Tail-Cuff Blood Pressure Measurement without External Preheating in Awake Rats

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SUMMARY A new photoelectric sensor capable of detecting tail pulses even in unheated rats was tested for accuracy in indirect measurements of blood pressure. This sensor proved more sensitive than a Doppler ultrasonic flowmeter because it allowed detection not only of tail pulsations without preheating but also of peak oscillations usable for estimating mean arterial pressure. After blood pressures in anesthetized rats were elevated with norepinephrine or lowered with sodium nitroprusside, systolic pressures determined with the photoelectric sensor were almost identical with those recorded concurrently from femoral catheters (r = 0.939). Cuff pressure at peak oscillations in the tail correlated better with femoral mean pressure than with femoral diastolic pressure. However, similar comparisons in awake rats with chronically implanted carotid catheters showed that, although correlation between tail-cuff and carotid systolic pressures remained significant (r = 0.962), the correlation between peak tail oscillations and either mean or diastolic pressure was not. When systolic pressures were measured indirectly once a week for 7 weeks in unheated awake rats, normotensive rats could be easily distinguished from streptozotocin-diabetic and DOCA-salt hypertensive rats. (Hypertension 4: 898-903, 1982)

KEY WORDS • blood pressure • DOCA-salt hypertension • mean pressure • norepinephrine • sodium nitroprusside • streptozotocin-induced diabetes • systolic pressure • tail-cuff method • unanesthetized rats

To study experimental hypertension in rats, one must be able to measure blood pressure accurately and repeatedly for several weeks in awake rats. Direct recording from arterial catheters is undoubtedly the most accurate method now available. However, surgical implantation of indwelling catheters and subsequently keeping them open require considerable technical skill. Thus, many laboratories rely on indirect measurement with the tail-cuff method. With most methods, the arterial pulsations used as endpoints for indirect determination of systolic pressure can be detected in the tail only after vasodilation has been induced by preheating the rats. Because some investigators feel that externally applied heat falsely elevates blood pressure, however, they have used sensors that can detect tail pulsations in unheated rats. These sensors have without exception been custom-made until recently when IITC Inc (Landing, New Jersey) made available the photoelectric sensor described originally in Yen et al. Our studies evaluated this new IITC photoelectric sensor.

Methods

All experiments were done on male and female Sprague-Dawley rats weighing 200 to 300 g, purchased from SASCO Inc (Omaha, Nebraska). Chronic experiments involving repeated tail-cuff measurements were carried out on 32 rats of which eight were normotensive, 14 had streptozotocin-induced diabetes, and 10 had DOCA-salt hypertension. Diabetic rats were pretreated with streptozotocin, 50 mg/kg, injected intravenously. To produce DOCA-salt hypertension, silicon rubber molds containing DOCA (deoxycorticosterone acetate, 150 mg/kg) were implanted subcutaneously, the left kidney was removed, and drinking water was replaced with 0.9% sodium chloride solution.

For indirect measurements of blood pressure, a photoelectric sensor like that described by Yen et al. was used.
(manufactured by IITC Inc, Landing, New Jersey) was used for endpoint detection. The sensor, consisting of a miniature focused light and a photoresistive cell mounted in a 50 mm long inflatable rubber cuff, was placed on the base of the tail in rats that had been kept in a rat holder for at least 30 minutes. Room temperature was maintained constant at 27°C. Pulse signals from the photocell were fed into an amplifier (Model 47, IITC Inc) for regulation of gain, offset, and intensity of the light source. A programmed electrophysmomanometer (Narco Bio-Systems Inc, Houston, Texas) was connected to the rubber cuff to keep inflation-deflation rates constant and register pressure changes with a transducer. Signals from the photocell amplifier and cuff pressure transducer were recorded continuously on separate channels of an ink-writing recorder (figs. 1 and 2).

Other tail-cuff measurements were recorded similarly using a Doppler ultrasonic flowmeter (Parks Electronics Laboratory, Beaverton, Oregon) to detect arterial pulsations during deflation of a standard 20 mm tail-cuff. Initial comparisons were done by placing photoelectric and ultrasonic sensors alternately on the base of the tail in eight rats that had been trained to stay in the rat holder for 30 minutes. To record tail pulsations with both sensors, 12 other rats were preheated (to dilate their tail vessels) first for 15 minutes at 39°C in a warming box and then in a restrainer with the baseplate heated electrically to 38°C. Rectal temperature changes were determined using a digital rectal thermometer (M99, Heathkit, Benton Harbor, Michigan) and a 405 probe connected to a 73A temperature controller (Yellow Springs Instruments, Yellow Springs, Ohio) recorded ambient temperature in the rat holder.

For simultaneous recording of phasic arterial pressure during drug infusions, rats were anesthetized with amobarbital sodium (6.5 mg/100 g.i.p.), and catheters inserted into a femoral artery were connected to a low-volume-displacement pressure transducer (Model P23Gb Statham Instruments, Inc., Oxnard, California). Another catheter was inserted into a jugular vein. and freshly-prepared solutions of norepinephrine bitartrate (Levophed, Sterling Drug, Inc, New York, New York) or sodium nitroprusside (Sigma Chemical Company, St. Louis, Missouri) were infused continuously for 3 to 5 minutes at rates of 0.06 to 0.17 ml/min. Ensuing doses were 240 to 680 ng/min for norepinephrine and 150 to 300 ng/min for sodium nitroprusside. To allow simultaneous recording of phasic arterial pressure in awake rats, rats were transiently anesthetized with methoxyflurane (Metofane by inhalation) for surgical implantation of indwelling carotid catheters, and recordings were made 1 to 3 days later.

Data, expressed as average ± SEM, were usually analyzed using two-tailed t tests to compare means of independent samples. Differences at a 5% level (p < 0.05) were considered significant. Simultaneously recorded tail-cuff and femoral pressures were compared using regression analysis to determine the Pearson product-moment correlation, slope, and intercept for different pairs. For weekly tail-cuff measurements from three rats groups, an analysis of variance was used, and for F-ratios significant at 5% or less, Duncan’s multiple range test was applied to determine the significance of differences between pairs of means.

Results
Comparison of Photoelectric and Doppler Sensors
When each sensor was placed alternately for 30 minutes on the tail of rats that had not been preheated, pulsations were discerned in all rats with the photoelectric sensor, but in only one rat out of eight with the Doppler sensor. By contrast, in 12 other rats that had been preheated tail pulsations were easily detected with either sensor, but the tracings looked very different. The amplitude of the pulse oscillations registered with the photoelectric sensor increased progressively in a crescendo that peaked before fading (fig. 1 A) while that recorded with the Doppler sensor did not follow any regular pattern (fig. 1 B).

Even with the photoelectric sensor, however, pulsations could be detected without preheating only in rats that had been confined in the holder for 30 minutes. At a constant room temperature of 27°C, ambient temperature in the holder, initially at 27.3°C ± 0.1°C at the onset, increased almost equally whether preheating was applied or not; the average level attained was 32.2°C ± 0.2°C during measurements in preheated rats as compared with 32.5°C ± 0.3°C in those that had not

FIGURE 1. Arterial pulsations recorded from the tail of an awake, preheated rat using the photoelectric (A) and Doppler (B) sensors. Upward arrows indicate endpoints for reading systolic pressure while the downward arrow shows the cuff pressure level at peak arterial oscillation determined with the photoelectric sensor.
Simultaneous Recordings of Tail-Cuff and Femoral Arterial Pressures in Anesthetized, Preheated Rats

Tail-cuff and femoral arterial pressures were recorded simultaneously during intravenous infusion of norepinephrine (n = 6) or sodium nitroprusside (n = 7) into preheated rats anesthetized with amobarbital. Basal femoral pressures were: 130 ± 5 mm Hg systolic, 100 ± 5 mm Hg mean, and 85 ± 5 mm Hg diastolic. Systolic pressure measured with the photoelectric sensor averaged 128 ± 5 mm Hg and was, therefore, almost the same as femoral systolic pressure. Cuff pressure at peak tail-cuff oscillation, averaging 99 ± 6 mm Hg, was higher (p < 0.005) than femoral diastolic but almost identical with femoral mean pressure. Correlation between tail-cuff and femoral systolic pressures was highly significant (r = 0.939; fig. 3). Paired comparisons between peak tail-cuff oscillations and either diastolic or mean pressure in the femoral artery also gave significant correlation coefficients (r = 0.836 and 0.909, respectively), the correlation being better with mean than with diastolic pressure.
INDIRECT BLOOD PRESSURE MEASUREMENT IN RATS/Bunag and Butterfield

1 mean, and 75 ± 1 diastolic. Systolic pressures measured with the photoelectric sensor were exactly the same as those recorded from the carotid artery, averaging 137 ± 1 mm Hg. When 210 pairs of simultaneously recorded readings were compared, the resulting correlation coefficient of 0.962 (slope 1.023, y intercept −3.485) was highly significant (p < 0.001). Similar comparisons based on peak oscillations in 174 tail-cuff tracings, however, gave an average pressure level of 104 ± 1 mm Hg, which was much higher than either mean or diastolic carotid pressure (p values for both comparisons were < 0.001) and neither of the resulting correlation coefficients (0.489 for mean and 0.664 for diastolic) were significant.

Repeated Tail-Cuff Measurements as a Means for Separating Normotensive From Hypertensive Rats

To determine whether blood pressure could be measured chronically in awake rats without preheating, tail-cuff pressures in three groups of rats (8 normotensives, 14 streptozotocin diabetics, and 10 DOCA-salt hypertensives) were recorded once a week for 7 weeks, with the photoelectric sensor in a room where ambient temperature was kept constant at 27°C. Pressures recorded during three to five successive inflation-deflation cycles (Fig. 4) were averaged to obtain a single weekly reading.

Systolic pressures were lowest in normotensive rats and highest in the DOCA-salt hypertensives, with those in diabetics being in between (Table 1). Clear-cut tracings were obtained from all rats on Weeks 3, 4, and 7, but some others had to be discarded for various reasons. Tail pulsations could not be detected in half of the rats during Week 1, possibly because of vasoconstriction caused by fright during initial exposure to the procedure, and during Week 6 the inadvertent presence of barking dogs in a nearby room may also have...

![Figure 4: Three successive tracings of tail-cuff arterial pressures recorded without preheating in an awake diabetic rat. Upper tracing of cuff pressure with arrows marked as in fig. 1. Bottom tracing of arterial pulsations detected by the photoelectric sensor.]

### Table 1. Weekly Systolic and Mean Pressures (mm Hg) Determined Using the Photoelectric Sensor without External Preheating in Awake Rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Normotensive</th>
<th>Diabetic</th>
<th>DOCA-salt</th>
<th>F-ratio</th>
</tr>
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<tr>
<td>Systolic pressures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>110 ± 18 (3)</td>
<td>130 ± 5 (6)</td>
<td>148 ± 6 (7)</td>
<td>5.11</td>
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<tr>
<td>2</td>
<td>113 ± 3 (7)</td>
<td>136 ± 4† (14)</td>
<td>150 ± 6†† (10)</td>
<td>12.08</td>
</tr>
<tr>
<td>3</td>
<td>114 ± 5 (8)</td>
<td>142 ± 7† (14)</td>
<td>159 ± 5†† (10)</td>
<td>11.29</td>
</tr>
<tr>
<td>4</td>
<td>131 ± 3 (8)</td>
<td>141 ± 3†† (14)</td>
<td>166 ± 4†† (10)</td>
<td>28.21</td>
</tr>
<tr>
<td>5</td>
<td>126 ± 2 (8)</td>
<td>140 ± 4† (14)</td>
<td>157 ± 4†† (10)</td>
<td>15.90</td>
</tr>
<tr>
<td>6</td>
<td>120 ± 2 (3)</td>
<td>146 ± 3†† (5)</td>
<td>164 ± 4†† (10)</td>
<td>22.55</td>
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<tr>
<td>7</td>
<td>124 ± 2 (8)</td>
<td>148 ± 3†† (14)</td>
<td>165 ± 5†† (10)</td>
<td>25.42</td>
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<tr>
<td>Mean pressures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>91 ± 26 (2)</td>
<td>95 ± 3 (6)</td>
<td>120 ± 3 (4)</td>
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<td>101 ± 3† (13)</td>
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<tr>
<td>3</td>
<td>89 ± 3 (6)</td>
<td>96 ± 3 (12)</td>
<td>134 ± 5†† (10)</td>
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<tr>
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<tr>
<td>7</td>
<td>96 ± 2 (4)</td>
<td>111 ± 15 (3)</td>
<td>139 ± 5†† (10)</td>
<td>12.46</td>
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</tbody>
</table>

Data expressed as averages ± SEM from eight normotensive, 14 diabetic, and 10 DOCA-salt hypertensive rats. Number of rats with readable tracings enclosed in parentheses. With f1 = 2, and f2 = 13 to 29, F-ratios exceeding 3.80 are significant at 5% and those exceeding 6.70 are significant at 1%.

†R-value significant at 5% compared with normotensive group.
‡R-value significant at 5% compared with diabetic group.
provoked severe vasoconstriction. Despite missed readings, F-ratios and most R-values were significant at 5% so that three different rat groups could be separated on the basis of their systolic pressures.

A similar classification based on mean pressures was not possible, however, as differences between normotensive and diabetic groups were no longer pronounced (table 1). Although weekly F-ratios remained highly significant, only on Week 2 were R-values comparing the normotensive and diabetic groups significant at the 5% level. These results indicate that while both systolic and mean pressures can be measured with the photoelectric sensor without preheating, systolic pressures are more dependable than mean pressures for separating normotensive from hypertensive rats.

Discussion

The present method offers two distinct advantages over other tail-cuff methods: it allows indirect measurement of systolic pressure in awake rats without preheating, and it gives a reasonable estimation of mean arterial pressure. Of the two sensors compared here, the Doppler measures velocity of blood flow, and the photoelectric measures volume.11 Perhaps because Doppler sensors require blood flow beyond the occluding cuff while photoelectric sensors do not,12 peak oscillations can be registered with the photoelectric but not with the Doppler sensor (fig. 1). Although previous studies on isolated arterial strips imply that cuff pressure at the point of maximum oscillation closely approximates the true mean arterial pressure,13 our results show that in awake rats estimates of mean or diastolic pressure are neither as accurate nor as reliable as those for systolic pressure.

Rats use their tails to regulate body temperature, and their tail vessels dilate at environmental temperatures between 27° and 30° C;14 this may be the reason why measurements with the present method became unreliable whenever room temperature fell below 27° C. Adequate pulses occur in heated rats when rectal temperature is elevated by 0.8° C,15 and our results indicate that rectal temperatures become comparably elevated when rats are kept in a holder for 30 minutes. Blood pressure levels obtained when preheating is used may be artificially high because externally applied heat can have considerable pressor effects.16,17 Measurement errors can be minimized by applying just enough heat to increase pulse volume to 25% of the maximum obtainable,18 but other errors may occur particularly in rats with varying susceptibilities to thermal stress. For instance, pressures determined in spontaneously hypertensive rats with tail-cuff methods that require preheating may be spuriously elevated because spontaneously hypertensive rats are more susceptible to heat than their normotensive counterparts.19

Most difficulties during tail-cuff measurements result from failure to detect tail pulsations because of vasoconstriction. Accurate measurement is impossible whenever the rats are either uncomfortable or unfamiliar with surrounding conditions20 because vasoconstriction invariably occurs as a reaction to stress. In slightly warmed but unanesthetized rats, barely audible 1-second bursts of noise can elicit reproducible vasoconstriction.21 By using a heated baseplate in the rat holder to keep the tail vessels dilated,22 endpoints can be detected during measurements on preheated rats even when vasoconstriction occurs. With the present method, despite the elevated rectal temperature occurring in rats confined in the holder for 30 minutes, the ensuing vasodilation is evidently not strong enough to overcome subsequent stress-induced vasoconstriction. Hence, we obtained fewer readable tracings when the rats were frightened either by initial exposure to the procedure at Week 1, or by the threat of barking dogs at Week 6 (table 1).

To minimize inaccuracies in indirect measurement, any tail-cuff method should be validated under the same experimental conditions that would exist when it is used routinely later. Blood pressure data obtained with methods that were validated elsewhere will always be suspect simply because conditions in different laboratories are never exactly alike. Another useful precaution, particularly for small changes in blood pressure, would be to verify any such changes by direct measurement from indwelling arterial catheters.23,24

References

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