

Responsiveness vs. basal activity of plasma ANG II as a determinant of arterial pressure salt sensitivity

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Osborn, John W., Pilar Ariza-Nieto, John P. Collister, Sandra Soucheray, Benjamin Zimmerman, and Stephen Katz. Responsiveness vs. basal activity of plasma ANG II as a determinant of arterial pressure salt sensitivity. *Am J Physiol Heart Circ Physiol* 285: H2142–H2149, 2003. First published July 24, 2003; 10.1152/ajpheart.00200.2003.—Infusion of angiotensin II (ANG II) causes salt-sensitive hypertension. It is unclear whether this is due to the body's inability to suppress ANG II during increased salt intake or, rather, an elevated basal level of plasma ANG II itself. To distinguish between these mechanisms, Sprague-Dawley rats were instrumented with arterial and venous catheters for measurement of arterial pressure and infusion of drugs, respectively. The sensitivity of arterial pressure to salt was measured in four groups with the following treatments: 1) saline control (Con, $n = 12$); 2) administration of the angiotensin-converting enzyme inhibitor enalapril to block endogenous ANG II (ANG-Lo, $n = 10$); 3) administration of enalapril and $5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ANG II to clamp plasma ANG II at normal levels (ANG-Norm, $n = 10$); and 4) administration of enalapril and $20 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ANG II to clamp ANG II at high levels (ANG-Hi, $n = 10$). Rats ingested a 0.4% NaCl diet for 3 days and then a 4.0% NaCl diet for 11 days. Arterial pressure of rats fed the 0.4% NaCl diet was lower in ANG-Lo ($84 \pm 2 \text{ mmHg}$) compared with Con ($101 \pm 3 \text{ mmHg}$) and ANG-Norm ($98 \pm 4 \text{ mmHg}$) groups, whereas ANG-Hi rats were hypertensive ($145 \pm 4 \text{ mmHg}$). Salt sensitivity was expressed as the change in arterial pressure divided by the change in sodium intake on the last day of the 4.0% NaCl diet. Salt sensitivity (in $\text{mmHg}/\text{meq Na}$) was lowest in Con rats (0.0 ± 0.1) and progressed from ANG-Lo (0.8 ± 0.2) to ANG-Norm (1.5 ± 0.5) to ANG-Hi (3.5 ± 0.5) rats. We conclude that the major determinant of salt sensitivity of arterial pressure is the basal level of plasma ANG II rather than the responsiveness of the renin-angiotensin system.

renin-angiotensin system; sympathetic nervous system

LONG-TERM SALT SENSITIVITY of arterial pressure is a significant clinical problem in both the normotensive and hypertensive human populations. It is currently estimated that 25% of the normotensive population is "salt sensitive" in that their arterial pressures are abnormally sensitive to changes in dietary NaCl intake (29). This is clinically significant, because the salt sensitiv-

ity of arterial pressure may be a more accurate predictor of future cardiovascular disease and morbidity than the basal level of arterial pressure (29). Salt sensitivity is more prevalent in humans with essential hypertension; estimates are that 50–75% of these patients exhibit increased salt sensitivity of arterial pressure (28). Despite the clinical importance of salt-dependent hypertension, its pathogenesis is poorly understood.

Previous studies support the idea that impaired regulation of the renin-angiotensin-aldosterone system (RAAS) increases the salt sensitivity of arterial pressure. For example, several studies show that long-term infusion of angiotensin II (ANG II) causes salt-dependent hypertension in experimental animals (1, 7, 9, 11, 12, 16). However, the precise nature of the relationship between plasma ANG II and long-term salt sensitivity of arterial pressure remains unclear. It is logical to conclude that the salt sensitivity of this model is simply due to increased plasma levels of ANG II and aldosterone, since both enhance renal Na^+ retention. An alternate explanation is that exogenous ANG II administration prevents the suppression of the RAAS that normally occurs with dietary salt loading. Theoretically, the inability to suppress the RAAS during periods of high salt intake would attenuate the vasodilation and natriuresis associated with decreasing plasma levels of ANG II and aldosterone, respectively. Consequently, although the plasma levels of these hormones would not be elevated compared with normal conditions, they would be inappropriately high for an animal that is consuming a high-salt diet. In other words, it is not clear whether the basal level of plasma ANG II or the ability of the RAAS to respond to changes in salt intake determines the salt sensitivity of arterial pressure.

Figure 1 illustrates these theoretical relationships between dietary salt intake, plasma ANG II levels, and arterial pressure (Fig. 1A). Shown are the predicted responses in animals with a RAAS that is responsive to increases in dietary salt and animals in which plasma ANG II is "clamped" at low (ANG-Lo), normal (ANG-Norm), or high (ANG-Hi) levels (Fig. 1, B and C). Normally, an increase in dietary salt decreases plasma

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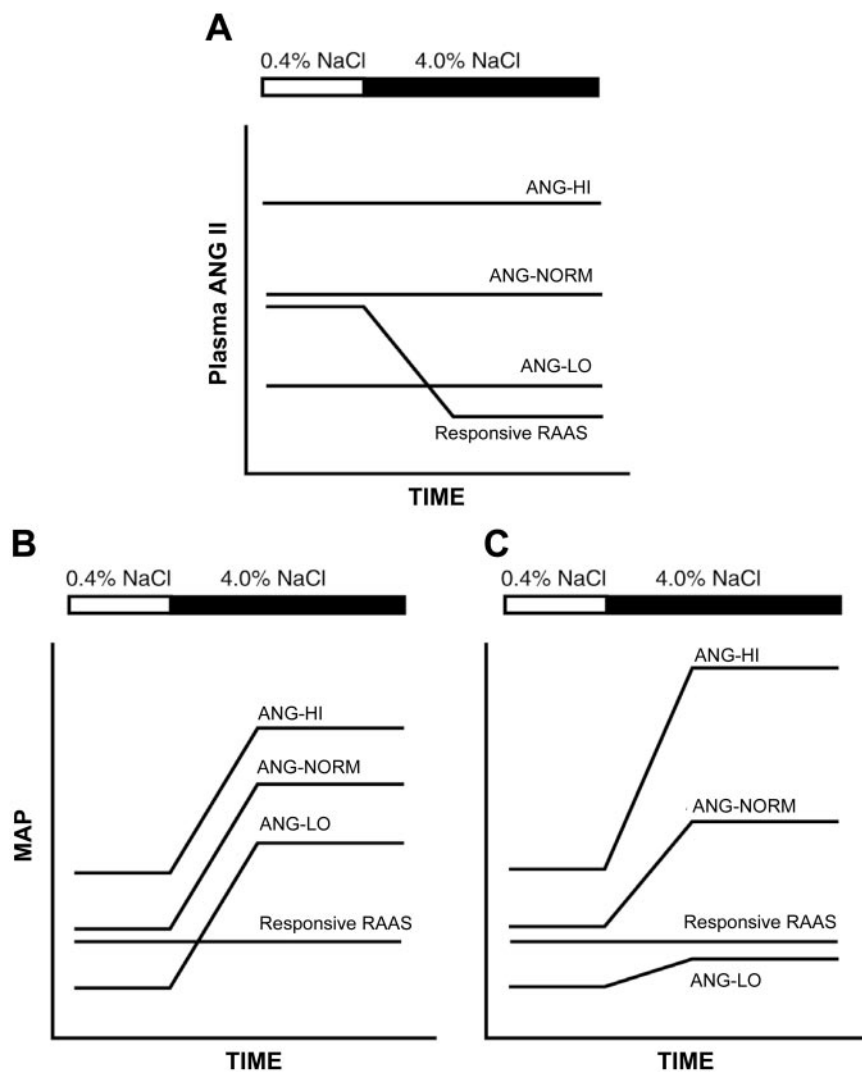


Fig. 1. Hypothetical relationship between dietary salt intake, plasma angiotensin II (ANG II), and mean arterial pressure (MAP) in rats with a responsive renin-angiotensin-aldosterone system (RAAS) and rats in which the RAAS is pharmacologically clamped at low (ANG-Lo), normal (ANG-Norm), or high (ANG-Hi) levels. *A*: hypothetical relationship between dietary salt content (bar, top) and plasma ANG II for all groups. *B*: hypothetical responses of MAP if the only determinant of arterial pressure salt sensitivity was the responsiveness of the RAAS. In this case, the increase in MAP from baseline level in response to a 4.0% salt diet is identical in all three ANG II-clamped groups. *C*: hypothetical response of MAP if the primary determinant of salt sensitivity of arterial pressure was the basal level of plasma ANG II before salt intake was increased.

ANG II in animals with a responsive RAAS (Fig. 1A), which serves to maintain constant arterial pressure in the presence of a high-salt diet. However, as shown in Fig. 1B, if the only determinant of the salt sensitivity of arterial pressure were the responsiveness of the RAAS, independent of basal plasma ANG II levels, then all three ANG II treatment groups would have the same magnitude of salt sensitivity of arterial pressure. In other words, the increase in arterial pressure induced by the same increase in dietary salt would be identical in all three groups. On the other hand, as shown in Fig. 1C, if the only determinant of arterial pressure salt sensitivity were the basal level of plasma ANG II, then there would be a direct relationship between plasma ANG II and the salt-induced increase in arterial pressure.

Studies on dogs have shown that chronic administration of an angiotensin-converting enzyme (ACE) inhibitor increases the salt sensitivity of arterial pressure to the same extent as administration of a subpressor dose of exogenous ANG II (17). Because ACE inhibitors decrease endogenous ANG II production, this observation supports the idea that inability of the RAAS to respond to changes in dietary salt intake,

rather than the basal level of the RAAS, is the primary determinant of salt sensitivity of arterial pressure. However, this hypothesis has not been thoroughly tested in other species, and the relationship between several different plasma levels of ANG II and salt sensitivity has not been carefully studied.

The present study was conducted to examine this issue in greater detail in rats, a species that is commonly used to study the role of the renin-angiotensin system in long-term regulation of arterial pressure and hypertension. Our objective was to determine the relative contributions of responsiveness vs. basal activity of the RAAS to the long-term salt sensitivity of arterial pressure. We compared the effects of increasing dietary salt on arterial pressure in rats with a normally responsive RAAS to those in which the RAAS was pharmacologically clamped at low, normal, or high levels.

METHODS

Surgical Instrumentation

Male Sprague-Dawley rats (275–300 g, Charles River Laboratory; Wilmington, MA) were prepared for catheter im-

plantation using aseptic techniques. All procedures were conducted in accordance with institutional and National Institutes of Health guidelines. For surgical procedures, animals were anesthetized with pentobarbital sodium (65 mg/kg ip) and were administered atropine (0.2 mg/kg ip). At this time, rats were given an injection of antibiotic (gentamicin, 2.5 mg im). Arterial and venous catheters were placed in the femoral vessels for measurement of arterial pressure and chronic infusion of vehicle or drugs, respectively. The catheters were passed subcutaneously to the top of the head and then through a lightweight flexible spring. The spring was attached to the skull surface with stainless steel screws and dental acrylic. The venous catheter was attached to a single-channel hydraulic swivel, and the arterial catheter was plugged until needed for direct measurement of arterial pressure. An injection of butorphanol tartrate (0.075 mg sc) was administered for analgesia at the end of the surgery. Rats were allowed to recover from anesthesia on a heating pad and were then placed in individual metabolic cages (Nalgene Nunc International; Rochester, NY). Rats were allowed to recover for 3 days before the experimental protocol started. A 0.4% NaCl diet (Research Diets; New Brunswick, NJ) and distilled water were provided ad libitum throughout the recovery period. During this period, daily bolus injections of ampicillin (15 mg iv) and tobramycin (4 mg iv) were administered.

Experimental Groups

The main objective of this study was to compare the salt sensitivity of arterial pressure in rats with a responsive RAAS to rats in which plasma ANG II was clamped at low, normal, or high levels. As illustrated in Fig. 1, we reasoned that if responsiveness of the RAAS were the sole determinant of salt sensitivity, then ANG-Lo rats would be as salt sensitive as ANG-Hi rats (Fig. 1B). On the other hand, as shown in Fig. 1C, if basal activity of the RAAS were the only determinant of salt sensitivity, then salt sensitivity would be lowest in the ANG-Lo rats and highest in ANG-Hi rats.

Preliminary experiments were conducted to establish chronic doses of enalapril to block endogenous ANG II production. These studies revealed that $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ of enalapril blocked the pressor response ($\sim 30 \text{ mmHg}$) to a bolus injection of ANG I (0.01 μg iv). In addition, this dose of enalapril decreased the baseline arterial pressure to levels similar to what we have reported for chronic angiotensin type 1 (AT₁) receptor blockade in normotensive salt-replete rats (5, 6, 17). We then established doses of exogenous ANG II that would normalize or elevate arterial pressure in rats chronically treated with enalapril. Based on these preliminary experiments, we established four treatment groups (Fig. 1).

1) *Responsive RAAS: saline vehicle.* This treatment was to determine salt sensitivity of arterial pressure in rats with a RAAS that is responsive to changes in dietary salt intake.

2) *ANG-Lo: $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ enalapril.* The aim of this treatment was to chronically clamp the RAAS at low levels. It was assumed that endogenous production of plasma ANG II was negligible with this treatment.

3) *ANG-Norm: enalapril and $5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ANG II.* The aim of this treatment was to chronically clamp the RAAS at normal levels. This dose of ANG II was chosen because it maintains arterial pressure at normotensive levels in enalapril-treated rats.

4) *ANG-Hi: enalapril and $20 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ANG II.* The goal of this treatment was to clamp the RAAS at elevated

levels. This dose was chosen because it produced hypertension in enalapril-treated rats.

On the day of the surgery, rats were randomly assigned to one of the four treatment groups and one of two experimental protocols (see below). Infusions were started once the surgery was complete, and animals were placed in their home cages. Sterile saline (0.9%) was used as the vehicle for experimental infusions. All solutions were infused through a 0.2- μm syringe filter at a volume flow rate of 7 ml/day.

Experimental Protocols

Protocol 1: effects of increased dietary salt in rats with a fixed and responsive RAAS. The experimental protocol was carried out in the four treatment groups described above: 1) responsive RAAS ($n = 12$), 2) ANG-Lo ($n = 10$), 3) ANG-Norm ($n = 10$), and 4) ANG-Hi ($n = 10$). The protocol consisted of a 3-day control period during which all rats were given a 0.4% NaCl diet followed by an 11-day period of a high-salt (4.0% NaCl) diet.

Throughout the protocol, mean arterial pressure (MAP), heart rate, food intake, and water intake were measured daily in conscious, unrestricted rats. MAP was measured directly by connecting the arterial catheter to a pressure transducer that was coupled to a polygraph (Grass Instruments; Quincy, MA). MAP was monitored daily between 0900 and 1000 for 15 min by computer at a sampling rate of 1 Hz as previously described (22). Rats were typically resting quietly undisturbed in their home cages during the measurement. Although food and water were not removed when MAP was monitored, rats did not eat or drink during the recording period. The resulting 900 data points were used to calculate the average MAP during the recording period. Heart rate was measured by increasing the chart speed and counting peaks on the pulsatile pressure tracing. Upon completion of the MAP and heart rate measurements, food and water were removed, and 24-h food and water intake measurements were determined gravimetrically. Na⁺ intake was calculated as the sum of Na⁺ received in the daily infusion (1 meq/day) plus the product of food intake and Na⁺ content of the food, which had previously been determined (0.4% NaCl, 0.07 meq/g; 4.0% NaCl, 7.0 meq/g).

Protocol 2: time-control experiments. This protocol was identical to protocol 1 with the exception that rats were kept on a normal-salt diet (0.4% NaCl) for the entire period. The same four experimental groups were studied: 1) responsive RAAS ($n = 6$), 2) ANG-Lo ($n = 6$), 3) ANG-Norm ($n = 6$), and 4) ANG-Hi ($n = 6$).

Test of ACE inhibition. To test whether enalapril blocked ACE activity, acute pressor responses to bolus injections of ANG I (0.01 and 0.1 $\mu\text{g}/\text{kg}$) were measured on day 3 of the control period. Responses were measured as the peak increases of arterial pressure. Arterial pressure was allowed to return to baseline levels between injections.

Measurement of plasma ANG II concentration. Upon completion of the study, some rats were anesthetized with pentobarbital sodium (50 mg/kg iv) before the abdominal aorta was isolated, and a 2-ml blood sample was taken for determination of plasma ANG II concentration. Blood was collected into a chilled stop solution to prevent ANG II metabolism, which was followed by centrifugation and application of the plasma to a 1-ml BondElute phenylsilyl column. The column eluants were evaporated in methanol using a Savant Speed Vac, and this was followed by HPLC of the reconstituted eluants (in 42% acetonitrile in 0.15% heptafluorobutyric acid), recovery of the ANG II HPLC fractions, and subse-

quent radioimmunoassay for ANG II as previously described (30).

Statistical Analysis

Data were analyzed by two-way ANOVA for the effects of treatments and time. A one-way ANOVA was used to compare salt sensitivity of arterial pressure and heart rate between groups on the final day of the 4.0% NaCl diet. A Fisher's post hoc test was employed when ANOVA revealed statistically significant effects of treatment or time. All values are reported as means ± SE, and statistical significance was set at $P < 0.05$.

RESULTS

Shown in Table 1 are the 3-day average control values for arterial pressure and heart rate for all experimental groups in each protocol. Compared with the saline vehicle group (responsive RAAS), rats treated with enalapril alone (ANG-Lo) were hypotensive in contrast to ANG-Hi rats, which were hypertensive. Arterial pressure values in ANG-Norm rats were not different from the responsive RAAS groups. Heart rate did not show statistical differences between the four treatment groups during the 3-day control period in either protocol.

The responses of arterial pressure and heart rate to increasing dietary salt from 0.4 to 4.0% NaCl are shown in Fig. 2. Data are expressed as the change from the 3-day control average (Table 1) during 11 days of 4.0% NaCl diet. Arterial pressure remained essentially unchanged during the 11 days of the high-salt diet in the responsive RAAS group. In contrast, clamping the RAAS increased the sensitivity of arterial pressure to increased salt intake in all groups. ANG-Lo rats had the smallest salt-induced increase of arterial pressure and were statistically higher than the RAAS group on days 1, 2, 5, and 8 of high salt intake. In contrast, both ANG-Norm and ANG-Hi rats had significantly higher arterial pressure increases than responsive RAAS rats throughout the 11 days of high salt intake. In addition, ANG-Norm rats had statistically higher increases in arterial pressure than ANG-Lo rats on days 2–4, 6, 8, and 9 of the high-salt period (not indicated in Fig. 2 for clarity). Moreover, salt-induced increases in arterial

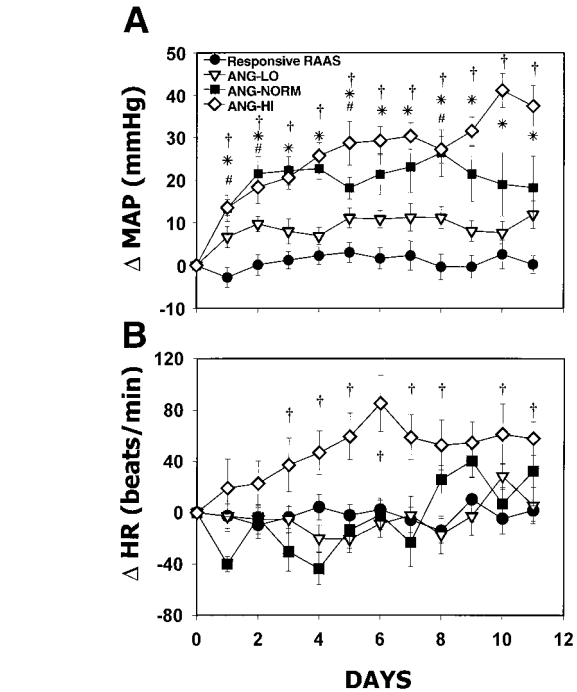


Fig. 2. Changes during 11 days of a 4.0% NaCl diet. A: MAP. B: heart rate (HR). Values for each day were calculated as the change from the 3-day 0.4% NaCl control period for each value (see Table 1). # $P < 0.05$, ANG-Lo vs. responsive RAAS; * $P < 0.05$, ANG-Norm vs. responsive RAAS; † $P < 0.05$, ANG-Hi vs. responsive RAAS.

pressure in ANG-Hi rats were statistically higher than for ANG-Norm rats on days 5, 10, and 11 of high salt intake (not indicated in Fig. 2 for clarity). Heart rate did not change during the 11 days of 4.0% NaCl intake in the responsive RAAS, ANG-Lo, or ANG-Norm groups. However, a statistically significant tachycardia was observed in the ANG-Hi rats on days 3–8, 10, and 11 of 4.0% NaCl intake. Finally, there were no statistically significant changes in arterial pressure or heart rate in any group for the time-control experiments (protocol 2; data not shown). Because no cardiovascular effects were observed in the time-control studies, data for these groups are not shown in subsequent sections.

Changes in Na⁺ intake during the 11 days of the 4.0% NaCl diet are shown in Fig. 3. Basal Na⁺ intake

Table 1. Basal mean arterial pressure and heart rate during 3-day 0.4% NaCl period

	Basal MAP, mmHg		Basal Heart Rate, beats/min	
	Protocol 1	Protocol 2	Protocol 1	Protocol 2
Responsive RAAS	101 ± 3	94 ± 3	391 ± 10	411 ± 15
ANG-Lo	84 ± 2*	80 ± 2*	402 ± 12	430 ± 10
ANG-Norm	98 ± 4	100 ± 4	402 ± 11	412 ± 12
ANG-Hi	145 ± 4*	138 ± 3*	386 ± 14	386 ± 14

Values are means ± SE of 3 days. Rats in protocol 1 were subsequently placed on a 4.0% NaCl diet, and rats in protocol 2 remained on a 0.4% NaCl diet. MAP, mean arterial pressure; RAAS, renin-angiotensin-aldosterone system; ANG-Lo, -Norm, or -Hi, groups of animals where ANG II was “clamped” at low, normal, or high levels, respectively. * $P < 0.05$ compared with responsive RAAS (within each protocol).

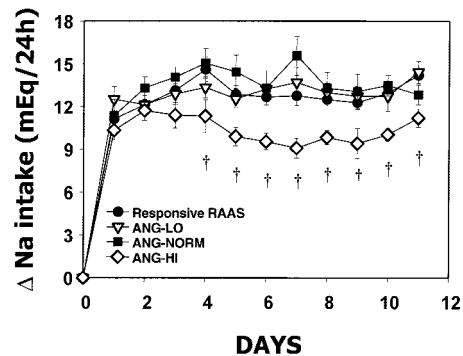


Fig. 3. Change in Na⁺ intake from the 3-day 0.4% NaCl control period during 11 days of 4.0% NaCl diet. † $P < 0.05$, ANG-Hi vs. responsive RAAS.

was not different between the responsive RAAS (2.5 ± 0.1 meq/24 h), ANG-Lo (2.5 ± 0.1 meq/24 h), ANG-Norm (2.3 ± 0.2 meq/24 h), or ANG-Hi (2.3 ± 0.1 meq/24 h) groups. During the 11 days of the 4.0% NaCl diet, Na⁺ intake increased similarly in most groups with the exception of the ANG-Hi group, which consumed significantly less Na⁺ than the responsive RAAS group from *days 4–11* of the 4.0% NaCl period.

Salt sensitivity of arterial pressure is defined as the change in arterial pressure induced by a given change in Na⁺ intake. Because the magnitude of dietary salt loading was not equivalent between the groups (Fig. 3), we normalized the changes in arterial pressure and heart rate during the 4.0% NaCl intake period to changes in Na⁺ intake. This was done by dividing the daily changes in arterial pressure or heart rate by the daily change in Na⁺ intake (Fig. 4). Expression of salt sensitivity in this manner suggested a further separation in the salt sensitivity of arterial pressure between groups. Compared with the responsive RAAS group, ANG-Lo rats had statistically larger salt-induced increases in arterial pressure on *days 1* and *2* of high salt intake, whereas ANG-Norm and ANG-Hi groups were statistically significantly higher over the entire 11-day period of the high-salt diet. Compared with ANG-Lo rats, the ANG-Norm group had larger increases in arterial pressure on *days 3, 4, and 8* of the 4.0% NaCl diet (not indicated in Fig. 4 for clarity). Finally, ANG-Hi rats had statistically larger increases in arterial pressure than ANG-Norm rats on *days 4–7* and

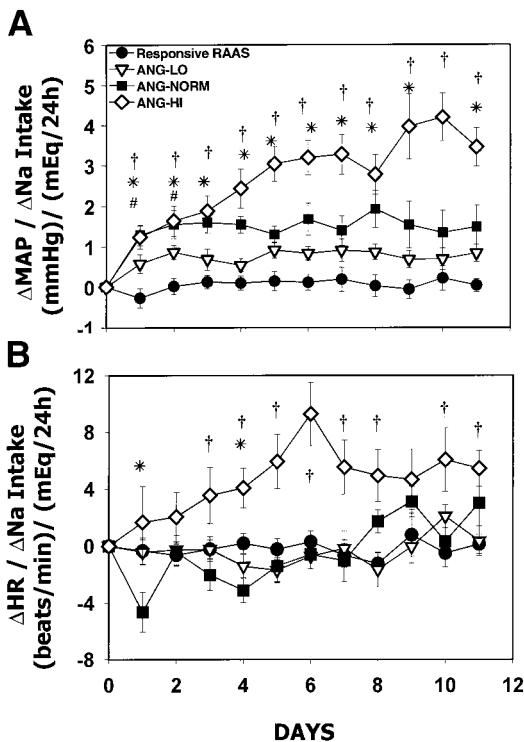


Fig. 4. Daily changes normalized by daily changes in Na⁺ intake. A: MAP. B: HR. Values were calculated by dividing the daily changes in MAP or HR from the 3-day 0.4% NaCl control period by the daily change in Na⁺ intake. Statistical symbols are as in Figs. 2 and 3.

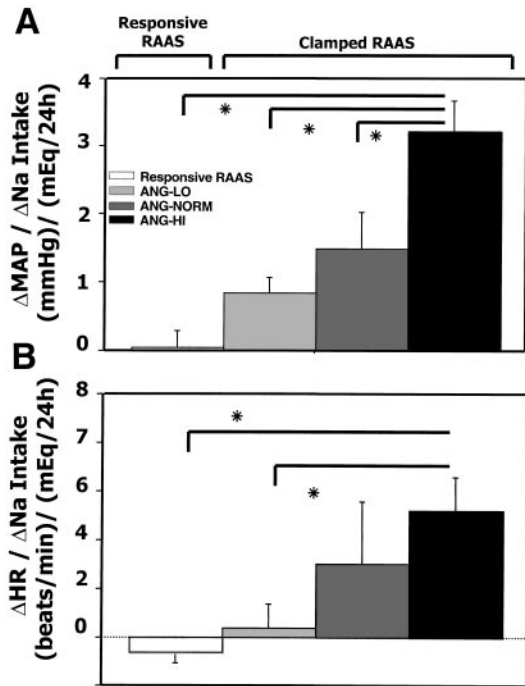


Fig. 5. Steady-state changes normalized for changes in Na⁺ intake. A: MAP. B: HR. Values were taken from the last day of the 4.0% NaCl period (*day 11* of Fig. 4). **P* < 0.05, ANG-Hi compared with groups shown.

9–11 of the high-salt period (not indicated in Fig. 4 for simplicity).

A subsequent analysis is shown in Fig. 5 in which the steady-state changes in arterial pressure and heart rate, normalized for changes in Na⁺ intake, were compared. Shown are data for the final day of the 4.0% NaCl diet. There was a statistically significant effect of treatment on salt sensitivity of both arterial pressure and heart rate. ANG-Hi rats were significantly more sensitive than the three other groups in terms of arterial pressure and more sensitive than responsive RAAS and ANG-Lo groups with regard to heart rate.

Shown in Fig. 6 are the 24-h water intake measurements for the four groups of rats for the 3 days of the

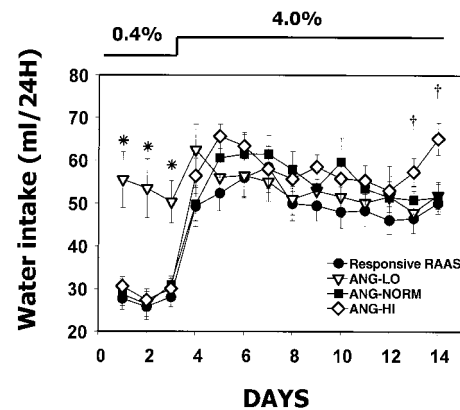


Fig. 6. Daily water intake measurements for the four groups studied during the 0.4% and 4.0% NaCl periods. **P* < 0.05, ANG-Lo vs. responsive RAAS; †*P* < 0.05, ANG-Hi vs. responsive RAAS.

0.4% NaCl and the 11 days of the 4.0% NaCl diets. Total water intake was the sum of that given by infusion of saline (7 ml/24 h iv) and ad libitum intake. With the exception of the ANG-Lo rats, all groups had similar water intake values during the 3-day control period: ~25–30 ml/24 h. Water intake in these same groups increased similarly during the 4.0% NaCl diet period to ~55 ml/24 h. In contrast, ANG-Lo rats had markedly elevated water intake measurements during the control period and surprisingly did not increase their water intake over the 11-day period of the 4.0% NaCl diet.

The pressor responses to bolus doses of ANG I were measured on the last day of the 0.4% NaCl intake period. Responses to a low dose of ANG I (0.01 µg/kg) were 32 ± 3 mmHg for the responsive RAAS group and 0 ± 0 mmHg for all three ANG groups ($P < 0.05$). Similarly, the response to the high-dose ANG I (0.1 µg/kg) was higher in the responsive RAAS group (52 ± 3 mmHg; $P < 0.05$) compared with the ANG-Lo (22 ± 4 mmHg), ANG-Norm (23 ± 3 mmHg), and ANG-Hi (16 ± 3 mmHg) groups.

Finally, shown in Table 2 are results of the assay for plasma ANG II. We did not take samples from all rats in *protocol 1*, and, as a result of complications with the assay, we did not obtain reliable results for rats in *protocol 2*. Despite these limitations, there was a clear relationship between the dose of intravenous ANG II and plasma ANG II concentration in the ANG-Lo, ANG-Norm, and ANG-Hi groups in *protocol 1*. In addition, plasma ANG II levels in the responsive RAAS and ANG-Norm rats were similar to one another and to those previously reported for conscious rats (23).

DISCUSSION

Salt sensitivity of arterial pressure is a clinically significant problem within the normotensive and hypertensive human populations. Although this condition occurs with less frequency in normotensive patients, it has been proposed to be a predictor of subsequent cardiovascular disease independent of resting levels of arterial pressure (29). The mechanisms that contribute to the salt sensitivity of arterial pressure remain poorly understood but most likely involve impaired neural and hormonal control of renal and/or vascular function (1). Whereas altered renal responses to changes in dietary salt intake would impair maintenance of extracellular fluid volume, impaired control

of the vasculature may result in inappropriate arteriolar and venous tone under conditions of high salt intake.

Several investigators have demonstrated that in experimental animals, chronic administration of exogenous ANG II results in salt-dependent hypertension (1, 7, 9, 11, 16). Although it is reasonable to conclude that salt sensitivity of this model is simply due to elevations of plasma ANG II, some have reported a similar degree of salt sensitivity in dogs treated with an ACE inhibitor alone (17). This has led to the hypothesis that salt sensitivity is determined by the ability of the RAAS to respond to changes in salt intake rather than by basal plasma concentrations of ANG II. We tested this hypothesis in the present study by comparing the steady-state arterial pressure responses to increased dietary salt between rats in which the RAAS was pharmacologically clamped at low, normal, and high levels to rats with a responsive RAAS.

Based on the results of our study, we conclude that the primary determinant of the salt sensitivity of arterial pressure in this model is the basal level of circulating ANG II. This conclusion is supported by the statistically significant relationship between the dose of exogenous ANG II administered (0, 5, or 20 ng·kg⁻¹·min⁻¹) in the presence of ACE inhibition and salt sensitivity of arterial pressure (see Figs. 4 and 5). However, we feel we cannot exclude the contribution of the responsiveness of the RAAS entirely, because there was a tendency for enalapril treatment alone to increase salt sensitivity of arterial pressure.

It is also important to note that there were differences in the basal levels of arterial pressure between groups that need to be considered in the interpretation of these results. Specifically, although the ANG-Hi group was the most salt sensitive in terms of absolute increase in arterial pressure, these rats also started at a higher baseline level of pressure. Similarly the ANG-Lo group, which was the least salt sensitive, had the lowest basal level of arterial pressure. However, calculation of the percent changes in arterial pressure based on the increase observed on the last day of the high salt diet and the 3-day control average of arterial pressure revealed a pattern similar to that observed from absolute changes in arterial pressure. The percent increase in arterial pressure progressed from rats with a responsive RAAS (0%) to ANG-Lo (14%), ANG-Norm (18%), and ANG-Hi (26%) rats.

Previous studies in dogs show that inhibition of ACE alone or administration of a subpressor dose of ANG II (5 ng·kg⁻¹·min⁻¹) increases salt sensitivity of arterial pressure to similar degrees (17). These investigators conclude that basal levels of circulating ANG II shift the renal function curve in a parallel fashion along the pressure axis such that the “set point” for arterial pressure changes, but salt sensitivity of arterial pressure (i.e., slope of the renal function curve) is independent of plasma ANG II. A notable difference between studies is that we examined salt sensitivity in rats treated with enalapril alone or in combination with ANG II infusion. We used this approach to ensure that endogenous ANG II production was minimal before we

Table 2. Plasma ANG II concentrations in four groups of protocol 1

	n	ANG II, pg/ml
Responsive RAAS	6	102 ± 28
ANG-Lo	4	39 ± 9
ANG-Norm	4	87 ± 7
ANG-Hi	6	436 ± 145*

Values are means ± SE; n, no. of rats. Samples were obtained at the end of the 11 days of the 4.0% NaCl diet period. * $P < 0.05$ compared with responsive RAAS.

infused exogenous ANG II. This also allowed us to compare the salt sensitivity of rats with ANG II clamped at low, normal, and high levels. Previous studies in dogs involved administration of exogenous ANG II only, with the assumption that endogenous ANG II production was suppressed under these conditions. Another explanation is that species differences may also account for differing results from these studies.

The mechanisms that underlie the increase in salt sensitivity of arterial pressure in animals in which the RAAS is clamped at normal or elevated levels is not clear, but two distinct possibilities exist. One possibility, as mentioned above, is that prevention of the shift of the renal function curve to lower pressure levels when dietary salt intake is increased impairs the animal's ability to excrete the Na^+ load, which results in blood volume expansion and hypertension. The idea that blood volume expansion contributes to this response is supported by studies on dogs in which volume expansion with salt loading was prevented by the servo control of body weight, a surrogate index for extracellular fluid volume (18). This prevented salt-dependent hypertension in dogs that were infused with a subpressor dose of ANG II (18). A second possibility is that clamping ANG II levels at normal or high levels interferes with a vasodilatory response to volume expansion that has been reported to occur in normal animals (15, 19) and humans (13, 24, 25) that consume a high-salt diet. Indeed, in a companion study to the servo control experiment in dogs (18), it was reported that a chronic Na^+ load increased cardiac output in normal dogs but also resulted in a simultaneous decrease of vascular resistance such that arterial pressure did not change (19). In contrast, vasodilation did not occur in dogs that were administered a chronic subpressor dose of ANG II (20). In contrast, a recent study from our own laboratory (10) showed that increasing dietary salt has no hemodynamic effects in rats with a responsive RAAS. However, we did observe that a high-salt diet does result in a sustained increase in cardiac output in rats with the RAAS clamped at normal levels (i.e., enalapril plus $5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ANG II). Taken together, these studies strongly suggest that blood volume expansion contributes at least in part to increased salt sensitivity of arterial pressure in this model.

Another possible mechanism by which circulating ANG II may increase salt sensitivity of arterial pressure is suggested by the heart rate responses to increased dietary salt in the present study. In ANG-Hi rats, elevated salt intake was associated with a marked tachycardia that was sustained throughout the high-salt period. By the last day of the high salt intake, heart rate was elevated ~ 50 beats/min above control level despite the fact that arterial pressure was also increased 40 mmHg above baseline level. Increased heart rate in the face of hypertension is consistent with the idea that dietary salt alters autonomic regulation of the heart in such a way that baroreceptor reflex control of heart rate is overridden. Because this was only observed in rats on the high dose of ANG II, this

suggests that elevated plasma ANG II may sensitize central autonomic responses to dietary salt, perhaps by an interaction with central osmosensitive sites (3, 4, 27). Offering further support for this hypothesis are numerous studies that suggest that the slow pressor model of ANG II hypertension, which is salt dependent, is mediated in part by activation of the sympathetic nervous system (2, 8, 12, 14, 21).

Finally, we measured water intake and found that rats treated with enalapril alone had markedly elevated water intake when they consumed a normal-salt diet (see Fig. 6). More surprisingly, water intake remained constant during the high-salt period in this group. The explanation for this polydipsia observed during the normal-salt period is not clear, but there are two distinct possibilities. One is simply that the diuretic actions of enalapril resulted in a secondary increase in water intake to maintain water balance. Alternatively, it has been shown that low doses of enalapril increase drinking (26). It has been suggested that this response is due to an increase in circulating ANG I that is converted centrally to ANG II to stimulate thirst (26).

In summary, the results of the present study suggest that the increased salt sensitivity of arterial pressure in rats in which plasma ANG II is pharmacologically clamped is largely due to the basal levels of plasma ANG II rather than loss of responsiveness of the RAAS. The mechanisms of the increased salt sensitivity are not clear but most likely involve altered renal, vascular, and neural responses to dietary salt loading.

DISCLOSURES

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