

Does whole body autoregulation mediate the hemodynamic responses to increased dietary salt in rats with clamped ANG II?

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Fine, Deborah M., Pilar Ariza-Nieto, and John W. Osborn. Does whole body autoregulation mediate the hemodynamic responses to increased dietary salt in rats with clamped ANG II? *Am J Physiol Heart Circ Physiol* 285: H2670–H2678, 2003. First published August 7, 2003; 10.1152/ajpheart.00395.2003.—The present study was conducted to test the hypothesis that salt-dependent hypertension, in rats with an unresponsive renin-angiotensin system, is characterized by a “whole body autoregulation” hemodynamic profile. To test this hypothesis, rats were chronically instrumented to continuously measure cardiac output (CO) and arterial pressure (AP). A venous catheter was implanted for infusion of saline vehicle (Veh; $n = 8$) or treatment [enalapril ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) plus ANG II: ANG-NORM ($5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ANG II, $n = 8$) or ANG-HI ($10 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ANG II, $n = 9$)] to pharmacologically clamp plasma ANG II. After a 10-day recovery period on a 0.1% NaCl diet, AP and CO were measured continuously for 5 days of control (0.1% NaCl), 7 days of high salt (4.0% NaCl), and 5 days of recovery (0.1% NaCl). Hemodynamics did not change in the Veh group at any time. AP increased by ~ 20 mmHg in the ANG-NORM and ANG-HI groups when NaCl was increased. Hypertension was mediated by an increase in CO of $\sim 12\%$ at steady state, with no change in total peripheral resistance (TPR) during the high salt period. AP returned to control levels when dietary sodium was decreased, mediated by a $\sim 10\%$ decrease in TPR, with CO remaining elevated. There was no difference in the hemodynamic responses to increased salt between the ANG-HI and ANG-NORM groups. We conclude that the whole body autoregulation hypothesis does not explain the hemodynamic profile of salt-dependent hypertension in rats with an unresponsive renin-angiotensin system. cardiac output; vascular resistance; salt sensitivity; arterial pressure regulation

FOR OVER 100 YEARS, cardiovascular disease has been the leading cause of death in the United States (29), and hypertension is one of the most important risk factors for the development of cardiovascular disease (15). Despite decades of intensive research into the mechanisms of hypertension, there is still no consensus regarding the underlying defects or even the sequence of hemodynamic events leading to hypertension (3).

Elevated dietary sodium has long been implicated in the pathogenesis of hypertension (26, 39). It is well established that cultures with very high dietary sodium levels have a much greater incidence of hypertension

than those with low-sodium diets (21, 28). Although animal models of salt-dependent hypertension have been developed, including genetic, pharmacological, and surgical methods (33), the mechanistic link between dietary salt and hypertension remains poorly understood.

A critical question that remains unanswered is as follows: what are the transient and steady-state hemodynamic mechanisms underlying salt-induced increases in arterial pressure (AP)? One prominent hypothesis proposes that the kidneys, through regulation of the sodium and water balance, play the predominant role in the long-term control of AP (12). In this model, hypertension results from an impairment in the renal sodium excretory ability. When confronted with a sodium load, this defect results in sodium and water retention and blood volume expansion, which increases cardiac output (CO) and, consequently, AP (2, 23). The resulting tissue hyperperfusion leads to an autoregulatory vasoconstriction resulting in a sustained increase in peripheral vascular resistance. This response, referred to as “whole body autoregulation,” assures that delivery of nutrients and removal of metabolic waste products to and from the tissues, respectively, matches the metabolic activity of the tissue. The sustained elevation in AP returns sodium and water excretion to normal (i.e., pressure natriuresis and diuresis), which tends to return CO to near-normal levels. Thus this model predicts that hypertension is initiated by an early increase in CO, but the steady-state hemodynamics are characterized by an increase in peripheral resistance with little contribution of CO. Although whole body autoregulation has been proposed to explain the hemodynamic profile of all forms of salt-dependent hypertension (12), this unifying hypothesis has not been thoroughly tested due to the technical challenges of acquiring continuous long-term hemodynamic data in chronically instrumented animals.

The present study was designed to test the hypothesis that whole body autoregulation mediates the hemodynamic responses to increased dietary salt in an established model of salt-dependent hypertension (32). To test this hypothesis, we developed hemodynamic monitoring techniques that allowed us to measure AP, CO, and heart rate (HR) continuously for up to 6 mo in chronically instrumented rats. Using these methods, we characterized the hemodynamic profile of salt-dependent hyper-

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tension in rats in which plasma ANG II was pharmacologically clamped at normal or high levels.

METHODS

General Procedures

Male Sprague-Dawley rats (275–375 g) were purchased from Charles River Laboratories (Wilmington, MA). Rats were initially group housed in the cardiovascular monitoring laboratory with controlled temperature conditions and a 12:12-h light-dark cycle. Distilled water and rodent chow were available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with institutional and National Institutes of Health guidelines.

Surgical Procedures

Rats were instrumented for long-term continuous monitoring of AP, HR, and CO. Rats were anesthetized using isoflurane in a gas induction chamber, intubated, and then placed on a Harvard rodent ventilator (model 683, Harvard Apparatus; South Natick, MA). Anesthesia was maintained with a mixture of oxygen and 2% isoflurane delivered by a Bickford vaporizer (model 61020WOB; Wales Center, NY). At the time of induction, rats received atropine sulfate (0.06 mg ip), butorphanol tartrate (0.5 mg sc), and gentomycin sulfate (2.5 mg im).

The femoral vein was surgically exposed and catheterized for chronic infusion of saline vehicle (Veh) or ANG II plus enalapril (see *Experimental Protocol*). The catheter was tunneled subcutaneously and exteriorized through an incision just caudal to the scapulae. After the placement of the venous catheter, 15 mg ampicillin sodium was administered intravenously.

A midline laparotomy was performed, and the abdominal aorta and caudal vena cava were exposed. The aorta and vena cava were bluntly dissected apart. The catheter portion of a radiotelemetry transmitter (model TA11PA-C40, Data Sciences; St. Paul, MN) was then inserted into the aorta using a 21-gauge needle as a catheter introducer. The catheter was advanced rostrally so that the tip was distal to the renal arteries. The insertion point was closed with cyanoacrylate glue and a small patch of absorbent paper. The body of the transmitter was sutured to the abdominal wall, and the incision was closed routinely.

A midline thoracotomy was then performed by dividing the sternum from the midportion of the manubrium to just above the xiphoid process. The fascia connecting the ascending aorta and main pulmonary artery was carefully dissected apart until there was sufficient space to pass the reflector portion of an ultrasonic transit-time flow probe around the aorta (model SB2.5, Transonic Systems; Ithaca, NY). The flow probe cable exited the chest at the cranial end of the incision and was tunneled subcutaneously over the shoulder and exteriorized at the same site as the venous catheter. The chest was closed in three layers, and a 23-gauge butterfly catheter was used to suction air out of the pleural space to reestablish negative pressure.

The rats were individually housed upon recovery from anesthesia. A lightweight steel spring and custom-made polyester jacket secured and protected the venous catheter and flow probe cable. The spring and cable were connected to an electrical swivel (model SL6C, Kent Scientific; Torrington, CT) above the cage.

Experimental Protocol

Before surgery, rats were randomly assigned to one of three different treatment groups (Veh, ANG-NORM, and ANG-HI).

Group 1: Veh group (n = 8). Rats in this group served as the control group and received an intravenous infusion of 0.9% saline.

Group 2: ANG-NORM group (n = 8). This group received an infusion of the angiotensin-converting enzyme inhibitor enalapril ($2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) and ANG II ($5 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) to suppress the endogenous production of ANG II and “clamp” ANG II levels at approximately normal plasma concentrations. We (32) have previously shown that this combination results in normal resting levels of AP, HR, and plasma ANG II.

Group 3: ANG-HI group (n = 9). Rats in the third group received enalapril ($2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) plus ANG II ($10 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) to clamp ANG II at elevated plasma levels. ANG II and enalapril were dissolved in 0.9% NaCl, and all infusions were delivered at a volume flow rate of 7 ml/24 h throughout the protocol.

The rats were given 10 days to recover after surgery. For the first 3 days postoperatively, they were treated with 15 mg ampicillin sodium and 1 mg tobramycin sulfate intravenously for antibiotic prophylaxis. They also received 0.015 mg buprenorphine hydrochloride intravenously for 5 days for analgesia. Distilled water and a 0.1% NaCl diet (Research Diets; New Brunswick, NJ) were available ad libitum. An intravenous infusion of 5% dextrose in water was given (7 ml/24 h) until food intake returned to normal (3–5 days postsurgery). Infusion of Veh or enalapril plus ANG II treatment was initiated on *day 6* of the recovery period to allow time for stabilization of basal hemodynamics before the protocol was initiated. A 5-day control period for recording of baseline hemodynamic parameters began on *day 10*. After this, rats were switched to a high-salt diet (4.0% NaCl, Research Diets) for 7 days. The protocol was then completed with a 5-day recovery period in which dietary NaCl was switched back to 0.1%.

Monitoring Techniques

The AP transmitter signal was monitored by a Data Sciences receiver (model RPC-1) mounted behind the cage and connected to a data exchange matrix. The CO signal was transmitted via the flow probe cable to a flowmeter (model T-206, Transonic Systems); the output was digitized with a Data Sciences analog-to-digital converter (model C11V) and then sent to the data exchange matrix. Data acquisition and analysis were performed using Dataquest ART version 2.2 software on a Dell XPS B866 computer. AP, CO, and HR were sampled for 10 s every 5 min throughout the entire protocol.

Daily food and water intake were measured. Sodium intake was calculated as the product of total food intake and sodium content in the diet (0.1% NaCl = 0.0175 mmol/g; 4.0% NaCl = 0.7 mmol/g) plus the sodium present in the daily infusion (1.078 mmol/day iv).

Data Analysis and Statistics

Twenty-four-hour averages of AP, CO, and HR were calculated for each animal from the raw data sampled from 12:00 AM to 11:59 PM daily. CO was normalized to body weight by dividing the measured CO by the daily body weight (in g). This adjusted for the increase in CO that occurs normally with growth. The daily weight gain (in g/day) was calculated by subtracting the beginning body weight from the ending body weight and then dividing by the number of days

in the protocol. Total peripheral resistance (TPR) was calculated from the measured AP and CO ($TPR = AP/CO$).

A two-way ANOVA with repeated measures was used to compare the hemodynamic effects of dietary sodium manipulation in rats with a responsive renin-angiotensin system (Veh group), fixed normal plasma ANG II levels (ANG-NORM group), and fixed elevated plasma ANG II levels (ANG-HI group). The Dunnett's multiple-comparison test was used to evaluate differences within the groups, comparing the average of the 5-day control period (0.1% NaCl) versus the high-salt period (*days 6–12*, 4.0% NaCl) and the recovery period (*days 13–17*, 0.1% NaCl). Percent changes in hemodynamics during the high-salt and recovery periods were calculated using the average of the 5-day control period. The absolute hemodynamic values for each day of the high-salt and recovery period were subtracted from the average of the 5-day control, and the product was then divided by the 5-day average. Day-by-day comparisons between groups were performed using the Tukey-Kramer multiple-comparison test. All values are reported as means \pm SE. Analyses were performed using statistical software (NCSS 2000; Kaysville, UT). A P value ≤ 0.05 was accepted as significant.

RESULTS

Hemodynamic Responses of Treatment Groups to Increased Dietary Salt

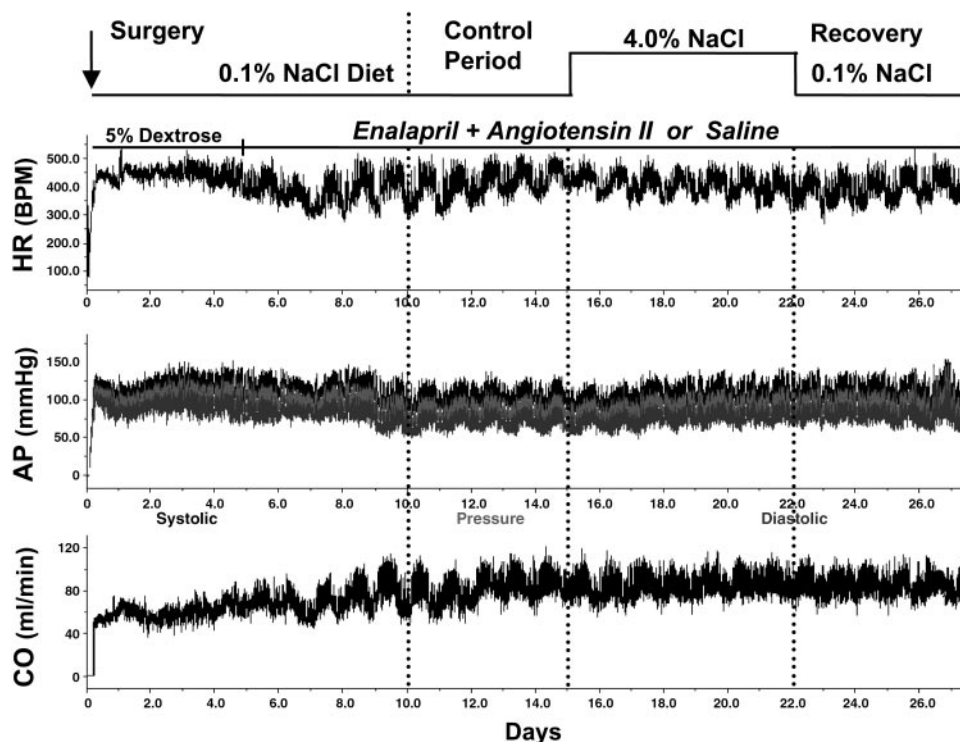
Figure 1 diagrams the experimental protocol and shows representative, unfiltered traces for HR, AP, and CO from a single Veh rat during the 27-day study. Note the prominent 24-h circadian rhythm present in the HR. Circadian rhythms were also present, although less pronounced, in the AP and CO traces. The circadian rhythms were absent or only poorly defined until ~ 6 –8 days postsurgery. This demonstrates the importance of having a surgical recovery period that is

of sufficient length to allow for the return of normal physiological parameters. The peaks in HR, AP, and CO all occurred during the night period, when rats are the most active and consume the majority of their daily food and water intake. There was no significant change in AP when dietary sodium was increased to 4.0% in this rat, which is the expected response in an animal with a normally functioning renin-angiotensin system (32).

Measurements of 24-h sodium and water intake were performed (Fig. 2); 24-h sodium intake during the control period was similar in all groups, ~ 1.5 mmol/day. Sodium intake increased to a similar steady-state level in all three groups (~ 15 mmol/day) when dietary NaCl was increased to 4.0%. Sodium intake returned to control levels in all three groups during the recovery period. Similarly, there were no statistically significant differences in ad libitum water intake during the control, high-salt, or recovery periods for the three groups.

Figure 3A shows the 24-h averages of mean AP (MAP) for each treatment group during the control, high-salt, and recovery periods. There was no change in MAP in the Veh group in response to changes in dietary sodium. MAP of the ANG-NORM group was not different from the Veh group at baseline (0.1% NaCl). In contrast, MAP of the ANG-HI group was increased ~ 20 mmHg over that of the other two groups during the control period. After 4 days on the high-salt diet (4.0% NaCl), MAP in both ANG II treatment groups had increased by ~ 20 mmHg. This increase was significant on *days 2–7* of high salt in both groups. AP in both ANG II groups rapidly returned to the respective baseline levels during the recovery period (0.1%

Fig. 1. *Bottom*: representative tracings of the complete heart rate [HR; in beats/min (BPM)], arterial pressure (AP), and cardiac output (CO) data from a saline vehicle-treated (Veh) rat during its entire 27-day protocol. Of particular note are the prominent circadian rhythms in these traces. The peak in each hemodynamic parameter occurred during the night, which is when rats are the most active. Circadian rhythms emerged 6–8 days after surgery. *Top*: diagram of the experimental protocol.



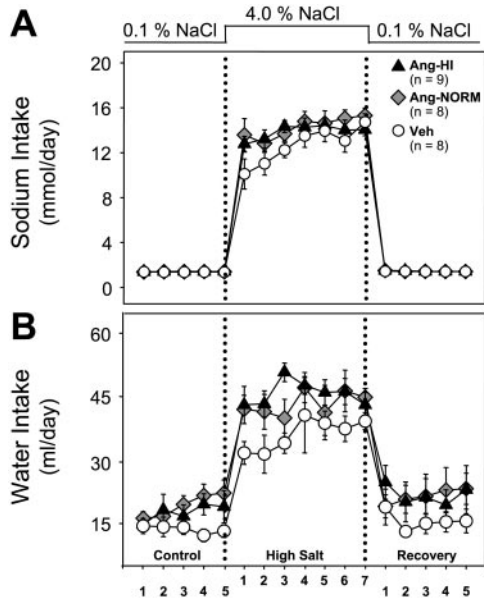


Fig. 2. Twenty-four-hour measurements of sodium intake (A) and water intake (B) during the control (0.1% NaCl, 5 days), high-salt (4.0% NaCl, 7 days), and recovery periods (0.1% NaCl, 5 days) for the Veh and ANG II-treated rats (ANG-NORM and ANG-HI). *Top*, diagram of the dietary protocol. There was no difference in sodium or water intake among treatment groups at any time during the protocol.

NaCl). Between-group comparisons showed that MAP of the ANG-HI group was significantly higher than that of the Veh group at every time point except the last 2 days of the recovery period. Similarly, MAP of the ANG-HI group was increased over that of the ANG-NORM group at every point except the last day of recovery.

In contrast to AP, there were no statistically significant differences in basal HR among the Veh (431 ± 33 beats/min), ANG-NORM (413 ± 12 beats/min) or ANG-HI (414 ± 28 beats/min) groups (mean of the 5-day control period). In addition, there were no statistically significant changes in HR in any group during the high-salt or recovery periods ($P = 0.17$, data not shown).

As shown in Fig. 3B, CO was not different among the three groups during the control period. However, during the high-salt period, CO increased significantly in both the ANG-NORM and ANG-HI groups but not in the Veh group. In the ANG-HI group, the increase in CO was statistically significant on *days 1–6* of the high-salt period and on *day 5* of the recovery period compared with the control period. CO was significantly increased in the ANG-NORM group on *days 3–5* and *day 7* of the high-salt period and *day 4* of the recovery period compared with the control period.

TPR was significantly elevated in the ANG-HI group compared with the ANG-NORM group at all comparison points in the study except for *day 1* of the high salt period and the last 3 days of the recovery period (Fig. 3C). More importantly, there were no statistically significant changes in TPR in response to increased dietary salt in any of the groups. Surprisingly, however,

both ANG II groups exhibited a vasodilatory response when switched back to a 0.1% NaCl diet. This response was not observed in the Veh group. This resulted in a statistically significantly lower TPR in the ANG-NORM group relative to the Veh group on *days 1–5* of recovery. Within the ANG-HI group, TPR actually decreased below the control period on the last 3 days of the recovery period.

Percent Changes in Hemodynamic Responses to Increased Dietary Salt

Analyses were performed on the percent change in hemodynamics to distinguish results that were primarily ascribable to alterations in the dietary salt levels. Figure 4 shows the responses of MAP, CO, and TPR during the high-salt and recovery periods as the percent change of the measurements from control. The

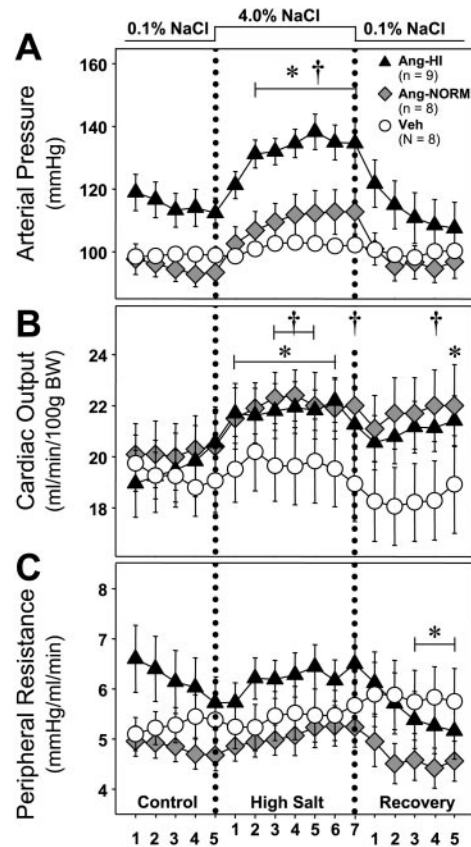


Fig. 3. Twenty-four-hour averages of mean AP (MAP; A), CO (B), and total peripheral resistance (TPR; C) during the control (0.1% NaCl, 5 days), high-salt (4.0% NaCl, 7 days), and recovery periods (0.1% NaCl, 5 days) for Veh, ANG-NORM, and ANG-HI rats. *Top*, diagram of the dietary protocol. BW, body weight. *ANG-HI versus the 5-day average of the control period; †ANG-NORM versus the 5-day average of the control period. Significant differences in MAP between groups are as follows: ANG-HI versus Veh, control period average, high-salt *days 1–7*, and recovery *days 1–3*; and ANG-HI versus ANG-NORM, control period average, high-salt *days 1–7*, and recovery *days 1–4*. Significant differences in TPR between groups are as follows: ANG-HI versus ANG-NORM, high-salt *days 2–7* and recovery *days 1* and *2*; ANG-HI versus Veh, high-salt *days 2* and *5*; and ANG-NORM versus Veh, recovery *days 1–5*. $P < 0.05$ for all significant comparisons.

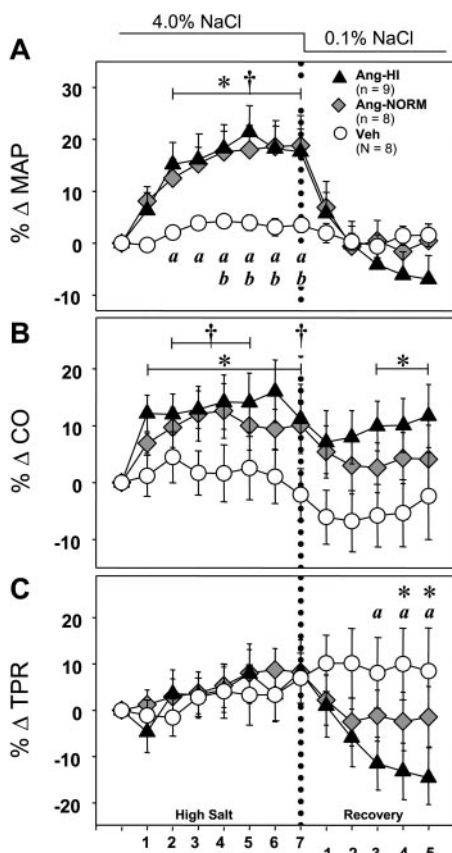


Fig. 4. Percent change in the 24-h averages of MAP, CO, and TPR during the high-salt (7 days) and recovery periods (5 days). *Top*, diagram of the dietary protocol. *ANG-HI, significant percent change within group; †ANG-NORM, significant percent change within group; ^aANG-HI versus Veh; ^bANG-NORM versus Veh. $P < 0.05$ for all significant comparisons.

percent change in MAP of the ANG-NORM and ANG-HI groups was essentially identical during the 7 days of increased salt intake and the 5-day recovery period. In both groups, the percent increase in MAP was significant on all but the first day of the high-salt period. Between-group comparisons showed that the percent change in MAP of the ANG-HI group was significantly higher than that of the Veh group on *days* 2–7 of high salt. Similarly, the percent change in MAP of the ANG-NORM group was significantly increased over that of the Veh group on *days* 4–7 of high salt (Fig. 4A).

The percent change in CO during the 7 days of salt loading was not significantly different among the three groups (Fig. 4B). However, within the ANG II groups, there were significant increases in the percent change in CO during the high-salt period. Despite the rapid recovery of AP during the 5-day recovery period (Fig. 4A), CO did not immediately normalize during this time. Indeed, when expressed as the percent change from control, CO remained elevated above control during the final 3 days of the recovery period in the ANG-HI group.

As seen in Fig. 4C, despite the fact that TPR did not change during the high-salt period in any of the

groups, the rapid fall of AP during the recovery period was the result of a decrease in TPR in both ANG II-treated groups. Indeed, TPR fell below control values during the last 2 days of the recovery period in the ANG-HI group.

DISCUSSION

Hemodynamic Profile of Salt-Dependent Hypertension in Animals With a Clamped Renin-Angiotensin System

In 1963, Borst and Borst-de Geus (1) and Ledingham and Cohen (22) independently introduced the concept of whole body autoregulation. Since then, Guyton (12) has proposed this theory to explain the hemodynamic profile of all models of hypertension. This hypothesis, elegant in its simplicity and logic, has rarely been tested using continuous long-term recordings of AP and CO in conscious unrestrained animals. Such an experimental approach is critical because anesthesia has profound effects on the neurohormonal control of cardiovascular function, and short-term intermittent hemodynamic measurements are likely to miss transient changes crucial to the understanding of the pathogenesis of hypertension.

The present study was conducted to test the hypothesis that salt-dependent hypertension, in rats with an unresponsive renin-angiotensin system, is characterized by a whole body autoregulation hemodynamic profile (1, 22). Several aspects of the experimental protocol were designed to ensure that hemodynamic measurements were made under optimal experimental conditions. First, rats were given a considerable recovery period (10 days) after the surgical instrumentation to ensure they were in a stable hemodynamic state. Recovery was indicated not only by the day-to-day stability of the variables measured but also by the emergence of normal circadian rhythms of these variables. Second, AP, CO, and HR were monitored continuously, 24 h/day, throughout the protocol in conscious unrestrained rats. Finally, the 17-day experimental protocol included 5-day control and recovery periods bracketing the 7-day high-salt period, further enabling us to assess the hemodynamic stability of these animals.

The results of this study are not consistent with the whole body autoregulation theory, which predicts that salt-induced increases in AP result from an initial increase in CO, followed by a slow increase in vascular resistance secondary to autoregulatory vasoconstriction and return of CO to normal (22). In contrast, we observed that the salt-induced increase in AP was mediated entirely by a sustained elevation of CO, whereas vascular resistance remained unchanged during the 7-day period of high salt intake. This finding suggests that CO was not regulated at the expense of AP, as suggested by the whole body autoregulation theory. This conclusion is further underscored by the observation that CO remained elevated during the recovery period, when dietary salt was switched back to normal levels. Finally, the fact that AP promptly

returned to control levels at this time, secondary to a decrease in vascular resistance, suggests that the regulation of AP prevailed over CO during the recovery period. This is in direct conflict with the whole body autoregulation theory.

In contrast to our findings, a previous study (20) of salt-dependent hypertension in dogs infused with a subpressor dose of ANG II ($3 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) showed a hemodynamic profile consistent with the theory of autoregulation. Hypertension induced by a 7-day period of high salt was mediated by an initial increase in CO, followed by a more gradual rise in vascular resistance. CO remained significantly elevated throughout the 7-day high-salt period but did show a tendency to decline toward control values. The increase in CO was attributed to volume expansion secondary to retention of sodium and water. This conclusion was supported by a subsequent study (18) demonstrating that salt-sensitive hypertension did not occur if blood volume expansion was prevented by a servo controlling body weight (a surrogate measure of total body water) when salt intake was increased. Neither of these studies included a control group in which the hemodynamic responses to salt loading were measured in normal dogs. Rather, these measurements were reported in a third study (19), which showed that CO was chronically elevated in normal dogs during salt loading but that AP remained constant as a result of systemic vasodilation. This hemodynamic profile in normal dogs is not consistent with the model proposed by Guyton (12), which predicts that salt loading will have no long-term hemodynamic consequences because the renal function curve is normal, and therefore volume homeostasis should be maintained.

Taken together, the three studies by Krieger and colleagues (18–20) clearly show that salt loading increases blood volume and CO similarly in normal dogs and dogs infused with a subpressor dose of ANG II. More importantly, although a salt-induced expansion of blood volume and an increase in CO are necessary for hypertension to develop, the mechanism whereby ANG II causes salt-dependent hypertension in the dog is by preventing a normal vasodilatory response to increased blood volume and CO. In other words, the difference between normal dogs and dogs infused with ANG II is not the response of CO to salt loading but rather the response of the vasculature to blood volume expansion and increased CO. Normal dogs exhibit a vasodilatory response to this stimulus, whereas dogs infused with ANG II fail to decrease vascular resistance. This distinction is critical because it points to a vascular rather than renal mechanism of salt-dependent hypertension in the dog model.

The reasons for the divergent results between our study and those of Krieger and colleagues are not clear, but possibilities include species differences, the magnitude and route of salt loading, and the presence or absence of an angiotensin-converting enzyme inhibitor. On a millimole per kilogram basis, the dogs in the studies of Krieger and colleagues received a lower dose of sodium ($\sim 8 \text{ mmol/kg}$) than the rats in the present

study ($\sim 50 \text{ mmol/kg}$). However, all water and virtually all sodium was administered via intravenous infusion in dogs compared with oral administration in the present study. Experiments conducted in rats, rabbits, and humans have shown that intravenously administered sodium loads are excreted more slowly by the kidneys than orally administered loads (24, 25, 30). Although the magnitude and route of salt loading differed between our study and those of Krieger and colleagues (18–20), it is important to note that the magnitude of the hypertensive response to increased salt intake was similar. Thus, although it may not be appropriate to compare the hemodynamic profiles based on salt loading alone, it is clear that despite similar increases in AP, the hemodynamic profiles underlying salt-induced hypertension were very different. Finally, another difference between our study and those of Krieger et al. was that we coadministered the angiotensin-converting enzyme inhibitor enalapril to block endogenous production of ANG II. Theoretically, it is possible that the elevated tissue levels of the vasodilator bradykinin, in response to converting enzyme blockade by enalapril, buffered vasoconstrictor responses mediated by whole body autoregulation. Although we cannot dispute this possibility, we are unaware of any studies that support this hypothesis.

Mechanisms of Increased CO in Salt-Dependent ANG II-Mediated Hypertension

At any moment, CO is the product of stroke volume and HR (31). We did not observe any statistically significant changes in HR over the duration of the protocol and therefore conclude that salt-induced increases in CO were secondary to an increase in stroke volume. Stroke volume increases as a result of either an increase in preload or cardiac contractility. Although we cannot rule out a sympathetically mediated increase in cardiac contractility, this does not seem likely because the HR did not increase. Preload is directly related to mean circulatory filling pressure, which is determined by the ratio of blood volume to venous capacitance (12). We did not measure blood volume and therefore cannot make any definitive conclusions in that regard. Similarly, because we did not measure the mean circulatory filling pressure, we cannot determine the contribution of venoconstriction to changes in CO in the present study. As discussed above, previously described studies of ANG II infusions and salt loading in dogs have demonstrated that blood volume expansion is the most likely cause of increased CO in this model (18, 20). Whether this is also true in the rat model remains to be determined.

Theoretically, the sustained elevation of CO may have resulted from a persistent rise in metabolism stimulated by the increase in dietary sodium. This would be consistent with whole body autoregulation. This explanation seems unlikely given that CO remained elevated during the recovery period when dietary sodium was returned to control levels. In addition, a salt-induced increase in oxygen metabolism

would also lead to vasodilation, which was not observed. In fact, the opposite was seen during the recovery period when vasodilation occurred after a decrease in dietary sodium intake. However, definitively ruling out a metabolic affect on CO requires the assessment of oxygen consumption, which was not performed.

Mechanisms Mediating "Off Transients" to Salt in Rats With a Clamped Renin-Angiotensin System

When dietary salt was returned to 0.1% NaCl during the recovery period, AP rapidly returned to control levels. Surprisingly, this was not the result of a return of CO to normal but a fall in TPR in the ANG II groups. The mechanism responsible for the persistent elevation in CO is not clear. One possibility is that maintaining the ANG II clamp during the recovery period did not permit excretion of the sodium load. This would have served as a sustained stimulus for volume retention, resulting in continued maintenance of the elevated CO. However, sodium balance was not measured in this study, and therefore it is not possible to know whether volume retention actually mediated the persistent increase in CO during the recovery period.

More importantly, the fact that AP returned to control levels, in the face of a persistent elevation of CO, further challenges the whole body autoregulation theory, in which CO is regulated at the expense of AP (13). Indeed, these results imply that the opposite is true.

Role of Whole Body Autoregulation in Other Models of Salt-Dependent Hypertension

This study was conducted to test the hypothesis that salt-dependent hypertension in rats in which the renin-angiotensin system is clamped exhibits the hemodynamic profile predicted by the whole body autoregulation theory. Our results were not consistent with that hypothesis. Additionally, although the model of Guyton (12) predicts that all forms of hypertension exhibit a whole body autoregulation hemodynamic profile, that hypothesis has not been supported by all studies.

Early research using sodium loading in dogs that had undergone renal reduction surgery yielded hemodynamic results that provided much of the initial support for the whole body autoregulatory hypothesis (2, 8, 23). Interestingly, a study (18) of rats with reduced renal mass (RRM), whose total fluid volume was prevented from expanding by servo control of water delivery, still became hypertensive when salt loaded, unlike the similar study (18) performed in dogs. Blood volume and CO were not measured in the rodent study, but a decrease in hematocrit was seen in the servo-controlled rats, which suggested that a shift of intracellular fluid into the circulatory compartment had occurred. This, they suggested, could have effectively increased blood volume in the absence of a net retention in fluid. However, plasma sodium was also increased markedly in these rats, which potentially elevated sympathetic activity as well (4).

While surgical models of renal dysfunction appear to generally conform well to the autoregulatory theory of

hypertension, this is not necessarily the case with other models of salt-dependent hypertension such as the Dahl salt-sensitive (Dahl-S) rat. Greene and colleagues (10) reported that the salt-resistant strain of the Dahl rat (the Dahl-R rat), when placed on a high-salt diet, has a sustained increase in blood volume and CO, but AP does not increase as a result of peripheral vasodilation. Dahl-S rats exhibited an identical increase in blood volume and CO but failed to reduce peripheral vascular resistance, resulting in hypertension. This is almost identical to the hemodynamic profiles reported by Krieger et al. in normal dogs (19) and dogs infused with ANG II, as discussed above (20).

Other mechanisms mediating salt-dependent hypertension in the Dahl-S rats have also been demonstrated. Greene and colleagues (10) argued that volume expansion was necessary for hypertension to develop and showed that when the intravenous delivery of water was servo controlled to prevent weight gain (and therefore volume expansion), salt-dependent hypertension was prevented (18). In contrast, Qi et al. (34) found that hypertension did develop in Dahl-S rats whose body weight was servo controlled, but the delivery of sodium and water occurred through oral intake. No change in plasma volume or hematocrit was seen, arguing against the possibility of a shift of intracellular fluid into the circulatory system. This protocol, they concluded, represented a more physiological presentation of sodium and supported a role for the influence of elevated plasma sodium on regulation of AP, via the sympathetic nervous system.

When the importance of whole body autoregulation is assessed in hypertension, it is critical to clearly distinguish the transient and steady-state hemodynamic phases and to carefully evaluate the temporal relationships between CO and vascular resistance. In the present study, we evaluated the hemodynamic response to a 7-day high-salt period in rats with fixed renin-angiotensin systems. There were several reasons why we chose this time frame to investigate whole body autoregulation in this model. First, as described by Guyton (12), the classical autoregulatory hemodynamic profile occurs within the period of time required to reach a new steady-state level of AP. On the basis of this logic, and the fact that we (32) had shown that AP reaches a steady-state within the first week of salt loading in this model, we designed the protocol around a 1-wk period of high salt. Second, as discussed above, Krieger and co-workers (18, 20) reported that whole body autoregulation occurs within this 7-day period of salt loading in dogs infused with a subpressor dose of ANG II. Finally, this time frame was chosen to allow us to specifically examine whole body autoregulation mediated by metabolic signals and avoid responses that were potentially due to structural remodeling of the vasculature secondary to hypertension itself.

Nonetheless, other studies have used longer exposures to high-salt diets to address this question, and some have observed hemodynamic patterns that were consistent with whole body autoregulation. Simchon and colleagues (36), using the microsphere method to

measure CO in restrained rats, reported that 4 wk of an 8% NaCl diet increased blood volume, CO, and AP in Dahl-S rats with no change in TPR. However, after 8 wk of an 8% NaCl diet, despite a persistent increase in blood volume, CO returned to control levels and hypertension was maintained by an increase in vascular resistance, whereas AP continued to increase (36). These observations are not entirely consistent with the whole body autoregulation theory because CO returned to normal despite a sustained increase in blood volume. In addition, it was also reported that AP and vascular resistance increased in Dahl-S rats maintained for 46 wk on a normal salt diet (1% NaCl), suggesting that perhaps time-dependent increases in vascular resistance in Dahl-S rats are not necessarily due to increased dietary salt and whole body autoregulation.

Hinojosa-Laborde and colleagues (14) characterized the hemodynamic profile of rats with RRM exposed to 6 wk of a high-salt diet (4.0% NaCl). Hemodynamic variables were measured acutely in restrained conscious rats 2 days after the implantation of an aortic flow probe. Between-group comparisons of hemodynamic variables were made after 2, 4, and 6 wk of high-salt intake. They reported that CO was increased after 2 but not 4 and 6 wk of a 4.0% NaCl diet in RRM rats and that hypertension was sustained by a gradual increase in vascular resistance over the 6-wk period. However, the statistical comparison of hemodynamic variables in RRM rats on a 4.0% NaCl diet was with sham-operated rats consuming a normal salt diet rather than with RRM rats on a normal salt diet. Thus it is difficult to establish to what extent the hemodynamic profile was caused by renal mass reduction itself or increased dietary salt.

Perspectives

The occurrence of whole body autoregulation is difficult to demonstrate in humans with essential hypertension due to the need to perform long-term serial monitoring of cardiovascular hemodynamics. However, hemodynamic patterns consistent with whole body autoregulation have been shown to occur in some forms of secondary hypertension (1, 35).

Studies that have addressed the issue of sodium sensitivity in humans have demonstrated hemodynamic profiles similar to those seen in the studies by Krieger et al. (18–20) and Greene et al. (10). That is, in the salt-sensitive groups, the increase in AP is mediated by volume expansion and elevated CO, with peripheral resistance unchanged. The salt-resistant groups showed a similar increase in CO, but this was offset by a proportional decrease in resistance, which prevented AP from rising (37, 38).

Numerous studies have evaluated the hemodynamic profiles of humans that are considered borderline hypertensive. Borderline hypertension is of particular interest because it is assumed that this is the initiating phase of essential hypertension (6, 27). The hemodynamic profiles of borderline hypertension have been

found to be remarkably complex and heterogeneous, defying attempts to develop simple classification schemes such as “high output” and “high resistance” hypertension. Multiple combinations of elevated, normal, and decreased CO and resistance have been seen in many studies (5, 9, 16, 17, 27).

The results of this study do not support the whole body autoregulation hypothesis of hypertension. Salt-dependent hypertension was mediated entirely by an increase in CO with no change in vascular resistance, and, importantly, AP returned to control levels during the recovery period despite the persistent elevation in CO.

Although certain forms of hypertension fit the hemodynamic profile of whole body autoregulation, neither our results nor those of numerous other studies support a universal role for this hypothesis. Indeed, the literature suggests that at least three different hemodynamic profiles may mediate salt-dependent hypertension: 1) whole body autoregulation (2, 7, 12, 22); 2) increased CO in all subjects, but hypertension occurring in salt-sensitive individuals as a consequence of impaired vasodilation (10, 18–20); and, as seen in this study, 3) elevated CO in the salt-sensitive group, with no change in resistance.

Understanding of the hemodynamic response to salt loading in experimental models of hypertension is a crucial step in elucidating the underlying pathophysiology of essential hypertension. Essential hypertension clearly has multiple etiologies (race, diet, genetics, age, etc.), and evidence suggests that no single hemodynamic profile underlies this condition.

DISCLOSURES

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