Comparison of simultaneous measurement of mouse systolic arterial blood pressure by radiotelemetry and tail-cuff methods

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Whitesall, Steven E., Janet B. Hoff, Alan P. Vollmer, and Louis G. D’Alecy. Comparison of simultaneous measurement of mouse systolic arterial blood pressure by radiotelemetry and tail-cuff methods. Am J Physiol Heart Circ Physiol 286: H2408–H2415, 2004. First published February 12, 2004; 10.1152/ajpheart.01089.2003.—Radiotelemetry of mouse blood pressure accurately monitors systolic pressure, diastolic pressure, heart rate, and locomotor activity but requires surgical implantation. Noninvasive measurements of indirect systolic blood pressure have long been available for larger rodents and now are being reported more frequently for mice. This study compared mouse systolic arterial blood pressure measurements using implanted radiotelemetry pressure transducer with simultaneous tail-cuff measurements in the same unanesthetized mice. The pressure range for comparison was extended by inducing experimental hypertension or by observations of circadian elevations between 3 AM and 6 AM. Both trained and untrained tail-cuff operators used both instruments. Every effort was made to follow recommended manufacturer’s instructions. With the initial flow-based tail-cuff instrument, we made 671 comparisons (89 sessions) and found the slope of the linear regression to be 0.118, suggesting poor agreement. In an independent assessment, 277 comparisons (35 sessions) of radiotelemetry measurements with the pulse based tail-cuff instrument were made. The slope of the linear regression of the simultaneous measurements of systolic pressures was 0.98, suggesting agreement. Bland-Altman analysis also supported our interpretation of the linear regression. Thus although reliable systolic pressure measurements are possible with either tail-cuff or radiotelemetry techniques, in our hands some tail-cuff instruments fail to accurately detect elevated blood pressures. These data, however, do not distinguish whether this instrument-specific tail-cuff failure was due to operator or instrument inadequacies. We strongly advise investigators to obtain an independent and simultaneous validation of tail-cuff determinations of mouse blood pressure before making critical genotyping determinations.

rodent blood pressure; indirect and direct blood pressure measurement technique

THE MANIPULATION OF THE MOUSE GENOME has provided cardiovascular research many new disease models. The extraction of the maximum information from these models requires systems that can reliably record changes in the mouse’s cardiovascular function. Radiotelemetry of mouse blood pressure, while initially invasive, has been shown to accurately monitor systolic pressure, diastolic pressure, heart rate, and locomotor activity (16). Noninvasive measurements of indirect systolic blood pressure have long been available and accepted for large rodents. Much of the previous arterial pressure data obtained for the mouse used tail-cuff procedures or carotid artery cannulation in anesthetized or restrained animals (7, 11, 17). Despite increased use of mice for cardiovascular studies, there still is insufficient evidence supporting the idea that all tail-cuff instruments consistently produce acceptable measurement of systolic pressure. Recently, a novel ultrasound-based system for mice was developed and validated that uses pulse Doppler flow sensing. This system for the first time measures both systolic and diastolic pressure noninvasively in mice (17). Such custom-made systems illustrate the current need for better, validated, noninvasive instrumentation for mice. Unfortunately, not all the currently available commercial instruments have been independently validated nor have they shown to be reliable in conscious, anesthetized mice.

Protocols have been designed that are successful at continuous conscious monitoring of arterial pressure and heart rate in mice using an indwelling arterial catheter tethered to an outside transducer (14, 15). Short-term catheter patency and restricted animal mobility limit the usefulness of these techniques. At the same time, implanted blood pressure transducers have been miniaturized for use in the mouse.

This study used state-of-the-art (9) radiotelemetry to conveniently and precisely monitor cardiovascular parameters including systolic, diastolic, mean arterial pressure, and heart rate, 24 h a day, 7 days a week (8, 10, 16). This technique circumvents problems with previously used measurement techniques by avoiding tethering, handling, or heating of the animals (7, 11, 14, 15). The modified implantation technique described here has improved our survival rates in mice smaller than the ~27-g limit suggested by the telemetry manufacturer. While appreciating some of the strengths and weaknesses of commercially available tail-cuff and radiotelemetry, we decided to make a simultaneous set of measurements to determine the extent of agreement between these two methodologies.

METHODS

Animals and Housing

Experiments were performed on male CD-1 and C57Bl/6J mice (Charles River) weighing 17–30 g at the time of radiotelemetry implant. In the return-to-flow (RTF) arm, 13 mice (8 CD-1 and 5 C57Bl/6J male mice) were used, and in the return-to-pulse (RTP) arm, 6 (4 male and 2 female C57Bl/6J) mice were used. Multiple simultaneous measurements were made with each mouse as defined below.

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After instrumentation, each animal was housed individually in a standard polypropylene cage placed on a radio receiver. Mice were maintained in a 12:12-h light-dark cycle, fed standard rodent chow, and given drinking water ad libitum. All procedures were approved by Institutional Animal Care and Use Committees (Approval No. 8252), and mice were cared for in accordance with the standards established in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Direct Measurement of Blood Pressure: Radiotelemetry Methods

Radiotelemetry system (TS)-derived blood pressure requires the surgical implantation of a catheter into an artery of the mouse, generally the left common carotid artery or the infrarenal abdominal aorta. The catheter is attached to a combination pressure transducer, transmitter, and battery, all encapsulated in an implantable microminiaturized electronic monitor (PA-C20, Data Sciences International; St. Paul, MN).

Common carotid cannulation with transmitter in abdomen. Surgical anesthesia was induced by placing the mouse in a 100-ml chamber through which 5% isoflurane in oxygen was flushed at 1 l/min for 90 s. When the righting reflex was lost, the mouse was placed on its back on the operating table with the nose secured carefully in a mask. Sutures over the upper incisors extended the incision and stabilized the head. Isoflurane was adjusted (0.75–1.5%) to maintain a surgical plane of anesthesia. The depth of anesthesia was monitored by withdrawal from toe pinch and a decreased respiratory rate to between 30 and 40 breaths/min. Eyes were coated with ophthalmic lubricant (Puralube Vet, Pharmaderm; Melville, NY), and skin on the ventral body wall was shaved from the chin to pelvis. The skin was further treated with chemical hair remover (Nair, Carter Wallace; New York, NY), washed, and wiped clean with topical antiseptic and alcohol. The hind limbs were extended and taped down. The remaining procedures were all done with the aid of a dissecting stereomicroscope.

A midline skin incision 2–3 cm long from pelvis to xiphoid process was made with scissors. The skin was undermined for at least 1 cm around the edges of incision. A second incision from chin to manubrium exposed the salivary glands. The surrounding skin was undermined, and a subcutaneous channel from the neck site to the abdominal site was made by blunt dissection. The linea alba was identified and incised, with care taken to avoid puncture of the viscera. A 16-gauge trocar was passed from the abdominal cavity, through the lateral aspect of the left rectus muscle and under the skin to the neck. The trocar was withdrawn from the neck incision, with care taken not to dislodge the protective sheath covering the implant’s catheter. The body of the implant was secured in the midline of the abdominal cavity with six to eight interrupted nonabsorbable sutures (4-0 braided polyester) with alternating sutures engaging the three eyelets of the implant. The skin was closed with staples and tissue adhesive (Vetbond, 3M; St. Paul, MN), and topical antiseptic was applied.

Each forelimb was secured laterally to a 1-cm high platform, and the salivary glands were retracted laterally by blunt dissection. Medial and lateral hook retractors were used to expose the left carotid artery from the bifurcation of internal and external carotid toward the heart. About 1 cm of the common carotid artery was isolated, and a snare (nylon 6-0) was placed proximally. A silk ligature (7-0) was tied at the bifurcation. An additional tie was placed midway on the vessel for securing the catheter and hemostasis. Distal and proximal traction stabilized the vessel. An arteriotomy adjacent to the bifurcation was performed with the bent tip of a 25-gauge needle allowing cannulation toward the heart. The proximal traction was loosened, and the catheter was placed such that 1 cm entered the vessel and the tip lay at the opening of the common carotid into the aorta. The pulsing radio signal confirmed a functional implant by a distinctive high-frequency oscillating tone on a portable AM radio set at 500 kHz. A 1-cm mark on the catheter was aligned with the distal tie, and the catheter was set in place with tissue adhesive and secured with the middle tie. The salivary glands were returned to their natural location over the top of the catheter and secured with a drop of adhesive. The skin was closed with four to five interrupted 6-0 nylon sutures. Approximately 1 ml of normal saline was injected subcutaneous into two or more sites to assure adequate postoperative hydration, and the animal was kept in a ventilated and warmed environment for 24 h with continuous blood pressure monitoring. Most animals were fully ambulatory within 30 min with return of drinking, eating, and bowel function within 1 h. An antibiotic (ampicillin, Polyflex, Port Dodge Animal Health; Fort Dodge, IA; 100 mg/kg sc) was administered at the time of the operation and twice daily during recovery. An analgesic (buprenorphine, Buprenex, Reckitt Benckiser Pharmaceuticals; Richmond, VA; 0.1–0.5 mg/kg sc) was provided if the animal behavior suggests the presence of pain by attention to wound site, lethargy, or aggression. Detailed guidelines set forth by the Animal Use and Care Committee for survival of rodent surgery were followed.

Aortic cannulation with transmitter in abdomen. This procedure was modified from Mills et al. (16). In an effort to facilitate retraction of the intestines and access to the aorta, the mice undergoing the aortic cannulation were fasted for 12 h before surgery but were allowed free access to water. General anesthesia and abdominal wall preparation were as described above. The remaining procedures were all done with the aid of a dissecting stereomicroscope as previously published (16). The abdomen was closed, and hydration, analgesics, and antibiotics administered were as described above.

Murine DOCA hypertension model. In an effort to better assess correlation between methods, we expanded the pressure ranges available for comparison by experimentally inducing hypertension (>200 mmHg). A restricted range of pressures about normal (+/-20 mmHg) makes correlation assessments and evaluation of differences from the mean more difficult to interpret. Approximately 1 wk after telemeter implantation, when cardiovascular parameters were returning to normal circadian patterns, the mice were prepared for the hypertension model. Mineralocorticoid hypertension was produced in these mice by unilateral nephrectomy combined with subcutaneous deoxycorticosterone acetate (DOCA) (Sigma D-7000) implant and drinking water containing 0.9% NaCl, 0.2% KCl, and 5% glucose. Sham-operated control mice received only unilateral nephrectomy.

Mice were anesthetized with isoflurane as described above, and the left flank skin from the rib cage to the iliac crest was shaved and treated as described above. A 1-cm incision through the skin was made and used to undermine the dorsal skin from the shoulders to the hips. This subcutaneous skin pocket was used for placement of a DOCA-impregnated silicone rubber slab ~5.0 × 25.0 mm containing ~50 mg of DOCA. The left kidney was exposed, and the pedicle was double ligated and sectioned. Muscle was closed with two interrupted nonabsorbable sutures. The DOCA implant was inserted subcutaneous, and the skin was stapled closed and secured with tissue adhesive. The DOCA implant was made from a 2:1 ratio mixture of silicone rubber (Corning 3110 RTV) and DOCA (Sigma D-7000). The silicone rubber base was weighed into flat dish, and DOCA was slowly added and stirred to assure complete incorporation. Once the DOCA was incorporated, one drop of catalyst (Dow Corning Catalyst No. 4) was added and quickly mixed to a uniform consistency. The mixture was pressed down with a block to form a uniform sheet and allowed to dry overnight. The fully cured sheet was cut into strips of appropriate weight and dimension for each animal.

Indirect Systolic Blood Pressure Measurements: Noninvasive Tail-Cuff Methods

Tail blood flow detection. The procedure used for this instrument was modified after Krege (11) and the manufacturer’s instruction manual. For 5–7 days, mice were acclimated to restraint and tail-cuff inflation. The restraint platform was maintained at ~33–34°C. For
each session, the mouse was placed in a metal box restraint with its tail passing through the optical sensor and compression cuff and finally taped to the platform. A traditional tail-cuff occluder was placed proximal on the mouse’s tail, which was then immobilized with tape in a V-shaped block between a light source above and a photosensor below. On inflation, the occluder stopped blood flow through the tail, and on deflation the return of blood flow (RTF) was detected by the sensor. An initial series of inflation-deflation cycles was used to set amplifier and instrument controls. This instrument (model BP2000 Blood Pressure Analysis System, Visitech Systems; Apex, NC) automatically takes 10 30-s measurements using proprietary software (BP2000 Software Beta Version 03/10/97). If at least 8 of 10 readings were acceptable, the highest and lowest readings were discarded, and the remaining readings were averaged for a single session value. Telemetered blood pressure measurements were acquired simultaneously by placing the receiver pad adjacent to the tail-cuff device and synchronizing instrument clocks such that 10-s telemetered segments were obtained throughout the duration of each tail-cuff session. The corresponding TS systolic pressures were averaged and used for comparisons.

External tail pulse detection. This procedure was similar to a method previously described by Johns et al. (7). To obtain an accurate blood pressure reading, mice must have remained still and unper- turbated throughout the measurement period. Mice were conditioned to the restraint and the warming chamber for 10–20 min/day for at least 3 days before measurements. After this 3-day training period, mice typically remained relatively still and unper- turbated when placed in the restrainer on the day of testing. Conditioning occurred more readily when mice were handled gently and not forced to enter the restraint. The chamber (model 306 warming chamber, IITC Life Science; Woodland Hills, CA) was kept at 31–33°C, and a darkened nose cone helped calm and secure the mouse in the restrainer (model 84 mouse restrainer, IITC Life Science). An integrated sensor-cuff occluder (model B60-1/4, IITC Life Science) operated to stop tail pulsation on inflation and to detect the return of tail pulsations (RTP) passing through the occluder cuff on each deflation cycle. This system does not depend on light passage through the tail to detect blood flow, and the mouse’s tail was not taped. The RTP-computerized blood pressure monitor (model 6M 229 6 channel mouse system, IITC Life Science) was set for desired sensitivity, number of cycles, the maximum tail-cuff inflation pressure, the rate of deflation, and the interval between cycles. The maximum pressure of inflation was set 20–40 mmHg above anticipated systolic pressure. The instrument was set for a maximum inflation pressure of 200 mmHg for mice that were expected to have pressures in the normal range and was increased to 250 or 300 mmHg for markedly hypertensive mice.

After 5–10 min of stabilization in the chamber, a typical run involved 10 repetitions of the automated inflation-deflation cycle. Although the program can generally detect systolic pressure, we routinely examined the tracings and confirmed the computer selection, discarding obviously aberrant tracings due to movement or artifacts. With the original software, a run was accepted if at least 6 of 10 repetitions were adequate (free of gross artifacts and having detectable pulses). If less than six repetitions were adequate, the entire run was discarded and the procedure was repeated at another time. The current version of the software (model 31 NIBP software) allows immediate operator assessment of artifactual tracings. Poor tracings were immediately discarded and measurements were repeated to obtain up to 10 values per session. When requisite determinations were obtained, the average was calculated and used as the single systolic value for that session. Telemetered blood pressure measurements were acquired simultaneously by placing the receiver pad under the tail-cuff device and synchronizing instrument clocks such that 10-s telemetered segments were obtained throughout the duration of each tail-cuff session. The corresponding telemetered systolic pressures were averaged and used for the averaged least.

Statistical Analysis

Assessment of agreement between methods. Correlation coefficients and slopes of regression lines were calculated for the average session data for each RTF, RTP, and simultaneous TS sets of measurements. This analysis, although visually intuitive, is not the most rigorous approach to assessing agreement between methods. We have used in addition an analysis proposed by Bland and Altman (1–3) involving calculating the mean of the paired measurements (plotted on the x-axis) versus the difference between the same two measurements (plotted on the y-axis) along with ±2 SD and the overall mean difference. This gives a more compelling statistical analysis for measurement agreement in method comparison studies.

Effects of restraint on measurements. Tail-cuff measurements require restraint. To assess the effect of restraint on systolic blood pressure and heart rate, an undisturbed period of telemetered blood pressure was compared with the measurement period immediately after placement in the tail-cuff restraint. Six-minute averages were collected for eight periods (total: 48 min) just before the housing room was entered and the mice disturbed. All values were expressed as a percentage of the 48-min undisturbed average. Three 6-min periods during restraint incorporated the typical tail-cuff measurement period and in most animals included the multiple inflation-deflation cycles required for the tail-cuff measurements.

RESULTS

During the RTF-TS simultaneous comparison of systolic blood pressure, 8 CD-1 and 5 C57Bl/6J male mice were used in 89 averaged sessions computed from 671 individual simultaneous systolic arterial blood pressure measurements or an average of 7.5 determinations/session. During the RTP-TS simultaneous comparisons of systolic pressure, 4 male and 2 female C57Bl/6J mice were used in 35 averaged sessions computed from 277 individual simultaneous arterial systolic blood pressure measurements or an average of 7.9 determinations/session. No attempt was made to separate carotid artery pressures from infrarenal aortic pressures in any of the comparison analyses.

Recovery from Implantation

Figure 1 shows the gradual return of the circadian pattern of arterial pressure and heart rate in the 1–2 wk after transmitter implantation. The peak pressures, appearing in days 9–14, occur between 10 PM and 6 AM. All comparisons with tail-cuff measurements were done once circadian patterns were fully established. The RTF and RTP comparisons, however, were performed at various times to obtain a wider range of pressures by taking advantage of the circadian patterns shown in Fig. 1.

Restraint Effects

During the period just before restraint for tail-cuff testing, the TS heart rate and systolic blood pressure varied between 103% and 96% of the average for the 48-min undisturbed period (Fig. 2). Immediately on restraint, the TS systolic pressure decreased slightly (mean 92%) and by the third 6-min restraint period had returned toward the undisturbed TS value. TS heart rate increased on restraint and returned toward undis- turbed values by the third 6-min restraint period.

Tail Blood Flow Detection vs. Telemetered Systolic Pressure

Figure 3 plots on identical x- and y-axes the average and SD for 89 simultaneous sessions for determination of systolic
Pressure. The values plotted on the y-axis were recorded from the tail blood flow detection tail-cuff instrument and remain generally between 80 and 120 mmHg even though pressures in excess of 250 mmHg (plotted on the x-axis) were detected simultaneously from the implanted TS pressure transducer. The radiotelemetry acquisition system (Dataquest A.R.T., Data Sciences International) obtained up to 150 2-s values simultaneous with each tail-cuff session. Only those sessions with a SD of 10 mmHg or less were included. The solid symbols represent data obtained by an experienced tail-cuff operator, and the open symbols represent data obtained by an inexperienced tail-cuff operator. The solid line represents the line of...

Fig. 1. Postoperative recovery of circadian patterns: mean data ± SD (n = 4) for heart rate (A; in beats/min (bpm)) and blood pressure (B) for 2 wk after radiotelemetry implant. Each point is a 4-h moving average of data collected every 2 min. The return of circadian patterns begins to appear within a week, showing a 10 PM to 6 AM peak and a 10 AM to 6 PM valley in both blood pressure and heart rate.

Fig. 2. Effects of restraint on systolic pressure (B) and heart rate (A). Radiotelemetry reveals a minimum and opposite effect of restraint on systolic pressure and heart rate compared with the previously undisturbed hour.
Identity for the graph. The linear regression of the scattered points gives a slope of 0.118, suggesting little change in these tail-cuff-determined pressures, whereas the catheter measured pressure more than doubles. The $r^2$ value was 0.13.

External Tail Pulse Detection (RTP) Vs. Telemetered Systolic Pressure

Figure 4 plots the session averages for the simultaneous RTP and TS pressures. Similar data acquisition protocols to those used above collected the 35 averaged points (sessions) scattered about a linear regression line with a slope of 0.98. This linear regression slope suggests general agreement between the two measurement techniques. The $r^2$ value was 0.71. Figure 5 plots 277 individual RTP tail-cuff determinations against simultaneous systolic pressure-detected TS. This analysis assessed agreement of individual rather than averaged tail-cuff and telemetered measurements. The $r^2$ value was 0.63, and the slope was 0.99. Again, there is good agreement between the methods.

Difference Against the Mean Analysis of Method Agreement

Correlation coefficients can be inappropriate for determining whether two methods can be used interchangeably. Figure 6 shows a more compelling analysis of tail-cuff measurements compared with simultaneous radiotelemetered systolic pressure. For each panel, the mean of the paired (compared) measurements is plotted on the x-axis versus the difference between the same two measurements plotted on y-axis along with $\pm 2 \text{SD}$ of the mean difference. Figure 6A shows that the flow detection system consistently underestimates the direct systolic pressure measurement by radiotelemetry averaging a difference of 53 mmHg and the difference in methods increases markedly at higher pressures. Figure 6, B and C, shows the data for the pulse detection instrumentation and indicates overall excellent agreement (mean of 2 and 1 mmHg, respectively) in terms of the mean difference between methods. However, the SD of $\sim 20$ mmHg suggests that the RTP method should be restricted to situations where changes $> 20$ mmHg are anticipated.

DISCUSSION

Both radiotelemetry and tail-cuff determinations of systolic pressure have strengths and weaknesses. In the first arm of this comparison study, the tail-cuff determinations using the RTF detection system we evaluated failed to follow radiotelemetry of systolic arterial pressure (slope of 0.118). This was particularly true at pressures above the normal range (Fig. 3). Although the reproducibility of the RTF system we tested was acceptable at all pressures, the accuracy compared with the simultaneous TS measurements was unacceptable. Analysis of
differences against the mean (Bland-Altman analysis) clearly illustrates substantial disagreement between this RTF detection system and the direct measurement of systolic pressure by TS. The differences are even greater at the higher pressures that are more likely to be of interest to those studying hypertension. It is worth noting that recently Reddy et al. (17) used the same Bland-Altman analysis in evaluating the Doppler system they developed and validated for flow detection and obtained excellent agreement with catheter measurements of both systolic and diastolic pressures. Thus it is not the basic concept of flow detection but the actual execution of the detection that establishes its validity and agreement with catheter measurements.

In the second arm of this comparison study, radiotelemetry of systolic arterial pressure agreed with a slope of 0.99 and 0.96 with the commercially available RTF systolic pressure (Figs. 4 and 5). Thus we observed excellent agreement between the TS and RTF system we tested. The RTF instrument showed differences against the mean that were also consistent over the full range tested, again supporting the validity and reliability of this instrument over the pressures ranges evaluated. A broader look at tail-cuff and telemetry methodologies reveals several issues that should factor into an investigator’s choice of these methods.

Krege (11) reported the measurement of mouse pressures by tail-cuff and intravascular catheter and concluded there was an acceptable agreement. We have some concerns on examining the data used for his comparisons. The systolic pressure from a RTF system was compared with the mean arterial pressure recorded from an implanted-tethered catheter. The interval between tail-cuff and intra-arterial measurements averaged 9.3 days (range: 4–19 days). Krege (11) used the same RTF systems we used; however, with our simultaneous measurements (Fig. 3), we failed to detect an acceptable correlation between the methods. In fact, we observed excessive differences against the mean of the two measurements. This lack of agreement occurred despite our measurements comparing systolic to systolic pressure, on the same mouse, on the same day, and without any influence of general anesthesia. Krege (11) did the tail cuff in conscious mice and subsequently, an average of 9.3 days later, and 4 h after general anesthesia, did the intra-arterial determinations from tethered mice. Thus, although the data he presents do show a general pattern of agreement using the same tail blood flow detection systems we used, we were unable to reproduce the agreement he observed. Although correlations can be coincidental, the absence of correlation and excessive differences against the mean over a wide range of pressures suggests the methods in our hands are not measuring the same biological information.

A new method for radiotelemetry of mouse blood pressure was described in detail by Mills et al. (16), and they concluded it to be more efficient, reliable, and less labor intensive than either tail-cuff or exteriorized methods for mouse blood pressure. Additionally, because parameters are sampled continuously, changes in circadian rhythm (12) associated with specific pathologies or pharmacological manipulations can be more readily determined. Although these investigators reported consistently over 90% long-term survival achieved by one of the authors, they also indicated mouse average body weights of 30 g in one group and 28 g in another. Kramer and Kinter (9) reviewed radiotelemetry in small laboratory animals and had a similar positive assessment of its utility, characterizing it as state of the art for monitoring physiological functions in awake and freely moving laboratory animals. After extensive documentation of the advantages of radiotelemetry, these authors highlighted some of the disadvantages, including 1) initial equipment costs, 2) need for specialized surgical training, and 3) need for sophisticated data acquisition management, among others. Missing from this otherwise detailed radiotelemetry assessment was a comparison to the tail-cuff methodologies that arguably could be the most common alternative for systolic pressure measurement in mice.

Butz and Davisson (4) reported success with radiotelemetry on mice as small as 17 g (average 22 g) and female mice instrumented before and monitored during and after pregnancy. They avoided the abdominal aorta and abdominal cavity by placing the catheter in the left common carotid artery and passing the body of the transmitter to a subcutaneous pocket on the right flank. We chose, as they did, to avoid the increased probability of aortic obstruction in smaller mice and to utilize the accessibility of the left carotid. We, however, placed the transmitter body in the abdominal cavity because we wanted the more stable transmitter mounting afforded by securing to the midline body wall. With experience, our implant protocol can be completed in 45 min, and the mouse becomes fully ambulatory within 20 min. We combined the best aspects of each approach to obtain stable blood pressure telemetry in mice smaller than 22 g and found that the full range of movement in
these mice was unimpeded. In contrast, we found subcutaneous placement of the implant that tended to compromise limb movement.

Some investigators attempting to determine toxicity and drug doses or to characterize phenotypes require a higher throughput than can readily be obtained by radiotelemetry. In such a setting, noninvasive tail-cuff determinations of the mouse’s systolic pressure are more likely to be the method of choice. The external tail pulse detection system we used agreed well (Figs. 4–6) with our simultaneous radiotelemetry determinations of systolic pressure. The integral inflation cuff and sensor creates a stable relationship between the two and allows enhanced sensitivity without requiring light to pass through the mouse’s tail. This system required minimal warming to obtain an external tail pulse and no anesthesia or postoperative recovery is required.

With the use of radiotelemetry, Gross and Luft (6) observed the response to the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester could be mimicked by applying restraint equivalent to that used for tail-cuff measurements. These authors, concerned about the restraint response, recommended that tail-cuff measurements in mice only be used when responses >20 mmHg were anticipated. They also concluded that blood pressure and heart rate responses to restraint are similar regardless of conditioning over 10 days. In the data presented here comparing methods, the influence of stress on pressure is viewed very differently. Many of the above normal TS pressures were not obtained in a conditioned and relaxed mouse. The intent was to compare simultaneous measurements, not to make assessments of resting arterial pressure. It was thus not necessary, and indeed inappropriate, for us to wait until the animal had fully acclimated. As long as adequate tail-cuff signals were obtained, the comparison measurements were made.

Although tail-cuff determinations of systolic pressure in mice have the advantage that they are surgically noninvasive, they require significant training of both operator and mouse to be accurate and reliable. Figure 2 illustrates that a well-trained operator can restrain a mouse for tail-cuff measurements and be accurate and reliable. Figure 2 illustrates that a well-trained operator can restrain a mouse for tail-cuff measurements and be accurate and reliable.

The successful validation by Reddy et al. (17) with the ultrasound tail-cuff technique on anesthetized mice avoided the demonstrated weaknesses in other RTF systems like the one we tested. In addition, the ultrasound system is able to detect both systolic and diastolic pressure. The authors obtained their best ultrasound signals by using some anesthetic, even of short duration that in turn necessitated the control of body temperature. These aspects do detract from this otherwise elegant system for RTF assessment of arterial blood pressure noninvasively in the mouse.

Independent and simultaneous validation of the tail-cuff system chosen is critical to reliable systolic pressure measurements over the normotensive to hypertensive ranges. Lorenz (13) gave a comprehensive review of cardiovascular, respiratory, and renal methods for use in mice. He reported the simultaneous measurement of telemetered blood pressure in a mouse and a simultaneous tail-cuff determination using the tail blood flow detections system used by Krieger (11). For this measurement he reported “...very good agreement between arterial pressure and tail-cuff pressure...” Unfortunately, there were no statistical assessments of this observation, but caution was given that movement artifacts can result in erroneous measurements that should be discarded. No mention was made of the range over which comparison was made between these two measurement techniques.

Johns et al. (7) noted a comparison between intra-arterial tethered catheter measurements and those made on similar populations mice with the external tail pulse detection. The external tail pulse detection system they used was the same we used to obtain good agreement of simultaneous measurements (Figs. 4 and 5). While investigating several models of hypertension, they randomly selected mice from different groups that had either the tail-cuff measurement or the catheter measurement. These were population comparisons and not simultaneous measurements, or even measurements in the same animals, yet these 16 paired measurements closely correlated ($r = 0.876, P < 0.001$) tail cuff with direct measurements from tethered intra-arterial mean arterial pressure from the iliac artery (7). Differences against the mean were not reported.

While investigating the role of the SA gene in blood pressure regulation, Walsh et al. (18) utilized both the radiotelemetry system and external tail pulse detection system for indirect measurement of systolic blood pressure that we used. The presentation of the data suggests that these investigators consider these two methods completely equivalent and essentially interchangeable for systolic pressures. The indirect measurements with external tail pulse detection system were gathered as basal observations, and the radiotelemetry system was used to track and compare changes in blood pressure in response to several treatments over several weeks. No simultaneous comparisons were reported.

The study reported here utilized a modification of the radiotelemetry technique created and validated by others (5, 10, 16) and circumvents problems with previously used techniques by avoiding tethering (14, 15), handling, or heating of the animals. By simultaneously measuring systolic pressure with both the radiotelemetry system and two different tail-cuff systems, we gained new insights into the advantages and limitations of these methods. We conclude that reliable systolic pressure measurements are possible with either tail cuff or radiotelemetry, but in our hands some tail-cuff instruments fail to accurately detect elevated blood pressures. We consider it essential to have an independent and simultaneous validation of tail-cuff determinations of mouse blood pressure before making critical cardiovascular assessments including genotyping determinations.
GRANTS

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