Role of the renin-angiotensin system during alterations of sodium intake in conscious mice

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Cholewa, Brian C., and David L. Mattson. Role of the renin-angiotensin system during alterations of sodium intake in conscious mice. Am J Physiol Regulatory Integrative Comp Physiol 281: R987–R993, 2001.—The present studies were performed to quantify circulating components of the renin-angiotensin-aldosterone axis and to determine the functional importance of this system during alterations in sodium intake in conscious mice. Increasing sodium intake from ~200 to 1,000 μeq/day significantly decreased plasma renin concentration from 472 ± 96 to 304 ± 83 ng ANG I·ml⁻¹·h⁻¹ (n = 5) but did not alter plasma renin activity from the low-sodium level of 7.7 ± 1.1 ng ANG I·ml⁻¹·h⁻¹. Despite the elevated plasma renin concentration, plasma ANG II in mice on low-sodium level averaged 14 ± 3 pg/ml and was significantly suppressed to 6 ± 1 pg/ml by high-sodium intake (n = 7). Consistent with the modulation of ANG II, plasma aldosterone significantly decreased from 41 ± 8 to 8 ± 3 ng/dl when sodium intake was elevated (n = 6). In a final set of experiments, the continuous infusion of ANG II (20 ng·kg⁻¹·min⁻¹) led to a mild salt-sensitive increase in mean arterial pressure from 108 ± 2 to 131 ± 2 mmHg as sodium intake was varied from low to high (n = 7). In vehicle-infused mice, mean arterial pressure was unaltered from 109 ± 2 mmHg when sodium intake was increased (n = 6). These studies indicate that the physiological suppression of circulating ANG II may be required to maintain a constancy of arterial pressure during alterations in sodium intake in normal mice.

Genetically manipulated mice have become a popular tool for the study of cardiovascular/renal physiology. One of the cardiovascular regulatory systems, which has been the subject of intense investigation using transgenic/knockout technology in the mouse, has been the renin-angiotensin system (RAS). Manipulation of the genes encoding renin (4), angiotensinogen (15, 16, 19, 26–28), angiotensin-converting enzyme (ACE) (6, 7, 12, 26, 29), and the AT₁ (14, 20, 25, 30) and AT₂ receptors (11, 13) has produced profound effects on cardiovascular homeostasis. An additional finding in mice with manipulations of renin, angiotensinogen, ACE, and the AT₁ and AT₂ receptors has been abnormalities in renal development. It is therefore possible that the alterations in fluid and electrolyte homeostasis and arterial blood pressure, which were observed in these genetically manipulated animals, may have been attributable to the renal abnormalities rather than the direct effects of the RAS on renal/cardiovascular function.

Previous studies from our laboratory have demonstrated that conscious mice rapidly achieve neutral sodium balance following a step change in sodium intake (17). This occurs in the absence of any measurable change in arterial blood pressure or body weight. Furthermore, studies from a number of laboratories have demonstrated that plasma renin concentration (PRC) is extremely high in normal mice (3, 18, 31) and suppressed when mice are placed on a high-sodium diet (31). We were surprised to observe in preliminary studies that plasma renin activity (PRA) was unaltered as sodium intake was increased from low to high levels in conscious mice (unpublished observations). Because the use of transgenic and knockout mice is a powerful tool in hypertension research, it is important to understand the mechanisms whereby mice regulate their level of mean arterial pressure (MAP) during alterations in fluid/sodium intake; this is particularly true if the RAS behaves differently in mice than in other mammals.

The present studies were performed to first determine how the different components of the renin-angiotensin-aldosterone axis respond to alterations in sodium intake by measuring PRA, PRC, plasma aldosterone (Aldo), and plasma ANG II in blood drawn from freely moving, conscious mice. The second objective of this study was to examine the importance of suppression of ANG II during alterations in sodium intake by measuring the change in MAP in control mice and in mice continuously infused intravenously with ANG II as sodium intake was increased from low to high levels.

METHODS

Experiments were performed on 6- to 8-wk-old male Swiss-Webster mice (25–30 g) obtained from Taconic Farms. The mice were housed in the Animal Resource Center at the Medical College of Wisconsin with normal food and tap water provided ad libitum. All animal procedures were approved by the Medical College of Wisconsin Animal Care Committee, and the mice were closely monitored to ensure that none experienced undue stress or discomfort.

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The chronic catheterization was performed as we have previously described (17). Mice were preanesthetized with methoxyflurane and administered pentobarbital sodium (50 mg/kg ip) to induce anesthesia. Supplemental anesthesia was administered as needed. With the use of aseptic techniques, catheters were placed in the femoral artery for the measurement of arterial pressure and blood sampling; catheters were also placed in the femoral vein for infusions and blood volume replacement. The catheters were tunneled subcutaneously and exteriorized through a 10-cm piece of lightweight spring (McMaster Carr, Chicago, IL); the tethering spring was attached to the back of the animal by sewing a 1-cm diameter stainless steel button into the strap muscles between the scapulas. The free end of the spring was connected to a swivel device at the top of the cage. During the surgical procedure and recovery from anesthesia, the animals were kept warm on a heated surgical table. The mice were allowed to recover for 3–5 days before the experimental protocol. Any animal exhibiting pain or distress was euthanized with an overdose of pentobarbital sodium intravenously or intraperitoneally.

All mice were housed individually in metabolic cages within a specially designed chronic rodent hemodynamic monitoring facility. Coat quality, grooming patterns, activity, and food and water consumption were assessed daily as indexes of animal health. Any animal that was not considered in excellent health was eliminated from further study. Arterial pressures (systolic, diastolic, and mean) were recorded using solid-state pressure transducers (Argon Medical Technologies, Athens, TX). The output of the analog pressure signals was amplified (StemTec, GPA-4: Quinton, Menominee Falls, WI), low-pass filtered (30 s \(^{-1}\), 4 pole), and sampled at 360 s \(^{-1}\) (Data Translation, Marlboro, MA). The frequency response of the entire analog and digital system [catheter, transducer, amplifier, A/D, computer] was evaluated and found to be of second order with a damping ratio of 0.4, a roll-off frequency of >16 s \(^{-1}\), and an average amplitude ratio of 0.993 over the range of 0 to 35 s \(^{-1}\). The pulsatile blood pressure signals were reduced to periodic (1 min) averages of systolic, diastolic, and mean arterial blood pressure and heart rate.

**Blood Sampling/Replacement Protocol**

After a 3- to 5-day period of recovery in their home cages, experiments were performed to evaluate the renin-angiotensin-aldosterone axis in conscious mice. Unfortunately, a relatively large amount of blood was required for some of the assays (125 \(\mu\)l for PRA and PRC, 400 \(\mu\)l for Aldo, and 600 \(\mu\)l for ANG II). To minimize the disturbances of blood withdrawal on systemic hemodynamics and to permit repeated sampling from the same mouse, a simple protocol was performed in which the blood withdrawn from the arterial catheter was simultaneously replaced with donor blood infused into the venous catheter. The donor blood consisted of red blood cells obtained from littermate donor mice suspended in artificial plasma. The donor mice were deeply anesthetized with pentobarbital sodium (50 mg/kg ip), and whole blood was drawn for PRA and PRC measurements. The blood sample was collected in a tube containing 0.10 \(\mu\)l whole blood. The mice were then infused intravenously with isotonic saline at ~6 ml/day, which provided the mice with a sodium intake of 1,000 \(\mu\)eq/day, for an additional 5 days; a second blood sample was then drawn for PRA and PRC from the mice during the elevated sodium intake. To document the ability to measure a change in intake using this method, and this technique permits the precise control of intake and delivery of both sodium and pharmacological agents. Moreover, although the intake of volume is increased as well as sodium, the isotonic solution avoids potentially confounding effects of hypertonic or hypotonic solutions and should best mimic the normal changes in fluid consumption that occur when oral NaCl intake is altered.

After 5 days on ~200 \(\mu\)eq/day NaCl intake, an initial 150-\(\mu\)l sample of arterial blood was obtained for PRA and PRC measurement. The blood sample was collected in a tube containing 5 \(\mu\)l 0.3 M Na\(_2\)EDTA per 100 \(\mu\)l whole blood. The mice were then infused intravenously with isotonic saline at ~6 ml/day, which provided the mice with a sodium intake of 1,000 \(\mu\)eq/day, for an additional 5 days; a second blood sample was then drawn for PRA and PRC from the mice during the elevated sodium intake. To document the ability to measure a change in intake using this method, and this technique permits the precise control of intake and delivery of both sodium and pharmacological agents. Moreover, although the intake of volume is increased as well as sodium, the isotonic solution avoids potentially confounding effects of hypertonic or hypotonic solutions and should best mimic the normal changes in fluid consumption that occur when oral NaCl intake is altered.

**Biochemical Assays**

PRA and PRC were measured by RIA using a modification of the method of Sealey and Laragh (23) with nephrectomized rat plasma used as substrate for PRC. Aldo was measured by RIA using a kit from Diagnostic Products (Los Angeles, CA). Plasma ANG II was measured by RIA following HPLC separation of ANG I, ANG II, and the primary angiotensin metabolites as previously described (22). For the ANG II assay, as previously described (22), the strong antibody cross-reactivity with Ang II (2–8), Ang II (3–8), and Ang II (4–8) necessitated the HPLC separation to accurately quantify ANG II (1–8). We observed that the total concentration of immunoreactive ANG II-like fragments was as much as fivefold higher than ANG II (1–8) in plasma obtained from conscious mice.

**Protocol 1: Influence of Dietary Sodium Intake and Captopril and Furosemide Infusion on PRA and PRC in Conscious Mice**

Mice were surgically prepared as described above and placed on a low-sodium intake consisting of 0.1% NaCl food, tap water, and an intravenous infusion of isotonic saline at 1.0 ml/day for 5 days. We have determined that this rate of isotonic saline infusion provides a sodium intake of ~200 \(\mu\)eq/day. In each experiment in this manuscript, the sodium intake was varied by infusing differing amounts of isotonic NaCl solution. Previous experiments from our laboratory have demonstrated (17) that mice achieve neutral sodium balance within 2 days following a step change in intake using this method, and this technique permits the precise control of intake and delivery of both sodium and pharmacological agents. Moreover, although the intake of volume is increased as well as sodium, the isotonic solution avoids potentially confounding effects of hypertonic or hypotonic solutions and should best mimic the normal changes in fluid consumption that occur when oral NaCl intake is altered.

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Finally, the mice received an intravenous infusion of furosemide (50 mg/kg ip) to induce anesthesia. Supplemental anesthesia was administered as needed. With the use of aseptic techniques, catheters were placed in the femoral artery for the measurement of arterial pressure and blood sampling; catheters were also placed in the femoral vein for infusions and blood volume replacement. The catheters were tunneled subcutaneously and exteriorized through a 10-cm piece of lightweight spring (McMaster Carr, Chicago, IL); the tethering spring was attached to the back of the animal by sewing a 1-cm diameter stainless steel button into the strap muscles between the scapulas. The free end of the spring was connected to a swivel device at the top of the cage. During the surgical procedure and recovery from anesthesia, the animals were kept warm on a heated surgical table. The mice were allowed to recover for 3–5 days before the experimental protocol. Any animal exhibiting pain or distress was euthanized with an overdose of pentobarbital sodium intravenously or intraperitoneally.

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Protocol 3: Influence of Dietary Sodium Intake and Intravenous Aldo Infusion on Plasma Aldo in Conscious Mice

Mice were prepared as described above and placed on a low-sodium intake (200 µeq/day) for 5 days. An initial 400-µl sample of arterial blood was obtained (with a simultaneous replacement of drawn blood by intravenous infusion of donor red blood cells suspended in artificial plasma) for measurement of plasma Aldo. The blood sample was collected in a tube containing 5 µl 0.3 M Na2EDTA per 100 µl whole blood. The mice were then infused intravenously with isotonic saline at a rate to provide a high-sodium intake (1,000 µeq/day) for an additional 5 days; a second blood sample was then drawn for Aldo. To document the ability to measure a change in plasma Aldo, the mice then received an intravenous infusion of Aldo (50 mg·kg⁻¹·day⁻¹) for 24 h, and a final blood sample was drawn.

Protocol 4: Acute and Chronic Effects of Intravenous ANG II Infusion on Blood Pressure in Conscious Mice

Experiments in this protocol were performed to determine the sensitivity of blood pressure to acute intravenous bolus administration of ANG II and chronic intravenous ANG II infusion in conscious mice.

Acute effects of intravenous ANG II bolus administration. After a 3- to 5-day recovery period from surgery, the acute blood pressure response to the intravenous bolus administration of ANG II was determined. Blood pressure was measured during a 5-min control period, the average of which served as the baseline MAP. Mice were then injected with an intravenous bolus of saline vehicle (100 µl), and blood pressure was monitored for 5 min. This procedure was repeated with ANG II boluses (0.5, 1, 2, 5, and 10 ng) in saline. The maximum blood pressure change from the preceding control period was calculated for each dose for each mouse by subtracting the peak blood pressure response to the bolus from the baseline pressure.

Chronic effects of intravenous ANG II infusion in conscious mice. After recovery from surgery, the mice were maintained on 0.1% NaCl food and tap water. The venous line was continuously infused with isotonic saline at a rate to provide a normal sodium intake (500 µeq/day), and daily measurements of blood pressure were made during a 2- to 3-h period. After 2 stable control days, ANG II was added to the infusion at various concentrations during a constant intravenous isotonic NaCl infusion. The sodium intake in this protocol (~500 µeq/day) is equivalent to the intake of mice on standard 1% NaCl chow. The ANG II dose was successively increased to 10, 20, and 40 ng·kg⁻¹·min⁻¹ for 2 days at each dose. Daily blood pressure measurements were made during a 2- to 3-h period on each day of the experiment.
Protocol 2: Influence of Sodium Intake on Plasma ANG II in Conscious Mice

The influence of sodium intake and intravenous ANG II infusion on circulating ANG II levels in conscious mice is illustrated in Fig. 2, top. As sodium intake was increased from 200 to 1,000 mEq/day by intravenous infusion of isotonic saline, plasma ANG II was significantly decreased from 14 ± 6 to 6 ± 1 pg/ml in conscious mice (n = 7). When exogenous ANG II was infused (40 ng·kg⁻¹·min⁻¹ iv), circulating ANG II significantly increased to 72 ± 26 pg/ml.

Protocol 3: Influence of Sodium Intake on Plasma Aldo in Conscious Mice

The changes in plasma Aldo in conscious mice are illustrated in Fig. 2, bottom. As sodium intake was increased from 200 to 1,000 μeq/day by intravenous infusion of isotonic NaCl, plasma Aldo was significantly decreased from 41 ± 8 to 8 ± 3 ng/dl (n = 6). When exogenous Aldo was infused (50 ng·kg⁻¹·min⁻¹ iv), circulating Aldo significantly increased to 104 ± 17 ng/dl.

Protocol 4: Acute and Chronic Effects of Intravenous ANG II Infusion on Blood Pressure in Conscious Mice

The acute influence of ANG II on blood pressure in conscious mice is illustrated in Fig. 3, top. Baseline MAP averaged 115 ± 4 mmHg in the acute dose-response experiment. Blood pressure increased in a dose-dependent manner, achieving a statistically significant increase of 21 ± 2 mmHg with the 1.0-ng bolus of ANG II. The maximal blood pressure effect was observed following the 10-ng bolus of ANG II when arterial pressure increased by 40 ± 2 mmHg (n = 14). The effect of chronic ANG II infusion to conscious mice is illustrated in Fig. 3, bottom. Mean arterial blood pressure averaged 111 ± 5 mmHg in the control period, was significantly increased during the infusion of 10 ng·kg⁻¹·min⁻¹ ANG II (to 127 ± 6 mmHg after 2 days), and was not further significantly increased from that level through the remainder of the protocol averaging 135 ± 3 mmHg on the second day of 40 ng·kg⁻¹·min⁻¹ ANG II (n = 5).
Protocol 5: Influence of Fixing Circulating Levels of ANG II on Blood Pressure During Alterations in Sodium Intake in Conscious Mice

The influence of sodium intake on blood pressure in control mice and those continuously infused intravenously with ANG II (20 ng kg\(^{-1}\) min\(^{-1}\)) is illustrated in Fig. 4. As sodium intake was increased from low (200 μeq/day) to normal (500 μeq/day) to high (1,000 μeq/day) levels in conscious mice, MAP was unaltered from the low-sodium intake level of 109 ± 2 mmHg (n = 6). Heart rate was also unaltered and averaged 671 ± 16 beats/min during the low-sodium intake period. In contrast, MAP significantly increased from 108 ± 2 mmHg on a low-sodium intake (not significantly different from the control group on low salt) to 131 ± 2 mmHg on high-sodium intake when ANG II was fixed by continuous infusion (n = 7). Heart rate averaged 683 ± 12 beats/min on the low-sodium intake and was not significantly altered from that level throughout the ANG II-infusion protocol.

DISCUSSION

The present studies showed that the levels of PRC, plasma ANG II, and plasma Aldo were suppressed as sodium intake was increased in normal conscious mice. Evidence of the functional importance of suppression of ANG II levels in mice was the observation that blood pressure increased in a sodium intake-dependent manner when circulating ANG II levels were fixed by exogenous infusion. Although ANG II infusion had a minimal influence on arterial blood pressure in mice maintained on a low-sodium intake, MAP directly increased as sodium intake was increased in these animals. These experiments therefore indicate that the RAS is of importance in the regulation of fluid and electrolyte homeostasis in conscious mice, despite the findings that these animals have higher PRC values compared with other animal models under resting conditions.

The baseline values for PRA, PRC, and ANG II as well as the modulation of PRC with changes in sodium intake observed in the present study are similar to those recently reported in mice (3, 18, 31). It is notable that the...
normal levels of PRA, plasma ANG II, and plasma Aldo in mice are similar to the levels of these parameters in rats (9, 21, 22), despite the extremely high mouse PRC, which is ~100-fold higher than PRA. The elevated PRC with relatively normal PRA indicates that renin is in excess of renin substrate in the normal mouse. It is interesting that PRA was unaltered as sodium intake was increased, yet PRC, ANG II, and Aldo all decreased following salt loading. The mechanism(s) whereby ANG II levels are modulated in the absence of changes in PRA in the normal mouse are currently unexplained.

Coincident with the modulation of ANG II levels, circulating Aldo was also significantly decreased when sodium intake was increased. Because we did not detect a change in plasma potassium during sodium loading, we assume that the change in Aldo level was not due to alterations in extracellular potassium but rather caused by the change in circulating ANG II. The relative contribution of ANG II and extracellular potassium in the regulation of Aldo in this model remains to be determined. In addition, we cannot at this time determine the relative contribution of ANG II and Aldo to long-term fluid and electrolyte homeostasis and blood pressure regulation in the mouse, although ANG II, either by direct or indirect effects, appears critical in this process.

The arterial blood sampling in this protocol was performed with the simultaneous intravenous infusion of donor blood. This technique was used to control the possible changes in systemic hemodynamics that would be predicted to lead to alterations in the levels of different components of the RAS. Because a 30-g mouse would be estimated to have a blood volume of ~2 ml, the withdrawal of 600 μl of whole blood would have a significant impact on arterial blood pressure. In preliminary experiments, we observed that blood pressure transiently fell as much as 50 mmHg during such a blood draw, which was restored with the immediate replacement of an equivalent volume that was removed. To maintain total blood volume and hence minimize the disturbances in arterial pressure, it became necessary to simultaneously infuse donor blood intravenously while drawing an arterial blood sample.

One drawback with the simultaneous intravenous infusion of donor blood during arterial blood sampling is the possibility of diluting the plasma and thereby lowering the absolute level of the hormones measured. Although this is clearly possible, especially with the larger volumes of blood drawn to quantify plasma Aldo and ANG II, the experiments were performed in a paired fashion. Any error introduced by infusion of donor blood should therefore be consistent between the different conditions in which the plasma samples were drawn. Moreover, we performed additional experimental manipulations to each group of animals to ensure that our blood sampling method and biochemical assays would be capable of quantifying a change in each parameter. Overnight treatment with the ACE inhibitor captopril led to a significant increase in both PRA and PRC. An additional day of infusion of furosemide led to a further increase in PRC but no additional change in PRA. Similarly, increases in plasma ANG II and plasma Aldo were measured in mice continuously infused intravenously with exogenous ANG II or Aldo. We are therefore confident that our blood collection and assay system can detect changes in these parameters in conscious mice.

The present data indicate that the normal mouse has approximately the same sensitivity to acute ANG II bolus administration as rats (1, 24). A consistent, dose-dependent rise in MAP was observed in the mice to acute ANG II bolus infusion. With sustained infusion of ANG II, it was observed that MAP was increased at all doses of ANG II compared with the control period. The sensitivity of arterial blood pressure to chronic infusion of ANG II is similar to that observed in rats (8) and dogs (5). Interestingly, although MAP significantly increased during administration of the initial dose of ANG II, MAP did not increase further when greater doses of ANG II were infused.

The present results demonstrate that suppression of PRC, Aldo, and ANG II not only occurs in conscious mice, but they also indicate that the suppression is of functional importance in the adaptation to increased dietary sodium intake. When conscious mice were infused with gradually increasing amounts of isotonic saline, there was no significant alteration in MAP. This observation is in good agreement to that which we had previously reported (17), where mice achieved neutral sodium balance by the third day following a change in isotonic saline infusion without any significant changes in MAP. In contrast, when ANG II levels were fixed by the infusion of a large dose of exogenous ANG II (20 ng·kg⁻¹·min⁻¹), arterial blood pressure significantly increased as the daily sodium intake of the mice was increased from low to normal levels. This observation is similar to that made in rats (2) and dogs (10). The sodium-dependent increase in arterial blood pressure during ANG II infusion, combined with the suppression of PRC, ANG II, and Aldo in the normal mice during increased sodium intake, indicates that suppression of the renin-angiotensin-aldosterone axis is important for normal mice to attain neutral sodium balance during changes in sodium intake. In contrast, when ANG II levels were fixed by the infusion of a large dose of exogenous ANG II (20 ng·kg⁻¹·min⁻¹), arterial blood pressure significantly increased as the daily sodium intake of the mice was increased from low to normal levels. Because the infusion of ANG II at 40 ng·kg⁻¹·min⁻¹ to mice increased circulating ANG II to over 70 pg/ml while the normal level of ANG II in low-salt mice is ~14 pg/ml, it is likely that the dose of ANG II infused when sodium intake was altered (20 ng·kg⁻¹·min⁻¹) elevated circulating ANG II above that observed in control mice. It is possible that the infusion of exogenous ANG II at a lower rate, which would clamp circulating ANG II at 14 pg/ml as sodium intake was altered, would not lead to an alteration in arterial pressure with increased sodium intake. The blood pressure effects of lower dose ANG II during high-salt intake remain to be determined.

In summary, the present experiments demonstrate that suppression of the RAS is critical for the maintenance of arterial blood pressure during alterations in...
sodium intake in normal mice. The circulating levels of renin are decreased when mice are placed on a high-sodium diet. Accompanying the decrease in PRC was a significant decrease in circulating ANG II and Aldo. Finally, we demonstrated that the level of MAP increased as sodium intake was increased while ANG II was continuously infused. These hormonal and functional data indicate that suppression of circulating ANG II levels occurs in conscious mice, despite an elevated resting PRC, and may be important for the maintenance of arterial blood pressure in normal mice during alterations in sodium intake. In conclusion, although there have been severe renal abnormalities reported in RAS knockout mice, the changes in MAP and fluid/electrolyte homeostasis that were observed in those mice may not have been due to the renal problems alone but indeed by the alterations in the RAS.

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