A transfer function method for the continuous assessment of baroreflex control of renal sympathetic nerve activity in rats

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Kanbar R, Oréa V, Chapuis B, Barrès C, Julien C. A transfer function method for the continuous assessment of baroreflex control of renal sympathetic nerve activity in rats. Am J Physiol Regul Integr Comp Physiol 293: R1938–R1946, 2007.—The present study examined whether the gain of the transfer function relating cardiac-related rhythm of renal sympathetic nerve activity (RSNA) to arterial pressure (AP) might serve as a spontaneous index of sympathetic baroreflex sensitivity (BRS). AP and RSNA were simultaneously recorded in conscious rats, either baroreceptor-intact (control, n = 11) or with partial denervation of baroreflex afferents [aortic baroreceptor denervation (ABD; n = 10)] during 1-h periods of spontaneous activity. Transfer gain was calculated over 58 adjacent 61.4-s periods (segmented into 10.2-s periods). Coherence between AP and RSNA was statistically (P < 0.05) significant in 90 ± 3% and 56 ± 10% of cases in control and ABD rats, respectively. Transfer gain was higher (P = 0.0049) in control [2.39 ± 0.13 normalized units (NU)/mmHg] than in ABD [1.48 ± 0.22 NU/mmHg] rats. In the pooled study sample, transfer gain correlated with sympathetic BRS estimated by the vasoactive drug injection technique (R = 0.75; P < 0.0001) and was inversely related to both time- (standard deviation; R = −0.74; P = 0.0001) and frequency-domain [total spectral power (0.0028–2.5 Hz); R = −0.82; P < 0.0001] indices of AP variability. In control rats, transfer gain exhibited large fluctuations (coefficient of variation: 34 ± 3%) that were not consistently related to changes in the mean level of AP, heart rate, or RSNA. In conclusion, the transfer function method provides a continuous, functionally relevant index of sympathetic BRS and reveals that the latter fluctuates widely over time.

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IN BOTH HUMANS AND RATS, THE BAROREFLEX CONTROL OF SYMPATHETIC NERVE ACTIVITY (SNA) IS COMMONLY ASSOCIATED WITH THE SO-CALLED PHARMACOLOGICAL METHOD THAT IS BASED ON THE MEASUREMENT OF SNA CHANGES IN RESPONSE TO ARTERIAL PRESSURE (AP) CHANGES EVOKED BY THE INTRAVENOUS ADMINISTRATION OF VASOACTIVE DRUGS, USUALLY PHENYLEPHRINE AND SODIUM NITROPRUSSE (9, 32, 45, 47). THIS METHOD, HOWEVER, HAS SEVERAL LIMITATIONS, AMONG WHICH ARE BARORECEPTOR FACILITATION BY ß1-ADRENERGIC STIMULATION (15) AND DIRECT MECHANICAL EFFECTS OF THE DRUGS ON BAROSENSITIVE AREAS (43). IN ADDITION, A PROPER DESCRIPTION OF BAROREFLEX CURVES REQUIRE THAT SEVERAL INJECTIONS BE PERFORMED UNDER STATIONARY CONDITIONS, WHICH MAKES IT DIFFICULT TO USE THIS TECHNIQUE FOR CONTINUOUSLY EVALUATING CHANGES IN THE CHARACTERISTICS OF THE REFLEX UNDER DAILY LIFE CONDITIONS. THESE LIMITATIONS HAVE PROMPTED SOME INVESTIGATORS TO DEVELOP OTHER METHODS USING SPONTANEOUS RATHER THAN EVOKED AP CHANGES. WHILE THESE METHODS HAVE MOSTLY BEEN USED FOR INVESTIGATING THE BAROREFLEX CONTROL OF HEART RATE (HR) (36, 39), A FEW STUDIES HAVE BEEN DEVOTED TO THE BAROREFLEX CONTROL OF SNA (20, 34, 44, 50, 51). IN THESE STUDIES, THE SYMPATHETIC BAROREFLEX SENSITIVITY (BRS) WAS ESTIMATED BY RELATING SNA TO BEAT-TO-BEAT VALUES OF AP RECORDED OVER SEVERAL HEART BEATS. OVER SUCH TIME SCALES, BAROREFLEX OPERATION IS CLOSED LOOP (4, 12), WHICH COMPLICATE THE INTERPRETATION OF SNA-AP RELATIONSHIPS (25). MOREOVER, IT IS LIKELY THAT RESPIRATORY-RELATED RHYTHMS OF AP AND SNA INTERFERENCE WITH THE OBSERVED RELATIONS, WHILE IT IS WELL ESTABLISHED THAT THE RESPIRATORY FLUCTUATIONS OF SNA HAVE A MIXED CENTRAL AND BAROREFLEX ORIGIN (1, 16, 30). IN BOTH HUMANS AND RATS, SNA CONTAINS AT LEAST TWO RHYTHMS THAT DERIVE FROM THE ACTIVITY OF THE ARTERIAL BARORECEPTOR REFLEX: A FAST ONE SYNCHRONOUS WITH THE CARDIAC BEAT (~1 Hz in humans; 5–7 Hz in rats), AND A SLOWER ONE ACOMPANYING THE VASOMOTOR WAVES OF AP USUALLY TERMED MAYER WAVES (~0.1 Hz in humans; ~0.4 Hz in rats). IT HAS BEEN SHOWN IN RATS THAT SINOARTIC BARORECEPTOR DENERVATION ABOLISHES BOTH RHYTHMS WHEN SNA IS RECORDED FROM A RENAL NERVE (22, 28, 42). HOWEVER, ONLY THE PULSE-SYNCHRONOUS OSCILLATION CAN BE REGARDED AS REFLECTING THE OPEN-LOOP OPERATION OF THE BAROREFLEX REFLEX BECAUSE OF THE LOW-PASS FILTER PROPERTIES OF THE RESISTANCE VASCULATURE THAT PREVENT FLUCTUATIONS OF SNA TO BE TRANSLATED INTO CORRESPONDING FLUCTUATIONS OF AP AT FREQUENCIES ABOVE 1 Hz (21, 23). IT IS, THEREFORE, TEMPTING TO SPECULATE THAT THE AMPLITUDE OF THE CARDIAC-RELATED OSCILLATION OF SNA DEPENDS BOTH ON THE AMPLITUDE OF THE AP PULSE AND ON THE SYMPATHETIC BRS. THE AMPLITUDE RATIO BETWEEN AP AND SNA FLUCTUATIONS AT THE FREQUENCY OF THE HEART BEAT WOULD THEREFORE PROVIDE AN INDEX OF THE SYMPATHETIC BRS.

The reference method for quantifying the amplitude of an oscillation in a cardiovascular signal is power spectral analysis (41). The ratio between the amplitude of two oscillations can be derived either from the power spectra of both signals or from the gain of the transfer function computed between the two signals. The latter method has the advantage of taking into account the degree of linear correlation between oscillations of the two signals, i.e., the coherence (48). We recently used this method to show that, in urethane anaesthetized rats, the transfer gain between AP and renal SNA (RSNA) computed in the highfrequency is insensitive to changes in the mean HR level in the 5.6–9 Hz frequency range (37).

The specific objective of the present study was to examine the possibility of deriving a continuous index of sympathetic BRS from the calculation of the gain of the transfer function...
between AP and RSNA at the frequency of the heart beat in conscious rats. In an attempt to enlarge the range of variation of sympathetic BRS, the study included rats with partial (aortic) arterial baroreceptor denervation (35). Rats with complete baroreceptor denervation were not included in the study because this procedure would have eliminated the cardiac-related RSNA oscillation, which would have resulted in low coherence values (42) and thus, would not have allowed the computation of reliable transfer gain values. Modeling analysis predicts an inverse relationship between AP variability and sympathetic BRS (8, 49). The ability of the new index to predict buffering of AP fluctuations was therefore examined and taken as an indication of its functional relevance.

METHODS

Animals and surgery. All experiments were approved by the local Animal Ethics Committee. Male Sprague-Dawley rats were purchased from Charles River Laboratories (L’Arbresle, France). They were 10- to 11-wk-old at the time of the first surgery. Surgical procedures have been previously described in detail (3, 5, 22, 24). Two weeks before the study, rats were anesthetized with a mixture of acepromazine maleate (12 mg/kg ip) and ketamine hydrochloride (120 mg/kg ip), and received a prophylactic injection of penicillin G (50,000 IU sc). They then underwent denervation of aortic baroreceptors (ABD group, n = 10) or a sham surgery (control group, n = 11). To alleviate postoperative pain and improve recovery, the nonsteroidal anti-inflammatory drug, ketoprofen (5 mg/kg sc) was given just after surgery and 24 h later. One day before the study, rats were anesthetized with halothane (2% in oxygen) and received an injection of penicillin G. Femoral arterial and venous polyethylene catheters were inserted for AP measurement and drugs administration, respectively. Rats were reanesthetized 4 to 6 h later, with pentobarbital sodium (60 mg/kg ip), and a branch of the left renal nerve was exposed retroperitoneally, placed on a bipolar electrode, and insulated with a silicone gel for RSNA measurement. Both catheters and electrode were led subcutaneously to exit at the back of the neck. Rats received an injection of ketoprofen (2.5 mg/kg sc) and were allowed 16–18 h to recover from anthesia.

Data collection and experimental protocol. AP was measured by using a pressure transducer (model TNF-R; Becton Dickinson, Sandy, UT) coupled to an amplifier (model 13–4615–52; Gould, Cleveland, OH). RSNA was band-pass filtered (300–3,000 Hz) and amplified with a gain of 50,000 (model P-511J; Grass, Quincy, MA). The RSNA signal was then full-wave rectified and low-pass filtered (cut-off frequency: 150 Hz) with a custom-made device. All signals were digitized by using a computer equipped with an analog-to-digital converter (model AT-MIO-16; National Instruments, Austin, TX) and LabVIEW 5.0 software (National Instruments). The AP and RSNA signals were resampled at 500 and 5,000 Hz, respectively.

AP and RSNA were continuously recorded in conscious, freely behaving rats for at least 3 h. The baroreflex control of RSNA was then assessed in the awake resting state by means of sequential injections of sodium nitroprusside and phenylephrine, as previously described (9, 24). Baroreflex testing was repeated after a time interval sufficient to allow complete return of cardiovascular variables to predrug levels, usually 30 min. Finally, the background noise level of RSNA was estimated as the residual electrical activity obtained after administration of the ganglionic blocker chlorisondamine (2.5 mg/kg iv). This activity incorporated possible movement artifacts and, as the nerve was not cut distal to the electrode, afferent renal nerve activity, if present. On completion of the experiments, rats were euthanized with an intravenous overdose of pentobarbital sodium.

Data analysis. Offline processing of data was performed with LabVIEW 6i software. AP and RSNA were resampled at 50 Hz by averaging over consecutive 20-ms periods. These 50-Hz time series were split into data segments of 512 points (10.24 s). Cross-spectral techniques using a fast Fourier transform algorithm were employed to calculate the coherence and transfer functions between AP (input signal) and RSNA (output signal) over 11 segments overlapping by half, which corresponded to a total duration of 61.4 s. The fast Fourier transform was also used to compute autospectra of AP that served to locate the frequency at which maximum spectral power density occurred in the frequency band encompassing HR (usually 5–8 Hz). Coherence, gain and phase were then noted at this particular frequency. However, only gain and phase values associated with a significant coherence were retained for further calculations. The significance threshold for coherence is the limit above which the observed coherence is statistically different from zero, and thus, a linear relationship between oscillations of two variables can be assumed. It is determined by the number of nonoverlapping segments used for computing spectra (6 in this case) and the shape of the window used to taper data (Hamming window in this case) (2). In the present study, the significance threshold for coherence was 0.348 at P < 0.05. Mean values of AP, HR, and RSNA were calculated over the same 61.4-s periods as those used for spectral analyses. HR was derived from the mean interbeat interval. From the total recording period, one continuous 60-min period free of artifacts was selected for analysis so that a maximum of 58 gain and phase values (depending on the coherence) could be obtained in each rat (Fig. 1). The mean of the transfer gain values was calculated and will be referred to as the spontaneous gain in the following text. The mean RSNA value calculated over the entire 60-min period was taken as the reference value for normalizing RSNA data in all computations [normalized gain = (raw gain × 100)/mean RSNA].

Pharmacological estimates of the sympathetic BRS were obtained by fitting a four-parameter sigmoid function to AP-RSNA data pairs collected from the maximum nitroprusside-induced fall in AP up to the maximum phenylephrine-induced rise in AP (18, 24). For this purpose, AP and RSNA time series were resampled at 1 Hz by averaging over consecutive 1-s periods. RSNA data were normalized by the mean RSNA value calculated over the 1-min period preceding baroreflex testing. This analysis provided the full range of RSNA variation (P1), a slope coefficient (P2), AP at half the RSNA range (P3), and the lower plateau of the curve (P4), where sympathoinhibition is maximal (Fig. 2). The first derivative of the sigmoid function was computed to determine the baroreflex gain across the full range of AP, including the maximum gain, which could also be calculated as −P1/P2/P4. However, for comparing with the transfer function gain, which takes no sign, only the absolute values of the maximum gain were used and are referred to as the pharmacological gain in the following text. In each rat, baroreflex parameters were usually the mean of two determinations.

The spontaneous cardiac BRS was estimated using the transfer function method (29). The purpose of this analysis was to compare the evolution over time of the spontaneous indices of cardiac and sympathetic BRS. Beat-to-beat mean AP and pulse interval were calculated and resampled at 50 Hz after spline interpolation. The cardiac BRS was estimated as the gain of the transfer function between mean AP (input signal) and pulse interval (output signal) in the midfrequency band (0.3–0.6 Hz). In this band, coherence between AP and HR oscillations has been shown to be mediated by the baroreceptor reflex because it is abolished after sinoaortic baroreceptor denervation (7). The transfer gain was calculated over the same 61.44-s periods as those used for sympathetic BRS computation. To include a sufficient number of full cycles, these periods were split into segments of 20.48-s segments overlapping by half. The maximum coherence in the band was detected, and the gain was taken at this frequency. Only gain values associated with a significant coherence were retained for
further calculations. The significance threshold ($P < 0.05$) for coherence was 0.631 in this case.

Indices of AP variability were obtained from the 60-min period used for transfer function analysis. A time-domain estimate of overall AP variability was obtained by calculating the standard deviation of beat-to-beat mean AP values. A frequency domain estimate was obtained by using the discrete Fourier transform to calculate a single autospectrum of AP. Total spectral power was then calculated by integrating power spectral density from 0.00028 to 2.5 Hz.

Fig. 1. Example of spectral and cross-spectral analysis of arterial pressure (AP) and renal sympathetic nerve activity (RSNA) in 1 control rat. Autospectra of AP (A) and RSNA (B) were computed from 58 adjacent 61.4-s periods (segmented into 10.2-s periods) taken from a continuous recording in a conscious, baroreceptor-intact rat. In A, error bars show SE for the upper and lower plateaus and for AP at midrange of the curve. In B, error bars show SE for the maximum gain. Note that the absolute value of the gain functions is shown.

Fig. 2. Effect of aortic baroreceptor denervation on AP-RSNA baroreflex function curves. Group-average parameters were used to generate baroreflex function curves (A) and their first derivative (B) in control ($n = 11$) and aortic baroreceptor denervated (ABD; $n = 10$) rats. In A, error bars show SE for the upper and lower plateaus and for AP at midrange of the curve. In B, error bars show SE for the maximum gain. Note that the absolute value of the gain functions is shown.
RESULTS

On the day of the recording session, control (n = 11) and ABD (n = 10) rats had similar body weights (359 ± 7 and 344 ± 9 g, respectively).

Effect of chronic aortic baroreceptor denervation on sympathetic baroreflex function curves, mean value, and variability of AP. As shown in Fig. 2 and Table 1, aortic baroreceptor denervation decreased the baroreflex pharmacological gain by almost 70%. This effect was a consequence of a decrease in the upper plateau of the baroreflex curve (Fig. 2A), and hence, in the range of RSNA variation (P1), combined with a decrease in the slope coefficient (P2).

The 1-h mean values of beat-to-beat mean AP and HR did not differ significantly between control (115 ± 2 mmHg and 394 ± 4 beats/min) and ABD (116 ± 3 mmHg and 404 ± 9 beats/min) rats. The spontaneous variability of AP was increased in ABD rats compared with control rats, considering either the standard deviation (9.6 ± 0.5 vs. 5.9 ± 0.4 mmHg, P = 0.0001) or the total spectral power (75 ± 6 vs. 32 ± 4 mmHg², P = 0.0002).

Effect of chronic aortic baroreceptor denervation on the AP-RSNA transfer function at HR frequency. The frequency at which maximum power spectral density was observed in the high-frequency band of 1-min AP spectra did not differ significantly between control and ABD rats (Table 2), thus confirming that the denervation procedure had no noticeable effect on HR. In control rats, coherence between AP and RSNA was significant in 90% of cases at that frequency and, consequently, transfer function gain and phase could be estimated at the 95% confidence level of 90% of the time. This percentage dropped to 56% in ABD rats. The mean transfer gain at HR frequency was significantly reduced by 38% in ABD rats compared with control rats, whereas the transfer phase remained unchanged (Table 2).

Linear correlation analysis. In the pooled study sample (n = 21), the spontaneous gain correlated positively with the pharmacological gain (Fig. 3A). The pharmacological gain (Fig. 3B) and the spontaneous gain (Fig. 3C) showed significant inverse relationships with overall indices of AP variability, considering either the standard deviation of beat-to-beat data or the spectral power computed from 50-Hz time series. The correlation coefficients tended to be higher for the correlations with the spontaneous gain, but this did not reach significance (31).

Variability of the spontaneous index of sympathetic BRS. In control rats, 1-min transfer gain values were plotted as a function of time (Fig. 4). Visual examination of these chronograms revealed that this index of sympathetic BRS varied widely over time without showing any obvious rhythmic patterns. The variation coefficient of these consecutive gain values (n = 52 ± 2; range: 40 to 58) was 34 ± 3%. To examine whether changes in AP, HR, or RSNA played any role in the fluctuations of the transfer gain, linear regressions between 61.4-s mean values of these variables and corresponding transfer gains were calculated in each control rat. It was found that the transfer gain was significantly (P < 0.05) related to the mean level of J) AP in five of eleven cases (4 negative correlations), 2) HR in five of eleven cases (all negative), and 3) RSNA in seven of eleven cases (all positive).

The spontaneous cardiac BRS was calculated using the transfer function method over the same 61.4-s periods as those used for computing the spontaneous sympathetic BRS, with the aim of comparing the time course of the two indices. The spontaneous cardiac BRS could be reliably estimated during 57 and 25% of the time in control and ABD rats, respectively. In control rats, the maximum significant coherence (0.77 ± 0.01) was detected at 0.42 ± 0.01 Hz. Linear regression analysis included gain values that were associated with a significant coherence for both indices. Consequently, this analysis included 30 ± 4 data pairs (from a maximum of 58) in control rats. There was no significant relation between the sympathetic and cardiac BRS in nine of eleven rats (R = −0.09 ± 0.05). In the remaining two rats, significant, but directionally opposite, correlations were disclosed (one positive correlation: n = 33, R = 0.476, P = 0.0051, and one negative correlation n = 16, R = −0.523, P = 0.0375).

DISCUSSION

In conscious, freely moving rats, the gain of the transfer function relating RSNA and AP at the frequency of the heart beat can be reliably estimated during ~90% of the time when it is computed over consecutive 1-min periods. The validity of the transfer gain as a spontaneous index of sympathetic BRS is supported by three lines of evidence: 1) in rats with partial baroreceptor denervation, the transfer gain either could not be determined or was markedly attenuated; 2) the transfer and

Table 1. Effect of ABD on the characteristics of the AP-RSNA baroreflex function curve in conscious rats

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<th>Control</th>
<th>ABD</th>
<th>P Value</th>
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<tbody>
<tr>
<td>n</td>
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<td>10</td>
<td></td>
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<tr>
<td>R²</td>
<td>0.91±0.02</td>
<td>0.77±0.02</td>
<td>0.0006</td>
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<tr>
<td>P1, NU</td>
<td>317±29</td>
<td>180±30</td>
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<tr>
<td>P2, mmHg⁻¹</td>
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<tr>
<td>P3, mmHg</td>
<td>98±2</td>
<td>109±7</td>
<td>0.6221</td>
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<tr>
<td>P4, NU</td>
<td>24±5</td>
<td>26±6</td>
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<tr>
<td>Gmax, NU/mmHg</td>
<td>10.0±0.9</td>
<td>3.2±0.7</td>
<td>0.0003</td>
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</tbody>
</table>

Values are means ± SE. ABD, aortic baroreceptor denervation; AP, arterial pressure; RSNA, renal sympathetic nerve activity; R², coefficient of determination (observed versus predicted values); P1, RSNA range; P2, slope coefficient; P3, AP at midrange; P4, lower plateau; Gmax, absolute value of the maximum gain; NU, normalized units. P values refer to comparisons between control and ABD rats.

Table 2. Effect of ABD on the coherence and transfer functions computed between AP and RSNA at HR frequency in conscious rats

<table>
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<tr>
<th></th>
<th>Control</th>
<th>ABD</th>
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<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>10</td>
<td></td>
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<tr>
<td>Frequency of AP spectral peak, Hz</td>
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<td>6.7±0.2</td>
<td>0.4813</td>
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<tr>
<td>Percentage occurrence of significant coherence values, %</td>
<td>90±3</td>
<td>56±10</td>
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</tr>
<tr>
<td>Mean of significant coherence values</td>
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<td>0.56±0.03</td>
<td>0.0007</td>
</tr>
<tr>
<td>Transfer function phase, degrees</td>
<td>−33±7</td>
<td>−29±10</td>
<td>0.5184</td>
</tr>
<tr>
<td>Transfer function gain, NU/mmHg</td>
<td>2.39±0.13</td>
<td>1.48±0.22</td>
<td>0.0049</td>
</tr>
</tbody>
</table>

Values are means ± SE. P values refer to comparisons between control and ABD rats.
pharmacological gains differed in absolute values but were positively correlated; 3) the transfer gain was inversely related to AP variability. The latter observation points to the functional relevance of the new index with respect to the short-term control of AP.

Effects of aortic baroreceptor denervation. As mentioned in the introduction, there is a strong rationale behind the hypothesis that the AP-RSNA transfer function gain at HR frequency might provide an index of sympathetic BRS in rats, simply because the cardiac-related RSNA rhythm is abolished by both acute (42) and chronic (28) sinoaortic baroreceptor denervation. Accordingly, chronically ABD rats of the present study had markedly reduced coherence and transfer gain values, which points to an overall decrease in the occurrence frequency and/or amplitude of cardiac-related oscillations of RSNA. On the other hand, phase between AP and RSNA at HR frequency did not differ between control and ABD rats, which indicates that the lead/lag relationship between AP and RSNA at HR frequency was not altered by the denervation procedure.

**Fig. 3.** Correlation analysis. A: relation between the spontaneous and pharmacological estimates of the sympathetic baroreflex sensitivity (BRS). B and C: relations between the overall indices of AP variability and the pharmacological and spontaneous estimates of sympathetic BRS, respectively. The pharmacological gain is expressed as the absolute value of the maximum gain. In each rat, indices of AP variability were obtained from a 1-h continuous recording and were computed as the standard deviation of beat-to-beat mean AP values and as the spectral power of AP in the 0.00028–2.5 Hz frequency range. Filled and open symbols show data from control (n = 11) and ABD (n = 10) rats, respectively.

**Fig. 4.** Example of chronogram of the spontaneous index of sympathetic BRS. Transfer function gain (A) and phase (B) were computed over 58 adjacent 61.4-s periods. C: horizontal dotted line shows the significance threshold (P < 0.05) for coherence. Data are from the same conscious, baroreceptor-intact rat as in Fig. 1. Gain was normalized by the 1-h mean RSNA value (1.67 μV in this case).

Effects of aortic baroreceptor denervation. As mentioned in the introduction, there is a strong rationale behind the hypothesis that the AP-RSNA transfer function gain at HR frequency might provide an index of sympathetic BRS in rats, simply because the cardiac-related RSNA rhythm is abolished by both acute (42) and chronic (28) sinoaortic baroreceptor denervation. Accordingly, chronically ABD rats of the present study had markedly reduced coherence and transfer gain values, which points to an overall decrease in the occurrence frequency and/or amplitude of cardiac-related oscillations of RSNA. On the other hand, phase between AP and RSNA at HR frequency did not differ between control and ABD rats, which indicates that the lead/lag relationship between AP and RSNA at HR frequency was not altered by the denervation procedure.
While there have been several reports on the effects of aortic baroreceptor denervation on the cardiac component of the baroreceptor reflex in rats (13, 14, 38), only one study has been devoted to the sympathetic component of the reflex (35). In the latter study, AP-RSNA baroreflex function curves were constructed in chloralose anesthetized rats after sequential transection of aortic depressor and carotid sinus nerves. It was found that aortic baroreceptor denervation alone produced a 58% decrease in the pharmacological BRS, while subsequent removal of carotid sinus baroreceptor influences abolished the remaining reflex RSNA responses. In ABD rats of the present study, the pharmacological and spontaneous estimates of sympathetic BRS were reduced by 68 and 38%, respectively. We therefore confirm that aortic baroreceptor denervation is a valid approach for achieving a partial reduction of the sympathetic BRS in rats. Moreover, in both control and ABD rats, there was a rather large scattering of pharmacological and spontaneous gain values, which resulted in some degree of overlap between the two sets of data. For this reason, correlations could be calculated using the pooled study sample.

Comparison between pharmacological and spontaneous gains. In both control and ABD rats, pharmacological gains were higher than spontaneous gains. There are at least two simple explanations for this difference. First, the pharmacological gain was defined as the maximum gain of the baroreflex curve, which is the most widely used procedure for estimating sympathetic BRS with the vasoactive drug injection technique (24, 32, 33). In conscious rats, however, the maximum gain is usually observed at AP values lower than the reference AP (9). Consequently, the gain measured at this reference AP is lower than the maximum gain, which was indeed the case in the present study. Gains corresponding to the 1-h mean values of AP were 3.9 ± 0.7 and 1.7 ± 0.3 NU/mmHg in control and ABD rats, respectively. These values are only slightly higher than the transfer gains, which by definition are computed at spontaneous AP values. The second explanation for the difference between pharmacological and spontaneous gains is that low-pass filter properties of arterial baroreceptors are important in the frequency range encompassing spontaneous HR (6, 27) and thus, probably contribute to attenuate RSNA responses to AP changes. As a consequence, transfer gains computed at these frequencies are lower than steady-state gains (37).

The correlation between pharmacological and spontaneous gains was far from being perfect. First, the linear regression line did not pass through the origin, contrary to what would be expected when one considers that rats with complete sinoaortic baroreceptor denervation have no measurable RSNA responses to drug-induced changes in AP (null pharmacological gain) and no cardiac-related oscillations of RSNA (null spontaneous gain) (42). The relationship between the two estimates of sympathetic BRS is thus probably curvilinear, and indeed, the correlation coefficient was slightly improved \((R = 0.82)\) when a curvilinear equation \((y = a + bx^2)\) was fitted to the data set. Secondly, some data pairs were clear outliers, especially in control rats. This might reflect the fact that pharmacological testing of the baroreflex was performed in resting rats, whereas the transfer function gain was an average value calculated over periods of rest and activity. Changes in behavioral and emotional states have been shown to alter the characteristics of sympathetic baroreflex curves (24, 32, 33) and are probably accompanied by changes in the spontaneous gain as well (see below). In summary, it appears that pharmacological and spontaneous estimates of sympathetic BRS both explore baroreflex function while not being interchangeable.

The correlation between spontaneous gain and AP variability. The spontaneous gain was inversely related to overall AP variability. AP variability is largely the result of the continuous interplay between hemodynamic perturbations and the corrective action provided by the sympathetic component of the baroreceptor reflex (8, 49). It is therefore not surprising that interanimal differences in BRS would account for part of interanimal differences in AP variability, especially large differences such as those observed when pooling data from control and ABD rats. In this regard, it is worth noting that both estimates of sympathetic BRS were inversely and similarly related to AP variability, which suggests that both indices are indicators of the AP buffering capacity of the baroreceptor reflex.

Two different indices were used for estimating AP variability: the standard deviation of beat-to-beat mean AP time series and the total spectral power calculated over one single autospectrum of AP. In the latter case, the upper frequency limit was set at 2.5 Hz. Therefore, both indices incorporated all AP fluctuations slower than the cardiac cycle. It must be recalled, however, that AP variability is mainly secondary to slow (i.e., low-frequency) fluctuations (8, 19, 22). Spectral power calculated in the low-frequency band (0.0028–0.27 Hz) accounted for 81 ± 3 and 92 ± 1% of the total power in control and ABD rats, respectively. For this reason, the correlation between AP spectral power and spontaneous gain was only marginally improved when considering low-frequency power instead of total power (data not shown). It should be mentioned that spectral power in the midfrequency band containing Mayer waves was not significantly related to the spontaneous gain. This means that the sympathetic BRS is not the sole determinant of Mayer wave’s amplitude, in accordance with a previous report in anesthetized rats (9).

Variability of the spontaneous gain. The spontaneous gain showed rather large fluctuations in control rats. This feature was not investigated in ABD rats because transfer gains were calculated, on average, during only one-half of the recording time, due to the constraint imposed by the coherence significance threshold. Spontaneous fluctuations of the transfer gain could not be ascribed to any specific behavioral changes as behavior was not rigorously monitored. It should be mentioned, however, that one important criterion for the selection of the 1-h periods used for analysis was that the rats displayed the normal pattern of common behaviors, as indicated by the routine watching of the animals during the recording sessions. Under these conditions, cardiovascular variables showed fluctuations that could be compared with those of the spontaneous gain. No systematic association could be found between the 1-min mean levels of AP, HR, and RSNA and the spontaneous gain computed over the same periods. The lack of correlation with HR fluctuations is not surprising. We have thoroughly investigated this question in anesthetized rats and reported that no relation exists between the transfer gain and HR at cardiac pacing frequencies ranging from 5.6 to 9 Hz (37), which is a frequency range relevant to the present study. On the other hand, a correlation with AP fluctuations could have been expected. In a previous study, we were able to show that there was a strong dependence of the amplitude of cardiac-related
oscillations of RSNA on the AP level in conscious resting rats (5). Specifically, changes in RSNA power at HR frequency were characterized by a bell curve reaching a maximum at 22 mmHg below basal AP. This relation was explained on the basis of the nonlinear properties of baroreceptor activity during the cardiac cycle, in particular, the existence of a threshold AP for baroreceptor discharge (5). The present study clearly indicates that forced and spontaneous AP changes exert differential effects on the cardiac-related RSNA rhythm. One tentative explanation would implicate a continuous resetting of baroreflex relationships linked to behavioral changes and locomotor activity. Such a resetting has indeed been observed in rats during exercise (32), grooming (33), and exposure to a mild emotional stressor (24). Finally, it is of note that the spontaneous gain was positively related to the RSNA level in seven of eleven rats. It is possible that in these animals, some stress reactions had contributed to this relationship (10). To examine the latter hypothesis, we reanalyzed our previously published data demonstrating an 80% increase in the pharmacological estimate of sympathetic BRS associated with an 86% increase in RSNA during air-jet stress in conscious rats (24). By using the method described in the present paper, the AP-RSNA transfer function gain at HR frequency was calculated and averaged over the five periods preceding, and the fifth to ninth periods following, the onset of the stress trial (Fig. 5). The transfer gain increased during stress [from 3.14 ± 0.32 to 5.12 ± 0.51 NU/mmHg; (n = 11); P = 0.0044]). This was accompanied by an increase in coherence (from 0.79 ± 0.03 to 0.89 ± 0.02; P = 0.0033) and a decrease in phase (from −27 ± 9 to −45 ± 9 degrees; P = 0.0208). The latter effect was probably secondary to the stress-induced increase in HR (24). The positive relationship between changes in pharmacological and spontaneous estimates of sympathetic BRS did not reach statistical significance (R = 0.50, P = 0.1196). The variations of the transfer gain were positively correlated with changes in the mean RSNA level (R = 0.71; P = 0.0152). These observations further confirm the value of the novel index for measuring variations in sympathetic BRS, and provide a potentially important mechanism responsible for spontaneous changes in sympathetic BRS.

Finally, as the cardiac and sympathetic vascular components of the baroreceptor reflex share some common determinants, the evolution over time of both spontaneous indices of baroreflex function was compared. The absence of relation between the two indices could result from the complex regulation of regional sympathetic outflows and from the differential control of the heart by the parasympathetic and sympathetic efferent limbs of the baroreceptor reflex. Indeed, Kawada et al. (26) have reported that the neural arc of the baroreceptor reflex may exert differential effects on the cardiac and renal SNA in response to dynamic baroreflex activation in anesthetized rabbits. In addition to the dissociation between cardiac and non-cardiac SNA, Simms et al. (46) showed that the cardiac parasympathetic baroreflex is active over a higher range of AP values in an artificially perfused in situ rat preparation, thus concluding that there is a hierarchical recruitment of the output limbs of the reflex with a sympathetic predominance at lower AP values. Moreover, it has already been shown by using the pharmacological method that efferent limbs of the baroreceptor reflex are differently affected by exercise [cardiac BRS is unaltered and sympathetic BRS is increased (32)], the rapid-eye-movement stage of sleep [cardiac BRS is unaltered and sympatheic BRS is decreased (33)], and air-jet stress [cardiac BRS is unaltered (17) and sympathetic BRS is increased (24)] in rats.

Methodological issues. The temporal resolution of the transfer function method is limited by physiological and computational factors. The amplitude of cardiac-related oscillations is rhythmically altered by AP Mayer waves (data not shown). Therefore, the duration of the segments used for computation must be at least equal to that of the period of Mayer waves, i.e., about 2.5 s. That would produce a frequency resolution of ~0.4 Hz, which is not appropriate for a precise calculation of the transfer gain in the 5–9 Hz frequency band. A minimum duration of 5 s is therefore advisable. A fundamental algorithmic requirement is the use of at least three nonoverlapping segments for computing coherence. Taken together, these considerations imply that the temporal resolution of the method has an absolute minimum of about 15 s.

Conclusion and Perspectives

By using the so-called “spontaneous” methods, it has been possible to show that the cardiac BRS exhibits large fluctuations in humans (11, 40) and in rats (36). The present study
provides a demonstration that the sympathetic BRS also exhibits large spontaneous fluctuations.

It is likely that in both humans and rats, the sympathetic vascular component of the baroreceptor reflex plays the most critical role in determining the potency of the reflex in buffering AP perturbations (8, 49). Fluctuations of sympathetic BRS are thus of great functional relevance to the short-term control of AP. Future studies will have to examine whether these fluctuations are linked to changes in behavioral and emotional states. It would also be important to prolong the recording periods to investigate slow and long-lasting changes in sympathetic BRS that might possibly be linked to changes in homeostatic states.

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