An Impedance Method for Blood Pressure Measurement in Awake Rats Without Preheating

SUNG-FENG WEN, JOSEPH M. TREMBLAY, MINGHAI QU, AND JOHN G. WEBSTER

SUMMARY The tail-cuff methods for measuring systolic blood pressure in the rat usually require preheating of the animal to obtain recordable pulse signals. To find a more sensitive method, we applied the principle of differentiated impedance (dZ/dt) to the tail-cuff measurement of systolic blood pressure. We obtained clear pulse signals from the tail in awake rats without preheating the animals, and the systolic blood pressure obtained by this method had an excellent correlation with the directly measured femoral artery pressure (correlation coefficient = 0.98). Heating the animals at 40°C for 5 minutes increased systolic blood pressure by a mean of 6 mm Hg as compared with that determined at the ambient temperature of 21 to 24°C. Mean systolic blood pressure in young female diabetic rats was 122 ± 3 mm Hg, which was significantly higher than the 111 ± 2 mm Hg of normal rats. It is concluded that the technique of electrical impedance as applied to the tail-cuff method is simple and highly sensitive and is suitable for measurement of tail systolic blood pressure in awake rats without preheating. (Hypertension 11: 371-375, 1988)

KEY WORDS • tail-cuff method • temperature effect • hypertension • diabetes mellitus

NONINVASIVE measurement of arterial pressure in small animals such as rats is hampered by the small size of the accessible peripheral arteries, which provide relatively weak pulse signals for detection. Thus, the commonly used tail-cuff methods for measuring systolic blood pressure (SBP) in the rat, which employ plethysmography1-3 or Doppler ultrasonic flowmeter,4 require preheating of the animals to enhance the amplitude of pulsation in order to record the pulse signals. Such a maneuver can artificially increase SBP5,6 and, therefore, can lead to less accurate determination of SBP. The photoelectric sensor technique,7-11 as described by Yen et al.10 and studied by Buşag and Butterfield,11 does not require preheating, but a high ambient temperature is needed for reliable measurements of SBP. In the present study, we developed a relatively simple method for the determination of SBP in awake rats without preheating using the technique of differentiated impedance. With this technique, we obtained an excellent correlation of the indirectly measured SBP with the directly measured values and showed a significant increase in SBP following preheating of the animals.

Materials and Methods

Forty-eight female Sprague-Dawley rats (Harlan Sprague Dawley, Madison, WI, USA) weighing 175 to 300 g were used for the measurement of tail arterial pressure. These animals were individually placed in a Lucite restrainer. The tail was cleaned, the hair shaved, and any lichenification was removed by gentle scraping against the direction of the scales. Approximately 5 minutes was allowed for the animals to stabilize, but prolonged restraint of the animals was avoided to prevent agitation. Figure 1 shows the arrangement of the impedance monitoring system in awake rats. For occlusion of the tail arterial blood flow, a pneumatic tail cuff 15 mm in width and 9 mm in diameter was placed at the base of the tail proximal to the segment of impedance monitor. A four-electrode system was used to minimize the effect of the skin-electrode interface impedance. The electrodes were made from 6.4 mm wide aluminum electrode backed by a wider Mylar adhesive tape 25 mm in width (M60001, 3M, Minneapolis, MN, USA). The aluminum electrode was cut in half, leaving half...
FIGURE 1. Tail cuff and four electrodes (numbered 1-4) as applied to the rat tail for the measurement of SBP and a block diagram of the impedance monitoring system. A constant current of 1.7 mA is passed to Electrodes 1 and 4. A full-wave precision rectifier demodulates the signal from Electrodes 2 and 3 to yield basal impedance, Z. A D/A converter automatically balances Z to yield impedance change (ΔZ), which is then differentiated to yield the derivative, dZ/dt. A bandpass filter further reduces the noise.

The impedance monitoring system is based on the variations of body impedance that occur during the cardiac cycle. As shown in Figures 1 and 2A, a high-frequency current of 1.7 mA root mean square from a 100-kHz source is passed between Electrodes 1 and 4. The impedance signal measured between two voltage electrodes, 2 and 3, is composed of three components. One (undesired) component is associated with the basal impedance (Z), which is dependent on the conductivity of the segment consisting of that of tissues, tissue fluid, and blood. The second (also undesired) component is associated with the respiratory activity and motion artifacts. The third (desired) component, ΔZ, is caused by the pulsatile expansion of the volume of the elastic tail artery that results from pulsatile blood pressure changes. The second component is usually greater in amplitude but slower in frequency than the third component. To reduce the effects of respiration and motion artifacts that constitute the second component, the derivative of the impedance signal, dZ/dt, is used for recording the pulse signals. Figure 1 also shows the block diagram of the impedance monitoring system. Figure 2 shows the components of the circuit diagram. The impedance signal between two voltage electrodes, 2 and 3, is amplified by an instrumentation amplifier, as shown in Figure 2B. A full-wave precision rectifier demodulates the signal to yield basal impedance Z of the segment of rat tail. A typical value of Z is about 450 Ω for a voltage electrode separation of 8 mm. In Figure 2C, a D/A converter automatically balances Z of the adhesive tape still attached. This remaining half of the adhesive tape was also cut in half, and the free cut piece was attached to the other side of the aluminum electrode to provide bilateral adhesive tape backing. The rat tail was encircled by four electrodes; two inner electrodes were about 5 to 7 mm apart, and the inner and outer electrodes were about 8 to 10 mm apart. To make low contact impedance, standard electrode gel was spread lightly on each electrode surface and the electrodes were pulled to fit snugly around the tail to make contact with the entire circumference. The better the contact was, the greater the change in impedance (ΔZ) measured, and the less was the effect of 60-Hz power line interference and motion artifacts.

FIGURE 2. The detailed circuit diagram (simplified from that by Shankar and Webster) provides a detailed description of the function of each element: constant current source (A), instrumentation amplifier and full-wave rectifier (B), autbalancing circuit (C), and differentiator and bandpass filter (D). A, B, Z = input or output points connecting the diagrams; MSB = most significant bit; LSB = least significant bit; ΔZ = change in impedance; dZ/dt = differentiated impedance.
to yield pulsatile impedance change $\Delta Z$, which is caused by the pulsatile expansion of the tail artery. $\Delta Z$ is typically 0.2% of $Z$, and $\Delta Z$ is differentiated to yield the derivative of the impedance signal, $dZ/dt$, as shown in Figure 2D. A bandpass filter passes frequencies from 0.5 to 3 Hz to further reduce the effect of respiration and high frequency noise.

Standard tail-cuff method was applied in the determination of SBP, and a pneumatic cuff was inflated to occlude the arterial blood flow. The cuff was then deflated slowly at about 2 mm Hg/sec, and the cuff pressure at which the characteristic pulse signals reappeared was taken as the tail SBP. All the procedures of SBP measurement were performed at a room temperature of 21 to 24°C, and no preincubation at a higher ambient temperature was needed. However, in about 10% of the rats, the pulse signals were blurred by agitation and a resting period of 5 to 10 minutes was necessary to record the signals. On rare occasions, the animals had to be returned to their cages and successful measurements of SBP were obtained later.

The effect of preheating the animals on tail SBP reading was examined by determining SBP in nine normal and 15 diabetic rats before and after heating. SBP was first obtained in the animals while they were kept at a room temperature of 21 to 24°C. They were then put into a thermostatically controlled chamber at 40°C for 5 minutes before the second SBP determination was made. For comparison of the simultaneous direct and indirect measurements of SBP, a cannula was placed in the femoral artery of four normal and six diabetic rats under light anesthesia with ether. The animals were then allowed to wake up, and the comparison of direct and indirect SBP was made in the conscious state. Four of the diabetic rats had been placed on an 8% salt diet to induce hypertension. Diabetic animals were chosen because of the propensity of the diabetic kidney to retain salt, especially with high salt intake. The determination of tail SBP by the impedance technique was performed with simultaneous monitoring of femoral SBP by connecting the arterial cannula through a saline-filled tubing to a pressure transducer (MP-15D, Micron Instrument, Los Angeles, CA, USA) and a strip recorder (recorder Model 350, Harvard Apparatus, Millis, MA, USA).

To test the sensitivity of our differentiated impedance method, we compared tail SBP in the awake normal and diabetic rats. Experimental diabetes mellitus was induced in eight young female rats by an intravenous injection of streptozocin, 55 mg/kg, and the presence of diabetes was confirmed by the presence of polyuria, polydipsia, and glycosuria. Six age-matched female rats were used as the normal controls, and all animals were placed on a regular rat chow diet with free access to water and food. Determination of the tail SBP by the impedance technique was made 8 weeks following the induction of diabetes.

The experimental data were expressed as means ± SEM and were analyzed statistically using paired or unpaired Student's $t$ test between the experimental groups or the experimental maneuvers tested. Linear regression analysis was made by the least-squares method for calculation of the correlation coefficient. 13

Results

The differentiated impedance method as applied to the measurement of tail SBP in the rat was simple but highly sensitive, and the time required for each measurement was 5 to 10 minutes. A representative tracing of the pulse signal $dZ/dt$ obtained in an unanesthetized, unheated rat with the simultaneous recording of the cuff pressure is shown in Figure 3. Occlusion of the tail arterial blood flow clearly flattened the pulse signals to allow the determination of the SBP. The remaining oscillations of $dZ/dt$ during the period of occluded tail blood flow were due to respiration and motion arti-

![Figure 3. A representative tracing of pulse signals that are obliterated by inflation of the tail cuff with simultaneous monitoring of the cuff pressure. The arrow indicates SBP. A tracing of the direct recording of the femoral arterial pressure is also shown. $dZ/dt =$ differentiated impedance.](http://hyper.ahajournals.org/issue/373/BLOOD-PRESSURE-MEASUREMENT-BY-IMPEDANCE-Wen-et-al.)
indirectly measured SBP in six diabetic and four normal awake rats. (Correlation coefficient \( r = 0.98 \); regression equation \( y = 1.05x - 5.43 \).) The dashed line indicates the line of identity, which is nearly superimposed on the regression line.

The accuracy of our indirect method of SBP determination was tested in six diabetic and four normal rats with and without increase in salt intake to obtain various levels of SBP ranging from 92 to 194 mm Hg. SBP was measured in the awake animals following direct cannulation of the femoral artery, and the values were compared with those obtained with the indirect tail-cuff method using the impedance technique. As shown in Figure 4, in 56 paired determinations there was an excellent correlation between SBP readings by the two methods, with a correlation coefficient of 0.98 \((p < 0.0001)\). The dashed line of identity is nearly superimposed on the regression line, further supporting their close correlation. The mean values for each group with respective correlation coefficients are shown in Table 1.

The effect of 5 minutes of preheating on tail SBP was tested in nine normal and 15 diabetic rats, and the results are shown in Figure 5 and Table 2. In both groups of rats, the points are scattered above the line of identity, indicating significant increases in SBP after heating, with the mean value increasing from 128 ± 2 to 134 ± 2 mm Hg.

To examine whether the impedance method is capable of demonstrating subtle differences in SBP among experimental animal groups, we compared SBP between eight young diabetic rats and six age-matched normal rats. The mean values for the two groups were 122 ± 3 and 111 ± 2 mm Hg (femoral SBP, 122 ± 2 and 110 ± 2 mm Hg), respectively, indicating a significant difference \((p < 0.05)\) between the two groups.

### Table 1. Comparison of Simultaneous Determinations of Indirect Tail and Direct Femoral SBP in Normal and Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of paired determinations</th>
<th>Indirect SBP (mm Hg)</th>
<th>Direct SBP (mm Hg)</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ((n = 4))</td>
<td>16</td>
<td>124.8 ± 2.2</td>
<td>125.4 ± 2.3</td>
<td>0.97</td>
</tr>
<tr>
<td>Diabetic ((n = 6))</td>
<td>40</td>
<td>147.0 ± 2.7*</td>
<td>146.1 ± 2.7*</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Values are means ± SEM. The animals were placed on normal or high salt intake. \( r \) = correlation coefficient. *\( p < 0.001 \), compared with the normal group.

### Table 2. Effect of Heating at 40°C for 5 Minutes on the Tail SBP in Normal and Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Control SBP (mm Hg)</th>
<th>SBP after heating (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ((n = 9))</td>
<td>123 ± 2.7</td>
<td>129 ± 3.1*</td>
</tr>
<tr>
<td>Diabetic ((n = 15))</td>
<td>132 ± 2.4f</td>
<td>137 ± 2.8*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *\( p < 0.001 \), compared with the control value. f\( p < 0.02 \), compared with the normal group.
significant increase in SBP by a mean of 6 mm Hg. This artifactual error in the measurement of SBP by preheating may become more significant in the hypertensive state, since greater increases in SBP in hypertensive rats following heating have been reported. With the photoelectric method, preheating is not necessary, but in the hands of some investigators this technique requires preincubation of the animals at 27 to 30°C for 30 minutes because it becomes unreliable whenever the room temperature falls below 27°C. Our technique of differentiated impedance appears to be more sensitive in that accurate SBP measurement can be made at the ambient temperature of 21 to 24°C and no preincubation period is required.

The SBP obtained by the impedance method has excellent correlation with that determined by direct cannulation of the femoral artery, thus validating the technique. The sensitivity is such that the small but significant difference in SBP between normal and diabetic rats can be easily demonstrated. The designs for recording the differentiated impedance are relatively simple and can be constructed by most electrical engineering laboratories.

In summary, a simple method to record the pulse signals in the rat tail using the technique of differentiated impedance is described. The technique can be used, along with the tail-cuff method, to measure SBP in awake rats without preheating. The method is highly sensitive and can be used at the ambient temperature of 21 to 24°C.

References
An impedance method for blood pressure measurement in awake rats without preheating.
S F Wen, J M Tremblay, M H Qu and J G Webster

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