Evaluation of Blood Pressure Measured by Tail-Cuff Methods (without Heating) in Spontaneously Hypertensive Rats

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Conventional noninvasive blood pressure in conscious rats or mice are typically measured using the tail-cuff method after heating the animal. The goal of this study was to assess the validity of a novel tail-cuff method without animal heating when compared with the conventional heating tail-cuff method (unanesthetized rats with heating), telemetry method (unanesthetized restrained rats without heating), or carotid arterial catheter method (unanesthetized rats, carotid arterial cannulation). The blood pressure and heart rate of spontaneously hypertensive rats were measured at 13:00—17:00 h for all experiments. Experiments demonstrated similar systolic blood pressure measurements when comparing the unheated-animal tail-cuff method and the telemetry method. Further, values obtained by both methods were lower than those obtained by the heated-animal tail-cuff method. Systolic blood pressure measurements obtained through carotid arterial cannulation were lower than those obtained by any other method. The heart rate was highest using the unheated-animal tail-cuff method when compared with the other methods. These data suggest that the novel unheated-animal tail-cuff method is a sensitive and accurate approach for the noninvasive measurement of blood pressure in conscious rats.

Key words: blood pressure; spontaneously hypertensive rat; tail-cuff method; telemetry method

Generally, blood pressure (BP) in rats and mice is measured using the tail-cuff method after heating the animal. However, this method yields an indirect measurement of BP, and the requirement for animal heating and restraint may cause stress-induced changes in BP. To overcome these limitations, a radiotelemetric monitoring system was developed to continuously measure cardiovascular parameters in freely moving rats.

Bazil et al. compared the cardiovascular parameters of spontaneously hypertensive rats (SHR) recorded by different methods (e.g., radiotelemetry device, cannulated arterial catheters, and indirect tail-cuff) and suggested that telemetric monitoring was a useful method of cardiovascular study that did not cause stress-induced changes in blood pressure. However, telemetric monitoring requires the implantation of a radiotransmitter in the abdomen, and the apparatus is expensive.

Recently, a novel device was developed for the tail-cuff method that does not require animal heating if the ambient temperature is greater than 23 °C. The goal of this study was to assess the validity of this novel tail-cuff method without animal heating (method A) when compared with the conventional heating tail-cuff method (method B; unanesthetized rats with heating), telemetry method (method C; unanesthetized restrained rats without heating), or carotid arterial catheter method (method D; anesthetized rats, carotid arterial cannulation).

MATERIALS AND METHODS

Animals and Operative Procedures Experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and Mukogawa Women’s University. Male rats at 11—12 weeks of age were used, and BP and heart rate (HR) were always measured between 13:00—17:00 h.

Tail-Cuff Method without Heating (Method A) Rats (SHR/Izm, Japan SLC, Inc., Shizuoka, Japan) were anesthetized with urethane (1 g/kg, i.p.), and underwent cannulation of the right carotid artery. Catheters were connected to pressure transducers (SBP-105, NEC San-ei Instruments, Ltd., Tokyo, Japan) without heating and were averaged from at least three consecutive readings obtained from each rat.

Tail-Cuff Method with Heating (Method B) In this experiment, we measured the blood pressure and heart rate of rats used in the experiment of method A. Rats were preheated in a chamber at 35 °C for 10 min, then placed in plastic restrainers. A cuff with a pneumatic pulse sensor was attached to the tail. Rats were allowed to habituate to this procedure for 7 d before experiments were performed. BP and HR values were recorded on a Model MK-2000 (Mucromachi Kikai Co., Ltd., Tokyo, Japan) without heating and were averaged from at least three consecutive readings obtained from each rat.

Telemetry Method (Method C) Rats (SHR/NCrJ, Charles River Japan, Yokohama, Japan) were implanted with a radio transmitter (TA11PA-C40, Data Sciences, St. Paul, MN, U.S.A.). Individual rats were placed in a plastic cage on top of a receiver (RLA1020, Data Science) for measurement of BP or HR. Data were continuously collected and analyzed by computer software (Data Quest Lab Pro, Data Science). Values measured at 13:30 were shown as results.

Carotid Catheter Method (Method D) Rats (SHR/Izm, Japan SLC, Inc., Shizuoka, Japan) were anesthetized with urethane (1 g/kg, i.p.), and underwent cannulation of the right carotid artery. Catheters were connected to pressure transducers (SBP-105, NEC San-ei Instruments, Ltd., Tokyo, Japan).
Japan), and BP was monitored on a PowerLab/800 (ADInstruments Pty Ltd., NSW, Australia).

**Effects of Urethane on Cardiovascular Parameters**
Rats (Wistar, Japan SLC, Inc., Shizuoka, Japan) were placed in plastic restrainers, and baseline BP and HR values were determined by method A. Rats were then removed from the restrainers and given urethane (1 g/kg) or vehicle (saline) by intraperitoneal injection, and BP and HR were recorded at 30 min after the injection.

**Effects of Nicardipine on Cardiovascular Parameters**
Rats (SHR/Izm, Japan SLC, Inc., Shizuoka, Japan) were placed in plastic restrainers, and baseline BP and HR values (0 h at am 10:00) were determined by method A or B. Rats were then removed from the restrainers and given nicardipine (30 mg/kg) by oral administration using a stomach sonde, and BP and HR were recorded at 1, 2, 4, 6, 8, 23 h after administration.

**Drugs**
Urethane and nicardipine were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. Other reagents were purchased from Wako Pure Chemical Ltd. (Osaka, Japan).

**Statistics**
All values are reported as the mean±S.E.M. For multiple comparisons, statistical analysis was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn test. Other data were evaluated for significance by Student’s t-test. When the variances of two groups were different, the Welch test was used. Probability of less than 0.05 was considered significant. Statistical analyses were performed with a computer software package (StatView 4.5, Abacus Concepts, Cupertino, CA, U.S.A.).

**RESULTS**

**Comparison of the Four Methods**
Systolic BP (SBP) and HR were measured in SHR using four different methods (Fig. 1). SBP values by method A were similar to those by method C. SBP values by method B were higher than those by the other methods. HR values by method A were higher than those by the other methods. HR values by method C were similar to those by method D.

**Effects of Urethane and Nicardipine on Cardiovascular Parameters**
The influence of anesthesia on SBP and HR of SHR was examined using urethane (1 g/kg, i.p.). Urethane induced a marked decrease in SBP and HR as recorded by method A (Fig. 2). The values of SBP and HR treatment with urethane were similar to those by method D.

BP and HR were also recorded in SHR after the oral administration of nicardipine (30 mg/kg) (Fig. 3). SBP and diastolic BP (DBP) values at 0 and 23 h were higher when using method B than when using method A. In contrast, SBP measured at 1 h (maximal hypotensive time) was significantly lower when measured by method B compared with method A. HR values at 0, 8 and 23 h were higher when using method A than when using method B.
DISCUSSION

Telemetry is a useful method of performing cardiovascular studies in conscious experimental animals. However, this method is limited by the need for radio transmitter implantation and by the cost of the telemetry apparatus. By contrast, the tail-cuff method is simple and inexpensive but carries the potential for stress-induced changes in blood pressure due to animal restraint and heating. Several groups have attempted to develop improved tail-cuff methods, such as integrating the use of a lamp lens to localize the heating stimulus to the tail rather than to the whole animal, or by employing electrical impedance to avoid animal heating altogether. However, these methods still require specialized apparatus, which limits their widespread use. The goal of this study was to assess the validity of a novel tail-cuff method that does not require animal heating (method A).

This study demonstrated that SBP values were similar when comparing methods A and C, and the SBP values from these two methods were higher than those obtained with method D and lower than those obtained by method B. By contrast, HR values were higher when measured with method A than when measured with methods B, C or D. The HR value measured by method D was low and almost equal to that measured by method C. The reason why HR values measured by methods C and D were lower may be the influence of sleep and anesthesia. Indeed, urethane decreased blood pressure and heart rate measured by method C. The reason why the HR value measured by method A was higher than that by method B is not clear. Yen et al. reported that heating stimulus resulted in increased SBP and decreased HR in SHR, likely because of a primary heat-induced elevation in SBP and a secondary baroreflex-mediated decrease in HR. Baroreflex or vasodilation by heating may be involved. The hypotensive action of nicardipine measured by method B was stronger than that measured by method A. This difference seems due to a difference in the BP level before nicardipine administration. Generally, it is accepted that the efficacy of vasodilator agents depends on the tension of vascular smooth muscle.

In conclusion, these data suggest that the novel unheated-animal tail-cuff method is a sensitive and accurate approach for the noninvasive measurement of blood pressure in conscious rats. Further research would be of benefit.

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REFERENCES