Carotid and aortic baroreflexes of the rat: I. Open-loop steady-state properties and blood pressure variability

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Dworkin, Barry R., Susan Dworkin, and Xiaorui Tang. Carotid and aortic baroreflexes of the rat: I. Open-loop steady-state properties and blood pressure variability. Am J Physiol Regulatory Integrative Comp Physiol 279: R1910–R1921, 2000.—To characterize the baroreflex in central nervous system-intact neuromuscular-blocked rats, we measured the vascular and cardiac responses and compared direct stimulation of the aortic depressor nerve (ADN) with a capacitance electrode (differentially activating either A or A + C fibers) to carotid sinus pressure with a micro-balloon (SINUS). One-thousand-two-hundred-ninety-seven open-loop measurements of systolic blood pressure (SBP), heart rate, venous pressure (VBP), and mesenteric (msBF), femoral (fmBF), and skin (skBF) blood flow were completed; the linear range of the effects was determined for each response and stimulus mode. The rats were sinoaortic denervated (SAD). The open-loop stimulation effect was very stable; e.g., the mean effect of 790 ADN stimulations during >7 days was −9.8 mmHg, with an average drift of +0.001 mmHg/h. In contrast, there was large variability of the SBP baseline (e.g., SD = ±10.9), which was due to SAD (±6.3 to ±16.3 mmHg, t = −13.9, df = 4, P < 0.0002) and was reversed by ganglionic block (±10.8 to ±2.9 mmHg, t = −12.9, df = 3, P < 0.001). The ADN stimuli produced larger depressor responses than sinus stimuli (−66 vs. −45 mmHg); all component responses paralleled the magnitude of the SBP effect, except interbeat interval (IBI), for which the ADN ΔIBI was = 10 times that of SINUS. For all stimuli, fmBF increased and msBF did not. Mesenteric and femoral vascular conductance both increased, whereas VBP decreased and skBF followed SBP. We found that for all baroreflex response components, with the exception of SINUS-elicited ΔIBI, there was an orderly, substantially linear, relationship between stimulus strength and response magnitude.

Baroreceptors; aortic depressor nerve; carotid sinus; sinoaortic denervation; noise; baroafferent stimulation

The baroreflexes are the major mechanism of blood pressure (BP) stabilization and are probably the most thoroughly studied example of a regulatory reflex. Two of the central observations in the reflex control of the circulation are that stimulation of the baroreceptors decreases BP (21, 30, 33) and that with destruction of the baroreceptors [sinoaortic denervation (SAD)], there is greatly increased BP variability (2, 4, 5, 20, 23, 32, 36, 38). It is likely that these phenomena are immediately related, but there have been no studies, within the same subjects, that actually reconcile the quantitative properties of the baroreflex with the effects of denervation. There is ample theory to support such an analysis: the mathematical relationship between the input and output spectra and transfer function are well defined for approximately linear systems. What is needed is an appropriate experimental model. The rat is increasingly an important species in the study of cardiovascular regulation; however, although the rat literature on SAD variability is extensive, the only detailed open-loop measurements of baroreflex properties have been acute with surgical-level anesthesia or in animals that were just operated (6). Previously, we described classical conditioning of cardiovascular responses (7, 8), including discriminative auditory conditioning of the aortic depressor nerve (ADN)-elicited baroreflex (8), in intensively maintained, unanesthetized, neuromuscular-blocked (NMB) rats. Because with NMB there is no skeletal muscle function and ventilation is mechanical, cardiovascular effects are not influenced by respiration or general activity. During 10–35 days of a typical NMB experiment, the rats have basal endocrine levels,1 normal vital signs, and typical patterns of BP variability, including diurnal rhythms and sleep cycles (7, 8). NMB rats can be instrumented for a full range of cardiovascular measurements as well as carotid sinus (SINUS) and ADN barostimulation. The ADN stimulation uses a special noncorrosive capacitance electrode, and the SINUS stimulation uses a volumetric balloon in a vascu-}

1Basal (BL) corticosterone levels are normal and rise in response to auditory disturbance showing that secretory function is not impaired. For two of the rats: BL = 7.88 μg/dl, and it increased to 32.3 μg/dl; BL = 3.9 μg/dl, and it increased to 20.1 μg/dl.

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compare, within the same subject, the linearity and general properties of the various response components at many levels of both SINUS and ADN stimulation. We determined the open-loop baroreflex effects of ADN and SINUS for systolic (SBP) and diastolic (DBP) BP; interbeat interval (IBI); intestinal (mesenteric artery, mBF), skeletal muscle (femoral artery, fmBF), and skin (paw, laser Doppler, skBF) blood flow; venous pressure (VBP); and vagus and peroneal nerve activity. The NMB rats were SAD, and the experimental procedures were entirely automated: barostimulation was prescheduled and administered at random. Thus over hours and days, the experimental stimuli interacted with the unattenuated (by the baroreflexes) effects of natural sources of BP variability. The mechanical SINUS and electrical ADN stimuli were related to one another using ΔSBP as an index of reflex activation and then comparing the subsidiary responses that produced similar ΔSBP. [In the companion paper (10), we give a new method for calibration of the SINUS balloon and ADN electrode stimuli to equivalent vascular pressure.] We found that for NMB rats 1) the patterns and determinants of variability were similar to what has been reported for freely moving rats, indicating that the sources of variability are endogenous to the central nervous outflow and not dependent on general behavior, 2) the variability was unattenuated by averaging in a way that indicated that it is not entirely random in time, and 3) for all baroreflex response components, with the exception of SINUS-elicited heart rate (HR) changes, there was an orderly, substantially linear, relationship between stimulus strength and response magnitude.

METHODS

Subjects

Fourteen female Long-Evans rats (CDBS-VAF, Charles River, Wilmington, MA), weighing 225–275 g, were obtained 3–4 wk before the start of an experiment and, after quarantine and examination by a veterinarian, they were housed in groups of four to six in an isolation cubicle at the Central Animal Facilities. The ventilated, central nervous system-intact, NMB rats were individually and carefully maintained with the use of monitoring, life support, and analgesic protocols as stringent as those that are accepted as adequate for critical care of human adults and infants. All actual surgery or physical manipulation was done under precisely controlled and carefully monitored, deep isoflurane anesthesia. The protocol is certified to be in compliance with National Institutes of Health Guidelines by the Pennsylvania State University College of Medicine Institutional Animal Use and Care Committee. The NMB rats are studied one at a time and attended around the clock.

General Procedures

All surgery and instrumentation was done with sterile techniques. During surgery, the anesthetic level was >1.5% isoflurane, which maintained the following states: 1) the electroencephalogram (EEG) was synchronized and dominated by high-voltage slow-wave (δ) activity; 2) mean arterial pressure <100 mmHg, HR <420 beats/min; and 3) no evident EEG, BP, or IBI responses to manipulation. Isoflurane was delivered into the inspiratory gas stream by a precision mass-flow controller. A low (analgesic 0.15–0.3%) level was maintained between surgical days, for 3– to 4-days postsurgery, and gradually reduced to zero on day 5, when the incisions were completely healed. During the experimental protocols, the rats were ventilated through a per os coaxial tracheal cannula at 72 breaths/min with an inspiratory and expiratory ratio of 1:2 and a minute volume of 180–200 ml and gas concentrations of 50% O2, 47% N2, and 3% CO2, delivered by a precision (±1%/wk) volumetric respirator. Intermittent hyperinflations (6 per hour at 15 cmH2O), positive end-expiratory pressure (1.5 cmH2O), and expiratory CO2 monitoring were continuous.

Surgery

Day 1. Under deep isoflurane anesthesia, the following procedures were performed. Precordial silver wire electrocardiogram (ECG) electrodes were implanted subcutaneously. An abdominal aortic catheter (28-gauge Teflon) was inserted via the left femoral artery, and to administer parenteral solutions and to record VBP, a 0.9-mm Renathane catheter was threaded into the inferior vena cava from the left femoral vein. Pulse transit time (PTT) flow probes (AP01IRS, Transonic Systems, Ithaca, NY) were applied to the right femoral artery and to the superior mesenteric artery or the caudal aorta. An ABLF21 laser Doppler flow probe was attached to the right paw. Two 0–80 screws were placed into the skull at lambda and bregma for EEG. Temperature was measured by an implanted intra-abdominal thermistor and servo-regulated at 37°C. Bipolar silver recording electrodes were applied to the cervical vagus and right peroneal nerve. A silicone cannula was inserted in the urethra to continuously record urine output. For the duration of the experiment, nutrition was maintained by infusion of high-nitrogen Vivonex (Vivonex TEN Novartis, Minneapolis, MN) through a surgically placed gastroduodenal feeding cannula (0.030 × 0.065 medical grade silicone).

Induction of NMB

Once a continuous display of IBI, BP, respiratory rate, and expired CO2 had been established, the vital signs stabilized within normal limits and computer alarm routines were set to monitor the depth of anesthesia, 100 μg of α-cobrotoxin (Biotoxins, Miami, FL), a specific neuromuscular-blocking agent, were injected intra-arterially. Within 20–30 min, as the drug took effect, mechanical ventilation was begun. NMB was maintained by continuous infusion of α-cobrotoxin (250 μg/day).

Parenteral Solutions

For the duration of the experiment, the following solutions were infused intra-arterially (0.37 ml/h): 50 ml H2O, 50 ml 0.5 N lactated Ringer, 500 IU heparin Na, 1.25 g oxacillin Na, 2.8 mg α-cobrotoxin, 0.3 mg vitamin K (Synkavit), and 20 meq K+ (as KCl). The following solutions were infused intravenously (0.45 ml/h): 50 ml H2O, 50 ml 0.5 N lactated Ringer, 300 IU heparin Na, 1.25 g oxacillin Na, and 0.5 g ticarcillin disodium.

Baroreceptor Surgery

Day 2. The left ADN was identified, and the Ta-Ta2O5 capacitance electrode was affixed; the left sinus was denervated, the right ADN was cut, and the stainless steel and silicone balloon was inserted into the right carotid sinus (See APPENDIX).
ADN Stimulation

Stimulus trains were generated by a computer-controlled pulse generator (Master-8vp, A.M.P.I., Jerusalem, Israel).

Electrical parameters. Fan and Andresen (12) described parameters that differentially activate A and A + C fibers; our implementation was as follows. On day 6, thresholds were determined by measuring ΔSBP to 60 s 2- and 40-impulses/s test stimuli of progressively increasing strength presented at six per hour until the ΔSBP of a five-trial average was at least −10 mmHg for three successive replications.

Current and duration. For all rats, A-fiber parameters were 15–50 μA and 100-μs pulse width (PW). A + C-fiber parameters were 80–100 μA and 300-μs PW; thus the A-to-A + C power ratio was ∼1.6, which is comparable to what was described (12, 27). The rate for A was 1–50 impulses/s, whereas the rate for A + C was 1–16 impulses/s.

Sinus Stimulation

The maximum depressor effect was at a volume of <3.5 μl. Because >4 μl could permanently damage the sinus and statistically reliable differences in depressor effect occurred with 0.25-μl increments, test volumes were limited to 0.5 μl greater than a maximum depressor effect.

Schedules

Stimulus presentations were automated and, except during routine maintenance, continuous (stimulation did not affect sleep patterns, and hyperinflations were postponed during trials).

Timing and sequence. Each of the six randomized trials per hour had the following pattern: 2-min baseline, 1-min stimulation, and 2-min baseline. The trials were separated by a pseudorandom (mean = 5 min) intertrial period. The last 30 s of the prestimulus baseline and the first 30 s of stimulus were used for the response calculations.

Stimuli

For SINUS, initially, only <2.25-μl stimuli were presented; the magnitude was gradually increased to identify the maximum test volume. Finally, a random sequence of volumes, of 1 μl to the maximum, was presented. For ADN, the stimuli were initially presented at widely spaced frequencies; then, additional rates were interpolated to locate the maximum depressor effect and to define the linear region (see Fig. 8). Where applicable, ADN-A, ADN-A + C, and SINUS stimuli were intermixed within the same hour to minimize bias in cross-modal comparisons. Complete determinations for all three stimulus modes required ∼7–9 days for each rat.

Data Acquisition

Single-beat and 2.5-s resolution data were acquired continuously throughout the experiment; 6-kHz digital audio tape (DAT) recordings of all raw signals (see Fig. 5) were acquired during all stimulation trials. SBP, DBP, VBP, urine flow, and temperature were directly processed in the data acquisition computers; low-level signals (ECG, EEG, peroneal nerve, vagus nerve) were preprocessed by AC preamplifiers (XCELL-3 × 4 40-#40–8B, FHC, Bowdoinham, ME). Additionally, ECG was conditioned by an amplifier-rectifier-integrator circuit. EEG was analyzed online into four power bands: δ (0.5–3 Hz), θ (6.5–7.5 Hz), α (8.5–18 Hz), and β (20–45 Hz) (#79–78–5, FHC).

Protocols

Response stability. An ADN of two NMB rats was repeatedly stimulated over extended time. To optimize sensitivity to changes in the threshold and response magnitude, the current strength and impulse rate of the test stimuli were set well below saturation; but, to stringently test for fatigue or long-term damage, maximal stimuli were intermixed.

Effects of denervation on variability. In five NMB rats, SBP variability was assessed before and after denervating the baroreceptors.

Effects of ganglionic block on variability. In four chronically SAD NMB rats, SBP variability was assessed before and during 2.5-mg·kg−1·h−1 infusions of chlorisondamine.

Cardiovascular mechanisms of baroreceptor stimulation. Five NMB rats were implanted with ADN electrodes, of these three also had balloons inserted into an isolated carotid sinus. Graded electrical and hydraulic stimuli were applied. With each kind of stimulation, ΔSBP, ΔmsBF, ΔfmBF, ΔskBF, ΔIBI, and ΔVBP were simultaneously measured. The ADN was stimulated to differentially select A or A + C fibers.

RESULTS

Response Stability

The response measure was the ΔSBP between the 30-s baseline mean and 30-s stimulation mean. In the first rat, during 109 h, the mean baroreflex SBP response (ΔSBP) to a 4-impulses/s stimulus (40 μA, 1 ms, 120 s) was −4.8 ± 11.9, and to a 100-impulses/s (maximal) stimulus, it was −39.0 ± 14.7 mmHg. [LANOVA 4 impulses/s: m = −0.009 mmHg/h, r² = 0.001, not significant (NS); 100 impulses/s: m = 0.014 mmHg/h; r² = 0.001, NS]. In the second rat (see Fig. 1A), there were 496 stimulations at 100 μA, 300 μs, 20 impulses/s; and there were 294 stimulations at 100 impulses/s during 173 h. The 20-impulses/s response changed from −9.9 to −9.7 mmHg (LANOVA: 0.001 mmHg/h, r² = 0.000, NS).

The stability of the mean change contrasted with its large variance; thus, to measure the variability of the baseline, interleaved null “trials” were also analyzed. In these trials, no stimulus was administered, but the effect was calculated exactly as in the actual test trials. Null trials thus show the unbiased sampling properties of the measurement procedure. For the first rat, the null trial SD was ±11.8 mmHg (compared with ±11.9 for the 4-impulses/s stimulus); for the second rat (Fig. 1B), SD (null) = ±10.9 (compared with ±8.7 mmHg for the 20-impulses/s stimulus). This result indicates that the large variability score was due to baseline variability not to erratically generated stimuli or variability of the baroreflex.

For the SINUS, stimulations at fixed volume also yield consistent average responses even when separated by hundreds of inflations. For example, for a 3.5-μl stimulus, the initial response was −43.2 ± 20.07 mmHg. After 225 h of two 1.5- to 3.5-μl peak-to-peak 5-min sinusoidal inflations per hour (total = 2,250 min), the mean response was −41.8 ± 23.9 mmHg (LANOVA: 0.007 mmHg/h, r² = 0.00, NS). However, the SD of the SINUS responses was twice that of the ADN, whereas the SINUS SD (null), ±8.98, was simi-
lar. Thus, unlike the ADN, the additional variability from the SINUS-elicited baroreflex response increased the overall ΔSBP variance.

Effects of Denervation

Denervation increases variability, and the above rats were effectively SAD (no pulse synchronous activity rostral to the ADN cuff and no detectable bradycardia to 10 μg phenylephrine). Figure 2 shows the effect of SAD on SBP variability in five additional rats. The mean SBP SD before surgery was 6.3 ± 3.5, and after surgery it was 16.3 ± 2.4 mmHg.

Effects of Ganglionic Block

The result in Fig. 3 suggests that much of the increased SBP variability is related to neural control. The observations were made on four additional surgically similar rats that were given 2.5 mg·kg⁻¹·h⁻¹ infusions of chlorisondamine, which blocks both the parasympathetic and sympathetic ganglia. The measurements were made more than 5 days after the surgery was completed; thus without isoflurane. The mean preblock baseline SBP of 140.2 decreased to 84.7 mmHg during the block; the mean baseline SBP SD of ±10.8 decreased to ±2.9 mmHg.

Statistical Analysis of the Variability

For NMB rats, respiration, temperature, and general environmental stimuli are tightly controlled. For example, over the entire 7 days of Fig. 1, baseline SBP was 107.8 ± 16.5 mmHg, core temperature was 37.13 ± 0.06°C, inspiratory pressure was 5.27 ± 2.70 cmH₂O, and expired CO₂ was 43.88 ± 0.90 mmHg. Variation in these and other recorded variables were not statistically related to the null trial or baroreflex-induced ΔSBP. Typical of all rats described, absolute SBP and absolute arousal level, as estimated by EEG (δ) power, were correlated, but the 30-s difference samples were not (>36% of the baseline SBP variance could be accounted for by EEG (δ); only 8% of ΔSBP could be accounted for by ΔEEG (δ)). The relationship between variance and mean, evident in Fig. 1, characterizes all of the subjects that we have studied, and in addition to ΔSBP, all component response measures and all modes of stimulation. Figure 4 gives the baseline coefficient of variation for major baroreflex mechanisms for the rats in Table 1. The only measure that appears substantially more stable than SBP is IBI; however, this is misleading, because the physiological range of the IBI scale is comparatively constrained,
and, relative to the baseline, the baroreflex-ΔIBI effects are much smaller.

Extensive regression and factor analytic exploration have not identified a combination of baseline measures that, when applied to the baroreflex-response data, substantially reduced the variance. For example, for the rat shown in Fig. 1, with the use of a stepwise regression analysis of nine baseline variables, an optimum model was identified that included SBP, IBI, fmBF, and EEG (δ) power. When the model was applied to the ΔSBP responses in Fig. 1 and the distributions before and after regression correction, compared with the use of a Kolmogorov-Smirnov test, no difference was found (χ² = 0.581, df = 2, P > 0.9999). In a parallel analysis for a second rat, an independent stepwise regression procedure identified the same suite of variables, and the result of applying the regression-correction procedure was also similar (χ² = 1.03, df = 2, P > 0.9999). The results with four additional rats and other regression methods were comparable; thus so far, we have been unable to identify any variable, or linear combination, or multiplicative dyad of variables that attenuates the ΔSBP SD by >10%.

Analysis of the Effects of Barostimulation

The individual component responses including mesenteric vascular conductance (msVC), femoral vascular conductance (fmVC), skBF, IBI, and VBP were measured in the same subjects during each kind of stimulation. A typical high-resolution record of the initial 6 s of a 3.25-μl SINUS trial is shown in Fig. 5. The maximum response effects are in Table 1; the averages of the maximums over all rats are in the last two columns. For all three SINUS and ADN rats, the maximal ADN stimulus was substantially more effective than the maximal SINUS stimulus in producing SBP decreases; the individual component responses, with the exception of ΔIBI, generally reflect the magnitude of...
Table 1. Summary of the component response effects

<table>
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<tr>
<th>Component Response</th>
<th>Stimulus Mode</th>
<th>Slope</th>
<th>R²</th>
<th>P</th>
<th>Max</th>
<th>Slope</th>
<th>R²</th>
<th>P</th>
<th>Max</th>
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<th>P</th>
<th>Max</th>
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<tbody>
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<td>0.98</td>
<td>0.01</td>
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<td></td>
<td>ADN-A</td>
<td>0.51</td>
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<td></td>
<td>ADN-A+C</td>
<td>-0.83</td>
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<td>0.001</td>
<td>-83.4</td>
<td>0.43</td>
<td>0.92</td>
<td>0.01</td>
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<td>Interbeat interval, ms</td>
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<td>9.1</td>
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<td>Caudal aortic conductance, μl/min⁻¹·mmHg⁻¹</td>
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<td>0.01</td>
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<td>123</td>
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<td>Femoral conductance, μl/min⁻¹·mmHg⁻¹</td>
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<td>Skin flow, tpu</td>
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<td>-0.0025</td>
<td>0.97</td>
<td>0.01</td>
<td>-0.169</td>
<td>-0.0039</td>
<td>0.70</td>
<td>0.05</td>
<td>-0.273</td>
<td>-0.0011</td>
<td>0.87</td>
<td>0.02</td>
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<td>-0.0025</td>
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<td>0.005</td>
<td>-0.771</td>
<td>-0.0032</td>
<td>0.86</td>
<td>0.02</td>
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<td>-0.0021</td>
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<td>0.93</td>
<td>0.02</td>
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<td>0.04</td>
<td>0.71</td>
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<td>ADN-A</td>
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<td>0.858</td>
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<td>0.001</td>
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<tr>
<td></td>
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Conductances and flows are systolic. Max is the mean of all observations at the stimulus strength that gave the largest effect, and Slope is the regression determined change per cent of the linear systolic blood pressure (SBP) range (see RESULTS for the normalization methods). R² is from the LANOVA for the Slope, and P is its reliability. R² measures the linearity of each response component with respect to stimulus strength (within the threshold-to-saturation range of the SBP effect). Comparing within-rat and across-stimulus modes, a larger slope implies a greater relative effectiveness of a particular response component, indicating that the component contributes more to the depressor effect. The data for rats EF and EH were comprehensive for all response measures and stimulus modes. With only a few exceptions, the individual regression coefficients for each rat and response are statistically reliable.
the ΔSBP. Although for strong SINUS stimulation a small ΔIBI was evident in all rats, the maximum ΔIBI to ADN-A was on average five times that to SINUS. Because of ceiling effects, for an accurate comparison across stimulation modes, the strengths must be equated and restricted to physiologically realistic values. To do this, we used the ΔSBP as a criterion and modulus for two different response-normalization procedures. For the first procedure, to qualitatively compare response mechanisms across modes, we found a magnitude for both SINUS and ADN-A stimuli that produced a similar (∼40 mmHg) ΔSBP, and ensemble averaged 20 trials of each stimulus. Figure 6 shows the result for rat EF (similar results were obtained for each of the three rats with both SINUS and ADN). Most perspicuously, compared with the SINUS, the ADN produced a much larger ΔIBI. To visualize the relationship between vagus firing and ΔIBI, 20 maximal volume SINUS trials were split at the median ΔIBI response, and for each subset, the ensemble averages of ΔIBI and vagus firing rate were computed. Larger ΔIBI was accompanied by a larger increase in nerve activity, and the nerve firing anticipated the IBI response (Fig. 7; also see Ref. 10).

A second, more general normalization procedure was used for quantifying the relative contribution of each response mechanism by stimulus mode. For each of the five rats and for all completed stimulus sets, we determined the linear range of the SBP effect, mapped the stimulus domain corresponding to this range onto a standard scale, and used the scale to systematically compare the maximum size and linearity of the individual component responses. The linear range was determined as follows. The stimulus-response data were least-squares fit with first-, second-, and third-order polynomial functions (see Fig. 8). The ANOVA model-corrected regression coefficients for these expressions were calculated, and the order-of-fit that...
yielded the largest model-corrected squared coefficient was determined. For all of the SINUS data, probably because of combined stimulus and receptor mechanical factors, the best fit was cubic; for the ADN data in all cases, a quadratic fit was best. The obtained regression equations were differentiated, set to zero, and solved. For the SINUS, the two roots were taken as the minimum and maximum extent of the linear range; for the ADN, the minimum was defined as a stimulus of zero and the maximum as the single root.

To calculate the linearity and comparative sensitivity of the component-response effects for each rat and each stimulus mode, the minimum stimulus strength within the previously defined linear ΔSBP range was assigned a value of zero and the maximum a value of 100. (The scale is used in Ref. 10 to define the amplitude and offset of the modulation stimulus within the linear range.) Each response, including ΔSBP, was linear fit with respect to this standardized “percent of ΔSBP linear range” scale, slope, R², and ANOVA reliability entered in Table 1.

The slope directly compares the efficacy with which different stimulus modes activate particular component responses, and the regression statistics measure the consistency of the effect. Because percentage transformation of the stimulus scale is linear, R² measures the degree to which each baroreflex-component response is proportional to stimulus magnitude within the threshold-saturation limits of the ΔSBP. Figure 9 shows ΔIBI for ADN-A and SINUS, plotted on the corresponding standardized stimulus scale.

Comparison Between Balloon and Open SINUS

McKeown and Shoukas (26) described a chronic method for directly applying hydraulic stimulation to the carotid sinus. To compare volumetric (balloon) and hydraulic stimulation, we prepared two chronic NMB

Fig. 8. Polynomial curve fits for SBP vs. SINUS, ADN-A, and ADN-A + C magnitude; the regression coefficients are 0.56, 0.54, and 0.64 (rat EF). For all rats, the best fits to the SINUS data were with third order, and to the ADN data with second-order functions. The polynomial curve fits were used to define the stimulus domain of the linear SBP effect. The large scatter of measurements at each stimulus level is typical and chiefly due to baseline variability (see Fig. 1). In contrast to the individual measurement variability, the means at each stimulus level are consistent; e.g., for these same data, the coefficients for the linear regression of mean values, within the threshold-to-saturation stimulus range, are 0.88, 0.88, and 0.96 (see Table 1).
rats with isolated and cannulated sinuses. With the use of a saline column, the maximum ΔSBP (at 170–200 mmHg) were −30.1 and −40.4 mmHg (which were similar to those in Ref. 26 and to the balloon maximums in Table 1). The slope for the first rat was −0.22 mmHg/mmHg, and for the second rat it was −0.20 mmHg/mmHg (Fig. 10), which are both similar to −0.22 mmHg/mmHg reported for a double-open sinus in conscious Sprague-Dawley rats (26).

DISCUSSION

Baroreflex-Stimulation Methods

ADN. A silicone-embedded Ta-Ta2O5 electrode is an accurate and consistent method to chronically stimulate the ADN. Of the 13 NMB rats that had Ta-Ta2O5 electrodes, the median functional time for the electrode was 18 days (8–67 days), and 9 of the rats had stretches of >10 days during which the stimulation effects were constant to less than ±10%. Thus the electrode is practical for repeated quantitative stimulation of baroreflex pathways in complicated within-subjects experimental designs. SINSUS. Unlike the dog chronic reversible sinus (34, 35), rat sinus preparations do not restore the intramural circulation between sessions. The baroreceptors themselves have a stable blood supply from small vessels that travel with the glossopharyngeal and sinus nerve and are not rendered ischemic by sinus isolation (24, 25); however, with isolation, ischemia of the vessel wall causes degenerative changes, including necrosis, revascularization, and scar formation, which almost certainly affect elasticity and thus compliance. As the compliance of the isolated sinus changes with time, the proximal stimulus, i.e., receptor stretch, at a designated pressure also changes. Although balloon stimulation lacks ostensible BP equivalence, it also does not depend on the vessel modulus of elasticity, and thus in practice, the stimulus can be more accurately and repeatably applied.

Baroreflex Mechanisms

The skeletal, visceral, and cutaneous circulations subserve separate functions and are potentially differently affected by various modes or intensities of baroreceptor input. Within the technical constraints of one laser Doppler and two PTT channels, we chose representative vascular fields and measured changes in flow to barostimulation. In four of five of the subjects, we measured superior msBF. However, preliminary analysis of the data showed that although msVC increased substantially, the actual flow increment was negligible; consequently, in the fifth rat (EH), we placed the second PTT probe on the aorta, caudal to the superior mesenteric artery. This location, which included the femoral, inferior mesenteric, iliolumbar, and caudal arteries, gave a broad sample of skeletal and visceral abdominal flow, which together increased substantially to baroreceptor stimulation (Table 1). In anesthetized rats, the observations of Faber and Brody (11), using the entire superior laryngeal nerve, and Hebert and Marshall (16, 17), using strong carotid inflation (∼250 mmHg), closely accord with ours. With the use of ADN stimulation, Machado et al. (22) found msVC substantially decreased in anesthetized rats and slightly decreased in semichronic awake (24- to 48-h postsurgery) rats (6). Assuming that the plantar flow of the paw approximately represents skin, taken together, our results indicate that in the NMB rat, the skeletal circulation has a large, probably dominant role in the baroreflex regulation of BP.

Baroreceptor Mode

We use the terms “A fiber” and “A + C fiber” to describe low-current, high-rate and high-current, low-rate stimulation of the ADN (12, 27). Others have reported that A or C fibers could be differentially stimulated [Fan and Andresen (12) confirmed this by capsaicin block of the C fibers]. However, in fact, there is no straightforward way of entirely avoiding stimulation of A fibers [Fan et al. (14) describe the use of anodal block to prevent A-fiber activation, but the block duration is limited.] However, at C fiber effective rates, A fiber activation is probably minimal. With the use of parameters similar to Fan and Andresen (12), we obtained similar response curves, with high-current effects asymptotic at ∼10 impulses/s (Fig. 8) and low-current effects continuing to increase up to ∼40 impulses/s.

There were no obvious differences between the A and A + C fiber baroreflex-response patterns, and the SINUS vascular patterns were also similar. However, the SINUS and ADN-ΔIBI effects were quite different. Table 1 shows that the ADN maximum ΔIBI is from 2 to 25 times larger than that of the SINUS; and the ΔIBI is disproportionately larger than ΔSBP, which is 1.5 to 2 times larger for ADN than SINUS (Table 1). Figure 9 shows the relationship between the ΔIBI change and stimulus magnitude. Whereas the ADN effects are orderly and linear, the SINUS effects are scattered. However, in contrast to ΔIBI, the R² values

Fig. 10. The relationship between carotid sinus pressure (CSP) and ΔSBP in a rat with an open (without balloon), isolated sinus (LANOVA: m = −0.20 mmHg/mmHg, R² = 0.96, P < 0.003). The preparation followed McKeown and Shoukas (26).
for SINUS and ADN-ΔSBP effects are similar (Table 1). Fan et al. (13) reported differences in the ΔIBI for ADN and carotid sinus nerve in anesthetized rats (compare with their Figs. 3 and 6), and unanesthetized ADN-transected (sinus intact) rats show greatly attenuated ΔIBI responses to phenylephrine (3). Compared with vascular conductance changes, baroreflex-ΔIBI effects are quite small and are probably of little regulatory significance; however, because ΔIBI responses to vasoactive drugs are used extensively to measure baroreflex gain and to confirm denervation, they are of importance: we have relied on the “phenylephrine test” in the past (9), and in the present studies, so have others. Schreinhofer and Sved (32) have cautioned that taking the absence of ΔIBI as evidence of denervation is potentially misleading, and it seems increasingly clear that, under some circumstances, sinus stimulation can produce a substantial depressor response with practically no ΔIBI and, correlative, rats with almost no ΔIBI to pharmacologically induced BP change can have completely intact carotid sinus innervation.

Properties of BP Variability

The large random SBP variability, evident in Fig. 1, is not peculiar to NMB but is generally characteristic of unanesthetized SAD rats (see Refs. 13 and 29 for the effects of anesthetics). In freely moving SAD rats, Jacob et al. (20) reported that SAD increased the SBP SD by a factor of three over controls. Schreinhofer and Sved (32) reported preSAD SD = ± 4 and postSAD SD = ± 12 mmHg; Machado et al. (23) reported preSAD SD = ± 3.6 and postSAD SD = ± 13.6 mmHg; Trapani et al. (38) reported preSAD SD = ± 5 and postSAD SD = ± 15 mmHg; Buchholz et al. (4) reported preSAD SD = ± 6.2 and postSAD SD = ± 22.5 mmHg; and Alper et al. (2) reported preSAD SD = ± 6.5 and postSAD SD = ± 19.6 mmHg. These results compare with our values of ± 6.3 ± 3.5 and ± 16.3 ± 2.4 mmHg for pre- and postSAD SD. [For historical reference in 1973, Cowley et al. (5) obtained values of ±10.9 for normal and ±20.6 for SAD dogs.]

That equivalent variability persists with NMB indicates that the variability is not a trivial artifact of, for example, respiration, skeletal movement, or thermal-regulation; whereas ganglionic block (see Fig. 3) confirms that a major constituent depends on neural activity, again similar to freely moving rats, where Jacob et al. (20) found that chlorisondamine reduced postSAD variability by a factor of ~3 and Alper et al. (2) found that it decreased from ± 17 to ± 7 mmHg. (In intact rats, with block, there is somewhat increased variability; consistent with a fraction of the increased variability being due to nonneural mechanisms that are buffered by an intact baroreflex.)

Consequences of BP Variability

Because noise confounds experimental observations, the greatly increased variability with SAD has practical consequences for open-loop baroreflex studies. Our response measure, the difference between a 30-s baseline mean and a 30-s baroreceptor-stimulation mean, is similar to that used by many others (11–13, 16, 17, 28, 29, 37) and was chosen to allow the response to asymptote but not fatigue. For this measure, fluctuations of period < 10 s are subsumed in each average, and of > 120 s, canceled in the difference (see Ref. 10); but, in fact, we found that postSAD variability was relatively unaffected by this within-response averaging, and a mean of many separate responses was needed to accurately estimate baroreflex effects.

More fundamentally, it appears likely that the variability that emerges postSAD is normally attenuated by the negative feedback of the baroreflex. In a companion paper (10), we consider whether the frequency...
spectrum of the variability and the transfer function of the baroreflex are mutually consistent with this interpretation.

**Perspectives**

In explaining to medical students his and Cowley’s classic observations of the effects of the baroreceptors on BP variability, Guyton (15) wrote, “Note the extreme variability of pressure in the denervated dog caused by simple events of the day such as lying down, standing, excitement, eating, defecation, noises, and so forth.” Guyton’s explanation reflects the conventional notion of baroreflex operation; the baroreflex is engaged on those particular occasions when BP regulation is directly challenged by extrinsic demands, such as postural adjustments, exercise, or digestion. However, if the variability that emerges after SAD is, in an intact animal, normally attenuated by the negative feedback effects of the baroreflex, then it follows that the usual conception is not correct. Instead, the baroreflex is engaged frequently, repeatedly, usually randomly, and as the data from NMB rats presented here show, without any need of skeletal activity.

**APPENDIX**

**Isolation of the ADN**

We located the SLN at the thyroid cartilage and, at $\sim 20 \times$, dissected toward the bifurcation to $\sim 5$ mm from the cartilage, where the ADN enters the main trunk of the SLN via a small “delta” of nerve and connective tissue.

**Verification.** Stimulation (30–70 $\mu$A, 300-$\mu$s pulses at 2–50 impulses/s) under ($\sim 1.5\%$ isoflurane) anesthesia elicits a gradual (30–90 s to asymptote) monotonic impulses/s and current-dependent depressor response, which includes bradycardia, hypotension, and vasodilatation and does not convert to a pressor pattern with very strong ($>500 \mu$A) stimulation.

**ADN electrode.** The anodized Ta-Ta$_2$O$_5$ electrode (Fig. 11) is polarized and was driven by the positive terminal of an optical isolator (CCIU-8, FHC), and the cathode was 2-cm s/s wire and imbedded in an adjacent muscle. (Electrochemical reaction products at the cathode disburse harmlessly in the muscle.)

**Embedding.** After testing, s/s wing-shaped microhooks were placed in the neck muscle surrounding the electrode site to stabilize and prevent twisting of the nerve. The electrode was then repositioned under the nerve, $\sim 800 \mu$m above the muscle, and the field was thoroughly dried. The embedding compound (KWIK-CAST, WPI, Sarasota, FL), which was injected through a 25-gauge tip directly under the electrode, rises to engulf the electrode and nerve and seal to the silicone jacket.

**Isolation of the SINUS and Insertion of the Balloon**

A threader was passed through the caudal aspect of the right bifurcation; and 7–0 silk suture captured, pulled through the bifurcation, and tied, ligating the external carotid, caudal to the carotid body artery. An s/s cannula was introduced into the common carotid and preloaded with a pair of 0.635-mm 316 s/s balls (Salem Specialty Ball, Canton, CT), which were flushed in toward the bifurcation. The balls, which can pass the pterygopalatine and cervical portion of the internal carotid but not the posterior lacerated foramen or carotid canal, lodge snugly in each of the branches and isolate the sinus. After the cannula was removed, the balloon (Fig. 12) was introduced and gradually advanced to where the front of the back ferrule was adjacent to the external carotid ligature. A 4–0 suture was passed under the artery and secured behind the ferrule; the 7–0 suture was then tied to the 4–0 suture, accurately and permanently fixing the balloon position relative to the bifurcation. Typically, the SINUS response is depressed for 12–24 h after the surgery, and it reaches asymptotic sensitivity and stability within 3 days.

**Balloon stimulus control.** The volume was controlled by a servo, constructed from an (–3 dB at 7 Hz) analog plotter. Linear motion of the plotter was hydraulically transmitted, from the plunger of a rigidly fixed 10-$\mu$l syringe (Hamilton gastight 1700 series, Reno, NV) mechanically coupled to the x-axis via rigid Teflon tubing, to the balloon.

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**REFERENCES**


