Chronic Sodium Balance and Blood Pressure Response to Captopril in Conscious Mice

David L. Mattson, Kristyn R. Krauski

Abstract—The influence of chronic administration of the converting enzyme inhibitor captopril on blood pressure and sodium balance was evaluated in conscious Swiss Webster mice. Arterial pressure was measured with chronic indwelling catheters, and sodium balance was determined by infusing sodium intravenously in isotonic saline and collecting urine 24 h/d. Experiments to validate sodium balance measurements in mice demonstrated recovery of 100±3% of sodium intake under steady-state conditions (n=20 mice on 70 individual days, sodium intake range 160 to 1000 μmol/d). It was further demonstrated that mean arterial pressure, heart rate, and body weight were unaltered from 115±7 mm Hg, 646±12 bpm, and 34±0.6 g, respectively, as sodium intake was increased stepwise from 150 to 900 μmol NaCl per day. An additional validation group (n=7) demonstrated that daily and cumulative sodium balance can be accurately determined during and after the intravenous administration of an agent known to alter renal sodium handling (furosemide 50 mg·kg⁻¹·d⁻¹). Experiments were then performed to examine the influence of intravenous captopril infusion (40 mg·kg⁻¹·d⁻¹, n=7) in mice in which the daily sodium intake was fixed at ≈200 μmol/d. This dose of captopril was determined to significantly decrease the pressor response to a 10-ng bolus of angiotensin I (Ang I) from 24±5 in the control state to 6±2 mm Hg (n=5). After 5 days of infusion of the converting enzyme inhibitor, mean arterial pressure significantly fell from 114±3 to 58±2 mm Hg, body weight significantly decreased from 36±1 to 33±1 g, and cumulative sodium balance significantly decreased to −270±55 μmol. These parameters returned toward control during 5 postcontrol days. Results of this study demonstrate that accurate sodium balance measurements can be obtained from individual conscious mice over a 5-fold range of sodium intake. The experiments also indicate that converting enzyme inhibition has a potent influence to lower blood pressure in normal mice; the hypotensive response appears to be due in part to increased urinary sodium excretion. (Hypertension. 1998;32:923-928.)

Key Words: renin-angiotensin system • captopril • blood pressure • sodium

Techniques in molecular biology have allowed the development of a number of interesting transgenic and knockout models in which important cardiovascular/renal control systems have been genetically altered.¹ One cardiovascular regulatory system that has been the subject of intense investigation using transgenic technology in the mouse has been the renin-angiotensin system (RAS). Manipulation of the genes encoding renin,²⁻³ angiotensinogen,²⁻⁴ angiotensin-converting enzyme (ACE),⁵⁻⁷ and the AT₁⁻⁸ and AT₂ receptors¹⁰¹¹ has produced profound effects on cardiovascular homeostasis. One of the most potent effects observed with manipulation of the RAS has been the hypotensive effect of deletion of the ACE gene. Arterial blood pressure, measured by both tail-cuff plethysmography⁶⁻⁷ and direct arterial cannulation,⁸ has been reported to decrease by as much as 50% in ACE knockout mice. This hypotensive effect is extremely potent when compared with the blood pressure response to pharmacological ACE inhibition (ACEI) in normotensive humans,¹²⁻¹³ dogs,¹⁴⁻¹⁶ and rats.¹⁷ It is unclear whether pharmacological ACEI in the mouse will decrease mean arterial pressure (MAP) to levels similar to those observed in mice in which the ACE gene has been genetically deleted. It is also not clear from these experiments what changes (if any) occur in fluid and electrolyte homeostasis to lead to the steady-state level of blood pressure in mice lacking the ACE gene. The present studies were designed to examine the changes in blood pressure and sodium balance in conscious, chronically instrumented mice during pharmacological blockade of ACE with captopril.

To examine the influence of ACEI on blood pressure and sodium balance in conscious mice, it was necessary to deliver saline intravenously with and without captopril, measure sodium intake and output, and quantify arterial blood pressure. We previously reported a method that allows the long-term measurement of arterial pressure and continuous intravenous infusion in conscious mice.¹⁸ The goals of the present study were 3-fold. The first aim was to develop a strategy for the measurement of daily and cumulative sodium balance in conscious mice. The second aim was to determine the appropriate dose (continuously delivered intravenously)
of the ACE inhibitor captopril on MAP and the pressor response to angiotensin I (Ang I) in mice. The third aim was to determine the influence of chronic intravenous infusion of captopril on blood pressure and sodium balance in conscious mice.

Methods

Experiments were performed on adult Swiss Webster mice obtained from Taconic Farms (Germantown, NY). 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 and the Institutional Care Committee, and the mice were closely monitored to ensure that none experienced undue stress or discomfort.

Mice were surgically prepared for chronic studies as we have previously described. The animals were preanesthetized with methoxyflurane and administered sodium pentobarbital (50 mg/kg IP) to induce anesthesia. Supplemental anesthetic was administered as needed. Using aseptic techniques, we placed catheters in the femoral artery for the measurement of arterial pressure and in the femoral vein for infusion. The arterial catheters were filled with 500 U of heparin in saline, sealed, and opened only when they were used for recording. Arterial pressure data were collected using computerized data acquisition software as previously described.

The venous catheters were continuously infused with saline or saline with drug at the rates indicated in the individual protocols.

Protocol 1: Sodium Balance and MAP in Conscious Mice Maintained on Different Daily Sodium Intake

Mice were surgically prepared as described above and housed in stainless steel metabolic cages (14 cm wide, 20 cm long, and 10 cm high) with silicone-coated collection funnels. After the recovery period, the animals were maintained ad libitum on tap water and sodium-free liquid rodent chow (Dyets) by use of feeders fabricated in our laboratory. All sodium intake was delivered by intravenous infusion of sterile isotonic saline. The range of sodium intake was chosen based on preliminary experiments in which mice maintained on normal chow (1.0% NaCl) had an average daily sodium intake of ~530 μmol/d; this level of sodium intake was used as a reference to normal intake, with the lower and upper levels of sodium intake spaced around that value. Mice were initially infused with saline at 1 mL/d to provide ~150 μmol of sodium per day. After 3 days on this regimen, the intravenous infusion was increased to 3 mL/d (~450 μmol/d) for an additional 3-day period. Finally, the infusion was increased to 6 mL/d (~900 μmol/d) for the final 3 days of this protocol. This technique is an adaptation of a method we have used to perform sodium balance studies in rats.

The MAP and heart rate (HR) of the mice were measured on each day of the experiment during a 2- to 3-hour recording period. After the recording period, the daily urine volume was measured and the urine collection funnel on each individual cage was washed with distilled water. This wash volume was added to the urine, the total volume of the urine and wash was determined, and the sodium concentration of the solution was determined by flame photometry.

Protocol 2: Daily and Cumulative Sodium Balance in Conscious Mice After Furosemide Administration

Experiments in protocol 1 were performed to document the ability to measure sodium balance over a 6-fold range of sodium intake. Experiments in this protocol were designed to demonstrate the ability to quantitatively changes in daily and cumulative sodium balance during and after the administration of an agent known to alter renal sodium handling (furosemide). Mice were prepared as described above and infused intravenously with isotonic saline at ~3 mL/d (450 μmol/d NaCl). After 3 control days, furosemide was added to the intravenous infusate to deliver 50 mg · kg⁻¹ · d⁻¹ for 24 hours. The infusion was then returned to saline for the final 2 postcontrol days. MAP was measured from 5 of the mice during a 2- to 3-hour period each day, and daily measurements of body weight were obtained from all mice on each day of the protocol.

Protocol 3: Dose-Response Effect of Captopril on MAP and Pressor Response to Ang I in Conscious Mice

Mice were instrumented as described above with chronic indwelling femoral arterial and venous catheters. After a 5- to 7-day recovery from surgery, the blood pressure of the mice was measured during a 2- to 3-hour daily recording period. During the control period, the mice were infused intravenously with saline (3.0 mL/d) and maintained ad libitum on normal chow (1.0% NaCl) and tap water. After 2 stable control days of blood pressure measurement, the intravenous infusate was switched to captopril in saline to deliver 20 mg/kg per day for the first 24 hours, 40 mg · kg⁻¹ · d⁻¹ for the next 24 hours, and 80 mg · kg⁻¹ · d⁻¹ for the final 24 hours. Blood pressure was measured after a 24-hour infusion of each dose of captopril.

A similar protocol was performed to evaluate the pressor response to an intravenous bolus of Ang I. Preliminary studies demonstrated a dose-dependent effect of Ang I to increase MAP in doses from 1 to 10 ng in a 0.1-mL saline bolus. The bolus volume itself had a minimal influence on blood pressure (~<3 mm Hg). It was determined that the 10-ng Ang I bolus reproducibly increased MAP by ~<20 mm Hg. The increase in MAP after administration of a 10-ng bolus of Ang I was, therefore, evaluated on an initial control day and after 24 hours of infusion of captopril at rates of 20, 40, and 80 mg · kg⁻¹ · d⁻¹ on successive days in the same animal. An additional protocol was performed in a separate group in which the MAP response to an Ang I bolus was measured during a control day and after 5 days of intravenous captopril (40 mg · kg⁻¹ · d⁻¹). The Ang I bolus was administered twice each day, and the average of the 2 measurements was taken as the value for that day.

Protocol 4: Influence of Chronic Intravenous Captopril on MAP and Sodium Balance in Conscious Mice

Mice were instrumented and housed in individual metabolic cages as described above. The animals were given sodium-free liquid chow and tap water ad libitum. All sodium was delivered intravenously in saline (~1 mL/d). After 2 stable control days, captopril was added to the intravenous infusate to deliver 40 mg · kg⁻¹ · d⁻¹. After 5 days of captopril, the drug infusion was stopped and saline was infused for an additional 5 postcontrol days. Daily measurements of MAP, HR, sodium excretion, and body weight were obtained throughout this protocol.

Statistical Methods

Data are expressed as mean±SE. The within-group changes were evaluated using 1-way ANOVA for repeated measures with a Tukey post hoc test. Between-group comparisons were made with 2-way ANOVA. A probability level of $P<0.05$ was considered significant.

Results

Protocol 1: Sodium Balance and MAP in Conscious Mice Maintained on Different Daily Sodium Intakes

The MAP, daily sodium intake and output, and daily sodium balance values from the initial group of mice are illustrated in Figure 1. As shown in the middle panel, sodium intake was chosen based on preliminary experiments in which mice maintained ad libitum on normal chow (1.0% NaCl) and tap water ad libitum had the rates indicated in the individual protocols.
achieved in the mice during each of the 3 days in which they were maintained on the lower level of sodium intake. On the first day after the transition from low to normal sodium intake, 125 ± 44 m mol of sodium was retained (P, 0.05). By the second and third day at this level, sodium intake and output were again equal. The same general pattern was observed after the transition from the normal to high level of sodium intake. Sodium was retained (99 ± 23 m mol of sodium, P<0.05) on the initial day at the upper level of sodium intake, but the mice came into a steady state by the second day at this level of sodium intake. Despite the significant sodium retention on the initial day after the increase to both normal and high sodium intake, the calculated cumulative sodium balance was not significantly altered throughout the protocol. Cumulative balance averaged −53 ± 67 m mol of sodium on the third day on low sodium intake, a value not significantly different from the average of 146 ± 145 μmol observed on the third day of high sodium intake. MAP, HR, and body weight were also unaltered throughout the protocol, averaging 115 ± 8 mm Hg, 646 ± 12 bpm, and 33.9 ± 0.6 g, respectively, on the final day of low sodium intake and 118 ± 8 mm Hg, 683 ± 15 bpm, and 34.2 ± 0.7 g on the final day of the high sodium intake.

The daily sodium balance data from the final 2 days (steady-state conditions) at each level of sodium intake in this protocol and the control data from protocols 2, 3, and 4 (described below) were pooled to evaluate the ability to accurately collect sodium output in mice during steady-state conditions with intakes ranging from 160 to 1012 μmol/d (n = 20 mice on 75 individual days, Figure 2). The mean recovery (measured sodium output divided by intake) averaged 100 ± 3% for all days. A linear regression on these data had a correlation coefficient of 0.93, a slope of 0.96, and a y intercept of 31 μmol.

**Figure 2.** Relationship between sodium intake and collected sodium output in conscious Swiss Webster mice (see text for details). Data were obtained from 20 mice on 70 individual days during steady-state conditions in protocols 1 to 4. y-int indicates y intercept.

**Protocol 2: Daily and Cumulative Sodium Balance in Conscious Mice After Furosemide Administration**

Daily and cumulative sodium balance results from this experiment are presented in Figure 3. Daily sodium balance averaged 1 ± 24 μmol, cumulative sodium balance averaged −21 ± 28 μmol, and body weight averaged 42.2 ± 0.9 g on the third control day (n = 7). After 24 hours of furosemide infusion, daily sodium balance significantly decreased to −360 ± 31 μmol (P<0.01), cumulative sodium balance significantly decreased to −381 ± 40 μmol (P<0.01), and body weight significantly decreased to 39.0 ± 1.9 g (P<0.01). MAP measured from 5 mice averaged 117 ± 4 mm Hg on the third control day and decreased to 100 ± 1 mm Hg after furosemide (P<0.05). After 1 day of saline infusion after furosemide administration (postcontrol day 1), sodium balance averaged 332 ± 33 μmol and cumulative balance was not significantly different from control. By the second postcontrol day, daily sodium balance also returned to levels no different from control (−56 ± 84 μmol/d), and MAP averaged 111 ± 2 mm Hg.
Protocol 3: Dose-Response Effect of Captopril on MAP and Pressor Response to Ang I in Conscious Mice

Chronic intravenous captopril infusion led to a progressive decrease in MAP from a control value of 117 ± 5 mm Hg to 94 ± 4, 82 ± 5, and 77 ± 6 mm Hg as the daily infusion rate was increased from 20 to 40 and finally to 80 mg/kg/day, respectively, on 3 successive days (n=8, p<0.01). HR was not significantly altered from the control value of 62 ± 27 bpm throughout the protocol. The blood pressure response to a 10-ng bolus of Ang I averaged 22 ± 6 mm Hg during the control period and decreased to 13 ± 2, 11 ± 2, and 6 ± 1 mm Hg over this same dose range (n=8, p<0.01). In a final group of mice (n=5), it was determined that the pressor response to a 10-ng intravenous Ang I bolus was significantly reduced from 24 ± 5 to 6 ± 2 mm Hg after 5 successive days of captopril infusion at 40 mg/kg/day (p<0.05).

Protocol 4: Influence of Chronic Intravenous Captopril on MAP and Sodium Balance in Conscious Mice

The long-term influence of intravenous captopril infusion on MAP and daily sodium balance in mice maintained on a sodium intake of ~200 μmol/d is illustrated in Figure 4 (n=6). Daily sodium balance, cumulative sodium balance, MAP, and body weight averaged −7 ± 20 μmol, −16 ± 35 μmol, 114 ± 5 mm Hg, and 36.2 ± 1.7 g, respectively, on the second control day. MAP significantly decreased from 114 ± 5 mm Hg on the second control day to 95 ± 6 mm Hg after 1 day and to 58 ± 3 mm Hg on the fifth day of captopril infusion (p<0.01). The decrease in arterial pressure was accompanied by increased sodium excretion that led to a significantly negative sodium balance (−117 ± 21 μmol/d, p<0.05) on the third day of captopril infusion. As the experiment progressed, cumulative sodium balance became more negative and body weight significantly (p<0.01) decreased, averaging −270 ± 56 μmol and 33.1 ± 1.5 g on the fifth day of captopril infusion. Interestingly, when the captopril infusion was stopped, the daily sodium balance tended to become positive as MAP returned toward control values. It was observed, however, that both MAP and cumulative sodium balance were significantly (p<0.01) lower than the control values, averaging 83 ± 5 mm Hg and −177 ± 97 μmol of sodium after 5 post-control days.

Discussion

The present studies describe an experimental method in which accurate daily sodium balance can be obtained from conscious mice over a 5-fold range of sodium intake (200 to 1000 μmol/d). As demonstrated by the furosemide study, this technique can also be used to accurately quantify changes in cumulative sodium balance. This method was then used to...
examine the influence of ACEI in conscious mice. Continuous intravenous infusion of captopril (40 mg · kg⁻¹ · d⁻¹) decreased blood pressure response to an Ang I bolus by 75% and led to marked hypotension. The decrease in MAP was associated with a significant decrease in cumulative sodium balance, indicating that at least part of the hypotensive effect of ACEI in mice is because of increased sodium excretion and contraction of extracellular fluid volume.

The goal of the present experiments was to examine the renal and cardiovascular effects of pharmacological blockade of ACE in conscious mice. Despite the ability to chronically monitor pressure and infuse compounds intravenously into conscious mice, the accurate measurement of sodium balance was found to be extremely difficult. Our original strategy to determine balance in mice was to measure the amount of food consumed and to collect the urinary output. We have previously noted in rats, however, that accurate balance measurements are extremely difficult to perform when sodium intake is in solid chow.19 This is apparently because of difficulty in quantifying the amount of food consumed and because of fecal loss of sodium, problems that are even more exaggerated in mice. To avoid these problems, the mice were fed a sodium-free liquid diet ad libitum and all sodium intake was delivered intravenously. This method permitted the collection of an average of 100±3% of sodium intake over a 5-fold range, allowing accurate collection of urinary sodium output and precise control of sodium intake.

The results of protocol 2 are further evidence of the utility of this method to accurately measure sodium balance. In this experiment, cumulative sodium balance returned to a level not significantly different from control after the mice underwent a 6-day protocol that included 3 control days, a 24-hour intravenous infusion of furosemide (which led to a loss of >300 μmol of sodium for that day), and 2 postcontrol days. Despite daily sodium balance measurements that were significantly positive or negative on 2 of the 6 experimental days, the final cumulative balance point was not significantly different from control in this study. This experiment demonstrated that this approach can be used to account for most of the sodium that has been lost or gained in an individual protocol. Together, these validation data indicate that daily and cumulative sodium balance can be accurately measured in mice by use of this procedure.

In addition to the validation of the balance-measurement technique, several interesting observations arose from protocols 1 and 2. First, MAP, cumulative sodium balance, and body weight were not altered after the 9-day protocol in which sodium intake was successively increased from 150 to 900 μmol/d. The lack of sodium sensitivity of blood pressure in the normal mouse is similar to that observed in normotensive rats20 and dogs.14 A second interesting observation from these validation experiments was the relatively rapid adjustment of the renal/cardiovascular system to changes in dietary sodium intake or altered urinary sodium output. When sodium intake was incrementally increased from either low to normal or normal to high in protocol 1, or when intake was maintained constant and furosemide was administered in protocol 2, an alteration in daily sodium balance was observed on the initial day. Neutral sodium balance, however, was achieved in each case by the second day after the alteration in balance. The integrated renal, cardiovascular, neuronal, and hormonal regulation of fluid and electrolyte homeostasis appears to function very efficiently within a 1- to 2-day period after an alteration in sodium balance in the mouse.

The final experiments in this study evaluated the influence of a 5-day infusion of captopril (40 mg · kg⁻¹ · d⁻¹) on MAP and sodium balance in conscious mice maintained on a low (200 μmol/d) sodium intake. ACEI had an extremely potent influence on MAP, decreasing pressure from 114 to 58 mm Hg after only 5 days of infusion. This surprisingly potent effect on arterial pressure was accompanied by a cumulative loss of 270 μmol of sodium and a decrease in body weight that exceeded 3 g, indicating that a portion of the hypotensive response to ACEI was because of contraction of the extracellular volume. It was also observed, however, that blood pressure rapidly fell after only 1 day of captopril infusion before any changes were observed in sodium balance. It is therefore likely that part of the decrease in MAP in the conscious mouse was because of a decrease in total peripheral resistance.

The changes in arterial pressure after captopril observed in the present study compare favorably with the results previously obtained in mice in which the ACE gene was deleted. Systolic arterial pressure was reduced by 30 to 40 mm Hg when measured by tail-cuff plethysmography, and MAP was reduced by approximately 50 mm Hg as measured by direct arterial catheterization in conscious mice lacking ACE. Furthermore, the MAP response to a 100-ng/kg IV bolus of Ang I was decreased from ∼29 mm Hg in wild-type mice to ∼5 mm Hg in mice with the ACE gene absent.6 Both the hypotensive response (MAP decreased by 56 mm Hg) and the decreased pressor response to a 10-ng Ang I bolus (24 versus 6 mm Hg) after 5 days of intravenous captopril infusion in the present experiments qualitatively agree with the data generated in ACE gene deletion mice. Interestingly, the effect of ACEI in mice was relatively potent compared with that observed in other species. Though normotensive humans,12,13 dogs,14–16 and rats17 maintained on a low sodium intake or depleted of sodium exhibit a hypotensive response after ACEI, the magnitude of the hypotensive response was not as large as that observed in the present study. In studies in sodium-deprived dogs and rats,1 a decrease in MAP to approximately 70 mm Hg occurred after chronic ACEI. The mechanism of the hypotensive response in the mouse presumably involves blockade of the formation of Ang II from Ang I. However, it is possible that other mechanisms may also participate in this hypotensive response in the mouse. The decrease in blood pressure observed in mice in which the ACE gene has been genetically deleted and with the pharmacological blockade of ACE in this study is much greater than the decrease in blood pressure observed in mice with angiotensinogen gene deleted or the decrease in blood pressure observed in mice deficient in the AT₁ receptor. It is conceivable that kinins or other humoral and/or paracrine factors could participate in the potent blood-pressure-lowering effect observed in mice in which ACE activity has been
genetically or pharmacologically reduced. Experiments to explore this possibility remain to be performed.

In summary, the present experiments demonstrate a technique that allows the quantitative measurement of daily and cumulative sodium balance in conscious mice. This technique was used to demonstrate the changes in sodium balance that occur during chronic ACEI in conscious mice. The influence of sodium intake on the hypotensive response to ACEI in mice, the relative role of Ang II, kinins, or other humoral mediators in this response, and the mechanisms (renal, vascular, or mixed) of this hypotensive response remain to be examined. The pharmacological approach to study ACE in mice in this study complements the previously reported results obtained using genetic deletion to further lend to our understanding of the importance of ACE in blood pressure control. While the genetic manipulation approach permits a much more selective manipulation of ACE without the dose-sensitive effects and possible undesirable side effects of pharmacological agents, the approach used in the present study permits the examination of a single animal before and after inhibition of the targeted system and allows the examination of transient changes in hemodynamic and/or fluid and electrolyte balance that may ultimately contribute to the long-term changes in blood pressure.

Acknowledgments

This work was partially supported by grants HL-29587 and DK-50739 from the National Institutes of Health, Bethesda, MD. The authors thank Timothy Bachman for technical assistance with some of these protocols.

References


Chronic Sodium Balance and Blood Pressure Response to Captopril in Conscious Mice
David L. Mattson and Kristyn R. Krauski

*Hypertension*. 1998;32:923-928
doi: 10.1161/01.HYP.32.5.923

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/32/5/923