Continuous Monitoring of Arterial Pressure Indicates Sinoaortic Denervated Rats Are Not Hypertensive

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SUMMARY The mean arterial pressure (MAP) of nine sinoaortic denervated (SAD) and eight control rats housed in standard-sized metabolic cages was determined continuously via aortic cannulae and computerized data collection over 24 hours. These continuous measurements were compared with direct, mean aortic pressure measurements and indirect, tail-cuff systolic pressure determinations made while these rats were resting in a Lucite restrainer. Denervated rats were studied 1 month after debuffering. Both types of measurements made during restraint indicated that the SAD rats were hypertensive; the MAP averaged 145 ± 3.4 mm Hg (mean ± SEM) in SAD rats compared with 119 ± 2.8 mm Hg in the control group (p < 0.001), and the tail-cuff pressure in SAD rats was 156 ± 5.4 vs 121 ± 2.7 mm Hg in control rats (p < 0.001). In contrast, continuous monitoring showed that the SAD rats were normotensive; the MAP averaged 119 ± 3.1 mm Hg in the SAD group and 119 ± 2.8 mm Hg in the control group. Denervation increased pressure lability; the average 24-hour standard deviation of MAP was 19.0 ± 1.2 mm Hg in SAD rats vs 8.0 ± 0.7 mm Hg in control rats (p < 0.001). Apparently, arterial pressure is elevated during restraint in SAD rats because buffering by the baroreceptor reflex is absent, and pressure measurements made under these conditions give a false indication of hypertension. (Hypertension 3: 119-125, 1981)

KEY WORDS • baroreceptor denervation • sphygmomanometry • arterial pressure measurement

The effect of arterial baroreceptor denervation on arterial pressure is a subject of continuing controversy. Many early studies indicated that baroreceptor denervation in dogs results in hypertension. However, Cowley et al. determined that the MAP of baroreceptor-denervated dogs was only 11 mm Hg greater than that of control animals if the pressure was monitored 24 hours per day. A recent report indicates that these investigators have now recorded the MAP throughout the day in over 40 control and baroreceptor-denervated dogs, and the MAP in the denervated group averaged just 1 mm Hg greater than in the control. These SAD dogs had a much broader range of arterial pressure over a 24-hour period, but the MAP dropped to hypotensive levels as often as it rose to hypertensive levels. There are other reports, though, that baroreceptor denervation in dogs causes at least a mild hypertension.

The effect of baroreceptor denervation on the arterial pressure level of rats has been much less controversial, and in general the SAD rat has been accepted as a model for "neurogenic hypertension." Studies of SAD rats have focused on determining the mechanism of this "neurogenic hypertension." Krieger reported that cardiac output is normal in SAD rats, and that the hypertension is due to increased total peripheral resistance. Alexander et al. demonstrated that plasma dopamine beta-hydroxylase is elevated in these denervated rats, and that plasma volume is decreased during the first few days following denervation but is normal thereafter. Chalmers et al. reported that altered activity of central adrenergic and noradrenergic neurons may be important in initiating and maintaining hypertension in the SAD rat. However, the controversy about whether baroreceptor denervation of dogs produces hypertension suggested that the techniques used to measure pressure in SAD rats might be crucial in interpretation of these results.

This study was designed to quantitate the effect of sinoaortic denervation on the blood pressure of rats during nominal restraint and during residence in a normal laboratory environment. The MAP was monitored 24 hours per day in conscious rats maintained in standard-sized metabolic cages. These continuous MAP data were then compared with pressures determined by two techniques for pressure measurement in conscious but restrained rats: tail-cuff sphygmomanometry and acute monitoring of MAP via indwelling arterial cannulae.

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Methods

Experiments were performed on male Wistar rats (8 control rats and 9 SAD rats) weighing 340 to 440 g. Data were collected in the SAD rats 3 to 5 weeks following the surgical denervation of the baroreceptors.

Sinoaortic Denervation

The technique used to produce baroreceptor denervation was that reported by Krieger, except that the denervation was performed in two stages rather than in a single operation. The rats were anesthetized with chloral hydrate (approximately 30 mg/100 g body weight), and the cervical region was infiltrated with 4% lidocaine. The first stage of the surgery included denervation of the left carotid sinus by vascular stripping of all the nervous and connective tissue from the carotid bifurcation and sinus region and painting with a solution of 10% phenol in alcohol. Also, the aortic baroreceptors were partially denervated by sectioning the left superior laryngeal nerve and bilaterally sectioning the cervical sympathetic chains and the aortic depressor nerves when present. The rats were then allowed to recover for 1 to 2 weeks before completion of the sinoaortic denervation. For the second stage of the denervation, the rats were anesthetized as before, the right carotid sinus denervated, and aortic denervation completed by sectioning the right superior laryngeal nerve.

Experimental Protocol

Arterial catheters were implanted in the control and SAD rats 2 to 4 days prior to starting data collection (approximately 1 month after complete denervation in the SAD rats). The rats were anesthetized with sodium pentobarbital (40 mg/kg), and a polyethylene catheter (PE 10) was inserted into the lower abdominal aorta via the left femoral artery. The catheter was then tunneled subcutaneously and exteriorized between the scapulae. The catheter was protected by a lightweight metal spring and was attached to a Statham pressure transducer via a rotating adaptor so that the rat could move freely within its standard-sized metabolic cage. An infusion line was connected to the arterial catheter, and 0.5 ml/hr of Ringer's solution containing 30 units/ml of heparin was infused continuously by a syringe pump (Harvard Apparatus) to maintain patency of the catheter. The metabolic cages were located in a quiet experimental laboratory maintained at 70°–75°F and illuminated by sunlight and, in addition, by fluorescent lighting from about 8 a.m. to 5 p.m. The rats had free access to food (Purina Rat Chow) and water.

To accustom the rats to having pressure measured by the tail-cuff technique, each rat underwent at least two preliminary series of systolic pressure determinations during the week preceding data collection. Each rat in both the control and SAD groups then underwent three series of arterial pressure measurements: measurement during restraint, continuous measurement over a 24-hour period, and finally measurement again during restraint. During the morning of the first day of data collection, the rat was removed from its metabolic cage and placed in a small animal study unit (Lucite housing with a temperature-controlled base plate; Narco Biosystems, Inc.) for pressure measurements. Systolic pressure of each rat was determined by averaging three to four readings obtained by the tail-cuff technique with a programmed electrophygysmomanometer (Narco Biosystems, Inc.). For comparison with the pressures determined by the tail-cuff technique, mean arterial pressure was determined simultaneously via the aortic catheter. This MAP during restraint in each rat was calculated by averaging the mean pressures recorded at the time of each tail-cuff pressure determination. The rat was then returned to its metabolic cage, and MAP and heart rate were determined continuously from noon until noon of the following day. The rat was then removed again from its metabolic cage and tail-cuff pressures with simultaneous direct MAP were measured as before.

Continuous Mean Arterial Pressure and Heart Rate Measurement

Computerized data collection and analysis techniques were used during the 24-hour period of continuous MAP and heart rate measurement. The pulsatile pressure waveform was monitored on a Grass Polygraph recorder, and analog-to-digital conversion was accomplished using a Digital Equipment Corporation PDP 11/70 computer. The pressure waveform was sampled 500 times during 1 second (interval equal to 2 msec) of each minute over the 24-hour period. The pressure maximums of the digitized data were identified, and the MAP was obtained by integration over the largest number of complete pressure cycles in the 1-second sample of the pressure waveform. Heart rate was calculated from the average interval between the pressure maximums. The mean pressure and heart rate during each minute were then stored for later analysis.

Test of Acute Baroreceptor Reflex Activity

Baroreceptor function was tested in six control rats and in six SAD rats by determining the decrease in heart rate in response to an increase in MAP caused by bolus intravenous injections of phenylephrine (2 μg/kg). A jugular catheter was implanted either at the time of aortic catheterization or upon completion of the 24-hour period of MAP measurement. The response of each conscious rat was determined by averaging the results of three to four separate phenylephrine injections on the day after continuous MAP monitoring (3–5 weeks after denervation of the SAD rats).

Statistical Analysis

Unpaired Student's t tests were used to compare pressure and heart rate measurements of SAD rats.
with those in the control rats. Analysis of variance was used to determine if there were significant differences among the three types of arterial pressure measurements within each group. All data are reported as mean ± SEM.

Results

Acute Baroreceptor Reflex Activity

The average ratio of the decrease in heart rate in response to an increase in MAP produced by intravenous injection of phenylephrine was 2.41 ± 0.16 beats/min/mM Hg in six control rats. In six SAD rats this ratio was markedly attenuated to 0.30 ± 0.14 beats/min/mM Hg (p < 0.001), indicating successful baroreceptor denervation.

Pressure Measurements Made During Restraint

Tail-cuff pressure and simultaneous MAP (via aortic cannulae) were determined while the rats were resting in a Lucite restrainer both before and after the 24-hour period of continuous MAP monitoring. During restraint the MAP recordings were elevated and much less labile compared to recordings made during continuous monitoring while the rats were in metabolic cages. Inflation of the cuff during the sphygmomanometer readings caused no large or consistent fluctuations in the MAP records. The average of the MAP measurements made during restraint prior to the continuous recording period in the SAD rats was 150 ± 2.8 mm Hg in contrast to 119 ± 2.6 mm Hg in control rats (p < 0.001). The average tail-cuff pressure in the SAD rats (155 ± 4.4 mm Hg) was also significantly (p < 0.001) greater than that found in the control rats (123 ± 3.4 mm Hg). Likewise, pressure measurements made during restraint following completion of the continuous recording period indicated SAD rats were hypertensive. The MAP was increased (p < 0.01) from 120 ± 4.1 mm Hg in control rats to 141 ± 4.7 mm Hg in SAD rats, and tail-cuff pressure was increased (p < 0.001) from 119 ± 4.7 to 156 ± 7.6 mm Hg in denervated rats.

Mean Arterial Pressure and Heart Rate Determined by Continuous Monitoring Over 24 Hours

Representative MAP tracings during a 1-hour period for a control and a SAD rat are shown in figure 1. Pressure in the normal rat was very stable, while baroreceptor denervation resulted in marked lability of the arterial pressure level. It was observed that the MAP in the SAD rat could fluctuate as much as 100 mm Hg within less than 1 minute. In sharp contrast to what has been reported in SAD dogs, the MAP oscillations of the denervated rats did not appear to be correlated with extraneous laboratory activity near the rats.

The average MAP for the eight control rats and nine SAD rats from minute-to-minute over a 24-hour recording period are given in figure 2. All recordings were taken from noon until noon of the following day.
greatly increased variability of MAP in the SAD rats is to consider the average of the standard deviations of MAP over 24 hours in each rat, as shown in table 1. The average 24-hour standard deviation of MAP in SAD rats is 138% greater than that found for the control rats ($p < 0.001$).

The average minute-to-minute heart rates (dots = ± 1 SEM) over 24 hours in the control and SAD groups of rats are shown in figure 4. The average heart rate of the control group was 393 beats/min (table 1), and this was not changed by denervation (382 beats/min). The heart rate in both groups of rats was higher at night when the rats were more active.

Figure 5 gives the average frequency distribution (histogram bin width = 10 beats/min) of the heart rate over 24 hours in the two groups of rats. In both
SINOAORTIC DENERVATED RATS ARE NORMOTENSIVE/Norman et al.

FIGURE 4. Average heart rates (dots = ±1 SEM) over a 24-hour period in the control and baroreceptor denervated rats.

Discussion

Several lines of evidence indicate the rats in the SAD group were effectively denervated. The technique of surgical denervation utilized in this study was essentially the same as that reported by numerous investigators, and the values of the pressure measure-
ments made during restraint following this denervation procedure agree closely with those reported in previous studies.\textsuperscript{8, 10, 12} Also, the phentolamine tests demonstrate nearly complete attenuation of the heart rate response to changes in MAP. This commonly used test of baroreceptor reflex function assesses only changes in heart rate mediated by this reflex and not changes in vasomotor activity. However, results of continuous pressure monitoring indicate the extreme importance of the baroreceptor reflex in stabilization of arterial pressure. The widely fluctuating MAP recordings from SAD rats (fig. 1) indicate loss of this control as does the large increase in the width of the frequency distribution of the MAP (fig. 3). Sinoaortic denervation results in a 138\% increase in the standard deviation of MAP over 24 hours.

Table 2 compares the MAP determined by 24-hour recording in the control and SAD groups of rats with the average tail-cuff pressure and direct MAP recorded during restraint. Analysis of variance indicated no differences among the three types of pressure measurements in the control rats, but it did indicate that there is a significant difference ($p < 0.001$) among the types of pressure determinations in SAD rats. Both measurements made during restraint would indicate that the SAD rats are hypertensive. However, results of the continuous measurement of arterial pressure 24 hours per day in SAD rats maintained in metabolic cages indicate that baroreceptor denervation (at least by the Krieger\textsuperscript{a} technique) did not result in any change of MAP from control. Therefore, the SAD rat is probably not a good model for studies of "neurogenic hypertension." Removing the SAD rat from its normal laboratory housing and making pressure observations while the rat is under even loose restraint apparently excites the animal, and the arterial pressure response is not buffered due to absence of the baroreceptor reflex. This results in the determination of increased pressure levels and gives a false indication of hypertension.

The observation that continuously recorded pressure in SAD rats shows that they are normotensive is supported by results of a study by Vann Jones and Hallback.\textsuperscript{14} They reported that acute measurements in baroreceptor-denervated rats indicate a pressure elevation of about 15\%, but that these rats do not appear to have persistent hypertension since no structural cardiovascular changes were found 4 months following denervation. In addition, the results of the present study agree with continuous pressure measurements in baroreceptor-denervated dogs by Cowley et al.\textsuperscript{9} which suggested that SAD dogs are normotensive. However, SAD rats appear to be different from denervated dogs in that environmental stimuli have been reported to drastically influence pressure fluctuations and MAP in denervated dogs.\textsuperscript{6, 13} While the observations in this study indicate that activity in the laboratory surrounding the rats appears to have little or no influence on pressure fluctuations or mean pressure as long as the rat is not handled or removed from its cage.

Since both pressure measurements made during restraint (tail-cuff systolic pressure and direct MAP) in the control rats gave the same value, the full level of systolic pressure was not measured by the tail-cuff technique. In Krieger's original description of the production of "neurogenic hypertension," he reported that in both control and SAD rats use of the tail plethysmographic technique gave values 10 to 20 mm Hg lower than the MAP values obtained by simultaneous direct recording. These are examples of the general problem of obtaining consistent tail-cuff pressure determinations from one laboratory to the next, and the fact that indirect pressure measurement

| Table 1. Mean Arterial Pressure and Heart Rate Determined by Continuous Recording Throughout a 24-hour Period, and the Average of the 24-hour Standard Deviations of These Variables (mean ± SEM) |
|-----------------|-----------------|-----------------|-----------------|
| Rat                          | Mean arterial pressure (mm Hg) | Standard deviation of mean arterial pressure (mm Hg) | Heart rate (beats/min) | Standard deviation of heart rate (beats/min) |
| Control (n = 8)             | 119 ± 3.1        | 8.0 ± 0.7       | 393 ± 15.1      | 39.3 ± 2.4       |
| Sinoaortic denervated (n = 9) | 119 ± 4.7        | 19.0 ± 1.2*     | 382 ± 15.7      | 36.7 ± 2.5       |

*p < 0.001 compared with control.

| Table 2. Comparison of Arterial Pressures Determined by Continuous Recording in Rats Housed in Standard-sized Metabolic Cages With the Average Pressures Measured During Restraint (mean ± SEM) |
|-----------------|-----------------|-----------------|
| Rat                          | Continuous mean arterial pressure (mm Hg) | Restraint mean arterial pressure (mm Hg) | Systolic pressure (mm Hg) |
| Control (n = 8)             | 119 ± 3.1        | 119 ± 2.8       | 121 ± 2.7       |
| Sinoaortic denervated (n = 9) | 119 ± 4.7        | 145 ± 3.4*      | 156 ± 5.4*      |

*p < 0.001 compared with control.

\textsuperscript{1}p < 0.001 compared with pressure measurements made during restraint in the sinoaortic denervated rats.
depends on the location of the cuff on the tail, the cuff width, and the temperature of the rat.10-18 The important point to note in the present experiment, however, is that the same procedure for tail-cuff systolic pressure determinations was consistently applied to all rats (control and SAD). The fact that SAD rats appear to be hypertensive when their pressures are measured during restraint and that this may be due to excitement or increased reactivity to stress appears to be consistent with many observations that have been made on baroreceptor-denervated animals. SAD rats have been reported to have elevated dopamine beta-hydroxylase activity,10 and the increased blood pressure reported in denervated dogs has been linked to increased sympathetic nervous system activity.9 Also, many investigators have reported elevated heart rates in SAD rats.10, 12

Recent studies in both dogs7, 11 and rats5, 9 have indicated that aortic baroreceptor denervation is the most important step in producing "neurogenic hypertension." Denervation of the aortic baroreceptors, while leaving the carotid baroreceptors intact, has been reported to produce increased pressure but not the increased pressure lability seen after sinoaortic denervation. Lesions of the nucleus tractus solitarii11, 51, 52 of dogs, cats, and rats have also been reported to result in a hypertension but with increased arterial pressure lability comparable to that which has been reported in SAD rats. These lesions may result in a more complete baroreceptor denervation and might also destroy other pathways that normally inhibit sympathetic function. However, a recent report by Snyder et al.53 in which arterial pressure was measured for 1-hour periods in unrestrained rats indicated that nucleus tractus solitarii lesions result in greatly increased pressure lability but no chronic change in the level of MAP — a finding identical to that reported in our current study of SAD rats. The results of our study suggest that in these types of experiments, in which nervous mechanisms for arterial pressure control have been interfered with, determinations of the mean level and variability of arterial pressure depend critically on the techniques used for pressure measurement. In any animal that might be expected to exhibit increased reactivity to stress or that might have reduced ability to buffer disturbances in arterial pressure, pressure measurements made during restraint may not be reliable estimates of the average pressure throughout the day.

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