The low frequency power of heart rate variability is neither a measure of cardiac sympathetic tone nor of baroreflex sensitivity

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1Florey Institute of Neuroscience and Mental Health, University of Melbourne, Parkville, Victoria, Australia; 2Laboratory of Physiological Regulation in Sleeping Mice, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy; 3Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Australia; and 4Department of Physiology, University of Auckland, Auckland, New Zealand

Submitted 26 May 2014; accepted in final form 23 July 2014

Martelli D, Silvani A, McAllen RM, May CN, Ramchandra R. The low frequency power of heart rate variability is neither a measure of cardiac sympathetic tone nor of baroreflex sensitivity. Am J Physiol Heart Circ Physiol 307: H1005–H1012, 2014. First published August 1, 2014; doi:10.1152/ajpheart.00361.2014.—The lack of noninvasive approaches to measure cardiac sympathetic nerve activity (CSNA) has driven the development of indirect estimates such as the low-frequency (LF) power of heart rate variability (HRV). Recently, it has been suggested that LF HRV can be used to estimate the baroreflex modulation of heart period (HP) rather than cardiac sympathetic tone. To test this hypothesis, we measured CSNA, HP, blood pressure (BP), and baroreflex sensitivity (BRS) of HP, estimated with the modified Oxford technique, in conscious sheep with pacing-induced heart failure and in healthy control sheep. We found that CSNA was higher and systolic BP and HP were lower in sheep with heart failure than in control sheep. Cross-correlation analysis showed that in each group, the beat-to-beat changes in HP correlated with those in CSNA and in BP, but LF HRV did not correlate significantly with either CSNA or BRS. However, when control sheep and sheep with heart failure were considered together, CSNA correlated negatively with HP and BRS. There was also a negative correlation between CSNA and BRS in control sheep when considered alone. In conclusion, we demonstrate that in conscious sheep, LF HRV is neither a robust index of CSNA nor of BRS and is outperformed by HP and BRS in tracking CSNA. These results do not support the use of LF HRV as a noninvasive estimate of either CSNA or baroreflex function, but they highlight a link between CSNA and BRS.

heart; sympathetic nervous system; baroreceptor reflex; heart failure; heart rate variability

HEART PERIOD (HP) is one of the instrumental variables, such as stroke volume, cardiac output, and blood pressure (BP), which are subjected to continuous adjustments for the maintenance of fundamental variables such as arterial O2 and CO2 concentrations, body temperature, and osmolality of the intercellular fluid (4). This continuous adjustment, operated by the levels of activity in cardiac sympathetic and parasympathetic nerves, is what causes HP to fluctuate over time. The contributions of HP fluctuations at different frequencies to total heart rate (HR) variability (HRV) may be quantified by a power spectral analysis of the HP signal in the frequency domain (7).

An increase in the mean level of cardiac sympathetic nerve activity (CSNA) in pathological conditions such as hypertension or heart failure is detrimental as it can promote disease progression, arrhythmias, and sudden death (3, 11). Having a noninvasive measure of CSNA would be extremely beneficial in the assessment and management of these diseases. The lack of direct noninvasive approaches to measure CSNA has encouraged the scientific community to look for indirect estimates, the most popular of which has been the low-frequency (LF) power of HRV (7). In addition, the ratio of the power of HRV in the LF band to the high-frequency (HF) band (LF-to-HF ratio) has been proposed to represent sympathovagal balance (6).

There has been much debate regarding the pitfalls of LF HRV and other related estimates (for reviews, see Refs. 6 and 12). The arguments against the validity of LF HRV as an index of CSNA have been highlighted in a recent review article (8) that suggested that LF HRV is not a measure of the level of CSNA but rather of the modulation of cardiac autonomic outflows by baroreflexes. This hypothesis was mainly supported by evidence that LF HRV decreases with baroreflex impairment and correlates with the baroreflex sensitivity (BRS) of HP (17, 22). Accordingly, there is evidence that LF fluctuations of BP and HP originate from baroreflex buffering of BP perturbations because the baroreflex, which is a delayed negative feedback control, resonates in the LF band (2, 5).

In the present study, we directly tested the hypothesis (8) that LF HRV does not reflect the level of CSNA but rather baroreflex function. We performed simultaneous direct recordings of CSNA, HP, and BP in conscious sheep (10) and explored baroreflex function with the modified Oxford technique of drug-induced changes in BP (23). We studied control healthy sheep as well as sheep with pacing-induced heart failure (23), which is a condition of heightened CSNA tone with significant clinical implications (3, 11). We performed a cross-correlation analysis of spontaneous cardiovascular fluctuations (19, 21) to investigate whether in each individual, HP was coupled to CSNA and BP. We then analyzed correlations between the values of HP, LF HRV, CSNA, and BRS obtained in individual control sheep and sheep with heart failure.

METHODS

Adult Merino ewes (body weight: 35–45 kg) were housed in individual cages, fed a diet of oat chaff (800 g/day), and provided water ad libitum. Experiments were started when sheep were accustomed to laboratory conditions and human contact. All experiments were approved by the Animal Experimentation Ethics Committee of the Florey Institute of Neuroscience and Mental Health under guidelines laid down by the National Health and Medical Research Council of Australia.

Surgical procedures. Before the experiments, sheep underwent two aseptic surgical procedures separated by at least 2 wk. Anesthesia was induced with intravenous thiopental sodium (15 mg/kg) and maintained with 1.5–2.0% isoflurane/O2 after intubation. In the first sur-
surgery, all sheep were prepared with a carotid arterial loop. In the sheep to be paced into heart failure, a pacing lead was also inserted into the right ventricle under fluoroscopic guidance (23). In the second surgery, after thoracotomy, intraventricular recording electrodes were implanted in the left or right cardiothoracic nerves (24). Animals were treated with antibiotics (900 mg procaine penicillin, Troy Laboratories, NSW, Australia) at the start of each surgery and then for 2 days postoperatively. Postsurgical analgesia was obtained with flunixin meglumine (1 mg/kg im, Troy Laboratories) at the start of surgery and 4 and 16 h postsurgery. The day before implantation of recording electrodes, a catheter was inserted into the carotid arterial loop. Cannulae for measurement of arterial BP were connected to pressure transducers (TDXIII, Cobe, Lakewood, CA) tied to the wool on the back. Pressures were corrected to compensate for the height of the transducers above the level of the heart. In addition, four leads were attached at the four limb positions to collect an electrocardiographic signal, which was used to monitor heart rate (HR). Pressures were recorded simultaneously. The heart rate was determined by computing the mean of the absolute value of the data to reduce skewness of the distribution analyzed with the CCF analysis. The sign of the CCF was used for the purpose of ancillary analyses of HRV. As a measure of burst size, CSNA spike frequency was calculated as the number of CSNA spikes above threshold in each heart beat. The threshold was set just above background so that spikes from small bursts were counted (23). To determine CSNA burst incidence, the smallest CSNA burst was visually identified in the entire recording for each sheep, and the corresponding spikes above threshold were taken as the threshold for burst detection. For each sheep, the accuracy of burst detection was checked visually on raw tracings (18). The number of CSNA spikes in each heart beat was then divided by HP to yield CSNA spike frequency. BRS was estimated by plotting HP against systolic BP and fitting the data with a four-parameter sigmoidal logistic equation (SigmaPlot, version 8.0, SPSS) for each animal (23).

Analysis of cross-correlation functions (CCFs) and power spectra were performed with custom routines written in Matlab and its signal processing toolbox (MathWorks, Natick, MA) on beat-to-beat values resampled at 4 Hz (cubic spline interpolation). Cross-correlation functions were averaged over consecutive data windows of 60-s duration and 45-s overlap, limited to time shifts (τ values) between +10 s and −10 s, and normalized so that the autocorrelations at τ = 0 were identically 1. This CCF analysis yielded linear correlation coefficients (p values) between CSNA spike frequency and HP and between systolic BP and HP as a function of the respective τ values between these variables. The sign of the τ value indicated whether HP fluctuations preceded (positive sign) or were preceded by (negative sign) those of CSNA spike frequency or systolic BP. The sympathetic control of the heart is expected to cause a negative correlation between CSNA spike frequency and the following values of HP, i.e., a negative trough of CSNA versus HP CCFs at negative τ values. The baroreflex control of the heart is expected to cause a positive correlation between CSNA spike frequency and the following values of HP, i.e., a positive peak of systolic BP versus HP CCFs at positive τ values. For each sheep, we thus retained for analysis the minimum negative p value of the average CCF between CSNA and HP and the maximum positive p value of the average CCF between BP and HP together with the corresponding τ values.

The power spectrum of HRV was computed on HP data with the fast Fourier transform and averaged over consecutive Hanning-windowed data segments of 60-s duration and 45-s overlap. LF HRV spectral power was computed as HRV spectral power at frequencies between 0.05 and 0.15 Hz (23). In addition, the HRV LF-to-HF ratio was computed by dividing LF HRV by HRV spectral power in the HF range between 0.15 and 0.4 Hz (7). Ancillary analyses were performed by computing the indexes of LF HRV and LF-to-HF HRV based on time series of HR rather than on those of HP.

Statistical analysis. The values of τ yielded by the CCF analysis were reported as medians and intervals and analyzed with a binomial single-sample sign test (cutoff value of 0, probability parameter of 0.5) to evaluate whether they significantly clustered at negative or positive values in each group of sheep. Differences in median values of τ between groups were analyzed with a Mann-Whitney test. All other statistical tests were performed after logarithmic transformation of the absolute value of the data to reduce skewness of the distribution. Differences between groups were analyzed with an independent-sample t-tests. Pearson’s linear correlation coefficient was computed between LF HRV, measures of CSNA, and BRS. Correlations that were statistically significant taking all sheep into account were reported as medians and intervals and analyzed with a binomial single-sample sign test (cutoff value of 0, probability parameter of 0.5) to evaluate whether they significantly clustered at negative or positive values in each group of sheep. Differences in median values of τ between groups were analyzed with a Mann-Whitney test. All other statistical tests were performed after logarithmic transformation of the absolute value of the data to reduce skewness of the distribution. Differences between groups were analyzed with an independent-sample t-tests. Pearson’s linear correlation coefficient was computed between LF HRV, measures of CSNA, and BRS. Correlations that were statistically significant taking all sheep into account were reported.

Table 1. Mean values of cardiovascular and autonomic variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Sheep</th>
<th>Sheep With Heart Failure</th>
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<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>104 ± 4</td>
<td>89 ± 4 *</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>76 ± 3</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>HP, ms</td>
<td>892 ± 49</td>
<td>726 ± 39 *</td>
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<tr>
<td>LF HRV, m²s</td>
<td>858 ± 163</td>
<td>593 ± 216</td>
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<tr>
<td>LF-to-HF HRV, %</td>
<td>4.3 ± 1.4</td>
<td>8.2 ± 1.9</td>
</tr>
<tr>
<td>CSNA burst incidence, %</td>
<td>28 ± 5</td>
<td>84 ± 4 *</td>
</tr>
<tr>
<td>CSNA frequency, Hz</td>
<td>10 ± 3</td>
<td>50 ± 11 *</td>
</tr>
<tr>
<td>Baroreflex sensitivity of HP, ms/mmHg</td>
<td>33 ± 6</td>
<td>26 ± 5</td>
</tr>
</tbody>
</table>

Data are means ± SE in control sheep [n = 8 for baroreflex sensitivity (BRS) of heart period (HP) and n = 9 otherwise] and sheep with heart failure [n = 7 for BRS and n = 9 otherwise]. BP, blood pressure; HR, heart rate; LF HRV, spectral power of heart rate variability; LRV, low-frequency; HF HRV, high-frequency; CSNA, cardiac sympathetic nerve activity burst incidence (percentage of heart beats associated with bursts) and burst frequency (spike frequency of CSNA); *P < 0.05 vs. control sheep (by t-tests performed on logarithm-transformed data).
tested separately on each group of sheep. Data are reported as means ± SE, with n = 9 sheep/group with the exception of BRS, where n = 7 sheep with heart failure and n = 8 control sheep. Statistical significance was set at P < 0.05 in all tests.

RESULTS

Development of heart failure. Left ventricular ejection fraction and fractional shortening, measured in conscious sheep by echocardiography, gradually decreased over 8–10 wk of rapid ventricular pacing at 200–220 beats/min. In sheep with heart failure, at 1–2 days before implantation of recording electrodes, ejection fraction (34 ± 1%) and fractional shortening (17 ± 1%) were significantly reduced compared with prepping values (83 ± 2% and 52 ± 2%, respectively). Sheep with heart failure had significantly lower values of systolic BP and HP and significantly higher values of CSNA in terms of spike frequency and burst incidence compared with control sheep (Table 1). BRS, LF HRV, and LF-to-HF HRV indexes did not differ significantly between groups.

Spontaneous fluctuations of HP are coupled to those of CSNA and BP. Figure 1 shows representative raw tracings of the electrocardiogram, BP, and CSNA in control sheep and sheep with heart failure. Inspection of the cardiovascular time series suggested that the electrocardiogram appeared to be temporally linked to CSNA spike frequency. A link between HP and CSNA was evident between increases in CSNA and decreases in HP in control sheep (Fig. 2A). In sheep with heart failure, which had higher average values of CSNA spike frequency, the link between CSNA and HP was better evident as decreases in CSNA spike frequency in correspondence with high values of HP (Fig. 2B). These observations received quantitative support by the analysis of CCF between CSNA spike frequency and HP, which showed a negative trough at negative τ values (Fig. 2C and Table 2). This indicates a negative correlation between values of CSNA spike frequency and the subsequent values of HP. This pattern of coupling between CSNA spike frequency and HP did not differ significantly between control sheep and sheep with heart failure in terms of either ρ or τ values (Table 2). Inspection of the cardiovascular time series also suggested that the spontaneous fluctuations of HP were temporally linked to those of systolic BP. This correlation was evident as increases in HP that
Fig. 2. Physiological coupling between spontaneous fluctuations of heart period (HP), CSNA (spike frequency), and systolic arterial BP. A and B: representative tracings of HP and CSNA spike frequency in a control sheep (A) and a sheep with heart failure (B). C: cross-correlation function (CCF) between CSNA spike frequency and HP. The negative trough of the CCF at negative time shifts (τ values) indicates that HP correlated negatively with the previous values of CSNA spike frequency. D and E: representative tracings of HP and BP in the same animals as in A and B, respectively. F: CCF between BP and HP. The positive peak at negative τ values indicates that HP correlated positively with the previous values of BP. Data in C and F are shown as means (thick lines) ± SE (thin lines) in sheep with heart failure and control sheep, with n = 9 sheep/group.

followed increases in BP in control sheep (Fig. 2D) and sheep with heart failure (Fig. 2E). These observations were again supported by CCF analysis of the coupling between HP and BP, which showed a positive peak at negative τ values (Fig. 2F and Table 2). This indicates that the fluctuations of HP correlated positively with the previous values of BP, as expected based on cardiac baroreflex control, which increases HP in response to increases in BP. This pattern of coupling between BP and HP did not differ significantly between control sheep and sheep with heart failure in terms of the p value, whereas the τ value corresponding to the CCF peak was significantly more negative in sheep with heart failure than in control sheep (Table 2).

*LF HRV and LF-to-HF HRV are not indexes of CSNA tone.*

Figure 3 shows the results of spectral analysis of HRV, which evidenced an increase in HRV power spectral density (PSD) with decreasing frequency in control sheep and sheep with heart failure, as expected. A HF peak of HRV PSD was evident at frequencies between 0.3 and 0.4 Hz in control sheep but not in sheep with heart failure. This fits well with evidence showing that the eupneic respiratory rate is ~20 breaths/min (i.e., 0.33 Hz) in nonheat-stressed shorn sheep at rest (13).

CSNA spike frequency and burst incidence correlated negatively and significantly with the mean value of HP when all

Table 2. Quantitative analysis of CCFs between CSNA, HP, and systolic BP

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<th>Control Sheep</th>
<th>Sheep With Heart Failure</th>
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<tr>
<td></td>
<td>CCF of CSNA vs. HP</td>
<td>CCF of BP vs. HP</td>
</tr>
<tr>
<td>Trough</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ρ, s</td>
<td>−0.31 ± 0.03†</td>
<td>−0.35 ± 0.03†</td>
</tr>
<tr>
<td></td>
<td>CCF of BP vs. HP</td>
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<tr>
<td>Peak</td>
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<tr>
<td>ρ, s</td>
<td>0.38 ± 0.05†</td>
<td>0.46 ± 0.05†</td>
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Values are reported as means ± SE or as medians (minimum to maximum) in control sheep (n = 9) and sheep with heart failure (n = 9). ρ and τ, correlation coefficient and time shift, respectively, at the peak or trough or cross-correlation functions (CCFs) between CSNA spike frequency and HP or between systolic arterial BP and HP. Negative values of τ refer to the correlation between HP and the previous values of either CSNA or BP. *P < 0.05 vs. control sheep (by Mann-Whitney test); †P < 0.05 vs. 0 (by binomial test).
sheep were taken into account (Fig. 4, A and B), but these correlations lost statistical significance when computed separately on each sheep group. There was no significant correlation between individual values of CSNA spike frequency and burst incidence and LF HRV (Fig. 4, C and D) or LF-to-HF HRV (Fig. 4, E and F). Similar results were obtained in ancillary analyses performed computing LF HRV and LF-to-HF HRV based on HR rather than on HP (results not shown).

**LF HRV and LF-to-HF HRV are not indexes of BRS of HP.** Figure 5 shows representative examples of the baroreflex sigmoid functions, from which BRS was computed as the maximum baroreflex gain of HP in control sheep and sheep with heart failure. There was no significant correlation between individual values of BRS and either LF HRV (Fig. 6A) or LF-to-HF HRV (Fig. 6B). Similar results were obtained in ancillary analyses performed computing LF HRV and LF-to-HF HRV based on HR rather than on HP (results not shown).

**BRS of HP and CSNA are linked.** Individual values of BRS correlated negatively and significantly with CSNA spike frequency (Fig. 6C) when all sheep were taken into account. This correlation remained statistically significant when tested separately on control sheep, whereas it lost statistical significance when tested separately on sheep with heart failure.

**DISCUSSION**

Our study yielded three main findings. First, spontaneous fluctuations in HP were coupled to those of CSNA and BP in both normal animals and animals with heart failure. This is consistent with sympathetic and baroreflex control of HP, respectively. Second, despite such coupling, LF HRV and LF-to-HF HRV in different animals did not correlate with either CSNA tone or BRS of HP and, thus, could not be considered indexes thereof. Finally, we found evidence of a negative correlation between CSNA tone and BRS of HP in control sheep, which was lost in sheep with heart failure.

A unique feature of the present study was the comparison of LF HRV and LF-to-HF HRV with direct recordings of CSNA in conscious animals. Our results are partly at variance with those obtained by Piccirillo and coworkers (16), who measured left stellate ganglion activity before and after pacing-induced heart failure in ambulatory dogs. These authors found a significant yet weak ($r^2 = 0.10$) correlation between integrated left stellate ganglion nerve activity and LF HRV in control dogs but not in dogs with heart failure (16). However, it must be noted that left stellate ganglion activity contains activity from sympathetic neurons, which project to other destinations, such as the forelimb and the face, and the accuracy with which it tracks CSNA to the sinoatrial node is unclear. On the other hand, our present results confirm our previous findings that postsurgical changes in CSNA tone do not correlate with those

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**Fig. 4.** A–F: scatterplots of HP (A and B), LF power of HRV (C and D), and the index of LF-to-HF HRV, which is the ratio between LF HRV and HF power of HRV (E and F), versus CSNA [spike frequency (freq; A, C, and E) and burst incidence (inc; B, D, and F)]. Circles represent data in individual sheep. All data were logarithm transformed before plotting and analysis. Green lines represent the regression lines and their 95% confidence intervals for significant correlations computed taking all sheep into account irrespective of experimental group. $r^2$ is the square of Pearson’s correlation coefficient, which estimates the fraction of shared variance between the two signals included in the scatterplot. *$P < 0.05$ for the linear correlation.
in LF HRV in healthy sheep (10) and that sheep with heart failure differ from control sheep in terms of CSNA but not of LF HRV (23).

Our present negative finding regarding the correlation between LF HRV and BRS of HP contrasts with previous reports (14, 17) in which such correlation was clearly observed. One possible reason for this discrepancy is that these studies included patients with autonomic failure (14, 17), whereas the range of BRS values was smaller in our animals, which makes it more difficult to establish a significant correlation. Additionally, patients with autonomic failure have compromised vascular control such that the reduced LF HRV may be secondary to vasomotor failure. Another potential reason for the discrepancy is that these studies (14, 17) on humans estimated BRS in response only to reductions in BP, either induced by nitroprusside or during phase II of a Valsalva maneuver. Conversely, we estimated BRS of HP based on full baroreflex sigmoid curves obtained in response to graded increases and decreases in BP caused by multiple doses of phenylephrine and nitroprusside (cf. Fig. 5). On the other hand, we performed experiments after a relatively short period of recovery from open-chest surgery to prevent deterioration of the CSNA signal-to-noise ratio (cf. METHODS). Thus, an effect of surgery on baseline CSNA levels cannot be ruled out but was nonetheless insufficient to obscure the clear-cut differences in CSNA that we found between control sheep and sheep with heart failure (Table 1).

It might be argued that correlations between LF HRV and either CSNA or BRS of HP were not detected in our study because of physiological or technical (i.e., measurement noise) factors specific to our experimental preparation. For instance, the sampling rate of our electrocardiographic signal (100 Hz, cf. METHODS) was lower than that recommended in human subjects (> 250 Hz, cf. Ref. 7), which may interfere with the precision of QRS peak detection. To evaluate this alternative interpretation, we tested for the occurrence of detectable physiological relationships between the spontaneous fluctuations of HP, CSNA, and BP recorded in our preparation by performing a CCF analysis. The CCF analysis has proven successful in

Fig. 5. Beat-to-beat data (circles) of HP and systolic BP after intravenous infusions of sodium nitroprusside and phenylephrine, which were used to fit the baroreflex sigmoid functions (lines). Representative experiments in a control sheep and a sheep with heart failure are shown.

Fig. 6. A–D: scatterplots of LF power of HRV (A), the ratio between LF HRV and HF power of HRV (LF/HF HRV; B), and CSNA [in terms of spike frequency (C) or burst incidence (D)] versus the baroreflex sensitivity (BRS) of HP. Circles represent data in individual sheep. All data were logarithm transformed before plotting and analysis. Lines represent the regression lines and their 95% confidence intervals for significant correlations computed taking into account all sheep (green) or only control sheep (black). \( r^2 \) is the square of Pearson’s correlation coefficient, which estimates the fraction of shared variance between the two signals included in the scatterplot. *\( P < 0.05 \) for the linear correlation.
detecting linear relationships between spontaneous cardiovascular fluctuations in different species, including human subjects and sheep (19–21). We found that CCFs between CSNA and HP had a negative trough at negative $\tau$ values (Fig. 2C), which was superimposed in control sheep and in sheep with heart failure. This result indicates that the heart accelerated after CSNA had increased. This may correspond partly to the positive chronotropic effect of CSNA or, likely given the short time constant, may be due to inhibition of vagal activity linked to the increase in CSNA. Accordingly, the sinoatrial response to CSNA behaves as a delayed low-pass filter (1), which, together with the effect of concomitant parasympathetic cardiac control, likely accounts for the relatively low absolute value of the trough $\rho$ value between CSNA and HP (Table 2). Thus, the CSNA signal we measured was sufficiently robust to correlate with small changes in HP. We further tested whether the link between the spontaneous fluctuations of HP and those of BP was consistent with the expected arterial baroreflex control of HP. CCFs between systolic BP and HP had a positive peak at negative $\tau$ values, indicating that HP increased after increases in BP. This is consistent with cardiac baroreflex control, which behaves as a delayed negative feedback loop (21). The peak $\rho$ value between systolic BP and HP was similar between groups, whereas the corresponding $\tau$ value was significantly more negative in sheep with heart failure than in control sheep (Table 2). This is in line with experimental (19, 20) and theoretical (21) work that have shown that $\tau$, representing the effective time delay of the spontaneous cardiac baroreflex, is lengthened when sympathetic “central command” is relatively stronger. The HP and BP signals that we measured were sufficiently robust to correlate with each other comparably to results previously obtained in newborn lambs (19) and adult human subjects (20).

In light of these considerations, the most parsimonious explanation for our present findings is that LF HRV is not an adequate index of either CSNA tone or BRS of HP, as it may be unable to capture the physiological relationships among HP, CSNA, and BP in conscious animals. This negative result was obtained regardless of the details of LF HRV computation, i.e., in absolute values versus values normalized to the HF power of HRV. Thus, while the reason for the discrepancy between our study and those on humans (14, 17) remains unclear, our negative results at least cast doubt on the reliability of LF HRV and LF-to-HF HRV as markers of CSNA tone and BRS of HP in control subjects and in conditions of heart failure. As a matter of fact, LF HRV and LF-to-HF HRV were outperformed by a simple measurement of mean HP in tracking CSNA tone among different subjects (Fig. 4, A and B). However, the correlation between HP and CSNA tone was relatively weak ($r^2 = 0.26–0.31$ depending on the CSNA index) and mainly reflected differences between groups, being lost when computed separately in each group of sheep. This highlights the complexity of HP control, which depends not only on CSNA but also on parasympathetic nerve activity, the sinoatrial response to norepinephrine and ACh, and intrinsic HP.

Knowledge of CSNA tone in patients would clearly benefit the management of clinical conditions such as heart failure (3, 11). Thus, as Pagani et al. (15) have recently noted, it is important to develop and refine markers of CSNA tone. In this respect, our finding of a significant correlation between CSNA tone in terms of spike frequency and BRS of HP (Fig. 6C) is of interest because the pharmacological estimation of BRS of HP with the modified Oxford method, although cumbersome and potentially dangerous, may be performed in human subjects, whereas direct measurements of CSNA are not possible. However, the potential usefulness of BRS of HP to track CSNA tone in patients with heart failure is limited by our finding that the correlation between BRS and CSNA was significant in control sheep but became weaker when control sheep and sheep with heart failure were taken into account together, and lost significance altogether when tested separately in sheep with heart failure (Fig. 6C). Moreover, BRS of HP did not correlate significantly with CSNA tone in terms of burst incidence (Fig. 6D). The causal mechanisms that underlie the correlation between BRS of HP and CSNA spike frequency also warrant further experimental work. These mechanisms may include baroreflex restraint of CSNA, CSNA restraint of the vagal modulation of HP in response to baroreflex control, or parallel central autonomic modulation of CSNA and baroreflex circuits.

In conclusion, we have shown, using direct recordings of CSNA, that the LF power of HRV is neither an index of CSNA tone nor a measure of BRS of HP. Thus, our results support the first conclusion of a recent review by Goldstein et al. (8), namely, that LF HRV is not a measure of cardiac sympathetic tone, but do not support the second conclusion, namely, that LF HRV may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Perhaps it is time to accept that changes in HRV may never be a substitute for direct recordings of CSNA (6, 9).

ACKNOWLEDGMENTS

The authors acknowledge the expert technical assistance of Alan McDonald, Tony Dornom, and David Trevaks.

GRANTS

This work was supported by a National Health and Medical Research Council of Australia Grant and the Victorian Government’s Operational Infrastructure Support Program. R. Ramchandra was the recipient of National Health and Medical Research Council/National Heart Foundation Career Development Fellowship, and C. N. May was supported by National Health and Medical Research Council Research Fellowship 566819.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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