt hypertension

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Kuroki MT, Guzman PA, Fink GD, Osborn JW. Time-dependent changes in autonomic control of splanchnic vascular resistance and heart rate in ANG II-salt hypertension. Am J Physiol Heart Circ Physiol 302: H763–H769, 2012. First published November 23, 2011; doi:10.1152/ajpheart.00930.2011.—Previous studies suggest that ANG II-induced hypertension in rats fed a high-salt (HS) diet (ANG II-salt hypertension) has a neurogenic component dependent on an enhanced sympathetic tone to the splanchnic veins and independent from changes in sympathetic nerve activity to the kidney or hind limb. The purpose of this study was to extend these findings and test whether altered autonomic control of splanchnic resistance arteries and the heart also contributes to the neurogenic component. Mean arterial pressure (MAP), heart rate (HR), superior mesenteric artery blood flow, and mesenteric vascular resistance (MVR) were measured during 4 control days, 14 days of ANG II delivered subcutaneously (150 ng·kg⁻¹·min⁻¹), and 4 days of recovery in conscious rats fed a HS (2% NaCl) or low-salt (LS; 0.1% NaCl) diet. Autonomic effects on MAP, HR, and MVR were assessed by acute ganglionic blockade with hexamethonium (20 mg/kg iv) on day 3 of control, days 1, 3, 5, 7, 10, and 13 of ANG II, and day 4 of recovery. MVR increased during ANG II infusion in HS and LS rats but remained elevated only in HS rats. Additionally, the MVR response to hexamethonium was enhanced on days 10 and 13 of ANG II selectively in HS rats. Compared with LS rats, HR in HS rats was higher during the 2nd wk of ANG II, and its response to hexamethonium was greater on days 7, 10, and 13 of ANG II. These results suggest that ANG II-salt hypertension is associated with delayed changes in autonomic control of splanchnic resistance arteries and the heart.

Salt-sensitive hypertension; differential regulation of sympathetic outflow; splanchnic nerve activity; ganglionic blockade; hemodynamic measurement in conscious rats; angiotensin II

UNDER CERTAIN CONDITIONS, hypertension resulting from systemic administration of angiotensin II (ANG II-induced hypertension) is exacerbated by activation of the sympathetic nervous system (SNS). Our group and others have shown that salt intake is one such condition (19, 21). In rats fed a relatively high salt diet (2% NaCl), the level of blood pressure achieved in ANG II-induced hypertension is significantly higher than in rats fed a normal salt diet (0.4% NaCl); this is associated with an increase in whole body norepinephrine (NE) spillover (14) and enhanced mean arterial pressure (MAP) responses to ganglionic blockade (13, 15). In contrast, these measures of whole body sympathetic tone in rats fed a normal salt diet remain near control levels.

Despite increased “whole body” sympathetic tone in ANG II-salt (i.e., those fed a high-salt diet) hypertensive rats, we recently reported that sympathetic nerve activity (SNA) to the kidney and hind limb were reduced or unchanged, respectively (28). Suppression of renal SNA has also been directly measured during ANG II-induced hypertension in rabbits (2) and indirectly in dogs (4), suggesting that this suppression is not a salt-dependent effect, per se, but rather a baroreceptor-mediated phenomenon. Indeed, the chronic ANG II-decreased decrease in renal SNA is not observed in sinoaortic-denervated animals (17, 1). Additionally, ANG II-induced hypertension is unaffected by sinoaortic denervation, suggesting that baroreflex-mediated effects on renal SNA are not critical to the development of hypertension. Further evidence that renal SNA does not contribute to ANG II-induced hypertension is that renal denervation has no effect on the final level of ANG II-salt hypertension in rats (15), as well as ANG II-induced hypertension in rabbits (3). Combined, these latter results have been the major argument against the importance of the SNS in this form of hypertension (16).

In contrast to changes in sympathetic control to the kidney, relatively little attention had been given to a possible role for elevated SNA to nonrenal vascular beds in the pathogenesis of ANG II-induced hypertension. Recent studies by King and Fink suggest that the SNS contributes to ANG II-salt hypertension via an influence to the splanchnic vascular bed. Consistent with prior studies in ANG II-salt hypertensive dogs (29), mean circulatory filling pressure (MCFP) was found to be elevated in ANG II-salt hypertensive rats (13). Because the increase in MCFP was not associated with increased blood volume, this finding suggests that venomotor tone is elevated in ANG II-salt rats. Furthermore, the elevated MCFP was sensitive to ganglionic blockade and prevented by splanchnic sympathectomy via celiac ganglionectionomy. More importantly, this latter procedure attenuated ANG II-salt hypertension to levels similar to those observed in ANG II-induced hypertension in rats fed a normal salt diet (15). These findings suggest that the increase in MCFP during ANG II-salt hypertension is secondary to sympathetically mediated venoconstriction in the splanchnic vascular bed, causing a reduction in splanchnic vascular capacitance. Based on these findings, it has been proposed that the neurogenic reduction in splanchnic vascular capacitance contributes to higher levels of ANG II-induced hypertension in high-salt rats by redistributing blood volume from the venous to the arterial circulation (8).

Functional consequences of enhanced splanchnic SNA, however, are not restricted to veins. A question that remains unanswered is whether sympathetic vasoconstriction to splanchnic resistance arteries also is enhanced during ANG

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II-salt hypertension. This seems likely since labeling studies indicate that the majority of neurons in prevertebral ganglia (a major source of splanchnic sympathetic input) dually innervate arteries and veins (12). Thus, the proposed increase in sympathetic tone to the splanchnic vascular bed in ANG II-salt hypertensive rats may exert its impact on blood pressure via enhanced constriction of splanchnic resistance arteries, as well as veins.

We addressed this question in the present study by measuring arterial pressure (AP) and splanchnic blood flow continuously in conscious unrestrained rats before, during, and after ANG II administration in rats on a low- or high-salt diet. We hypothesized that ANG II-induced increases in splanchnic vascular resistance, as calculated from measures of AP and splanchnic blood flow, would be greater in rats consuming a high-salt diet compared with rats on a low-salt diet. Moreover, we predicted that the neurogenic contribution to splanchnic vascular resistance during ANG II administration, as assessed by the acute increase in splanchnic vasodilation during ganglionic blockade, would be greater in high-salt rats compared with low-salt rats.

MATERIALS AND METHODS

Animal subjects. Male Sprague-Dawley rats (Charles River Laboratories International, Wilmington, MA) weighing ~270–370 g (312 ± 4g) were used for these experiments. Animal care and experimentation were performed in accordance with the National Institutes of Health Animal Use and Care Guideline based on a protocol submitted to, and approved by, the University of Minnesota Institutional Animal Care and Use Committee.

Animal instrumentation and care. Rats were housed in a temperature-controlled environment with a 12:12-h light-dark cycle and acclimatized to a high-salt (HS, 2.0% NaCl) or low-salt (LS, 0.1% NaCl) diet (Research Diets, New Brunswick, NJ) for at least 7 days. On the day of surgery, rats were atropinized (0.2 mg/kg ip; Baxter International, Deerfield, IL) and anesthetized with isoflurane (2% end-expiratory concentration). At least 10 days were given for recovery. During this time, food and water intake were monitored along with signs of appropriate hydration. Surgery was performed using aseptic techniques. An AP telemeter [TA11PA-C40; Data Sciences International (DSI), St. Paul, MN] was implanted as previously described (27). A venous catheter made from a 7-cm segment of Silastic tubing (508 – 001, 0.3 mm ID, Saint-Gobain Performance Plastics, Akron, OH) was implanted subcutaneously and exteriorized through an incision made from the inferior vena cava via the left femoral vein. A 1-mm Transonic flow probe (MC-1PR5-JS; Transonic Systems, Ithaca, NY) was placed on the superior mesenteric artery approached retroperitoneally through an incision at the left flank. The venous catheter and the flow probe cable were tunneled subcutaneously and exteriorized through an incision made at the level of the scapula. A tether anchor made from a circular piece of surgical polyester mesh (PETKM14002; Textile Development Associates, Surgical Mesh Division, Brookfield, CN) attached to a silicone rubber catheter (51135K78; McMaster-Carr, Elmhurst, IL) was sutured to the skin at the incision over the scapula. A 38-cm stainless steel spring (Exacto Spring, Grafton, WI) was threaded halfway into the silicone portion of the tether anchor. The venous catheter and flow probe cables were externalized through this spring.

Rats were then housed individually in a custom made Plexiglas cylindrical cage and tethered via the spring attached to an electrical swivel (SVL6C; Kent Scientific, Torrington, CT) suspended above the cage. At least 10 days were given for recovery. During this time, food and water intake were monitored along with signs of appropriate recovery from surgery. A combination of ampicillin (50 mg/kg sid, iv; Sandoz International, Holzkirchen, Germany), tobramycin (3 mg/kg sid, iv; Teva Pharmaceuticals, Irvine, CA), and buprenorphine (0.05 mg/kg bid, iv; Reckitt Benckiser Pharmaceuticals, Richmond, VA) was given for antimicrobial prophylaxis and analgesia during the first 3 days of recovery.

Experimental protocol. The experimental protocol was conducted in the following two groups: HS (N = 10) and LS (N = 10) rats. AP and superior mesenteric artery blood flow (MBF) were continuously measured throughout the 22-day protocol. At the end of a 4-day control period, ANG II was administered for 14 days at a rate of 150 ng·kg⁻¹·min⁻¹ using ALZET osmotic pumps (2ML2; DURECT, Cupertino, CA) implanted subcutaneously. On the morning after the 14th day of ANG II, rats were anesthetized with isoflurane, the minipump was removed, and animals were returned to their cage for an additional 4 days of measurements (recovery period).

Assessment of autonomic control of MAP, heart rate (HR), and mesenteric vascular resistance (MVR) during ANG II infusion was performed by ganglionic blockade on days 1, 3, 5, 7, 10, and 13 of ANG II and compared with values obtained on the 3rd day of control and 4th day of recovery. Ganglionic blockade was achieved by intravenous administration of hexamethonium (H0879; Sigma-Aldrich, St. Louis, MO) at a dose of 20 mg/kg (23). Animals were weighed in the morning on the day of the experiment, and injections were performed in the afternoon between 3:00 and 6:00 P.M. At least 30 min after the injection, the intravenous catheter was flushed with saline containing 50 U/ml heparin (Sagent Pharmaceuticals, Schuamburg, IL).

Data acquisition and analysis. The AP and MBF signals were collected continuously at 500 Hz using DSI software (Dataquest ART version 4.0 Platinum; DSI). The AP signal was acquired using a wireless receiver (RPC-1; DSI), and the MBF signal was acquired using a dual-channel flowmeter (T206; Transonic Systems) connected to an analog-to-digital converter box (C11V; DSI). MAP, HR, and mean MBF were calculated on-line from consecutive 10-s segments of the pulsatile waveform and stored to disk. These data were imported into MATLAB (version R2009b; The Mathworks, Natick, MA) for calculating MVR, averaging daily hemodynamic values, and analyzing the ganglionic blockade data. MVR was calculated off-line from the MAP and mean MBF data as MAP/MBF. Twelve-hour averages of MAP, HR, MBF, and MVR were calculated starting from the beginning of the dark cycle on day 0 of the protocol after removal of the 4-h segments immediately following ganglionic blockade. To determine the response to ganglionic blockade, data were first smoothed with a third-order median filter. The peak/trough responses of MAP, HR, or MVR were determined on this smoothed dataset and subtracted from the average of the 10-min baseline period (see Fig. 1).

Statistical analysis. Variables in HS and LS rats were analyzed by factorial ANOVA. The effect of dietary salt intake and ANG II administration, on the 12-h mean hemodynamic values for each light-dark cycle, and response to ganglionic blockade were analyzed by two-way repeated-measures ANOVA using SigmaStat (version 3.5; Systat Software, San Jose, CA). A significant interaction or main effect was further analyzed by post hoc multiple comparisons with respect to the control samples for each factor (low salt for diet, and control day 3 for day of protocol) using the Holm-Sidak method. A P ≤ 0.05 was considered statistically significant.

RESULTS

Figure 1 shows a typical trace of the pulsatile AP and MBF waveform from a single HS rat taken during control. MBF data were of high fidelity (as shown by the pulsatile flow profile at each cardiac cycle) and allowed for determination of absolute flow rate to the mesenteric vascular bed throughout the entire 22 days of the protocol. Also shown in Fig. 1 are the 10-s MAP, MBF, and HR traces calculated from the waveform data and the MVR trace calculated from the 10-s MAP and MBF...
data points. These 10-s mean data were used to determine the responses to ganglionic blockade and changes in 12-h hemodynamics before, during, and after ANG II treatment in HS and LS rats.

**Effect of ANG II on 12-h hemodynamics in rats fed a HS or LS diet.** Figure 2 shows the daily 12-h average MAP, MBF, MVR, and HR for the entire 22 days of the protocol. Rats were allowed to recover from surgery (typically 10 days) until a distinct circadian rhythm was observed in every variable during baseline. There were no differences in the 12-h MAP, MBF, MVR, or HR during the 4-day control period between HS and LS rats. All variables recovered to control levels following 14 days of ANG II. There were no differences in the 12-h hemodynamic values during the 4-day recovery period between HS and LS rats except for the nighttime MAP on the 2nd day of recovery.

MAP was identical in both groups during the first 24 h of ANG II administration. However, whereas MAP reached a plateau in LS rats by the 1st day of ANG II infusion, MAP did not reach steady state until the 2nd wk of ANG II in HS rats. The steady-state level of MAP (averaged over days 10-13 of ANG II) was 162 ± 2 and 119 ± 2 mmHg for HS and LS rats, respectively, during nighttime and 149 ± 2 and 117 ± 2 mmHg for HS and LS rats, respectively, during daytime.

MBF decreased transiently during the initial stage of ANG II infusion in both HS and LS rats, and this decrease was similar in magnitude. Similarly, MVR increased to the same magnitude during this time in both groups. Following the acute drop, MBF gradually returned toward control levels in both HS and LS rats. Although there were no between-group differences in MBF on a day-to-day basis throughout the protocol, the recovery of MBF, compared with the within-group control levels, took 5 days in HS rats compared with 2 days in LS rats.

Changes in MVR following the peak at day 1 of ANG II deviated significantly between HS and LS rats. In HS rats, higher MVR levels persisted until removal of ANG II, whereas, in LS rats, MVR sharply dropped following the day 1 peak and gradually returned toward control levels. MVR in LS rats during ANG II infusion was no longer statistically significant from control after day 6 of ANG II. MVR averaged over days 10 through 13 of ANG II was 14.3 ± 0.7 and 9.5 ± 0.4 mmHg·min·ml⁻¹ in HS and LS rats, respectively, during nighttime and 14.9 ± 0.7 and 10.5 ± 0.5 mmHg·min·ml⁻¹ in HS and LS rats, respectively, during daytime. Removal of the minipump on day 14 of ANG II caused an acute drop in MVR toward control levels in HS rats.

Administration of ANG II resulted in an initial bradycardic response that was more pronounced in HS than LS rats. There was a clear trough for this response in HS rats at the end of day 4 of ANG II but then HR gradually rose toward control levels. In contrast, HR in LS rats remained at levels slightly below control throughout day 11 of ANG II. HR, both nighttime and daytime, on days 9, 10, 11, and 12 of ANG II was higher in HS rats compared with LS rats, although these levels in HS rats were not statistically distinguishable from their own control.

**Effect of ANG II on the hemodynamic responses to acute ganglionic blockade in rats fed a HS or LS diet.** Figure 3 summarizes the results from the ganglionic blockade experi-

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**Fig. 1.** Representative traces for the responses of arterial pressure (AP), mesenteric artery blood flow (MBF), mesenteric vascular resistance (MVR), and heart rate (HR) to ganglionic blockade in a single rat during control. AP and MBF signal was sampled and saved to disk continuously at 500 Hz (dark gray). Mean AP (MAP), MBF, and HR were calculated and saved every 10 s during acquisition (light gray for AP and MBF, dark gray for HR). MVR was calculated from the 10-s MAP and MBF data as AP/MBF. The peak responses of AP, MVR, and HR after injection of hexamethonium were determined with respect to baseline (average of 10 min immediately preceding injection). BPM, beats/min.
ments and shows the change in the contribution of autonomic tone to basal hemodynamics before, during, and after ANG II administration. During the control period, there was no difference in the depressor response to hexamethonium between HS and LS rats. However, the depressor response to hexamethonium in HS rats increased starting at day 3 of ANG II, with this trend continuing until day 13 of ANG II. In contrast, the depressor response to hexamethonium in LS rats was indistinguishable from control levels throughout the protocol. The depressor response to hexamethonium returned to control levels after removal of the minipumps in both HS and LS rats.

The MVR responses to hexamethonium showed a pattern similar to those observed with MAP in that responses increased during ANG II administration in HS rats but not in LS rats. Specifically, there were no differences during the control
period between HS and LS rats. There were no between-group differences in the MVR response to hexamethonium during the 1st wk of ANG II except for day 3 of ANG II where the response was significantly higher in HS rats. By days 10 and 13 of ANG II, the MVR response to hexamethonium was significantly higher in HS compared with LS rats. The response of MVR to hexamethonium returned to control levels by the 4th day of the recovery period and was not different between groups.

HR dropped in response to hexamethonium in both groups to a similar degree during control, and this was not different between groups. However, on day 3 of ANG II, the HR response to hexamethonium switched from a bradycardia to a tachycardia in HS rats in contrast to LS rats, which did not change. As observed in the responses of MAP and MVR, the HR response to hexamethonium during the 2nd wk of ANG II was significantly higher in HS rats compared with LS rats. In LS rats, the HR response to hexamethonium tended to gradually decrease in magnitude over time; however, these changes were not statistically different from control levels. The HR responses to hexamethonium in HS rats were greater than those measured in LS rats on days 7, 10, and 13 of ANG II; however, these values were not statistically different from the within-group control period. Finally, the HR response to hexamethonium measured on the 4th day of the recovery period was similar between HS and LS rats and not different from their respective within-group control periods.

**DISCUSSION**

Rats subjected to the ANG II-salt protocol, where they are fed a HS diet during continuous infusion of ANG II, have been shown to develop hypertension by multiple mechanisms, including a delayed increase in whole body sympathetic activity (13, 14). Consistent with these previous findings, ANG II-induced hypertension in our current study was significantly higher in HS compared with LS rats, and there was a delayed increase in the depressor response to hexamethonium in HS rats but not LS rats (13). Previous findings that celiac ganglionectomy attenuates the development of ANG II-salt hypertension suggested that the splanchnic vascular bed is a significant contributor to the neurogenic component of hypertension in this model (15). Furthermore, direct measurement of renal and lumbar SNA, which were previously found to be slightly below or unchanged from baseline (28), suggested that the sympathoexcitatory response in the ANG II-salt model is a regionally localized phenomenon. Although SNA to the splanchnic vascular bed has not been directly measured in ANG II-salt rats, the evidence reported here supports a role for splanchnic sympathetic nerves in mediating peripheral hemodynamic changes that ultimately contribute to the severity of hypertension in this model.

To date, the only finding that provides a mechanistic insight into the hemodynamic effect of a localized increase in sympathetic tone to the splanchnic vascular bed during ANG II-salt hypertension has been the observation that MCFP is elevated in this model, which has been shown to be progressively more sensitive to ganglionic blockade and dependent on an intact sympathetic innervation to the splanchnic vascular bed (13, 15). Our present study extends these findings by showing that continuous infusion of ANG II results in a sustained increase in MVR associated with an elevated MVR response to ganglionic blockade in HS but not LS rats (13). This new finding adds further support to the overall hypothesis that the higher level of dietary salt contributes to the severity of ANG II-induced hypertension in HS rats by increasing sympathetic vasoconstrictor tone to the splanchnic vascular bed. This appears to contribute to the hypertension by increasing splanchnic vascular resistance and reducing splanchnic vascular capacitance, and through their expected overall impact on total peripheral resistance and effective circulating blood volume. These combined effects would elevate AP by effectively translocating blood from the highly compliant venous compartment to the less compliant arterial compartment as suggested in a recent review (8).

The increase in sympathetic tone during ANG II-salt hypertension is delayed, as suggested by the timing at which measurable changes in whole body NE spillover occur (week 2 of ANG II) (14), but, more importantly, its contribution to AP is progressive, as suggested by the time course in which the depressor response to ganglionic blockade increases, starting at around day 3 of ANG II. The current study suggests that the neurogenic influence on the splanchnic vasculature also occurs in a time-dependent manner. The contribution of neurogenic tone to MCFP (which is largely determined by splanchnic venous capacitance) was increased as early as 1 day after initiating the infusion of ANG II (13). In the present study, although the magnitude of mesenteric vasodilation during ganglionic blockade was increased 3 days after the start of ANG II infusion, it was not consistently elevated until the 10th day of ANG II. This difference in the timing of the onset of sustained neurogenically mediated increases in MCFP (day 1 of ANG II) and MVR (day 10 of ANG II) may be the result of a gradually increasing peripheral sympathetic tone in ANG II-salt rats. For instance, the threshold for a functional response to increased sympathetic tone may be different in splanchnic veins and arteries. It has been reported that the frequency-response curve to nerve stimulation is shifted to the left (i.e., lower frequency) for splanchnic veins compared with splanchnic arteries (6). Thus, it is possible that splanchnic SNA increases gradually during ANG II infusion and that earlier and lower frequencies of sympathetic nerve discharge during ANG II infusion in HS rats affect mostly the splanchnic veins, whereas later and higher frequencies of sympathetic discharge affect both veins and arteries. Thus a progressive increase in the levels of SNA and differential responses of splanchnic resistance and capacitance vessels may be the underlying mechanism for the progressive rise in MAP seen in HS rats treated with ANG II. It is important to note, however, that there are several other possibilities to explain this observation, including changes in arterial reactivity to NE and release of vasodilators that may counter the early constrictor effects of increased SNA to splanchnic resistance arteries. Further studies are necessary to establish more certainly the mechanism behind the progressive change in the sympathetic contribution to AP during ANG II-salt hypertension.

In addition to changes in sympathetic vasoconstrictor tone in the splanchnic vascular bed, nonneurogenic vasoconstrictor mechanisms to the splanchnic and potentially other vascular beds appear to play a role in ANG II-induced hypertension. For instance, the MVR peak on day 1 of ANG II and the early differences in MVR between HS and LS rats starting at day 2.
cannot be explained by an elevated sympathetic vasoconstrictor tone, since MVR responses to ganglionic blockade were not consistently elevated until day 10 of ANG II. The initial increase in MVR in both HS and LS rats may be a result of direct vasoconstrictor effects of ANG II; the early differences in MVR between HS and LS rats may reflect differences in the compensatory responses triggered in response to the initial vasoconstriction and rise in MAP, or altered reactivity of mesenteric arteries to ANG II. It has been shown that HS intake can increase the reactivity of cremasteric arterioles to ANG II (9). Whether similar dietary salt-dependent sensitization occurs in mesenteric arterioles is unknown, but the initial increase in MVR in response to ANG II infusion was similar between HS and LS rats, suggesting that any effect of sensitization to ANG II-mediated vasoconstriction was small. Furthermore, the sustained increase in MAP in LS rats during ANG II infusion despite a return of MVR to control levels indicates that there is a persistent nonneurogenic vasoconstrictor stimulus to nonsplanchnic vascular beds. The effects of ANG II on the renal vascular bed are well known and likely play a prominent role in the nonneurogenic mechanisms underlying ANG II-induced hypertension.

In this study, we also assessed whether autonomic tone to the heart increases during ANG II-salt hypertension. Although HR was higher and the bradycardic response to ganglionic blockade more prominent in HS compared with LS rats during the 2nd wk of ANG II infusion, these levels in HS rats were not statistically higher compared with their own control levels. Similarly, the HR response to ganglionic blockade in LS rats during the 2nd wk of ANG II infusion was not statistically different compared with control, making it difficult to draw conclusions from the between-group differences found in this study. Nevertheless, it is possible that the difference in baseline HR between HS and LS rats during the 2nd wk of ANG II is due to an inappropriate balance of sympathetic and parasympathetic cardiac autonomic activity. Although further studies are necessary to determine the role of cardiac sympathetic nerves in ANG II-salt hypertension, preliminary findings in cardiac denervated rats and rats chronically treated with atenolol suggest that changes in sympathetic tone to the heart play little or no functional role in the neurogenic component of ANG II-salt hypertension (11). It is clear, however, that baseline HR is initially suppressed during ANG II-induced hypertension, as shown by the tachycardic response to ganglionic blockade on day 3 of ANG II in HS rats. This initial decrease in sympathetic tone (and/or increase in parasympathetic tone) to the heart is likely baroreflex mediated, which is consistent with previous studies showing that renal SNA is initially suppressed during ANG II-induced hypertension in a baroreflex-dependent manner (1, 17). Suppression of activity is not seen with sympathetic effects on splanchic resistance arteries and lumbar SNA (28), suggesting that there is a region-specific difference in the degree of baroreceptor inhibition of sympathetic tone in response to the initial phase of ANG II hypertension. This hypothesis of minimal baroreflex control of SNA to the splanchic vascular bed relative to neural control of the heart, kidney, or hind limb skeletal muscle needs to be tested in further studies.

Strengths and limitations of the study. To our knowledge, this study is the first to employ direct continuous long-term recording of superior mesenteric artery blood flow and AP in conscious unrestrained animals before, during, and after the induction of any model of experimental hypertension. The combination of this approach, with intermittent assessment of neural control of splanchnic hemodynamics using ganglionic blockade, has generated novel results regarding the role of neural control of this vascular bed in the pathogenesis of ANG II-salt hypertension. The strength of this approach is the ability to assess neural control of a specific vascular bed over a long period of time using a repeated-measures experimental design. Although we did not directly measure SNA to the splanchic vascular bed, a key variable of interest was calculated, i.e., MVR.

Ganglionic blockade has been a standard technique for determining the contribution of the SNS to AP and regional hemodynamics, and it has been shown that the peak response is relatively unaffected by compensatory release of hormones (e.g., vasopressin) in response to the rapid hypotensive effect (23). Nevertheless, results must be interpreted with caution (20). Although we feel that an increase in SNA to the splanchic vascular bed is the most likely explanation for the obtained results, other possibilities exist. One is that the response to ganglionic blockade is a reflection of a generalized increase in sensitivity to vasoconstrictors due to a “vascular amplifier” effect secondary to ANG II-induced vascular hypertrophy (10). Another possibility is a withdrawal of myogenic tone in response to a drop in pressure secondary to withdrawal of sympathetic tone elsewhere. Finally, ANG II may amplify nerve transmission at the sympathetic ganglia, postganglionic presynaptic nerve terminal, and vascular neuroeffector junction (22), which could result in sympathetic nerve-dependent changes in the splanchic vascular bed without an actual increase in nerve activity. Thus, further experiments, such as those measuring MVR in celiac ganglionectomized rats and directly recording splanchic SNA, are needed to determine whether enhanced sympathetic vasomotor nerve activity to the splanchic vascular bed was responsible for the observed changes in MVR.

However, several studies argue against these alternate possibilities. For example, it has been shown that prevention of vascular hypertrophy does not affect ANG II-induced hypertension, suggesting that ANG II-induced hypertension is not solely due to development of vascular amplifiers (5). Second, because sympathetic nerves to two major vascular beds, the hind limb and kidney, do not play a role in ANG II-salt hypertension (15, 28), the depressor response to ganglionic blockade is most likely due to inhibition of vasomotor tone to the splanchic vascular bed. Taken together, these findings strongly support the idea that a decrease in MVR following ganglionic blockade in ANG II-salt rats is due to a decrease in sympathetic tone rather than autoregulatory responses secondary to withdrawal of sympathetic tone to other vascular beds.

Although the ANG II-induced hypertension model has been a popular model for the study of both neurogenic and nonneurogenic mechanisms of hypertension, the protocols employed in the literature are highly variable both between species and within species. Importantly, the mechanisms that participate and predominate in the hypertension generated by infusion of ANG II appear to be highly dependent on the administered dose (24) and the level of dietary salt (19). Although our protocol generates a model of salt-sensitive hypertension dependent on a neurogenic mechanism at high levels of salt...
intake, others, such as that of Luft and coworkers (18), have reported a significant neurogenic component in rats given a higher dose of ANG II but fed a normal salt diet. It is possible that higher levels of ANG II would offset the threshold for dietary salt levels required to activate the neurogenic response. Despite these differences, it appears that the splanchnic sympathetic nerves play a prominent role when a neurogenic mechanism is activated during ANG II-induced hypertension, as shown by our studies and studies by Luft and colleagues (18), which reported increases in directly measured splanchnic SNA in their model of ANG II-induced hypertension.

**Perspectives.** What is the clinical relevance of a delayed change in autonomic tone in the ANG II-salt model of hypertension? In our view, it demonstrates that there is a clear role for the autonomic nervous system during an established phase of salt-sensitive forms of hypertension. Although several mechanisms contribute to hypertension in the ANG II-salt model, the neurogenic component activated not only exacerbates the hypertension but it also likely participates in inducing end-organ damage and augmenting cardiovascular risk in general. Furthermore, our studies highlight the fact that neurogenic mechanisms underlying some forms of hypertension may not be limited to effects on the kidneys alone. Our results in fact indicate that changes in sympathetic control of splanchnic vascular resistance and capacitance dominate the neurogenic component of ANG II-salt hypertension (8). Whether the splanchnic vascular bed is an important sympathetic target in vascular resistance and capacitance dominate the neurogenic mechanisms in ANG II-salt hypertension (8). The splanchnic vascular bed is an important sympathetic target in vascular resistance and capacitance dominate the neurogenic mechanisms in ANG II-salt hypertension (8).

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M. T. Kuroki generated the data and wrote the manuscript. P. A. Guzman conducted preliminary studies and provided surgical assistance. J. W. Osborn and G. D. Fink conceived the study and edited the manuscript.

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**DISCLOSURES**

None.

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