Recommendations for Blood Pressure Measurement in Humans and Experimental Animals: Part 2: Blood Pressure Measurement in Experimental Animals: A Statement for Professionals From the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research

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AHA Scientific Statement

Recommendations for Blood Pressure Measurement in Humans and Experimental Animals

Part 2: Blood Pressure Measurement in Experimental Animals

A Statement for Professionals From the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research

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Abstract—In experimental animals, as in humans, techniques for measuring blood pressure (BP) have improved considerably over the past decade. In this document, we present recommendations for measuring BP in experimental animals with the goal of helping investigators select optimal methods for BP monitoring in the research laboratory. The advantages and disadvantages of various BP measurement methods are discussed and specific recommendations are provided for selecting the optimal technique depending on the study objective. Although indirect techniques that permit only sporadic measurements of BP may be suitable for some purposes, methods for directly measuring BP are generally preferred because of their ability to monitor the highly dynamic nature of BP in a comprehensive fashion. Selection of the methods to be used should ultimately be guided by the study objectives to insure that the techniques chosen are appropriate for the experimental questions being explored. **(*Hypertension*. 2005;45:299-310.)**

Key Words: blood pressure ■ blood pressure determination ■ blood pressure monitoring ■ hypertension, experimental

Over the past decade, an impressive range of highly sophisticated scientific techniques have been developed in the areas of molecular and cellular biology, genomics, and proteomics that are now being actively applied to the study of hypertension and blood pressure (BP) regulation. Although many hypertension scientists have embraced these new technologies with enthusiasm, a surprising number of the same investigators continue to use suboptimal techniques for measuring BP in experimental animals. To address this problem and assist investigators in selecting optimal methods for BP monitoring, we have developed a set of recommendations for measuring BP in experimental animals. Comprehensive recommendations for BP measurement in humans are described in the companion statement "Recommendations for Blood Pressure Measurement in Humans and Experimental Animals. Part 1: Blood Pressure Measurement in Humans" by Pickering et al.[1]

The suitability of any research methodology is largely dependent on the investigative objective. Thus, a particular technique for measuring BP may be well suited for one type of study but less useful for another. Accordingly, in the current review, we focus on the advantages and disadvantages of various BP measurement methods with the goal of providing specific recommendations for selecting the optimal technique depending on the study objective. For example, if the primary objective is to determine whether a new drug protects against atherosclerosis or cardiovascular damage independent of any effects on BP, then the investigator should use a monitoring technique that provides a comprehensive measure of the total BP load

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on the vasculature. For this kind of study objective, techniques that provide only sporadic measurements of BP would be less useful or even potentially misleading no matter how accurate those measurements might be.

Techniques for measuring BP in experimental animals can be divided into indirect methods and direct methods. Most methods for measuring BP can be applied in a range of animals, although certain technical modifications may be required depending on the species undergoing study. Here, we consider the methods used for measuring BP in the animals most commonly used in hypertension research, with particular emphasis on mice, rats, and dogs. In most cases, the choice of method should be driven by the investigative objective rather than the species of animal being studied. It should be emphasized that regardless of the method used for measuring BP, systemic anesthesia should be avoided whenever feasible because of the well-documented effects of anesthetics on cardiovascular function.[2] It has long been recognized that commonly used anesthetics can affect multiple aspects of the circulatory system and that integrative cardiovascular responses often differ greatly in anesthetized versus conscious animals.[2]

Indirect Methods

In animals, the most commonly used indirect method for monitoring BP is the cuff technique in which BP is measured in a tail or limb by determining the cuff pressure at which changes in blood flow occur during occlusion or release of the cuff. A variety of methods have been used for sensing the point at which some type of change in blood flow occurs during manipulation of cuff occlusion pressure, including, but not limited to, photoelectric sensors, oscillometric sensors, Doppler sensors, chamber volume sensors, and acoustic sensors. Several improvements in sensor technology have occurred, but regardless of the type of sensor used, all of these methods share certain advantages and disadvantages that should be carefully considered when deciding whether to use an indirect technique in a particular study.

Advantages and Applications

Indirect methods have served a valuable role in experimental hypertension research for many years and continue to be useful in certain kinds of study designs. The indirect methods used in experimental animals share some of the same advantages and disadvantages of indirect office methods of blood pressure measurement that have been widely used in the clinical and epidemiological studies that form the scientific basis for current clinical practices in hypertension.

Whereas advances in direct BP measurement technology are shifting attention away from indirect techniques, these methods still provide a useful approach to the measurement of systolic blood pressure in some experimental circumstances (Table 1). Indirect methods are considered to have 4 main advantages[3–5]: (1) They are noninvasive and do not require surgery; (2) they can be used to obtain repeated measurements of systolic BP in conscious animals during studies of short or long duration; (3) they require less expensive equipment than some direct methods (eg, telemetry) and can also be less expensive to operate; and (4) they can be used to screen for systolic hypertension or substantial differences in systolic BP among large numbers of animals. Thus, indirect methods should be considered when an investigator wishes to noninvasively detect or confirm the presence of frank systolic hypertension, substantial differences in systolic BP between groups, or substantial changes in systolic BP over time, particularly when dealing with large numbers of animals (Table 1). For example, tail-cuff methods can be useful and cost-effective for large-scale, high-throughput genetic screens such as N-ethyl-N-nitrosourea (ENU) screens or genetic crosses in which hundreds of progeny are screened for substantial alterations in systolic blood pressure.

TABLE 1. Recommendations for the Use of Indirect Methods for Measuring BP in Animals

Recommended for noninvasive detection or screening for:
Frank systolic hypertension
Substantial group differences in systolic BP
Substantial changes in systolic BP over time
Systolic BP changes in large numbers of animals (eg, high throughput genetic screens)
Not recommended for:
Quantifying relationships between BP and other variables (eg, target organ damage)
Studying BP-independent effects of any intervention or variable (eg, drugs, diet, genotype, etc)
Ruling out intermittent or subtle forms of hypertension or changes in BP
Measuring BP variability
Measuring diastolic BP or pulse pressure in conscious rodents
Making inferences about BP in nonstressed, unrestrained animals

Disadvantages

Although indirect methods are clearly suitable for measuring BP in some circumstances (Table 1), they have 3 main disadvantages that markedly constrain their scope of usefulness in experimental studies of hypertension.

First, indirect methods only measure BP in a very small sample of cardiac cycles. Thus, indirect methods as conventionally used are incapable of assessing the average level of BP throughout the day and night over the course of a study. Because variability of BP is ordinarily quite large, the relatively small number of measurements typically obtained with an indirect method cannot be presumed to reflect an animal's true average BP. This problem greatly limits the value of indirect BP methods, regardless of how accurate such methods are thought to be in measuring systolic BP during an individual cardiac cycle. An obvious example of this problem is the fact that indirect BP measurements are typically performed during only a very brief portion of the day and provide no information whatsoever about BP during the night. Some animals, such as rodents, are more active during the night and BPs during normal daily activity may be important to the objectives of the experiment. Because of sampling limitations, indirect methods also cannot be used to assess the true fraction of BP values that may go above or below certain thresholds during a study. Although cuff devices for ambulatory BP monitoring are increasingly being used to indirectly mea-

sure BP over many cardiac cycles in humans, such approaches have not proven to be practical for measuring BP in animals.

Second, despite the noninvasive nature of indirect methods and well-intended efforts by investigators to train and acclimatize animals to undergo the procedures, these methods impose significant stress that disturbs multiple aspects of the cardiovascular system. The notion that one can truly acclimatize rodents to indirect tail-cuff procedures and effectively minimize the impact of the procedural stress on cardiovascular and endocrine function is doubtful.[6–8] Tail-cuff measurements of BP in rodents impose substantial amounts of thermal and restraint stress that are known to affect BP, heart rate, and stress hormones.[6–9] In fact, acute restraint has been shown to lead to acute BP increases and activation of vascular wall mitogen-activated protein (MAP) kinases comparable to those observed after acute infusions of angiotensin II or phenylephrine.[10] Moreover, the assumption that different experimental groups within a given study would be expected to demonstrate similar quantitative responses to restraint stress may also not be valid.[11–13] Thermal stress is caused by increased body temperature required to dilate the tail artery and allow sufficient blood flow into the tail. When the animals are confined to the restraining cages, substantial increases in body temperature occur even without intentional warming of the animal.[9] Tail-cuff measurements are also commonly performed during the day, which disrupts rodent sleep cycles.

Although it has been recommended to train or condition animals for 5 to 14 days before commencing tail-cuff measurements, some investigators have demonstrated that even 10 days of conditioning can fail to prevent the large changes in BP and heart rate induced by restraint stress.[6] To put this type of conditioning in perspective, one need only imagine conditioning human subjects for BP measurements by waking the subjects in the middle of the night, confining them in narrow boxes until their core body temperatures increase, and then recording their BPs. In other animals such as dogs and nonhuman primates, training protocols have also been used, but their efficacy in minimizing the cardiovascular effects of the procedural stress is not clear.[14,15]

Third, the accuracy of indirect BP measurement methods in animals, particularly tail-cuff methods, is open to question. Several studies have been published purporting to validate cuff methods based on correlations between indirect cuff measurements of BP and direct measurements of BP simultaneously or subsequently obtained with arterial catheters.[5,9,14,16–19] Most such validation studies have relied on simple correlation/regression analyses that can be misleading and obscure large individual differences or even systematic differences between measurement methods.[20] When more appropriate analytical techniques have been used, such as agreement analysis, BP measurements obtained by indirect methods have shown poor agreement with BP measurements simultaneously obtained by direct methods.[19,21] In some cases, tail-cuff measurements of systolic BP have appeared suspect because they have shown large differences with direct measurements of systolic pressure[19] or because they have shown minimal differences with direct measurements of mean arterial pressures.[5]

As noted by Reddy et al, another major limitation of most tail-cuff methods is that they are not well suited to measuring diastolic pressure.[19] Diastolic BPs measured with a custom-made instrument incorporating a pulsed Doppler tail-flow sensor have been reported to show good agreement with directly measured diastolic pressures[19]; however, this method requires the use of anesthesia, which has obvious drawbacks in cardiovascular research. Based on a validation protocol for evaluating automated sphygmomanometers that was developed by the Association for the Advancement of Medical Instrumentation, Jamieson et al compared systolic and diastolic BP results obtained with a commonly used tail-cuff instrument to those obtained by direct arterial recordings.[21] For both systolic and diastolic pressure measurements, the disagreement between the indirect and direct methods exceeded clinically acceptable standards, and 74% of diastolic pressures showed disagreements >5 mm Hg.[21] Finally, even if an indirect method can be shown to provide an accurate measurement of BP during a particular moment in time, this does not validate the method for assessing an animal's true average BP or for detecting the extent to which BP exceeds certain thresholds during the course of a study.

Given the major limitations of indirect BP measurement methods and particularly their inability to determine true average BP or the frequency of large BP fluctuations, these techniques are not recommended for studies intended to quantify the relationship between BP and other variables (eg, vascular damage, atherosclerosis, renal function, etc) (Table 1). Studies that use indirect measurements of BP to claim that a particular drug, condition, or genetic variant does not affect BP, or to quantify the extent to which such factors might affect BP, should be viewed with caution. For example, the use of indirect methods to investigate whether a particular drug or genetic variant can influence target organ damage independent of effects on BP or to claim that 2 different drugs have similar effects on BP is not recommended.[22] Because indirect methods impose substantial amounts of stress, are incapable of assessing true BP, and cannot determine how often BP exceeds various thresholds during the course of a study, such methods are not recommended for quantifying relationships between BP and any maneuver, phenotype, or genotype (Table 1).

Methodology Considerations

It is generally advised that investigators first establish the physical accuracy of an indirect method by calibration against a mercury column and by comparing indirect pressure measurements to simultaneously obtained direct measurements of arterial pressure.[14,23] The assessment of an indirect method should include attention to technical details, including cuff size,[17,23] and comparisons to a direct method should be performed using appropriate techniques of agreement analysis rather than simple correlation/regression analyses.[21] As discussed, such validation studies can test the accuracy of an indirect method but cannot establish that an indirect method is capable of measuring an animal's true average BP or justify its use for quantifying BP load on the vasculature.

Although it unclear how effective conditioning efforts are in reducing the stress of indirect pressure measurements, such conditioning efforts may serve to reduce movement by the animals and motion artifacts during the measurement procedure. It is usually recommended that the animals be exposed

to the measurement procedures every day for 7 to 14 days before the beginning of an experiment. Thus, notwithstanding the questionable value of conditioning protocols in eliminating stress, such efforts may improve the operational ability of investigators to use indirect methods to screen for substantial hypertension or to test for large effects on BP.

Investigators have also offered a variety of tips to reduce stress and improve measurement reliability including placement of a dark cover over the animals, use of a single technician to conduct the measurements at the same time each day, use of clean equipment free from foreign scent and blood odor, use of food rewards, and the like.[4,17,23] Again, although such tips may facilitate the ability to perform indirect BP measurements, their efficacy in eliminating stress is open to question and their use does not enable an indirect method to accurately quantify true BP in many circumstances, or to determine the average BP occurring throughout the day in a conscious, unrestrained animal.

Most protocols for indirect measurement of BP include recommendations to average the results of 3 to 10 measurements in a recording session. The practice of inspecting the results and discarding inconsistent values before averaging is open to observer bias and should be avoided unless an inconsistent result is definitely caused by a technical problem. In the absence of clear technical artifact, large variations could simply reflect substantial changes in BP that are known to frequently occur in humans and in animals. To minimize chances for biasing of results, it is advisable that the technician performing and analyzing the BP measurements be "blinded" with respect to the experimental groups and that the animals be tested in a randomized fashion. Given the limitations of tail-cuff BP measurement methods in animals, it has been recommended that study results obtained by this technique be verified by direct BP measurements.[3,23] However, in the absence of 24-hour direct BP recordings, such comparisons cannot be used to determine whether indirect measurements are accurately reflecting the true average BP.

Direct Methods

BP can be directly measured using radiotelemetry techniques or via indwelling catheters connected to externally mounted transducers. It should be emphasized that methods for measuring blood pressure through externally connected, fluid-filled catheters can provide nearly all of the same advantages as the more recently developed radiotelemetry techniques. Therefore, the recommendations on when to use external catheter systems and radiotelemetry techniques are almost identical and have been combined in a single table (Table 2). Nevertheless, in terms of specific advantages and disadvantages, there are a few distinctions between these direct methods that are worth noting. In addition, some important differences exist between these 2 approaches with respect to methodologic considerations. Therefore, we have divided the discussion of direct BP measurement methods into separate sections for radiotelemetry and for externally connected catheter systems.

Radiotelemetry

Advantages and Applications

The commercial availability over the past 10 to 15 years of reliable and easy-to-use wireless radiotelemetric technology for BP measurements in conscious freely moving laboratory animals has represented a significant advance in hypertension research.[24–26] The technique has been extensively validated and is now available for use in virtually all laboratory animals from mice to monkeys.[4,27–31] Not surprisingly, its greatest use thus far has been in the rat, the species that has most frequently been used in hypertension research.

TABLE 2. Recommendations for the Use of Direct Methods for Measuring BP in Animals

Recommended for:
Quantifying the magnitude of hypertension or of changes in BP
Quantifying relationships between BP and other variables (eg, target organ damage)
Studying BP-independent and BP-dependent effects of different interventions or variables (eg, drugs, diet, genotype, etc)
Identifying intermittent or subtle forms of hypertension or changes in BP
Measuring BP continuously over time
Measuring BP variability
Determining BP in unrestrained animals (telemetry)
Not recommended for:
Screening large numbers of animals for frank hypertension or big effects on BP

Implantable radiotelemetry has the advantage of allowing for continuous, direct measurements of BP without the need for restraint or the use of tethering devices. In some circumstances, such as with impaired baroreceptor function, BP variability is greatly enhanced and even mild stress associated with using a tail-cuff to measure systolic pressure can cause abnormally high BP. Because of their essentially unlimited capacity for continuous data acquisition over days, weeks, or months, radiotelemetry systems, like external catheter systems that include swivel devices, also provide the ability to measure BP around-the-clock for extended periods of time.[26,32–34] In nocturnal animals (eg, mice and rats), measuring BP during the usual working hours of most investigators may not provide an accurate reflection of average daily BPs. Direct measurements of BP, 24 hours per day, with catheters or telemetry permit these diurnal variations to be quantified. Another benefit of obtaining multiple, direct measurements of blood pressure by telemetry or external catheter systems is reduced variation in estimates of the mean. As reported by Van Vliet et al in a telemetry study of blood pressure in mice, the 95% confidence intervals for BP in 9 control mice ranged from 8 mm Hg for the 24-hour mean (ie, 4 mm Hg above and below the calculated mean value) to 14 mm Hg for a 30-minute mean, to 22 mm Hg for a single point estimate.[35] Clearly, the ability to obtain large numbers of measurements can have a substantial effect on the precision of the estimates and the number of animals required to accurately estimate BP.

Given the fundamental moment-to-moment lability of BP,[11,36] the ability to obtain continuous BP recordings is also quite valuable for investigating quantitative relationships between BP and other variables, particularly when small BP differences may have significant physiological or pathophysiological impact (Table 2).[32,37–39] A major advantage of such accurate and reliable BP phenotyping with direct measure-

ment techniques is that it is possible not only to compare groups but also to examine and correlate individual animal BP profiles with the phenotype of interest, thus providing a more rigorous and quantitative test for a given hypothesis.[11,33,38,40–42]

The convenience and reliability of BP radiotelemetry methodology is reflected in the increasing frequency of its utilization by more and more laboratories. In addition to its obvious attractiveness for definitively establishing the extent and duration of the cardiovascular and BP effects of old and new pharmaceutical agents,[26] it has been applied to address unresolved questions in which conventional indirect measurements had yielded inconsistent and often conflicting data.[33,38,43] More importantly, it has opened new areas of investigation, such as the long-term neurohormonal and cardiovascular regulation of BP and its variability,[25,34,44–46] that were previously beyond the reach of indirect measurement techniques in rats and mice.

Perhaps the clearest illustration of the superiority of BP radiotelemetry to address a controversial issue in cardiovascular research is provided by its application to the pathogenesis of hypertension-associated target organ damage. Although it is generally agreed that increased BP contributes to target organ damage, the quantitative relationships have remained controversial because target organ damage is a complex phenomenon thought to be influenced by both BP-dependent and BP-independent mechanisms and pathways. Identification of such BP-independent mechanisms of target organ damage has been a major focus of much research over the past 2 decades.

As a corollary, the relative efficacy of therapeutic agents and their relative impact on BP-dependent and independent mechanisms on target organ damage has also been extensively investigated. Given that the BP-dependent component of such target organ damage is expected to be a function of the 24-hour BP load, and because pressures are higher at night in nocturnal species such as rats and mice,[27,34,36] it is not surprising that the interpretations and conclusions of investigations based on sporadic indirect conventional BP measurements have often not been supported by more precise radiotelemetry quantification of 24-hour BP loads in the very same experimental animal models.[22,33,38,40,43] Such data have clearly demonstrated that accurate 24-hour chronic BP phenotyping not only is desirable but also is actually necessary for valid conclusions in investigating the relationship between BP and target organ damage (Table 2).

Through the use of BP radiotelemetry, it is possible to define both the BP thresholds and the slopes of the relationship between the 24-hour BP phenotype and target organ damage in individual animals and in different experimental animal models.[11,38,47] Such an analysis thus also allows for a critical examination of the effect of therapeutic interventions as well as the mechanisms by which such effects are mediated in these models. The data can also be analyzed to examine the significance of indices of BP lability, such as diurnal rhythms, standard deviation of the BP, or for the frequency of BP readings above a certain threshold on target organ damage.[11,32,33] Systems for direct measurement of BP throughout the day and night have now provided the tools necessary to investigate largely unexplored issues about hypertensive target organ damage such as how to accurately define "BP load," the relative importance of systolic, diastolic, mean and pulse pressures, the relative importance of pressure transients versus steady-state elevations in blood pressures, variation in susceptibility of different target organs to pressure-induced damage, and the potential contribution of genetic factors to susceptibility to hypertensive injury (Table 2).[37,39,48,49] In addition, simultaneous recording of BP and other parameters such as organ blood flow can be obtained in real-time to examine the real-time perfusion pressure flow relationships in conscious unrestrained animals before and after experimental interventions.[50–53] These and many other aspects of cardiovascular regulation are currently being investigated by several laboratories and would be impossible to reliably study with indirect measurement techniques.

BP radiotelemetry also provides a powerful methodology for investigating short-term and long-term regulation of BP and its variability. In addition to the investigation of the diurnal rhythm,[49,54,55] radiotelemetry has been used to determine the effects of locomotor activity on blood pressure. A recent study in endothelial nitric oxide synthase (eNOS) knockout mice demonstrated that the size of the activity effect (23 mm Hg in controls, 33 mm Hg in eNOS knockouts) was far greater than the day–night difference in BP, or even the effect of knocking out the eNOS gene itself.[35] These results could not have been uncovered using tail-cuff methods and indicate that locomotor activity can have profound effects on short-term BP levels in mice. Other telemetry studies of short-term and beat-to-beat BP variability have also been very productive.[36,44,45,56] For very detailed analyses, BP can be sampled continuously at 200 to 2000 Hz (depending on the species) for periods up to 24 hours at designated intervals to examine the differences in frequency distribution of BP power (energy/unit time) between models and/or animals and after pharmacological intervention. BP power spectra are determined by applying fast Fourier transforms to the obtained recordings. Other analytic methods for examining BP fluctuations in both the frequency and the time domain have also been developed and used.[37,57]

The high-fidelity BP phenotyping that is achievable with BP radiotelemetry and with direct measurements through external catheter systems has also provided an invaluable tool for the investigation of genetic determinants of BP regulation. Identifying quantitative trait loci for BP using genetic strategies has been of intense interest in recent years. The use of direct measurement techniques has enabled the mapping of chromosome regions involved in the regulation of multiple cardiovascular phenotypes including systolic and diastolic pressures, mean arterial pressure, and baroreceptor function.[37,58–61] Unfortunately, in many other studies, the sophistication of the genetic and molecular tools has not been matched by equally detailed and sophisticated BP phenotyping. This is of particular concern as the quantitative impact of individual genes and/or loci could be relatively small and difficult to detect by the conventional indirect methodologies. Moreover, different genes may be involved in the regulation of various aspects of blood pressure (eg, diurnal rhythms, diastolic pressures, etc) that cannot be evaluated by indirect techniques.

The more recent successful adaptations of radiotelemetry for chronic BP measurements in mice up to several months have been particularly promising for application in transgenic, knockout, and inbred mouse strains. For example, a recent report from Tang et al identified an important role for the RGS-2 gene (regulator of G-protein signaling-2) in vascular smooth muscle relaxation and BP regulation.[62] Telemetric recording of BP in RGS-$2^{-/-}$ mice revealed markedly elevated systolic and diastolic pressures compared with wild-type controls.[62] New roles for well-characterized genes have also been discovered through the application of radiotelemetry. For example, an important effect of eNOS on BP variability was reported,[35] and mice with targeted deletions of the angiotensin II type-2 receptor exhibited increased resting BP compared with controls.[63] BP effects of null mutations in the estrogen receptor (β) have also been detected by radiotelemetry measurements in mice.[64]

Application of radiotelemetry was essential in a recent report identifying a novel genetic mouse model of preeclampsia.[65] Continuous, stress-free recording of BP before, during, and after pregnancy in the inbred BPH/5 strain documented spontaneous development of hypertension in the last trimester that resolved on delivery—the same pattern of BP changes that occur in women with preeclampsia. Given that preeclampsia has a strong genetic component,[65] this inbred strain provides an excellent opportunity for defining the genetic determinants underlying this hypertensive disorder of pregnancy.

Disadvantages

The major disadvantage of BP radiotelemetry is the expense associated with it. Substantial expenditures are needed to initially acquire the equipment as well as for the periodic refurbishment and battery replacement for the implantable radio transmitters.[25,32] The lack of a competitive market has kept these costs relatively high. However, such cost considerations need to be balanced against the need for definitive and accurate BP measurements depending on the nature of the contemplated investigations. The savings that are usually achieved through a reduction of number of animals needed because of the consistency of the results and decreased interanimal variability should also be considered.[26] Nevertheless, given these cost considerations, BP radiotelemetry may not represent the optimal use of resources in all circumstances and, therefore, its use is not recommended for certain study objectives (Table 2).

Some investigators have succeeded in minimizing operating costs by reusing telemetry catheters multiple times before returning them to the manufacturer for refurbishment.[3] In the case of experiments in rats that require relatively brief (eg, several days) use in each animal, more than a dozen serial implantations per telemeter are feasible.[3] A relatively cost-efficient telemetry method for measuring BP in the mouse is to use a 2-week total implantation period, with BP being recorded in the final 72 hours.[35] With careful cleaning and great care in re-gelling of the catheter, a dozen or more serial implantations can be achieved, considerably reducing the refurbishment costs.

In addition to cost, other disadvantages include the need for some surgical skills and training, particular for smaller species such as mice.[4,26–28] Unlike direct catheter methods, it is not possible to check system calibration during an experiment, and tests for baseline drift at the conclusion of each study are also required. Like with any invasive methodology, there is a potential for complications from infections, particularly in diabetic or otherwise immunocompromised animals. However, with proper aseptic technique, such problems can be easily minimized. Additionally, dedicated space within the animal facility is required in which to conduct chronic BP radiotelemetry studies. For very small animals, such as mice that weigh <25 g, the size of the transmitter, which weighs >2 g, is likely to cause some stress to the animal, although it appears that even smaller transmitters may become available soon. In addition, some approaches for catheter implantation (eg, via the carotid artery) may adversely affect cardiovascular function. Finally, injudicious data gathering can create large amounts of data, leading to analysis problems.[26]

Methodology Considerations

In the rat, the traditional procedure consists of the placement of a gel-filled pressure-sensitive catheter into the abdominal aorta below the level of the renal arteries.[25,32] The transmitter body is then placed in the abdomen and sewn into ventral abdominal muscles. However, the practice of introducing the catheter directly into the aorta of the rat with the use of veterinary adhesive to seal it in place has been largely replaced by a simpler introduction of the catheter through the femoral artery and placement of the transmitter body subcutaneously along the flank, avoiding intraperitoneal invasion.

Alternatives to abdominal aortic implantation of telemeters were implemented early on for the mouse. Despite considerable miniaturization of the device, the catheter tip is relatively large compared with the abdominal aorta of the average-size adult mouse (20 to 30 grams) and may compromise blood flow to the lower extremities resulting in high rates of morbidity and mortality.[28,66] Therefore, implantation of the catheter into the thoracic aorta via the left common carotid artery has become a commonly used approach in mice, although some investigators have also succeeded with catheter placement in the femoral artery.[67] The carotid approach has proven to be a very reliable and successful method for obtaining high-fidelity recordings over weeks and months in mice that are in the average size range, but even as small as 17 grams.[26,28,65,66] Although the use of the carotid artery approach is technically quite reliable, occlusion of this vessel by the telemetry catheter may adversely affect cardiovascular function through several mechanisms, including impaired cerebral blood flow. It should be noted that some strains of mice have an incomplete development of the circle of Willis, and this should also be taken into consideration.[68,69]

With improved approaches for catheter implantation in mice came the challenge of finding a suitable site for placement of the transmitter body. Despite considerable miniaturization, the size of the device limits where it can be positioned in the mouse. Early studies used mid-scapular placement of the transmitter body[28,66]; however, this has been problematic because of perturbation of the wound and exte-

riorization of the probe as a result of scratching the area with the hind limbs.[28] More recently, it has been shown that, like in the rat, placement of the transmitter body subcutaneously along the inner flank of the mouse has proven quite effective. Because this is a region that is not easily accessed by the animal through scratching, etc, the implants remain relatively undisturbed over the months of implantation.[28] Furthermore, this approach makes the procedure less invasive by avoiding abdominal surgery. It should be noted that with inner flank placement of the transmitter, the transmitter segment ordinarily used for securing the device to the muscle wall during abdominal aortic implantation is no longer necessary. The design of this device is evolving to take this and other advances in miniaturization technology into account. In the event of battery failure or other problems, the telemeter can be removed and a correctly functioning device can be reimplanted, at least in the rat. The arterial catheter is withdrawn and the replacement transmitter is introduced at the same site, or alternatively into the contralateral femoral artery.[32] If the catheter is placed into the aorta directly, the replacement is more difficult and requires patience and surgical skill.

For both rats and mice, the animals are often housed individually and their cages are placed on top of the radio receiver. It is also possible to house animals together and then selectively activate the transmitters to obtain BP recordings from one of the animals in the cage at a time. The receivers are connected to a data exchange matrix that inputs to a software system designed for acquisition and analysis. Additionally, calibrated pressure analog adapters allow the simultaneous transmission of these data, as well as that of devices such as blood flow probes to be fed into other software systems simultaneously in real-time for analysis.[51,53] Animals should generally be allowed to recover for at least 5 to 7 days before initiating BP recordings, because of the time required for circadian rhythms and basal BP levels to return to normal after telemetry implantation surgery.[28] However, the exact duration of postsurgical recovery required may vary depending on the type of anesthesia, extent of surgery, the strain of animal being studied, and different amounts of time required for both BP and heart rate rhythms to return to normal.

The timing and frequency of the acquisition can be either intermittent or continuous, depending on the objective of the study. With the usual default setting, systolic, diastolic, and mean BP and heart rate can be intermittently assessed at 5- to 10-minute intervals, with each value being determined as the average of all pressures over a 10-second period. For example, the average BP during 50 to 60 heartbeats in the rat is recorded, with 144 such readings being obtained per rat per day over several weeks to months. However, as noted, rapid sampling rates of up to 2000 Hz can be used, depending on the species, to accurately capture and record continuous pressure wave forms and beat-to-beat variability.

Fluid-Filled Catheters

Direct recording of BP using fluid-filled catheters is the oldest and most widely used method of measuring arterial pressure. This method typically uses a catheter filled with heparinized fluid and inserted into a major artery. The distal end of the catheter is connected to a calibrated pressure transducer, which, in turn, is connected to an amplifier and recording device. This technique is very versatile and can be used effectively for acute studies in anesthetized animals or for long-term, continuous monitoring of arterial pressure in conscious animals. For chronic studies, the exteriorized portion of the catheter is housed in a protective sleeve and accessed whenever measurements of BP are needed. If long-term continuous recordings of BP are desired, the catheter can be connected to a pressure transducer via a swivel device and tether system that allows relatively free movement of the animal.[13,70–72] Servo-controlled turntables have also been used to allow free movement of rodents while maintaining the integrity of catheters, infusion lines, cables, or other connections between the animal and devices external to the cage.[73]

Advantages and Applications

The primary advantages and applications of this method for direct recordings of arterial pressure are similar to those described for telemetry, and the 2 methods can be recommended for largely the same purposes (Table 2). Thus, as with radiotelemetry, direct measurement of BP with an external catheter is accurate and reliable, and permits assessment of BP lability and diurnal variations in BP. In large animals such as dogs, arterial catheters can be maintained for 1 year or more with proper surgical implantation and meticulous care to prevent infections.[74] For smaller animals, the useful life of arterial catheters is shorter, although they can usually be maintained functional for 6 weeks in rats.[75] A few laboratories report maintenance of patent arterial catheters for 4 to 5 weeks in mice,[72] although most studies are performed between 5 and 14 days after implantation of the catheters.

There are some additional advantages of measuring BP with fluid-filled catheters. This is the most accurate technique for measuring mean arterial pressure directly because calibrations can be performed at any time, avoiding potential baseline drift or changes in sensitivity that might occur over long periods of time. This method is also relatively inexpensive. Pressure transducers and materials to make catheters are inexpensive, permitting almost any laboratory to use the method for acute or chronic experiments. The most expensive items needed include a suitable amplifier/recording device, as well as an analog–digital converter and computer if one wishes to use a computerized data collection system. These items are usually available in most hypertension research laboratories.

Another important advantage of the catheter-tether system for research studies is that it permits access to the arterial vasculature for infusions of various experimental agents. Also, with a tether system in place, blood vessels can be catheterized for chronic infusions of various fluids and pharmacological agents using external pumps that can be easily controlled. This is in contrast to indirect methods and other types of direct methods (eg, telemetry) that do not use a swivel–tether system and therefore must rely on subcutaneous injections or infusions, or implanting vascular catheters and connecting them with small nonadjustable, implanted pumps such as osmotic minipumps.

Tether–swivel systems for BP measurements can also be used in conjunction with other devices, such as electromagnetic or Doppler flow probes, that permit direct recording of cardiac output and blood flow in various tissues, 24 hours per day.[70,76] The electrical leads for implanted flow probes and other electrical devices, vascular occluders, venous catheters, and arterial catheters can all be attached to appropriate external devices through tether–swivel systems. Tether–swivel systems offer powerful methods for controlling fluid and electrolyte intake while at the same time recording arterial pressure and other hemodynamic variables continuously 24 hours per day using computerized methods. These systems also provide the opportunity to develop innovative approaches to important issues in hypertension research not possible with conventional methods. For example, this strategy has been used to servo-control renal perfusion pressure during chronic infusion of various hypertensive hormones, such as angiotensin II, aldosterone, norepinephrine, vasopressin, and others to directly determine the pressure-dependent and pressure-independent effects of these hormones.[77–79]

Disadvantages

There are some disadvantages of implantable catheters that must be considered when designing experiments, although many of these can be overcome with proper techniques. Tether–swivel systems may cause some stress to the animals, especially if the system does not permit free movement in all directions. However, with swivel and tether systems that allow free movement of the animal in all directions, long-term arterial pressure recordings can be obtained under conditions of low stress. There is potential for infection and minor disturbances to the animal caused by the surgery necessary for catheter implantation. Damping of BP signals or complete loss of catheter function can occur because of clotting or growth of fibrous tissue around the catheter tip. If the size of the catheter used is very small, there may be a limited dynamic response that prevents high-fidelity recording of pulsatile pressures, although accurate mean arterial pressure measurements may still be possible. In catheters larger than PE10, under-damping at the resonant frequency can also distort pulse pressures. Therefore, it is advisable to check the frequency response and critical damping ratio of fluid-filled recording systems before use. Substantial training and development of surgical skills are necessary for successful implantation and maintenance of catheters for chronic studies, especially in very small animals such as mice. Maintenance of catheters for chronic studies can be labor-intensive, and meticulous care of the catheters is required. Dedicated space is usually required to house the animals for chronic BP studies.

The contraindications for measuring BPs with catheters are similar to those described for telemetry. For example, implanted catheters do not provide a practical means of screening for major changes in BP in large numbers of animals, especially when the animals must be studied for several months or longer (Table 2).

Methodology Considerations

Most of the operational disadvantages of fluid-filled catheter systems can be overcome with appropriate procedures. For example, the potential for infection and catheter loss can be overcome by using the appropriate materials to make the catheters and by using sterile/aseptic techniques for surgical procedures, handling the tips of the catheters, and flushing the catheters.

Catheterization Procedures and Catheter Care

Appropriate catheter materials are critical for successful chronic recording of BP, especially in small animals. Although polyethylene tubing can be used for rats and larger animals, this material has not been suitable in mice because of its inflexibility and increased susceptibility to clotting.[72,80] Micro-Renathane tubing (Braintree Scientific; 0.025-in inner diameter; 0.040-in outer diameter) is advantageous for arterial catheters in mice and can also be very useful in rats. It can be stretched over heat to very small diameters ($\approx$300 μm), its flexible properties allow implantation without damage to delicate vessels, and there is diminished probability of clot formation.[72,80] In larger animals, such as dogs, other materials (eg, medical grade Tygon or Silastic) have also been used successfully for chronic arterial and venous catheters.[77–79]

For chronic use, regardless of species, catheters must be inserted into a rapidly flowing bloodstream. For example, a catheter inserted into a femoral artery should be advanced into the lower aorta to prevent vascular stasis and rapid clotting. Using nonsterile methods for catheter implantation is another common cause of catheter failure and violates the basic surgical principle that implantation of a contaminated foreign body almost always leads to infection and even death of the animal if the contaminated foreign body is allowed to remain in contact with the bloodstream.

Another potential source of infection is sinus track formation and subsequent infection caused by long-term penetration of the animal's skin by the exteriorized portion of the catheter. This source of infection can also be minimized by careful daily care of the exit wound and allowing sufficient time for it to heal before beginning the experiments. Also, attaching a sterile sleeve made of textured material (eg, Dacron) to the percutaneous portion of the catheter not only helps to protect the catheter but also permits mechanical attachment and has been reported to virtually eliminate sinus tract infections.[81]

Meticulous care of the catheters is necessary to prevent failure during long-term experiments. This means that the strict aseptic methods must be used when handling the tips of catheters and when they are flushed. Arterial catheters are usually filled with heparin, although some investigators have reported that adding antibiotics or proteolytic enzymes to inhibit platelet-mediated blood clotting and deposition of blood proteins may increase the useful life of the catheter.[74] The useful life of the catheter may also be extended by maintaining a slow continuous infusion into the arterial catheter while recording arterial pressure.

When catheters are filled with heparin and other substances for chronic studies, care must be taken to prevent repeated flushing of these substances into the animal. The catheter volume should be determined before implantation and the amount of heparin injected should be just enough to adequately fill the catheter lumen. When catheters are flushed, the filling volume should be completely withdrawn and discarded before the catheter is flushed with physiological solutions such as isotonic saline or 5% dextrose. For chronic studies in which daily electrolyte balances are measured, repeatedly flushing of large amounts of sodium chloride solutions or other electrolytes into the animal can significantly alter total electrolyte intake, unless one is careful to use only the amounts needed to fill the catheter. Also, other physiological solutions, such as 5% dextrose, can be used to flush the catheters to avoid altering electrolyte intake before refilling them with heparin for chronic recording of arterial pressure.

With training and practice, success rates for implantation and long-term maintenance of functional arterial catheters in large animals, such as dogs, is nearly 100%. The success rate usually is lower with smaller animals, although 90% success rates over a period of 5 to 6 weeks can be achieved in rats.[75] Mice present an even greater challenge, and currently there are only a few laboratories that have significant experience in maintaining functional arterial catheters in mice for more than ≈1 to 2 weeks. In mouse studies in which functional catheters were maintained for several weeks, an outbred strain was used and the animals were considerably larger (≈40 grams) than the average adult mouse of more typical genetic backgrounds.[72]

There is stress associated with anesthesia and with even the most minor surgical procedures. Therefore, as for radiotelemetry, and depending on the type of animal studied, it is generally recommended that BPs obtained for the first 4 to 7 days after surgical implantation of catheters not be used to estimate BP under unstressed conditions. Waiting a sufficient period of time after surgery to obtain measurements of BP is especially important when more extensive surgical procedures, such as abdominal surgery, are used.

Calibration and Data Collection

As discussed, one advantage of direct recording of arterial pressure with fluid-filled catheters is that calibrations of the recording system can be conducted whenever needed. This is especially important in long-term experiments to avoid potential baseline drift or changes in sensitivity of the recording device. In most cases, calibrations of the recording systems should be conducted daily using a mercury manometer.

Many commercial data collection systems are available and customized software systems have been developed that permit beat-by-beat analysis of arterial pressure and other hemodynamic variables (eg, cardiac output), 24 hours per day, for long periods of time.[82,83] The BP sampling rate depends on the species used and the objectives of the experiment. For small animals with high heart rates, such as mice, the BP sampling rate needed is greater than what is required for animals with lower heart rates, such as dogs. Sampling rates of 200 samples per second in 10- to 15-second bursts each minute are usually sufficient for high-fidelity determination of arterial pressures in most animals. The digital data from the computer can be compressed, stored, and analyzed with software according to the objectives of the experiment.

Transducer-Tipped Catheters

Arterial pressure can be measured directly with catheters containing high-fidelity miniature pressure transducers at the tip. These catheter transducers are relatively expensive but provide a very high-fidelity means of measuring arterial pressure with an excellent dynamic response. This method is limited mainly to use in acute experiments because of significant baseline drift that can occur during chronic studies. Also, the size of these catheter transducers precludes their use for routine measurement of arterial pressure in very small animals (eg, mice). Recently, however, miniaturized transducers have been introduced with sizes as small as 0.8 French.[83] This makes it possible to measure very accurately, for example, pressures in the left ventricle of mice in acute experiments. However, for most other pressure measurements, fluid-filled catheters are more than adequate and provide adequate fidelity and a more reliable method for long-term measurements of arterial pressure.

Environmental Influences on BP

Regardless of the method used to measure BP, it is important to keep in mind that a host of environmental factors can affect BP, including, but not limited to, ambient room temperature, light cycle, noise levels, duration of human contact, number of animals per caging unit, proximity to other animals undergoing experimental procedures, cage unit size and design, and access to supplemental items such as toys, treadmills, and hiding spaces within the cage unit. As emphasized in the introduction, systemic anesthetics can also exert major effects on cardiovascular function and should be avoided whenever possible. To illustrate the potential impact of a particular environmental factor on blood pressure, it is instructive to consider the effects of the ambient room temperature where the recordings are conducted. Room temperatures below the thermoneutral range (29°C to 30°C in rodents) tend to increase metabolic rate, food intake, and BP.[84,85] In C57BL/6J mice, increasing room temperature from 23°C (the usual temperature at which most animal facilities are maintained) to 30°C for several days reduced light-phase mean arterial pressure and heart rate, measured by telemetry, by 14 mm Hg and 184 bpm, respectively.[84] Similar results have also been found in several strains of rats.[85,86] The increase in food intake, metabolic rate, and BP caused by room temperature below the thermoneutral range are probably because of a generalized increase in thermogenic sympathetic nervous system activity. Because different species and strains of animals may have variable sympathetic nervous system and thermogenic responses to reductions in temperature, a low ambient room temperature may cause different effects on arterial pressure, heart rate, and other cardiovascular variables. Unfortunately, most studies are conducted at room temperatures that are more comfortable for the investigator than for the experimental animals in which BP is being measured. Regardless of the method used to measure blood pressure, it is important to keep in mind that many environmental factors can have substantial effects on cardiovascular function.

Summary

To fully capitalize on the scientific opportunities in hypertension and cardiovascular research afforded by recent advances in biology and biotechnology, it is important that investigators also use optimal methods for measuring BP. The techniques for measuring BP in experimental animals have improved considerably over the past decade, and several methods are available that allow routine monitoring of BP profiles throughout the day and night over prolonged periods of time in conscious, unrestrained, unstressedanimals. Although indirect techniques that permit only intermittent measurements of BP may be suitable for some purposes, methods for directly measuring BP are generally preferred because of their ability to monitor the highly dynamic nature of BP in a comprehensive fashion. Selection of the methods to be used should ultimately be guided by the study objectives to insure that the techniques chosen are appropriate for the experimental questions being explored.

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