Transfer function analysis between arterial pressure and renal sympathetic nerve activity at cardiac pacing frequencies in the rat

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Oreá V, Kanbar R, Chapuis B, Barrès C, Julien C. Transfer function analysis between arterial pressure and renal sympathetic nerve activity at cardiac pacing frequencies in the rat. J Appl Physiol 102: 1034–1040, 2007. First published November 22, 2006; doi:10.1152/japplphysiol.01064.2006.—This study examined the possible influence of changes in heart rate (HR) on the gain of the transfer function relating renal sympathetic nerve activity (RSNA) to arterial pressure (AP) at HR frequency in rats. In seven urethane-anesthetized rats, AP and RSNA were recorded under baseline conditions (spontaneous HR = 338 ± 6 beats/min, i.e., 5.6 ± 0.1 Hz) and during 70-s periods of cardiac pacing at 6–9 Hz applied in random order. Cardiac spontaneous HR rats, AP and RSNA were recorded under baseline conditions (systolic pressure (AP) at HR frequency in rats. In seven urethane-anesthetized function relating renal sympathetic nerve activity (RSNA) to arterial function analysis between arterial pressure and renal sympathetic nerve activity (RSNA) to arterial pressure (AP) at HR frequency in rats. In seven urethane-anesthetized function relating RSNA to AP at the frequency of the heartbeat (4). Because of the low-pass filter characteristics of the AP response to RSNA (6, 26), the peripheral path of the baroreflex negative-feedback loop is virtually opened at the frequency of the heartbeat. This might justify the calculation of the open-loop transfer function gain under baroreflex closed-loop conditions and, hence, the use of this gain as an index of the sympathetic baroreflex sensitivity. However, because of the spontaneous lability of HR in the conscious rat (16, 24), a prerequisite step in the assessment of the validity of this index is the evaluation of the influence of HR variations on the AP-RSNA transfer gain.

The aim of the present study was, therefore, to determine the effect of controlled, stable changes in HR induced by cardiac pacing on the gain of the AP-RSNA transfer function in the rat. The study was carried out in anesthetized rats to avoid the possible confounding influence of changes in sympathetic baroreflex sensitivity associated with changes in behavioral and emotional states (10, 21). Rats were anesthetized with urethane, which has been shown to exert little effect on the baroreflex control of RSNA (27).

METHODS

Animals and surgery. Male Sprague-Dawley rats (Charles River Laboratories, L’Arbresle, France) weighing 330–360 g (10–11 wk of age) were used. All experiments were performed in accordance with the guidelines of the French Ministry of Agriculture for animal experimentation and were approved by the local Animal Ethics Committee.

Under isoflurane anesthesia (2% in oxygen), one polyethylene catheter was inserted into the abdominal aorta via the left femoral artery for AP measurement, and two catheters (a heat-stretched section of PE-10 tubing fused to a PE-50 extension) were inserted into the inferior vena cava via the ipsilateral femoral vein for the administration of drugs (4). The rat then received an intravenous injection of urethane (1.5 g/kg, supplemented with 0.1 g/kg iv as needed) and was placed on a heating blanket to maintain rectal temperature at 37°C. A unipolar platinum catheter electrode was advanced into the right of its first harmonic, i.e., between 6 and 12 Hz. The main limitation of this study, however, is that the experimental model excluded the arterial baroreceptors themselves, insofar as aortic afferents were directly stimulated, while it is known that rat aortic baroreceptors show dynamic properties that might be important at frequencies >1 Hz (5).

The amplitude of the cardiac-related oscillation of RSNA depends on the size of the triggering signal, i.e., the arterial pressure (AP) pulse (11), and on the gain of the transfer function relating RSNA to AP at the frequency of the heartbeat (4). Because of the low-pass filter characteristics of the AP response to RSNA (6, 26), the peripheral path of the baroreflex negative-feedback loop is virtually opened at the frequency of the heartbeat. This might justify the calculation of the open-loop transfer function gain under baroreflex closed-loop conditions and, hence, the use of this gain as an index of the sympathetic baroreflex sensitivity. However, because of the spontaneous lability of HR in the conscious rat (16, 24), a prerequisite step in the assessment of the validity of this index is the evaluation of the influence of HR variations on the AP-RSNA transfer gain.

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THE MOST PROMINENT RHYTHM in sympathetic nerve activity is the cardiac-related rhythm (17, 22). It is still not clear whether cardiac-related bursts of sympathetic nerve activity (SNA) result from entrainment of centrally generated bursts or from periodic inhibition of a randomly generated activity by pulse-synchronous baroreceptor activity (18). The latter hypothesis is usually favored in the case of renal SNA (RSNA) in rats, because it has been shown in this species that the cardiac-related rhythm is abolished and RSNA is fully desynchronized in the high-frequency range (4–20 Hz) acutely (23) and chronically (14) after sinoaortic baroreceptor denervation. Furthermore, clear oscillations of RSNA can be evoked in urethane-anesthetized, sinoaortic-denervated rats by application of sinusoidal stimulation to the aortic depressor nerve at frequencies close to spontaneous heart rate (HR) (23). In the latter study, the amplitude of RSNA oscillations showed a strong but complex dependence on the frequency of aortic nerve stimulation, especially in the vicinity of the heartbeat frequency and
Data acquisition and experimental protocol. For measurement of AP, the arterial catheter was connected to a precalibrated pressure transducer (model TNF-R, Ohmeda, Bilthoven, The Netherlands) coupled to an amplifier (model 13-4615-52, Gould, Cleveland, OH). RSNA was amplified (×50,000) and band-pass filtered (300–3,000 Hz; model P-511J, Grass, Quincy, MA). All signals were digitized using a computer equipped with an analog-to-digital converter (model AT-MIO-16, National Instruments, Austin, TX) and LabVIEW 5.1 software (National Instruments). The AP signal was sampled at 500 Hz. The RSNA signal was sampled at 10,000 Hz without analog preprocessing and at 50,000 Hz after full-wave rectification and low-pass filtering (150-Hz cut-off frequency).

For pacing the heart, 2-ms impulses were delivered by the computer through the analog output of the converter board at a fixed voltage intensity (1.10 ± 0.15 V in the 7 rats of the study). The stimulation signal was sampled at 10,000 Hz. To avoid contamination of RSNA by an artifact due to the stimulation, all signals generated or recorded by the computer passed through an isolation device [models SC5MB49 (for stimulation) and 5B41 (for data acquisition), National Instruments]. HR was varied according to a randomly chosen sequence of values ranging from 6 to 9 Hz (360–540 beats/min) with an increment of 0.25 Hz (15 beats/min). Pacing trials lasted 70 s and were separated by ≈70 s of recovery.

For verification that the baroreflex control of RSNA was preserved under the particular experimental conditions of the study, AP-RSNA baroreflex function curves were constructed using the pharmacological method (8, 10). Briefly, AP was first decreased with a bolus injection of sodium nitroprusside (100 μg/kg iv) and then increased with an infusion of phenylephrine (50 μg·kg⁻¹·min⁻¹ iv) for ~60 s.

At the end of the recording session, the long-acting ganglionic blocker chlorisondamine was administered (2.5 mg/kg iv), and the heart was paced at 7 Hz to verify the absence of contamination of the RSNA signal by the stimulus (Fig. 1). On completion of the experiment, the rat was euthanized with an intravenous overdose of pentobarbital sodium.

Data analysis. The background noise of RSNA (residual activity after chlorisondamine administration) was subtracted from the rectified RSNA signal, which was then resampled at 50 Hz by calculation of average values over 100 consecutive points. Under baseline and pacing conditions, 62-s periods were selected for further analysis, which consisted of 1) beat-to-beat calculation of mean values of systolic AP, diastolic AP, mean AP, pulse pressure, and HR, and 2) calculation of coherence and transfer functions. In the latter case, each 62-s period was split into 11 data segments of 512 points (10.24 s) overlapping by half. The frequency resolution was 0.0976 Hz, which is sufficient to discriminate between cardiac pacing frequencies. Cross-spectral techniques using a fast Fourier transform algorithm were used to calculate coherence, gain, and phase between AP (input signal, resampled at 50 Hz) and RSNA (output signal) at HR frequency. The significance threshold (P < 0.001) for coherence was 0.627 (2). Gain values were normalized by the mean RSNA value calculated over baseline periods and are therefore expressed in normalized units (NU) per millimeter Hg.

The RSNA-AP baroreflex relation was assessed as previously described (8, 10). AP and RSNA time series were resampled at 1 Hz by an averaging procedure, and a four-parameter sigmoid function was fitted to AP-RSNA data pairs collected from the maximum nitroprusside-induced fall in AP to the maximum phenylephrine-induced rise in AP by an iterative least-squares procedure (SigmaPlot 2000, SPSS, Chicago, IL). The maximum gain was estimated as the slope of the tangent at the inflection point of the sigmoid curve.

Statistics. Values are means ± SE. One-way ANOVA for repeated measures was used to evaluate the stability of baseline values over time, as well as the effect of cardiac pacing frequency. Paired comparisons (pacing vs. baseline) were performed using the Wilcoxon signed rank test. P < 0.05 was taken to indicate statistical significance.

RESULTS

Baseline values of cardiovascular variables. Spontaneous AP and HR showed very little variability over time (Table 1). RSNA displayed slightly larger variations, but these fluctuations were not reproducible among animals. Only pulse pressure exhibited reproducible, albeit small, fluctuations, as revealed by repeated-measures ANOVA (Table 1). Baseline coherence values computed between AP and RSNA at spontaneous HR were high (0.957 ± 0.007) and varied little over time [variation coefficient (VC) = 1.66 ± 0.31%] without a systematic trend between rats (P = 0.520), which demonstrates a strong, reproducible coupling between fluctuations of both variables at this frequency (5.6 ± 0.1 Hz). The gain of the transfer function relating AP and RSNA at spontaneous HR was 2.44 ± 0.28 NU/mmHg. It was relatively stable over time (VC = 8.98 ± 1.02%) and did not show consistent fluctuations among animals (P = 0.806). The phase of the transfer function computed at the same frequency was −33 ± 10°. It tended to fluctuate over time (VC = 17.6 ± 5.7%), but these fluctuations were not reproducible between rats (P = 0.376).

Effect of cardiac pacing on cardiovascular variables. Cardiac pacing efficiently set HR at the desired frequency, as illustrated by Fig. 1 and demonstrated by the linear regression analysis performed between the frequency of impulses delivered by the computer and the actual HR measured beat-to-beat over the 62-s periods used for analysis (R² = 1, HR = impulse frequency + 0.0007 Hz).

Cardiac pacing slightly but significantly (P = 0.0014) increased mean AP, and this effect was mainly due to an increase (P < 0.0001) in diastolic AP (Fig. 2A). The group-average slope of the linear regression line relating mean AP to HR was 0.8 ± 0.2 mmHg/Hz. Cardiac pacing decreased the pulse pressure (P < 0.0001, slope = −3.6 ± 0.3 mmHg/Hz; Fig. 2B) while leaving the RSNA level essentially unaltered (P = 0.680; Fig. 2C).

Coherence between AP and RSNA was not affected by cardiac pacing (P = 0.149) and remained high (0.959 ± 0.006) across the HR range of 6–9 Hz (Fig. 3A). The transfer gain was also unaffected by changing HR (P = 0.185; Fig. 3B), and its mean value was 2.46 ± 0.27 NU/mmHg, which does not differ significantly (P = 0.800) from the baseline value. By contrast, phase decreased as a function of frequency (P < 0.0001; Fig. 3C). In most cases, this tendency was linear at frequencies ≥6.75 Hz (R² = 0.981 ± 0.005, n = 10 ± 1 data points), which indicates the presence of a fixed time delay (97 ± 6 ms) between AP and RSNA.

Baroreflex control of RSNA. The RSNA response to drug-induced changes in AP could satisfactorily be fitted by a four-parameter sigmoid function (R² = 0.972 ± 0.006; Fig. 4A). The maximum gain reached 5.7 ± 0.2 NU/mmHg (Fig. 4B). Resting mean AP lay close to mean AP at the midrange of the curve (Fig. 4B), so that the operational gain (5.0 ± 0.5 NU/mmHg) was close to the maximum gain.
DISCUSSION

The main finding of the present study is that the gain of the transfer function relating RSNA to AP at the frequency of the heartbeat is almost constant from 5.6 to 9 Hz. The practical implication of this finding is that, in this frequency range, HR fluctuations do not confound the interpretation of the transfer gain, especially if it is intended to serve as an index of sympathetic baroreflex sensitivity.

Methodological aspects. Cardiac pacing with a jugular electrode catheter very quickly and efficiently set HR at the desired

Fig. 1. Original 1-s recordings of impulses (imp) delivered to the heart through a jugular electrode, arterial pressure (AP), and renal sympathetic nerve activity (RSNA) in 1 conscious rat. Data were collected under baseline conditions (A) and during cardiac pacing at 7 Hz, before (B) and after (C) ganglionic blockade achieved with chlorisondamine administration. Note cardiac-related bursts of RSNA at spontaneous and paced heart rate. Note also disappearance of these bursts after ganglionic blockade, demonstrating the absence of contamination of the RSNA signal by the electrical stimulus.
frequency, and the return of HR to its spontaneous level was almost immediate after the cessation of stimulation. However, pacing the heart at frequencies below spontaneous HR invariably resulted in the appearance of ectopic beats, and thus, in most animals, the AP-RSNA transfer function could not be studied at frequencies below 5.6 Hz. We are not aware of a simple method to address this question, because decreasing HR by any pharmacological (e.g., /H9252-adrenoceptor blockade and cholinergic muscarinic stimulation) or nonpharmacological (e.g., hypothermia and electrical vagal stimulation) means would also potentially alter RSNA and its baroreflex control, either directly or indirectly, through changes in AP (12, 25).

With use of the present method, HR could be increased up to 9 Hz without evoking significant change in the mean RSNA level. This observation would be consistent with the recent finding that the mean discharge of aortic and carotid sinus baroreceptors of anesthetized rabbits changes very little in the presence of substantial changes in HR (3). However, pacing-induced tachycardia was accompanied by hemodynamic changes. The progressive increase in mean AP, although of modest amplitude, would be expected to induce a reflex decrease in the RSNA level, which was not observed. On the other hand, there was an HR-dependent reduction of the pulse pressure during cardiac pacing. It is possible that this decrease in the pulse pressure might have affected the amplitude of the systolic-diastolic variations in aortic and carotid artery diameters, such that it would have compensated for the effect of the slight increase in mean AP. Changes in the deformation of baroreceptive areas have indeed been shown to occur and engage the baroreceptor reflex, even in the absence of noticeable changes in AP, e.g., during nonhypotensive hypovolemia (15, 29).

An important observation was that the rhythmic bursting of RSNA at HR frequency was not an artifact of the electrical stimulus delivered through the jugular electrode, because it was absent after ganglionic blockade. A strong indication that cardiac-related oscillations of RSNA were of baroreflex origin derives from the analysis of the phase function between AP and RSNA during cardiac pacing. The time delay that was estimated from the linear fitting of these phase values (~97 ms) was very close to the time delay (~101 ms) calculated when a three-element model was applied to the phase of the transfer function relating RSNA to aortic depressor nerve stimulation in the 0.03- to 20-Hz frequency range (23). This observation is consistent with the previous report that arterial baroreceptors respond with a negligible time delay to AP changes (7).

Table 1. Baseline cardiovascular values

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Variation Coef, %</th>
<th>P</th>
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<tbody>
<tr>
<td>AP, mmHg</td>
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<tr>
<td>Systolic</td>
<td>143.0 ± 3.7</td>
<td>0.94 ± 0.17</td>
<td>0.6261</td>
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<tr>
<td>Diastolic</td>
<td>83.5 ± 2.1</td>
<td>1.09 ± 0.22</td>
<td>0.9634</td>
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<tr>
<td>Mean</td>
<td>107.9 ± 2.5</td>
<td>0.91 ± 0.18</td>
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</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>59.5 ± 2.0</td>
<td>1.41 ± 0.17</td>
<td>0.0117</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>338 ± 6</td>
<td>1.29 ± 0.25</td>
<td>0.7665</td>
</tr>
<tr>
<td>RSNA, NU</td>
<td>100</td>
<td>4.49 ± 0.80</td>
<td>0.9104</td>
</tr>
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</table>

Values are means ± SE (n = 7 rats). AP, arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; NU, normalized units. Variation coefficient quantifies variations over time of baseline values (n = 13 in each rat). Statistical significance (P) of these variations was determined by 1-way repeated-measures ANOVA.

Fig. 2. Effect of cardiac pacing at frequencies ranging from 6 to 9 Hz on systolic, mean, and diastolic AP (A), pulse pressure (B), and RSNA (C) in 7 conscious rats. ○, Spontaneous unpaced (U) values. RSNA data were normalized [normalized units (NU)] by the mean RSNA value calculated over baseline periods. Values are means ± SE.
Effect of cardiac pacing on the transfer function. A study performed on anesthetized rabbits has suggested that the amplitude of the cardiac-related oscillation of RSNA might be inversely related to HR (28). However, this conclusion was based on selective observations made after administration of a single dose of propranolol and isoprenaline to a single rabbit. A detailed analysis of the transfer function of the neural limb of the baroreceptor reflex (from carotid sinus pressure to left cardiac SNA) has been carried out in anesthetized rabbits (13). In this study, it was reported that the transfer gain increased up to 0.8 Hz and then declined beyond this frequency. However, coherence approached zero at frequencies >3 Hz, and thus the transfer function could not be studied in the frequency range encompassing spontaneous HR in conscious rabbits (3–5 Hz). In anesthetized rats, the transfer function from carotid sinus pressure to RSNA has been described from 0.01 to 1 Hz (26).

Fig. 3. Effect of cardiac pacing at frequencies ranging from 6 to 9 Hz on coherence (A), transfer gain (B), and phase (C) computed between AP and RSNA at the frequency of the heartbeat in 7 conscious rats. ●, Spontaneous unpaced (U) values. Transfer gain was normalized by the mean RSNA value calculated over baseline periods. Values are means ± SE.

Fig. 4. Baroreflex relation between mean AP and RSNA determined during sequential injections of sodium nitroprusside and phenylephrine. Group average (n = 7) parameters were used to generate baroreflex function curves (A) and their first derivative (B). ●, Gain observed at reference mean AP.
In the latter study, the transfer gain increased up to 0.8 Hz. As far as we know, the behavior of the transfer function relating SNA to baroreceptor pressure has not been described at frequencies >1 Hz in rats. This transfer function combines the properties of the transfer function from baroreceptor pressure to baroreceptor afferent nerve activity and those of the transfer function from afferent nerve activity to efferent SNA. The latter has been described by studying the effect of rhythmic electrical stimulation of the aortic depressor nerve on RSNA in anesthetized rats (23). The study has revealed that central nervous pathways of the baroreceptor reflex show derivative properties that result in amplification and acceleration of RSNA responses in the 0.03- to 1-Hz frequency range. At higher frequencies, low-pass filter properties become predominant, so that the transfer gain declines regularly with a slope of −20 dB/decade, which is the hallmark of a first-order low-pass filter or the combination of a derivative gain and a second-order low-pass filter. However, this decrease in gain is interrupted between 6 and 12 Hz, so that the gain function shows a local maximum near 10 Hz. This phenomenon might be related to the properties of brain stem neuronal networks responsible for the generation of SNA, as described by Barman et al. (1) in anesthetized cats. In summary, it is difficult to predict from the existing data in the literature the shape of the gain function of the neural limb of the baroreceptor reflex in the frequency range encompassing spontaneous HR in the rat. By using an isolated rat aortic arch preparation, Brown et al. (5) were able to study the gain of the transfer function relating baroreceptor discharge to baroreceptor pressure from 0.1 to 20 Hz. The transfer gain increased from 1 to 10 Hz for myelinated baroreceptor fibers, whereas it decreased monotonically from 0.1 Hz for unmyelinated fibers. In the present study, the transfer gain was relatively stable between 5.6 and 9 Hz, which was unexpected if one considers the particular properties of the central transfer function mentioned above. A tentative explanation would be that the low-pass filter properties of arterial baroreceptors cancelled the amplification of the gain provided by central nervous structures at these frequencies. This hypothesis would be consistent with reports of the predominance of unmyelinated over myelinated axons in the carotid sinus (19) and aortic depressor (9) nerves of the rat (≥4:1 axon ratio). Another observation supports this hypothesis. In the aortic nerve stimulation experiments (23), the transfer gain in the 6- to 9-Hz frequency range was near or slightly above the static gain, i.e., the gain measured at the lowest stimulation frequency (0.03 Hz). In the present study, the transfer gain in the same frequency range was about one-half of the static gain measured by the pharmacological method, pointing to a significant attenuation of RSNA responses.

The influence of nonlinearities in baroreflex relations might also be considered. For example, an increase in pulse pressure is supposed to decrease the transfer gain due to the saturation effect, and vice versa (11). However, the importance of this effect depends on the location of resting AP on the baroreflex function curve. In the present study, resting AP was close to the midpoint of the curve (Fig. 4). It is thus unlikely that changes in the pulse pressure would have markedly affected the transfer gain.

Limitations of the study. As mentioned above, the pacing technique used in this study did not allow assessment of the transfer function at low HR values (<330 beats/min). This might appear as a serious limitation of the study, because the HR of a conscious, quiet rat frequently falls below 330 beats/min, especially when it is measured using a radiotelemetry system (16). However, it must be recalled that the HR of a rat equipped with a renal electrode rarely shows such slow values, even when recordings are performed several days after surgery (20). Therefore, the limitations of the present study will grossly coincide with those of the techniques available for direct recording of RSNA in conscious rats.

Conclusions and perspectives. The present study indicates that HR does not affect the gain of the AP-RSNA transfer function at HR frequency in the upper part of the physiological range of HR variations in the anesthetized rat. It remains to be determined whether the transfer gain is insensitive to spontaneous HR fluctuations under physiological conditions, i.e., in the conscious, freely behaving rat.

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