Long-Term Telemetric Recording of Arterial Pressure and Heart Rate in Mice Fed Basal and High NaCl Diets

Scott H. Carlson, J. Michael Wyss

Abstract—Research examining the control of arterial pressure in mice has primarily relied on tail-cuff plethysmography and, more recently, on tethered arterial catheters. In contrast, the radiotelemetry method has largely become the “gold standard” for long-term monitoring of arterial pressure and heart rate in rats. Whereas smaller telemetry probes have recently been developed, no published studies have used radiotelemetric monitoring of arterial pressure in mice, largely because of a relatively low success rate in small mice (ie, <30 g body weight). We report on the development of a protocol for the use of these probes to continuously monitor arterial pressure and heart rate in mice as small as 19 g body weight. To test the accuracy and reliability of this method, adult C57/BL6 mice were monitored for 3 weeks during exposure to a basal followed by a high NaCl diet. The results demonstrate that carotid and aortic placements of the telemetry probe provide equally accurate monitoring of arterial pressure and heart rate, but the carotid placement has a much greater rate of success. Exposure to a high NaCl diet increases both the amplitude of the arterial pressure rhythm (+ 6.0±0.6 mm Hg, ~32%) and the average mean arterial pressure (+ 8.6±1.1 mm Hg, ~8%), as would be predicted from previous studies in NaCl-resistant rats. Thus, the data demonstrate that telemetric recording of long-term arterial pressure and heart rate provides a powerful tool with which to define the mechanisms of cardiovascular control in mice. (Hypertension. 2000;35:e1-e5.)

Key Words: telemetry • salt • circadian rhythm • transgenic mice

Studies into the mechanisms underlying cardiovascular control have increasingly employed transgenic mouse models, in which genes encoding for various products (eg, receptors, hormones, proteins, etc.) are functionally altered or deleted. Initial studies that have monitored arterial pressure and heart rate (HR) in mice primarily used the tail cuff plethysmography method or acute catheterization techniques. Recently, protocols have been developed to monitor arterial pressure on a long-term basis in catheterized mice, allowing for analysis of 24-hour arterial pressure and HR rhythms; these protocols, however, require mice to be tethered to an outside transducer.

Studies monitoring long-term arterial pressure and HR control in rats have been greatly facilitated by the development of radiotelemetric recording, a method in which an implanted transducer and radio transmitter continuously monitors and transmits arterial pressure and HR without the stress of heating, handling, or tethering. Newly developed telemetry probes for mice have the promise of similarly facilitating accurate monitoring of arterial pressure and HR over extended periods of time, but to date no studies have used this new system in mice, primarily because of a high mortality rate and a general inability to perform studies reliably in small mice (ie, <28 g, the typical size of most transgenic mice). The goal of the present study was to develop a technique for implantation of mouse telemetry probes that allows for the reliable, continuous measurement of arterial pressure and HR in conscious, untethered mice as small as 20 g body weight. To test the usefulness of this technique, we examined the circadian rhythm of arterial pressure and HR in C57/BL6 mice (19 to 30 g) on a basal and high NaCl diet. We predicted from previous data in rats, that in NaCl-resistant animals, a high NaCl diet would primarily affect the amplitude of the circadian rhythm of arterial pressure and have less effect on the average daily mean arterial pressure (MAP).

Methods

Animals

Female C57/BL6 mice were bred at the University of Alabama at Birmingham’s animal facilities and housed in individual cages in a sound attenuated room at constant humidity (60±5%), temperature (24±1 °C), and light cycle (6AM to 6PM light). All mice were allowed access ad libitum to either basal (0.6% NaCl; diet # 8746, Teklad,) or high (8% NaCl; diet #5008, Teklad) NaCl diet and water. All animal protocols were approved by the University of Alabama at Birmingham’s Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide on the Humane Treatment of Experimental Animals.

Carotid Artery Implantation

The mice were anesthetized by intraperitoneal injection (0.1 mL/20 g) of a mixture of ketamine (100 mg/kg; 2.0 mL) and xylazine (15
implantation was performed on a heating pad, and telemetry probes were heated in sterile saline (0.9% NaCl) to body temperature. Mice were anesthetized with Isoflurane (inhalant). Hair was removed from the abdomen and the incision area was cleaned as described above. A midline abdominal incision was made, and the intestines were retracted with a moistened gauze. A portion of the aorta (from the iliac bifurcation extending to the renal arteries) was exposed, and connective tissue and fat were then gently removed with sterile cotton applicators. With the use of fine-tipped vessel dilation forceps (#14114, World Precision Instruments), the aorta were separated from the vena cava at both the iliac bifurcation and the renal arteries, and ligatures (5-0 silk) were placed around the aorta at both sites to occlude blood flow. Before the occlusion of the aorta, 1 to 2 drops of lidocaine (2%) were dripped onto the vessel to dilate it. The tip of the telemetry probe catheter was gently grasped with vessel cannulation forceps (#00608-1, Fine Science Tools, Inc), and the aorta lumen was punctured above the iliac bifurcation with a 26-gauge hypodermic needle on which the tip had been bent at a 90-degree angle. The tip of the catheter was inserted into the aorta with the use of the needle as an introducer, and the needle was then withdrawn and the tip of the catheter advanced to the point of occlusion at the renal vessels. The area was dried with a sterile cotton applicator, and the catheter was secured with a single drop of Vetbond, applied through a 30-gauge needle to the catheter just above the vessel and allowed to wick down around the site of entry into the aorta. The occluding ligatures were then released and the area was checked for leakage. A small piece of cellulose patch was placed over the catheter and secured to the catheter with Vetbond. The area was then irrigated with 0.9% saline, and the skin incision was closed with surgical staples. The mice were then placed directly on a heating pad for initial recovery, as above, and then returned to their cages, which were placed with one half of the cage on the heating source, for 12 hours. The animals were then returned to the telemetry room and allowed a 1-week recovery period before experimentation.

**Experimental Protocol**

After recovery, mice were maintained on a basal (0.6%) NaCl diet for 1 week, after which the telemetry probes were magnetically turned on, and MAP and HR were monitored for 4 days. The probes were then turned off, and the mice were fed a high (8%) NaCl diet for 1 week, after which the probes were turned on, and MAP and HR were monitored for 4 days.

**Simultaneous Arterial Pressure Measurements**

In 7 anesthetized mice, changes in femoral arterial pressure were recorded in response to occlusion of the right carotid artery. Mice were implanted with a femoral arterial catheter as described above. Arterial pressure was subsequently measured via this probe before, during, and after ligation (5-0 silk suture) of the right common carotid artery.

In a second group of 5 mice, arterial pressure was recorded simultaneously from the common carotid artery and lower abdominal aorta. Mice were implanted with a femoral arterial catheter as above, and arterial pressure was monitored from the femoral catheter during the implantation of the telemetry probe into the right carotid artery. The mice regained consciousness and then recovered for at least 4 hours, after which arterial pressure was recorded simultaneously in awake, freely-moving mice via both probes for at least 30 minutes. The output of the probes was compared by correlational methods.

**Data Acquisition and Analysis**

MAP data were collected and analyzed as described previously. Circadian rhythm analysis of the individual hourly MAP and HR data was performed with the nonlinear, least-squares fitting program PHARMeFIT, and the “best fit” model was defined as the one with

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**Figure 1.** Sample tracing of mean arterial pressure (MAP) recorded telemetrically in the common carotid artery in a mouse fed a 0.6% and 8% NaCl diet. The dark bars indicate lights off (6PM to 6AM).
the lowest number of harmonics that had a confidence value of \([mteq]0.05\), as determined by the subprogram SYNOPS. All PHARMFIT analyses were based on data for 4 consecutive days, thus allowing comparisons of the harmonic patterns in each group and the mean (MESOR = average 24 hours arterial pressure, ie, midline estimating statistic of rhythm), amplitude and acrophase (clock time of peak amplitude) of the 24-hour adjusted rhythm.

Statistical Analysis
All data were evaluated by ANOVA (significance criteria of \(P<0.05\)) with appropriate post hoc tests (Newman-Keuls) to determine the source of main effects and interactions.

Results
Body weights of the mice averaged 28.4±2.1 g and ranged from 19.5 g to 40.2 g. Basal MAP was 112.8±1.2 mm Hg (Figures 1 and 2, Table 1), and displayed a 24-hour rhythm characterized by several nighttime (the mouse’s active period) peaks, the first of which occurred immediately at the start of the dark (lights off) period (Figure 2). The peak (acrophase) MAP of the rhythm (122.5±0.01 mm Hg) occurred at \(\approx 4\) AM, while the nadir MAP (97.6±0.01 mm Hg) occurred at \(\approx 1\) PM. Compared with the carotid measurements, basal MAP was significantly lower (\(\approx 14\) mm Hg) when measured in the lower abdominal aorta (Figure 2, Table 1), and the amplitude of the rhythm was reduced, although the rhythm pattern and acrophase of the rhythm were similar.

When the mice with the carotid probes were exposed to the high NaCl diet, the MESOR of MAP significantly increased (\(\approx 8\)%), as did the peak MAP (\(\approx 16\)%) and the amplitude of the 24-hour MAP rhythm (\(\approx 32\)%; Figure 2, Table 1). In contrast, the acrophase of the rhythm was not significantly altered. While the dietary NaCl-induced increases in MAP (\(\approx 4\)%) and peak MAP (\(\approx 5\)%) were also recorded from the mice with the aortic (versus carotid) probes, these changes were significantly smaller, and the amplitude and acrophase of the rhythm was unaltered by the high NaCl diet (Figure 2, Table 1).

Basal HR was similar in the mice with carotid artery versus aorta implantation. In the carotid group, basal HR was 569±9 bpm and displayed a peak that occurred at the onset of the nighttime period whereas the nadir occurred near the end of the nighttime period (\(\approx 5\) AM; Figure 3). The high NaCl diet significantly decreased basal HR (\(\approx 11\)%) and the amplitude of the 24-hour HR rhythm (\(\approx 47\)%) and shifted the acrophase of the HR rhythm (\(\approx 4\) hours; Figure 3, Table 2).

In 7 mice, arterial pressure was recorded from the femoral artery before, during, and after carotid ligation. Occlusion of the carotid artery produced a transient elevation in femoral arterial pressure (\(\approx 7.7\) mm Hg; \(P<0.05\)), that returned to baseline within 30 seconds. During the subsequent monitoring period, the arterial pressure of these mice was not significantly different from the baseline.

In 5 mice, arterial pressure was simultaneously recorded from the descending aorta (via the femoral catheter) and the common carotid artery (via telemetry). There were no significant differences in arterial pressure between the carotid artery and the descending aorta (75.7±9.5 versus 72.5±7.2 mm Hg, respectively), and there was a >80% correlation between the arterial recordings from the 2 methods.

Discussion
The present study demonstrates that radiotelemetry offers a reliable and consistent method for arterial pressure and HR assessment in mice that weigh 20 to 40 g, ie, well within the range of most adult transgenic mice. Additionally, the radio-telemetry data demonstrate that in the mouse, as in other nocturnal animals, MAP displays a 24-hour circadian rhythm that is highest during the nighttime when mice are active and lowest during the daytime when mice are usually quiescent. This finding is consistent with a recent study by Li et al, in which MAP and HR were continuously monitored via the common carotid artery in chronically catheterized, tethered mice. The present results are in good agreement with the arterial pressure data continuously recorded in tethered mice by Li et al and Mattson; compared with the carotid arterial
pressures in the present study, however, the basal MAP and the amplitude of the MAP rhythm were slightly lower in the Li et al study. The differences between these studies may be attributable to differences in the animals used or in the experimental procedures (eg, Li et al continuously infused mice with heparinized saline). The HR results (ie, mean HR and acrophase) of the present study are also consistent with those of Li et al and of studies using Data Science International’s implantable HR telemetry probes. The present study also demonstrates that the telemetry method is sensitive to stimulus-induced changes in arterial pressure in mice. Exposure to a high NaCl diet increases basal MAP and decreases HR in mice.

Compared with the descending aorta placement initially recommended for telemetry in mice, the carotid artery cannulation method offers a more effective procedure. Whereas both implantation techniques (ie, the carotid artery and the abdominal aorta placements) yielded comparable circadian MAP rhythms, MAP was consistently slightly higher in the carotid artery-cannulated mice. Simultaneous measurements of MAP from the carotid artery and the aorta indicate that there is no significant difference in the MAP recorded from the 2 sites, suggesting that the pressure wave does not significantly dissipate as it travels to the lower aorta. In the telemetry studies, the MAP recorded by the aortic versus the carotid telemetry probes was consistently lower; this, however, is likely due in part to the small number of animals in the abdominal aorta group and/or the disruption due to the more invasive abdominal surgery and the presence of the probe in the gut.

The success rate of the carotid artery placement was >90%, compared with a <20% success rate with the abdominal aorta implantation method. This higher success rate is attributable to 4 factors. First, the carotid placement does not occlude blood flow to any major area. The results demonstrate that complete occlusion of the right carotid artery causes only a transient rise on arterial pressure and no significant chronic effect. Additionally, the mice with carotid catheter placements display no associated morbidity, suggesting that collateral flow from the left carotid artery is sufficient to perfuse the right side of the head and face. In contrast, the abdominal aorta implantation can significantly occlude blood flow to the hindquarters, often leading to death within 24 to 48 hours. Because of the potential ischemia after the aorta placement of the probes, Data Sciences International recommends the use of telemetry probes for mice that have a body weight >30 g; in our hands, however, even in these heavier mice the aorta placement is often associated with ischemia. Additionally, great care must be taken during the aorta implantation to limit occluding spasms, and the Vetbond (used to secure the catheter) must be applied very carefully (<1 drop from the 30 gauge needle) to avoid limiting blood flow.

Second, mice are susceptible to hypothermia, especially when anesthetized, and the telemetry transmitter can act as a major heat sink, especially if the probe is not warmed to body temperature prior to implantation. Further, the choice of anesthesia can affect the ability of the mice to maintain body temperature. Therefore, care must be taken during the recovery period to maintain the body temperature by allowing the mice to freely move on and off a heated surface to assist in thermoregulation. For the abdominal implantation method, gas anesthesia (Isoflurane) is highly recommended, because both the abdominal approach and the probe placement chal-

<table>
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<tr>
<th>Table 1</th>
<th>Mean Arterial Pressure in C57BL/6 Mice Maintained on a 0.6% and 8% NaCl Diet</th>
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<tbody>
<tr>
<td></td>
<td>Carotid Implantation (n=7)</td>
</tr>
<tr>
<td></td>
<td>0.6% NaCl</td>
</tr>
<tr>
<td>MESOR, mm Hg</td>
<td>112.8±1.2</td>
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<tr>
<td>Amplitude, mm Hg</td>
<td>18.8±0.5</td>
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<tr>
<td>Acrophase, hour</td>
<td>0.1:11±0.7</td>
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*P<0.05 vs the same group on 0.6% diet; †P<0.05 vs the carotid implantation group on same diet. Lights on=6:00 to 18:00. MESOR indicates 24-hour average MAP; and acrophase, clock hour at which rhythm peaks.

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<th>Table 2</th>
<th>Heart Rate in C57BL/6 Mice Maintained on a 0.6% and 8% NaCl Diet</th>
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<tbody>
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<td></td>
<td>0.6% NaCl</td>
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<tr>
<td>MESOR, bpm</td>
<td>569.4±8.57</td>
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<tr>
<td>Amplitude, bpm</td>
<td>37.5±12.3</td>
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<tr>
<td>Acrophase, hour</td>
<td>17.34±0.7</td>
</tr>
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*P<0.05 vs the 0.6% diet. Lights on=6:00 to 18:00. Abbreviations are the same as in Table 1.

Figure 3. 24-hour HR rhythm recorded in the common carotid artery in mice fed a 0.6% and 8% NaCl diet. The dark bars indicate lights off (6PM to 6AM).
lenge body temperature regulation, making rapid recovery vital. In contrast, injectable anesthesia works well for the carotid placement, thus limiting the researcher’s potential exposure to and reducing the expense of the gas anesthesia. Using an injectable anesthetic (ketamine-xylazine) and the carotid probe placement, we experienced no anesthesia- or thermoregulatory-related mortality in our mice.

Third, for the abdominal implantation method, the catheter tip must be extremely smooth to prevent subsequent obstruction of flow. Although the catheters are designed to occlude <50% of the aorta, we found that the aorta in a 30 g mouse appeared to be nearly the same size as the probe tip. This greatly limits the ability to reuse probes. In contrast, with carotid implantation, the catheter tip does not need to be completely smooth since flow is intended to be completely obstructed in the common carotid artery on the side of the implant. Probe reuse in carotid implantation was primarily limited to battery life, ie, we could reuse probes 3 to 5 times before refurbishment.

The carotid placement is not without complications. The greatest challenge for this placement is securing the body of the probe on the back. We initially anchored it in the mid-scapular region but observed that in smaller mice (<30 g) head movement was restricted and the probe occasionally either externalized through the skin or slid down the side of the back. We found that the addition of a suture that passed around the probe body and attached to the muscle layer on the right side of the back allowed for normal movement of the mouse, limited slippage down the side of the mouse, and eliminated externalization of the probe body. Additionally, although it appears that the head is appropriately perfused on the side of the carotid occlusion, this method potentially causes at least short-term alterations in blood flow to the brain and other areas of the head. In preliminary experiments involving activity and maze testing, it does not appear that the carotid placement causes any gross behavioral abnormalities; further testing, however, is required.

In summary, these results demonstrate the usefulness of long-term telemetric recording of arterial pressure and HR in mice. Baseline arterial pressure and HR telemetry recordings are consistent with data from tethered methods,1,2,5 the circadian rhythms recorded are similar to those observed in rats3,5 and rabbits,12 and the telemetry approach is sensitive to relatively small, NaCl-induced changes in the amplitude and average MAP and HR. Also, the results demonstrate that in C57/BL6 control mice, a high NaCl diet increases the amplitude of (and slightly increases the average) MAP. These results demonstrate a method by which reliable, reproducible, and consistent data on arterial pressure and HR can be continuously recorded from unanesthetized mice as small as 19 g, thus facilitating the study of the mechanisms of cardiovascular control in transgenic mice.

Acknowledgments

This work was supported by the National Institutes of Health grant HL-37722 (J.M.W.). The authors are indebted to Dan Hueetten, Tom Vytlacil, and Gary Miller from Data Sciences International for their assistance and advice. The authors also wish to express their appreciation to Xinhua Feng for his technical assistance.

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Hypertension. 2000;35:e1-e5
doi: 10.1161/01.HYP.35.2.e1

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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