Chronic Low-Dose Angiotensin II Infusion Increases Venomotor Tone by Neurogenic Mechanisms

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Abstract—The purpose of this study was to identify changes in venomotor tone in the chronic low-dose angiotensin II (Ang II) model of hypertension and to establish the contribution of sympathetic nerve activation to these venomotor tone changes. Male Sprague–Dawley rats were acclimatized to a 0.4% or 2.0% NaCl diet for 7 days and then catheterized to allow chronic and repeated measures of arterial pressure, central venous pressure, and mean circulatory filling pressure (MCFP), an index of venous smooth muscle tone, in conscious undisturbed rats. After 4 days of recovery and a 3-day control period, an Ang II or physiological saline-filled osmotic minipump was implanted subcutaneously to deliver Ang II (150 ng/kg per minute) or vehicle control for 14 days. MCFP was measured in duplicate before and after acute ganglionic blockade with hexamethonium (30 mg/kg IV) on control day 2 and Ang II infusion on days 1, 3, 7, and 14. Blood volume was also measured on these days and was unchanged for the duration of the study in all of the groups. Arterial pressure was increased for the duration of Ang II infusion in rats on both 0.4% and 2% NaCl diets, but the increase was significantly greater in the 2% NaCl group and completely abolished by hexamethonium. MCFP was significantly increased for the entire Ang II infusion period only in rats fed 2% NaCl, and this increase was completely abolished by hexamethonium. We conclude that the combination of chronic low-dose Ang II infusion and high dietary salt intake engages the sympathetic nervous system to increase venomotor tone. (Hypertension. 2006;48:927-933.)

Key Words: angiotensin II • venomotor tone • mean circulatory filling pressure • sympathetic nervous system • salt

Emerging evidence suggests that sympathetic nervous system activation may represent a common neurogenic mechanism of hypertension in both human essential hypertension and many experimental animal models. Angiotensin II (Ang II) has been identified as a humoral factor underlying hypertension and the pressor response to infusion of chronic low-dose Ang II in animals has been shown, at least in part, to be sympathetically driven. Advances have been made in the elucidation of the central pathways involved in mediating this sympathoexcitatory effect, suggesting that systemically delivered Ang II likely activates critical circumventricular organs with efferent projections to brain centers known to influence sympathetic nervous system activity. Oxidative stress may mediate this central sympathoexcitatory effect. However, there is still substantial uncertainty as to the critical peripheral target and the hemodynamic response to this increased sympathetic activity.

In this study, we investigated the possibility that the venous circulation may be an important target for Ang II–induced sympathetic nervous system activation. The venous system contains ~70% of the blood volume, mostly in the small veins and venules, and has been shown to be more sensitive to sympathetic activation than arterioles. In particular, the splanchnic venous bed is densely innervated by the sympathetic nervous system and represents the most important active capacitance bed in the body. It has been shown that increases in splanchnic sympathetic nervous system activity cause a translocation of blood toward the heart, increasing cardiac diastolic filling and cardiac output.

It is well established that chronic low-dose Ang II infusion is a salt-sensitive model of hypertension, and there is evidence to suggest that the additional hypertensive effect of salt is mediated by sympathetic nervous system activation. Although the most compelling evidence for sympathetic nervous system activation in response to salt is described in rats, it has also been demonstrated recently in rabbits using repeated assessment of response to ganglionic blockade. More importantly, there is considerable evidence that neurogenic mechanisms may play a role in the pathophysiology of salt sensitivity in human essential hypertension. A recent study measuring spontaneous arterial baroreflex sensitivity convincingly demonstrated abnormalities in autonomic control of the cardiovascular system in association with salt sensitivity, supporting the hypothesis that salt sensitivity is at least in part neurogenically driven. In this study, we test the hypothesis that chronic infusion of low-dose Ang II increases venous smooth muscle tone by activation of the sympathetic nervous system in a salt-sensitive manner.
Mean circulatory filling pressure (MCFP) is the pressure measured in the vasculature 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. We used repeated measures of MCFP in conscious, undisturbed rats fed a normal (0.4%) or high (2%) NaCl diet to investigate venomotor tone changes in chronic low-dose Ang II-induced hypertension.

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, Portage, Mich), weighing 225 to 250 g at the beginning of the study, were acclimatized to a 0.4% or 2% NaCl diet (Research Diets) for 7 days before catheterization. During this time, rats were housed 3 per cage in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. Free access to food and distilled water was allowed.

Catheterization
Catheterization was similar to that described previously. After 7 days of dietary acclimatization, rats were chronically instrumented with catheters for the measurement of arterial pressure (AP) and central venous pressure (CVP), to produce brief circulatory arrest, and to allow drug administration and blood sampling. The rats were premedicated with atropine (0.04 mg/kg IP), and anesthesia was produced with sodium pentobarbital (40 to 50 mg/kg IP). Silicone-tipped catheters were inserted into the abdominal aorta, thoracic vena cava, and abdominal vena cava through the femoral artery or vein. A silicone right atrial balloon catheter (Vesta) was inserted through the right jugular vein. Optimal balloon catheter positioning was confirmed by a rapid decline in AP (to <30 mm Hg within 2 to 3 seconds) and simultaneous rise in CVP (to 6 to 8 mm Hg) in response to balloon inflation with 0.25 mL of saline. The catheter was secured in this location. The ends of all of the catheters were tunneled subcutaneously and exited the rat through the scalpula 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. Rats recovered from anesthesia, under close observation, on a heating pad. The rats were then loosely tethered in individual plastic cages to allow continuous access to all of the catheters without handling or disturbing. 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
Hemodynamic measurements were similar to those described previously. AP was determined by connecting the arterial catheter to a pressure transducer (TxD-300, Micro-Med) linked to a digital pressure monitor (BPA-400, Micro-Med) that provided measurements of systolic, diastolic, and mean arterial (MAP) pressures and heart rate (HR) at a sampling rate of 1000 Hz. The pressure monitor was connected to a computerized data acquisition program (DMSI-400, Micro-Med). The central venous catheter was connected to a separate pressure transducer and monitor to allow simultaneous recordings of CVP. The pressure transducers were calibrated daily against a column of water. MCFP measurements were made according to established methods for the rat. 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 CVP, both of which quickly plateau. This method results in “trapping” of blood on the low compliance arterial side, preventing full equalization of pressure throughout the circulation. To correct for this, MCFP was computed from arterial plateau pressure (APP) and venous plateau pressure (VPP) using the following formula: MCFP=VPP+(APP−VPP)/60. Each measurement of MCFP was taken 10 minutes after the previous measurement.

Volume Measurements
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: BV=PV/[1−Hct(0.8)/100].

Experimental Protocols
After 4 days of recovery and a 3-day control period, an Ang II (Sigma) or physiological saline-filled osmotic minipump (2ML2, Alzet) was implanted subcutaneously to deliver Ang II (150 ng/kg per minute) or vehicle control for 14 days. AP was measured at the same time daily for the duration of the experiment and recorded as a 10-minute average. MCFP was measured in duplicate before and starting 5 minutes after acute ganglionic blockade with hexamethonium (30 mg/kg IV) on control day 2 and Ang II infusion days 1, 3, 7, and 14. Before the MCFP measurements, the catheters were flushed and connected to pressure transducers. Rats were then allowed to sit undisturbed for 20 minutes. 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 0.4% NaCl or 2% NaCl diet and distilled water.

Statistical Analysis
Within-group differences were assessed by a 1-way repeated-measures ANOVA with post hoc multiple comparisons using Dunnett’s procedure (GraphPad Instat 3). Between-group differences were assessed by a 2-way mixed-design ANOVA, and post hoc testing at each time point was performed using Bonferroni’s procedure to correct for multiple comparisons (GraphPad Prism 4). A P value of <0.05 was considered significant. All of the results are presented as mean±SE.

Results
A total of 24 rats were studied in 4 different groups: 0.4% NaCl diet + vehicle ([NV] n=4), 0.4% NaCl diet + Ang II ([NA] n=8), 2% NaCl diet + vehicle ([HV] n=4), and 2% NaCl diet + Ang II ([HA] n=8). The MAP and HR response to chronic subcutaneous infusion of Ang II (150 ng/kg per minute) or saline vehicle is shown in Figure 1. MAP was not different between the 4 groups during the control period (NV 101±3 mm Hg, NA 100±2 mm Hg, HV 99±3 mm Hg, and HA 101±2 mm Hg). Similarly HR was not different between the 4 groups during the control period (NV 437±7 mm Hg, NA 413±12 mm Hg, HV 394±25 mm Hg, and HA 416±8 BPM). Additionally, MAP (NV 101±1 mm Hg versus HV 100±2 mm Hg on day 14 of vehicle infusion) and HR (NV 391±12 mm Hg versus HV 391±14 BPM on day 14 of vehicle infusion) did not change in response to vehicle infusion. Ang II caused a significant increase in MAP for the entire duration of infusion, irrespective of salt diet; however, the magnitude of the increase was much greater in rats on a 2% NaCl diet (NA 117±3 mm Hg versus HA 155±7 mm Hg on day 14 of Ang II infusion). HR was significantly decreased...
on Ang II infusion days 3 to 6 in rats on a 2% NaCl diet but was no different from rats fed a 0.4% NaCl diet for the remainder of the infusion period.

Figure 2 shows BV and MCFP responses to chronic subcutaneous infusion of Ang II or saline vehicle measured on control day 2 and infusion days 1, 3, 7, and 14. BV tended to be higher during the control period in rats fed a 0.4% NaCl diet (NV 27 ± 1 mL and NA 26 ± 1 mL) compared with rats on a 2% NaCl diet (HV 25 ± 1 mL and HA 24 ± 1 mL); however, this was not statistically significant. Also, there were no statistically significant changes in BV in response to vehicle or Ang II infusion in any group. MCFP tended to be higher during the control period in rats fed a 0.4% NaCl diet (HV 25 ± 1 mL and HA 24 ± 1 mL); however, this was not statistically significant. Also, there were no statistically significant changes in MCFP in response to vehicle infusion in rats fed either diet (NV 7.0 ± 0.4 mm Hg and HV 6.6 ± 0.2 mm Hg on day 14 of infusion) or in response to Ang II infusion in rats fed a 0.4% NaCl diet (NA 6.9 ± 0.3 mm Hg on day 14 of infusion). However, there was a significant and marked increase in MCFP in response to Ang II infusion in rats fed a 2% NaCl diet. This increase was significant on day 1 (8.5 ± 0.3 mm Hg) of Ang II infusion, and MCFP was further increased on days 3 (8.5 ± 0.8 mm Hg), 7 (9.8 ± 0.7 mm Hg), and 14 (9.8 ± 0.8 mm Hg) of Ang II infusion.

Peak MAP response to acute ganglionic blockade with hexamethonium (30 mg/kg IV) is shown in Figure 3 along with the MCFP response 5 and 15 minutes after hexamethonium administration. MAP response to hexamethonium was no different between the 4 groups during the control period (NV 47 ± 3 mm Hg, NA 41 ± 2 mm Hg, HV 42 ± 4 mm Hg, and HA 41 ± 2 mm Hg) and did not change in response to vehicle infusion in rats fed either diet (NV 47 ± 4 mm Hg and HV 43 ± 5 mm Hg on day 14 of infusion) or in response to Ang II infusion in rats fed a 0.4% NaCl diet (NA 49 ± 6 mm Hg on day 14 of infusion). However, there was a significant and marked increase in MAP response to hexamethonium, in response to Ang II infusion, in rats fed a 2% NaCl diet. This increase was significant on day 1 (−80 ± 6 mm Hg) of Ang II infusion, remained statistically

Figure 1. Heart rate (A) and MAP (B) response to chronic subcutaneous infusion of Ang II (150 ng/kg per minute) or saline vehicle started immediately after C3 time point in rats fed either a 2% or 0.4% NaCl diet. C, control day; A, Ang II or vehicle infusion day. *Significant difference (P < 0.01) between specific Ang II infusion day and control period average (C1, C2, and C3). #Significant difference (P < 0.01) between specific Ang II infusion day of rats fed 2% NaCl vs rats fed 0.4% NaCl.
significantly increased on day 3 (−63±4 mm Hg), and was further increased on days 7 (−90±4 mm Hg) and 14 (−93±4 mm Hg) of Ang II infusion. MCFP response 5 minutes after hexamethonium was no different between the 4 groups during the control period (NV −3.3±0.8 mm Hg, NA −2±0.3 mm Hg, HV −2.7±0.6 mm Hg, and HA −2.5±0.3 mm Hg) and did not change in response to vehicle infusion in rats fed either diet (NV −2±0.7 mm Hg and HV −2.7±0.4 mm Hg on day 14 of infusion) or in response to Ang II infusion in rats fed a 0.4% NaCl diet (NA −2.2±0.3 mm Hg on day 14 of infusion). However, there was a significant and marked increase in MCFP response to hexamethonium in Ang II infused rats fed a 2% NaCl diet. This increase was significant on day 1 (−5±0.4 mm Hg) of Ang II infusion, remained statistically significantly increased on day 3 (−5.3±0.6 mm Hg), and was further increased on days 7 (−6.4±0.7 mm Hg) and 14 (−6.2±0.8 mm Hg) of Ang II infusion. MCFP response 15 minutes after hexamethonium was very similar to 5 minutes (Figure 3).

Discussion

Consistent with previous studies, we have demonstrated a salt-sensitive hypertension in response to infusion of chronic low-dose Ang II. This dose of Ang II, when administered subcutaneously via osmotic minipump, has been shown to increase plasma Ang II levels ~2-fold and results in plasma concentrations within the pathophysiological range. More importantly, our results support the conclusion that the additional hypertensive response to salt is sympathetically driven, indicated by increased AP responses to acute ganglionic blockade. AP response to ganglionic blockade did not change during Ang II infusion in rats fed a normal-salt diet. This indicates that the rise in AP to Ang II in rats on a normal-salt diet may not be neurally mediated. However, the expected response to increased AP is baroreflex-mediated sympathoinhibition. The finding that the AP response to ganglionic blockade did not decrease in this group suggests that Ang II may prevent this sympathoinhibition. This is consistent with a previous report, which found that plasma
norepinephrine levels were unchanged by Ang II alone but markedly increased when administered in combination with a high-salt diet.\textsuperscript{13}

The major new finding in this study was that MCFP was increased in response to Ang II infusion, beginning on day 1 and increasing further on days 3, 7, and 14, only when administered in combination with high dietary salt. In the absence of a change in blood volume, increases in MCFP represent an increase in venous tone.\textsuperscript{21,41,42} In the present study, blood volume was unchanged. Therefore, we have demonstrated the ability of Ang II, in combination with high dietary salt, to chronically increase venous smooth muscle tone. This increase in MCFP was essentially abolished by acute ganglionic blockade with hexamethonium, suggesting that the increase in venomotor tone was neurogenically driven.

Interestingly, the sympathetic nervous system activation demonstrated in the Ang II-infused group fed a high-salt diet seemed to be regionally heterogeneous. Venous sympathetic activity clearly increased, as indicated by a hexamethonium-sensitive increase in MCFP; however, this occurred in the absence of any evidence of increased cardiac sympathetic activity. HR can provide a useful index of cardiac sympathetic activity,\textsuperscript{45} and no tachycardia was observed over the 14-day infusion period. The finding of regionalized sympathetic activation is consistent with other experimental findings demonstrating differential control of lumbar and renal sympathetic nerve activity in rabbits.\textsuperscript{46} Furthermore, by using regional norepinephrine spillover techniques, Esler and colleagues\textsuperscript{47–51} have elucidated the importance of regionalized sympathetic activation in human cardiovascular disease. However, the apparent regionalized sympathetic activation seen in this study may be because of the limitation of using HR as an index of sympathetic nerve activity or may be a result of temporal differences in the activation pattern.

Figure 3. Hemodynamic response to acute ganglionic blockade with hexamethonium (30 mg/kg IV). Peak fall in MAP (A) and decline in MCFP measured 5 minutes (B) and 15 minutes (C) after hexamethonium. C, control day; A, Ang II or vehicle infusion day. *Significant difference ($P<0.01$) between specific Ang II infusion day and control period average (C1, C2, and C3). #Significant difference ($P<0.01$) between specific Ang II infusion day of rats fed 2% NaCl vs rats fed 0.4% NaCl.
Reduced vascular capacitance has been documented as a feature of human essential hypertension and in many experimental animal models, including deoxycorticosterone acetate (DOCA) salt hypertension, spontaneously hypertensive rat, angiotensin-dependent 2-kidney, 1-clip hypertensive rat, and in 1-kidney, 1-clip Goldblatt hypertension. The increase in venous constriction and subsequent decrease in whole body venous capacity is commonly neurogenically driven in these models, and blood volume tends to be normal or decreased. Also, it has been shown recently that the salt sensitivity of DOCA salt hypertension is not mediated primarily by volume-related mechanisms but rather an increased sensitivity of blood pressure to total body water content. This again emphasizes the importance of reduced vascular capacitance in salt-sensitive hypertension. Vascular capacitance is strongly controlled by the sympathetic nervous system and is predominately influenced by the compliance of the venous system, suggesting that sympathetically driven increases in venous constriction and subsequent decrease in whole body venous capacity is commonly neurogenically driven in these models, and blood volume tends to be normal or decreased. Also, it has been shown recently that the salt sensitivity of DOCA salt hypertension is not mediated primarily by volume-related mechanisms but rather an increased sensitivity of blood pressure to total body water content.

It has been shown previously that acute infusion of Ang II increases venous tone in rats by both direct and neurogenic mechanisms. Also, chronic infusion of Ang II, in dogs fed a high-salt diet, increased MCFP and resulted in an increase in total peripheral resistance and a decrease in cardiac output. This marked increase in MCFP occurred in the absence of blood volume changes, indicating a reduction in vascular compliance mediated by venoconstriction. However, the salt sensitivity, temporal profile, and contribution of sympathetic nervous system activation to venomotor tone changes in this well-characterized model of chronic low-dose Ang II infusion in rats has not been investigated.

We propose that increases in venomotor tone, particularly in the active capacitance bed of the splanchnic circulation, contribute to Ang II-salt hypertension by increasing the driving force for venous return to the heart resulting in a translocation of venous blood to the less compliant arterial system. Guyton’s group have suggested that increases in MCFP will result in chronic hypertension only when associated with a simultaneous impairment of renal excretory function. It has been well documented by this same group that the salt sensitivity of DOCA salt hypertension is not mediated primarily by volume-related mechanisms but rather an increased sensitivity of blood pressure to total body water content.

What is the physiological impact of reduced vascular capacitance in hypertension? Blood volume generally is not increased, so reduced vascular capacitance accounts for the increased “effective blood volume” characteristic of established hypertension. That is, hypertensive individuals behave as if they are volume expanded: for example, they exhibit greater increments in cardiac output and natriuresis to acute volume loads and larger hypertensive responses to diuretic drug treatment. Reduced vascular capacitance, therefore, makes a significant contribution to the circulatory physiology of hypertension. The sympathetic nervous system, by virtue of its influence on splanchnic venous smooth muscle tone, is the principal factor regulating vascular capacitance. A better understanding of sympathetic control of capacitance vessels, especially in the setting of excess salt intake, could provide new approaches to the treatment or prevention of high blood pressure.

References
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