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Abnormal autonomic cardiac response to transient hypoxia in sickle cell anemia

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Abstract

The objective of this study was to non-invasively assess cardiac autonomic control in subjects with sickle cell anemia (SCA) by tracking the changes in heart rate variability (HRV) that occur following brief exposure to a hypoxic stimulus. Five African–American SCA patients and seven healthy control subjects were recruited to participate in this study. Each subject was exposed to a controlled hypoxic stimulus consisting of five breaths of nitrogen. Time-varying spectral analysis of HRV was applied to estimate the cardiac autonomic response to the transient episode of hypoxia. The confounding effects of changes in respiration on the HRV spectral indices were reduced by using a computational model. A significant decrease in the parameters related to parasympathetic control was detected in the post-hypoxic responses of the SCA subjects relative to normal controls. The spectral index related to sympathetic activity, on the other hand, showed a tendency to increase the following hypoxic stimulation, but the change was not significant. This study suggests that there is some degree of cardiovascular autonomic dysfunction in SCA that is revealed by the response to transient hypoxia.

Keywords

heart rate variability (HRV); sickle cell anemia (SCA); hypoxia; autonomic control; model

1. Introduction

Sickle cell anemia (SCA) is a genetic disorder where regular adult hemoglobin (HbA) has mutated into sickle hemoglobin (HbS) (Frenette and Atweh 2007). It is characterized by acute vaso-occlusive episodes in which normally flexible red blood cells (RBC) are transformed from their regular round shape to rigid, sickle shape and occlude microvasculature. The substitution of valine for glutamic acid at position 6 of the beta globin chain of hemoglobin causes hemoglobin S to polymerize in the de-oxy state and results in the marked reduction in flexibility of the red cell as oxygen is released in the tissue. This process reverses when the red cell returns to the lung and is reoxygenated. Adverse events such as acute pain, acute chest syndrome, multi-organ dysfunction, stroke, or renal dysfunction are common among this cohort of patients and lead to increased mortality (Platt *et al* 1994, Quinn *et al* 2007).

While this disorder is often characterized as episodes of sickling crisis between periods of normalcy, the sickling process is occurring continually. What triggers the transition from low-rate sickling to widespread crisis is not known. Hypoxia and decrease in local perfusion are known to trigger crisis. Furthermore, the frequency of transient 15–30 s duration episodes of

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hypoxia during sleep is strongly correlated with the frequency of crisis and development of overt stroke (Hill *et al* 2006, Hargrave *et al* 2003, Kirkham *et al* 2001).

Studies suggest that there are some degrees of cardiovascular autonomic dysfunction among SCA patients and sickle cell trait carriers (Connes *et al* 2006, Romero Mestre *et al* 1997, Romero-Vecchione *et al* 1995, Pearson *et al* 2005). Although acute atherosclerosis-related myocardial infarctions are rare in SCA patients, diverse cardiovascular anomalies—including heart murmur, cardiomegaly, biventricular hypertrophy and abnormalities in the cardiac conducting system—which sometimes correlate to sudden death in SCA patients, have been described by multiple researchers (James *et al* 1994, Assanasen *et al* 2003, Norris *et al* 1991, Pavlu *et al* 2007, Manci *et al* 2003, Batra *et al* 2002). Transient cardiac dysfunction and myocardial ischemia have also been reported during sickle cell crisis (Norris *et al* 1991, Deymann and Goertz 2003). Interestingly, up to 40% of the deaths in adults with sickle cell disease are the so-called sudden death events with no detectable cause found at autopsy (Darbari *et al* 2006, Perronne *et al* 2002, Platt *et al* 1994).

In past decades, interest in heart rate variability (HRV) as a non-invasive method of assessing the autonomic nervous activity has grown extensively (ESC/NASPE Task Force 1996). It is commonly accepted that parasympathetic (vagal) activity is a major contribution to the high-frequency (HF, 0.15–0.4 Hz) components of HRV. Consequently, the power of HRV in this frequency band has widely been used to quantitatively represent the level of vagal activity. On the other hand, the low-frequency (LF, 0.04–0.15 Hz) components of HRV can be due to both vagal and sympathetic activities (Eckberg 1997). Thus, the ratio between LF and HF spectral powers has been used by researchers broadly as an index of ‘sympathovagal balance’ (Cerutti *et al* 2001, ESC/NASPE Task Force 1996).

Although cardiovascular autonomic dysfunction in SCA patients has been reported by several groups, the causal relationship of the dysfunction to a known risk factor for crisis in this group of patients has not yet been reported. In this study, we introduce hypoxia as a potential stimulant of vaso-occlusion. Cardiovascular autonomic control following a brief-controlled episode of hypoxia was assessed non-invasively using spectral analysis of HRV.

We applied a time-varying modification of the traditional manner of HRV computation to determine the changes in autonomic function following transient exposure to hypoxia to be able to detect rapid changes in HRV. This method is based on a recursive autoregressive algorithm used by our group in previous studies on obstructive sleep apnea patients (Blasi *et al* 2003a, 2003b).

Furthermore, we extended the analytical method to compensate for variability in respiration. Since HRV is known to be confounded by intrasubject and intersubject differences in respiration pattern (Berntson *et al* 1997, Hirsch and Bishop 1981), we propose a technique to eliminate the effect of fluctuation in respiration patterns from the conventional HRV parameters. This technique is based on a previously proposed method (Khoo *et al* 1999), which partitions HRV into respiration-correlated and respiration-uncorrelated components. In doing so, the technique produces HRV indices that are substantially less confounded by the variability in respiratory pattern.

2. Methods

In this preliminary study, five SCA patients and seven normal control subjects were recruited to participate in the studies, carried out at Childrens Hospital Los Angeles (CHLA), Los Angeles, CA. Informed consent was obtained prior to each study. The protocol for the studies was approved by the institutional committee on human experimentation at CHLA. Four out of five sickle cell patients were treated with hydroxyurea. The subjects had no symptoms of sickle

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crisis within two weeks before the measurements, and had no blood transfusions in the past two months. The individual subject characteristics are displayed in table 1.

2.1. Experimental protocol

All subjects were spontaneously breathing through a face mask connected to a one-way valve which allows switching from room air to 100% nitrogen (N_2). Subjects were comfortably awake in a supine position and breathed room air for at least 15 min before the valve was switched to breathing in 100% N_2 for five breaths, following which the inhalate was returned to room air. The subject underwent a second five breath exposure to N_2 for 5–10 min later after the oxygen saturation and vital signs had returned to baseline. The duration and magnitude of the hypoxic stimulus were designed to mimic the episodes of hypoxia that occur naturally during sleep, while at the same time, taking into account considerations for subject's safety. The whole process was controlled so that the subjects were not aware of the change from room air to N_2. During each test respiratory patterns, electrocardiogram (ECG), oxygen saturation (SaO_2) and end-tidal CO_2 were continuously monitored by Lifeshirt® physiological monitoring system (VivoMetrics, Ventura, CA). The ECG was sampled at 200 Hz. The other signals were sampled at 50 Hz.

Immediately prior to the experiment, a blood sample was obtained from each subject for determination of the O_2 saturation curve. Oxy-hemoglobin dissociation curves were measured at 37 °C using a Hemox-Analyzer model B (TCS Scientific Corporation, New Hope, PA). The measured oxy-hemoglobin saturation curve determines the relation between the percentage binding of oxygen to hemoglobin, or SaO_2, and the partial pressure of oxygen (PO_2), which is a direct measure of hypoxia. This curve is different for normal hemoglobin and sickle hemoglobin. The SaO_2 is the parameter directly measured by the pulse oximeter in these experiments.

2.2. Data analysis

Each N_2 breathing session produced a transient bout of hypoxia, which could be observed by measuring the SaO_2 drop (figure 1). The session which caused maximum SaO_2 drop was selected from each patient for analysis. The time at an onset of the SaO_2 drop was marked as $t = 0$ for each set of data. Minute ventilation (vent) was calculated from breath period and tidal volume of each breath. All data were subsequently resampled to 2 Hz using spline interpolation (de Boor 1978) for further processing.

2.3. Computation of partial pressure of oxygen

The oxy-hemoglobin dissociation curves for HbS and HbA were used to estimate the PO_2 form the SaO_2 data in these experiments. To accomplish this, the O_2 dissociation data collected from the Hemox analyzer were fitted to a sigmoidal curve using the least-squares method for each patient. The relationship between SaO_2 and PO_2 is

$$\mathrm{PO_2} = \ln\left[\left(\frac{A_1 - A_2}{\mathrm{SaO_2} - A_2}\right) - 1\right] * \mathrm{d}x + x_0, \tag{1}$$

where A_1, A_2, x_0 and dx are parameters that characterize the shape of each sigmoidal O_2 dissociation curve. For subjects with multiple runs of O_2 dissociation curve measurements, we used the average value of all the runs to represent the parameters for that particular subject. We then used the parameters for each subject to convert SaO_2 to PO_2. The dissociation data fit this equation for each patient with an $R^2 > 0.99$. Thus, using the set of parameters, A_1, A_2, x_0 and dx for each patient, we could calculate the PO_2 for each time series and remove the

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differences between patients and controls due to different oxygen binding properties of their respective hemoglobin types.

2.4. Computation of HRV power spectral density

R-wave to *R*-wave intervals (RRI) were extracted from the ECG data using a threshold-based algorithm. These data then were resampled to 2 Hz to match other datasets. Time-varying spectra of RRI were then computed from an autoregressive model (Bianchi *et al* 1993, Eckberg 1997) using the same method as previously described by our group (Blasi *et al* 2003b). This method allows a new estimate of the RRI spectrum to be calculated with each successive time step. An important model parameter for this time-varying technique is the forgetting factor (λ), ranging from 0 to 1, which reflects the memory of the adaptive filter. When $\lambda = 1$, all data before the present time are used in computing a power spectral density (PSD) estimate. Small λ implies that the most recent data points are weighted much more heavily. A sample of PSD during a transient hypoxia episode is shown in figure 2.

From this estimate of the running RRI spectrum, we calculated the traditional HRV parameters, including high-frequency power (HFP) and the ratio between LF and HF spectral powers (LHR), on a time-varying basis, using the same technique described in Blasi *et al* (2003b).

2.5. Computation of respiration adjusted HRV indices

To adjust for the effect of respiration in HRV indices, we first applied a recursive autoregressive model with an exogenous input (RARX) model (Ljung 1999) to describe the relationship between past and present changes in the respiration (denoted as x) and the temporal changes in RRI (denoted as y):

$$y(n) = -\sum_{j=1}^{p} a_j y(n-j) + \sum_{k=0}^{r} b_k x(n-k) + e(n), \tag{2}$$

where p and r represent the orders of the model for the autoregressive and exogenous input parts, respectively. a_j and b_k are the model parameters which were estimated using the least-square minimization algorithm. $e(n)$ represents the residual error of the model estimation at the time point n.

Next, we partitioned y into respiration-correlated and respiration-uncorrelated components, denoted by y_{rc} and y_{ru}, respectively:

$$y(n) = y_{rc}(n) + y_{ru}(n), \tag{3}$$

where

$$y_{rc}(n) = \frac{\sum_{k=0}^{r} b_k q^{-k}}{1 - \sum_{j=1}^{p} a_j q^{-j}} x(n) \tag{4}$$

and

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$$y_{\mathrm{ru}}(n)=\frac{1}{1-\sum_{j=1}^{p}a_j q^{-j}}e(n). \tag{5}$$

Thus,

$$y(n)=\frac{\sum_{k=0}^{r}b_k q^{-k}}{1-\sum_{j=1}^{p}a_j q^{-j}}x(n)+\frac{1}{1-\sum_{j=1}^{p}a_j q^{-j}}e(n), \tag{6}$$

where q^{-m} represents a backward shift of m time steps.

From equation (6), the following expression for the RRI power spectrum ($S_y(f)$) was derived:

$$S_y(f)=S_{\mathrm{yru}}(f)+|H(f)|^2 S_x(f), \tag{7}$$

where $H(f)$ is the transfer function which translates respiration to a dynamic of RRI at frequency f, and can be computed from

$$H(f)=\frac{\sum_{k=0}^{r}b_k\,\mathrm{e}^{-\mathrm{i}2\pi f k\mathrm{T}}}{1-\sum_{j=1}^{p}a_j\,\mathrm{e}^{-\mathrm{i}2\pi f j\mathrm{T}}}. \tag{8}$$

where $\mathrm{i}=\sqrt{-1}$ and T is the sampling frequency. In equation (7), $S_x(f)$ is the spectrum of the respiration time series, and $S_{\mathrm{yru}}(f)$ is the spectrum of the uncorrelated component of HRV. We defined the respiratory sinus arrhythmia gain (G_{rsa}) as the average of the magnitude of $H(f)$ in the frequency range from 0.15 to 0.4 Hz,

$$G_{\mathrm{rsa}}=\frac{1}{0.4-0.15}\int_{0.15}^{0.4}|H(f)|\mathrm{d}f. \tag{9}$$

Equation (7) shows quite clearly that the HRV spectrum is influenced directly by the respiration spectrum, $S_x(f)$, which is determined by the ventilation level and the ventilatory pattern. Thus, changes in the ventilatory pattern alone can lead to changes in HF and LF power, thereby complicating the interpretation of these indices of HRV as measures of autonomic activity even within the same individual.

To overcome this problem, we introduce the notion of ‘respiration-adjusted’ indices in the following way. For each subject, we manually selected a 10 breath segment of data, in which ventilation was relatively uniform and breathing was occurring spontaneously, from the section prior to the start of application of the hypoxic stimulus and used this segment as the baseline for comparison of subsequent changes. Subsequently, we calculated the power spectral density, $S_{x0}(f)$, of this segment of the respiratory signal, $x_0(n)$, and used it to replace $S_x(f)$ in equation (7), in the subsequent sections of data following the start of the hypoxic stimulus.

Therefore, we defined the power spectrum of the adjusted RRI as

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$$S_{\mathrm{yra}}(f)=S_{\mathrm{yru}}(f)+|H(f)|^2 S_{x_0}(f). \tag{10}$$

$S_{\mathrm{yra}}(f)$ was then used to compute the respiration-adjusted high-frequency power, aHFP, and low-frequency to high-frequency ratio, aLHR, in the same way the corresponding conventional HRV indices were calculated.

2.6. Statistical methods

We used the Student's *t*-test to compare the maximum SaO_2 and PO_2 decreases following the hypoxia stimulus in SCA versus control subjects.

In order to perform statistical analysis on the time courses of each parameter, we reduced the number of samples by down sampling to 5 s intervals. For each subject, all parameters were normalized to their own baselines which were obtained from averaging the parameters 20 s prior to hypoxia onset. A normalized data sequence between the hypoxia onset $t = 0$ and $t = 180$ s was used in this analysis.

Two-way repeated measures analysis of variance (2W RMANOVA) was performed on all HRV indices. Three hypotheses were consequently tested:

- there is no change in each of the HRV indices after a hypoxia stimulus from its baseline level;
- changes in each of the HRV indices in SCA and control subject groups are not different;
- there is no interaction between the change of each of HRV indices over time after the hypoxia stimulus and the subject group (SCA or control).

Post-hoc pairwise comparisons with the Holm–Sidak method were conducted to determine the time points following the hypoxia onset at which (a) each HRV index differed significantly from its baseline value for each group of subjects, and (b) the values of the HRV index from the two groups of subjects were different from each other.

3. Results

3.1. Degree of hypoxic stimulus

The maximum SaO_2 drop following the hypoxia stimulus was compared between five SCA and five control subjects in which O_2 dissociation curve data were acquired. The differences in maximum decreases in SaO_2 (in raw units) between subject groups were significant (*t*-test, table 2(A)). However, when the decreases were compared in terms of percentage changes from baseline or after conversion of SaO_2 for each individual into equivalent change in PO_2, these subject group differences were no longer significant. The baseline levels of both SaO_2 and PO_2 are lower in SCA patients than in control subjects as shown in table 2(B). Figure 3 displays the time courses of SaO_2 and calculated PO_2 in terms of percentage (%) change in these parameters from their corresponding baselines (figure 3).

3.2. Changes in RRI

The two subject groups showed significant differences in time courses of the RRI response to transient hypoxia (figure 4). In the SCA subjects, RRI displayed a significant decrease of ~8–15% from baseline levels between 10 and 45 s post-hypoxia onset; no change in RRI related to hypoxia however was detected in the control group (figure 4). 2W RMANOVA revealed a significant *time* × *group* interaction ($P = 0.013$) in the responses of RRI to hypoxia (table 3).

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Post-hoc pair-wise testing (Holm–Sidak method, $P < 0.05$) showed that the differences in RRI between the two groups became significant between $t = 5$ and $t = 50$ s following the onset of hypoxic exposure.

3.3. Changes in parasympathetic HRV indices

HFP in SCA subjects displayed a tendency to decrease in the first minute during hypoxic exposure, whereas it was little changed in the controls (figure 5(*a*)). The difference between groups was significant between $t = 25$ and $t = 50$ s following the onset of hypoxic exposure. The time × group interaction in HFP from 2W RMANOVA, however, was only marginally significant. After correction for the effect of respiratory patterns on HRV, the differences in the time courses of aHFP became noticeably more pronounced (figure 5(b)). The aHFP of SCA subjects dropped to about 40–50% from the baseline level between $t = 20$ and 90 s post-hypoxia onset; while no change in the aHFP was detected in the control group. The time courses of G_{rsa}, which characterize the average magnitude of the transfer function from the respiratory signal to a change in RRI in the 0.15–0.40 Hz frequency band, for the control and SCA groups further emphasized the differences that were displayed in the time courses for aHFP (figure 5 (c)). 2W RMANOVA (table 3) indicated a significant time × group interaction ($P = 0.002$) and post-hoc pairwise testing revealed significant differences ($P < 0.05$) between the two groups from $t = 20$ to $t = 110$ s following the onset of hypoxia.

3.4. Changes in sympathovagal balance

The individual time courses in LHR for controls and SCA subjects were substantially more variable than the corresponding time courses in HFP. Figure 6(a) shows that there was an initial tendency for LHR to increase in the SCA subjects following the hypoxia onset, whereas there was little change in the controls. Adjustment for respiration led to an enhanced delineation of the post-hypoxic responses between the two subject groups. The tendency for aLHR in the SCA subjects to increase above the baseline remained relatively sustained; in contrast, the controls displayed little changes from the baseline. Due to the substantial variability, 2W RMANOVA (table 3) failed to reveal any significant differences, but post-hoc pairwise comparison of the points in the time courses between the groups showed that aLHR was higher in the SCA subjects relative to controls at various times following exposure to transient hypoxia (figure 6(b)).

4. Discussion

Our preliminary results show that the cardiovascular response to hypoxia in SCA patients is accompanied by marked reductions in the parasympathetic modulation of heart rate and possible increases in sympathovagal balance, whereas no significant changes were noted in the normal controls. Furthermore, our technique to adjust for the effect of ventilation pattern to HRV made the changes in both parasympathetic and sympathetic indices following the hypoxia stimuli in SCA much more apparent, making the study of non-respiratory evoked autonomic changes possible. These findings suggest that vagal tone is easily reduced in subjects with SCA following transient exposure to hypoxia. These responses could be responsible for the substantial increases in heart rate observed in this cohort after the hypoxic stimuli. This is also consistent with a report from Pearson *et al* (2005) that children with greater parasympathetic withdrawal during challenges showed more clinical severity of sickle cell disease.

This hypersensitivity of HRV response to hypoxia in SCA patients could be the result of a compensatory mechanism designed to increase oxygen delivery to tissues due to chronic hypoxemia. As shown in table 2(B), both SaO_2 and PO_2 baselines are lower in SCA patients and normal controls, suggesting that mild chronic hypoxemia occurs in this group of patients.

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Researchers have shown some degree of physiological cardiovascular adaptation in SCA patients, including elevations in heart rate and declines in cardiac output, primarily results from the associated chronic hemolytic anemia (Lester *et al* 1990, Batra *et al* 2002). Furthermore, Weiskopf *et al* reported linear increases in heart rates in healthy unmedicated human subjects as a result of acute isovolemic anemia (Weiskopf *et al* 2003). Thus, in SCA patients the sickling of erythrocytes induced by hypoxia leading to higher degree of anemia could have caused the further increases in sensitivity of the response.

In normal subjects, peripheral chemoreceptor stimulation by acute hypoxia activates both ventilatory and cardiovascular responses (Shoemaker 2004). Researchers have reported increases in sympathetic activity in rats submitted to chronic intermittent hypoxia (Zoccal *et al* 2007) and in sleep apnea patients (Jo *et al* 2003, Somers *et al* 1995) who also suffer from chronic intermittent hypoxia. The sickling process occurs continually in subjects with SCA resulting in sub-clinical vaso-occlusion. This results in chronic regional hypoxia. We speculate that the chronic sub-clinical vaso-occlusion may affect the sensitivity of chemoreceptors in SCA patients in a way analogous to the effects of chronic intermittent hypoxia observed in experimental animals and sleep apnea patients.

Acute hypoxia has been shown to enhance sympathetic drive while decreasing parasympathetic activity, leading to an increase in LHR, the ratio representing sympathovagal balance (Buchheit *et al* 2004, Iwasaki *et al* 2006). Although this view has been widely accepted, the time course of the changes remains ambiguous. Most studies in this area were focused on the steady-state effect of the hypoxia response during a longer period of hypoxia (>1 min). Much shorter periods of hypoxia (5 breaths) were used in our experiments, out of safety concerns for the SCA subjects. This duration of exposure was clearly insufficient to induce significant changes in HRV in our normal control subjects, but appeared sufficiently strong to evoke autonomic responses in SCA patients.

The premise that HRV can provide useful indices of cardiac autonomic control is based largely on the original study of Katona and Jih (1975), which demonstrated in an animal preparation a linear relationship between respiratory-related fluctuations in RRI and vagal firing rates. Their latter result was obtained under conditions in which respiration was relatively well controlled. Subsequent validation studies in humans employing pharmacological interventions to alter autonomic tone were also conducted under conditions in which respiration was controlled (Berntson *et al* 1997). Although some studies have underscored the important effect of respiratory rate and tidal volume on the HRV spectrum (Grossman *et al* 1991, Brown *et al* 1993), this confounding influence of respiration has largely been ignored in the HRV literature. Brown *et al* (1993) showed that depending on breathing frequency, changes in respiration within a given individual can substantially alter estimates of both high-frequency and low-frequency powers of the RRI spectrum. Our present study highlights the importance of correcting the autonomic indices derived from HRV for the respiratory changes that accompany a brief exposure to hypoxia. The approach we have introduced of ‘computationally correcting’ for these respiratory-related distortions of the HRV spectrum is analogous to the well-accepted statistical technique of ‘adjusting’ for confounding variables.

5. Conclusions

While this study is limited by the relatively small number of subjects, the preliminary findings we have reported indicate quite clearly that there are significant differences in autonomic reactivity to transient hypoxia in SCA subjects relative to normals. In particular, SCA subjects display a significant reduction in RRI and the parasympathetic indices of HRV following exposure to five breaths of N_2, in contrast with normal controls who demonstrate little or no response. Furthermore, the signal-processing technique employed here allows us to study the

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non-respiratory derived components of autonomic dysfunction, which we suspect may be important in the fundamental pathology of SCA. Given that otherwise unexplained sudden death accounts for up to 40% of the mortality in SCA and that autonomic dysregulation has clearly been associated with sudden death in other settings (Hathaway *et al* 1998, Sathyaprabha *et al* 2006, Piepoli and Capucci 2007), the application of the experimental and analytical methodology introduced in the current study may yield important insights into the pathophysiology and perhaps the clinical management of sickle cell disease.

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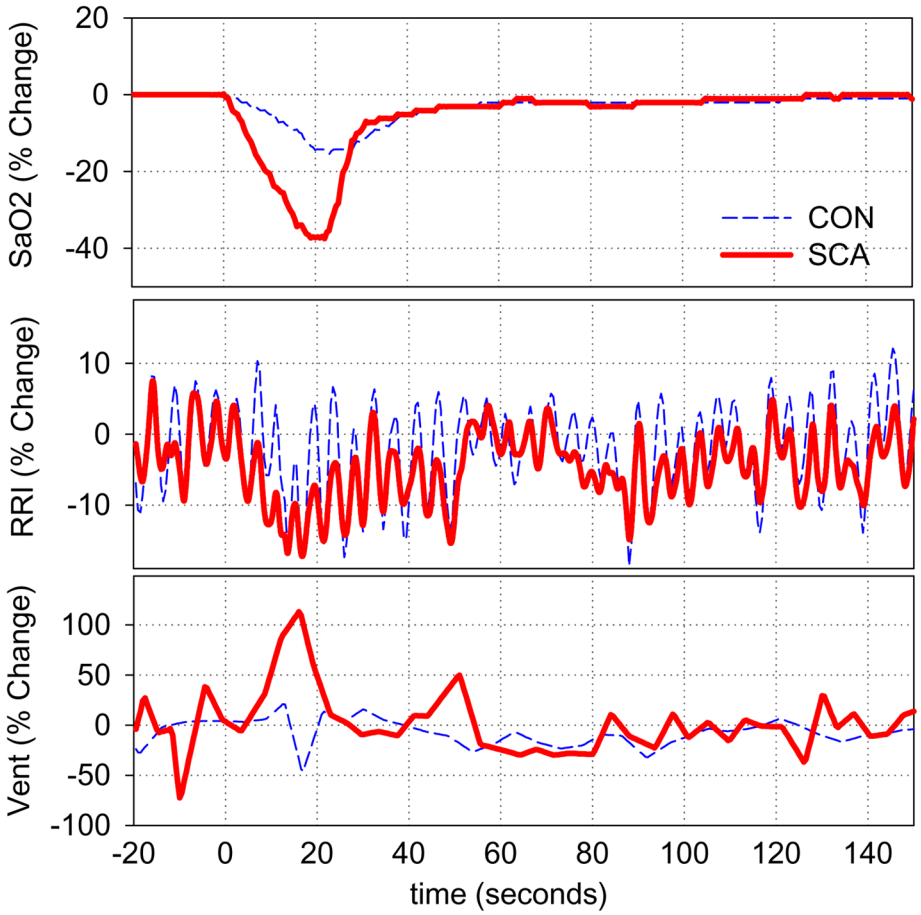

Figure 1.

Representative time courses of oxygen saturation (SaO_2), *R*-wave to *R*-wave interval (RRI), and minute ventilation (vent) from a sickle cell anemia patient (SCA) and a normal control subject (CON) after transient episode of hypoxia. Time $t = 0$ indicates the onset of the oxygen saturation drop after nitrogen breathing.

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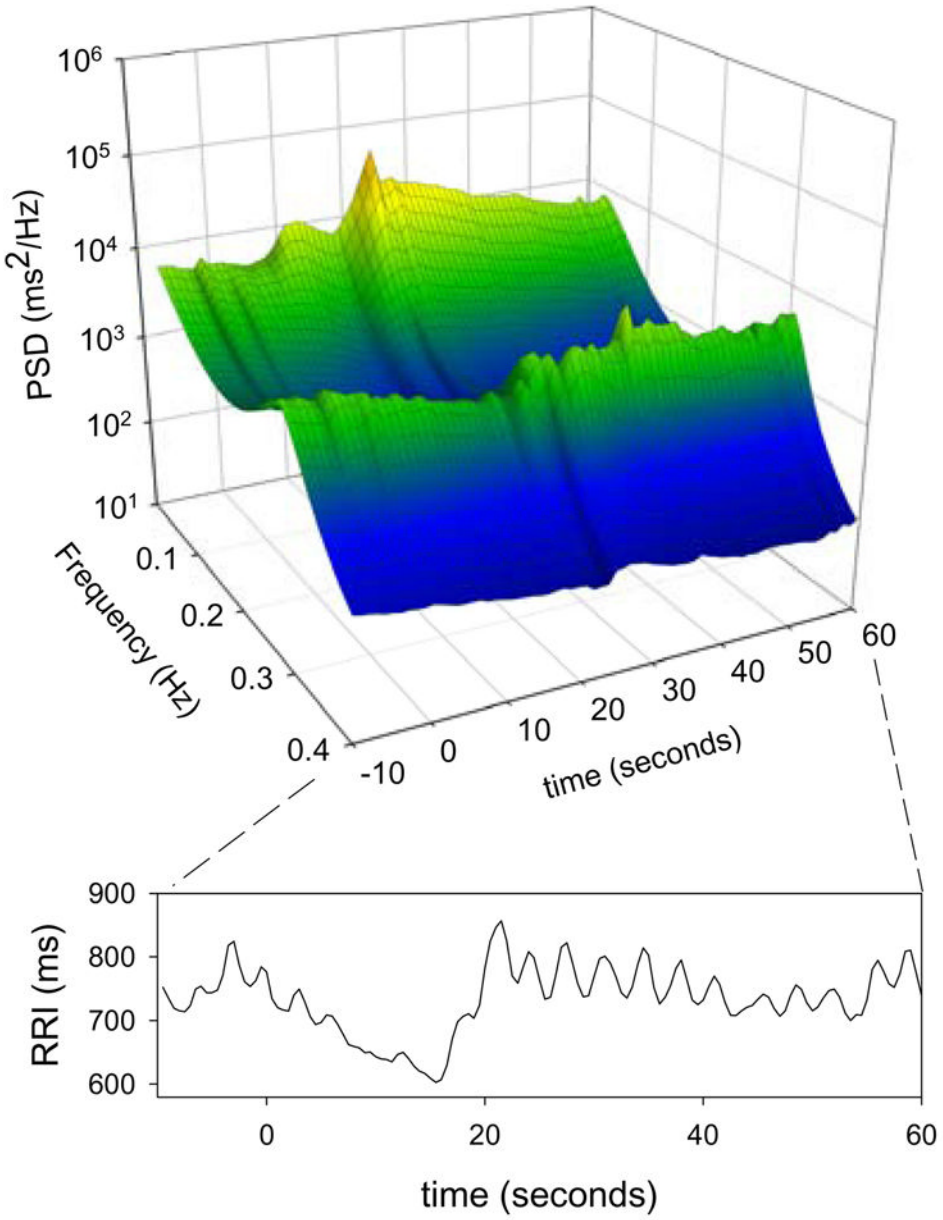

Figure 2.

Sample of PSD and its corresponding RRI from a five-breath 100% N_2 breathing study. Note: time $t = 0$ indicates the onset of the SaO_2 drop.

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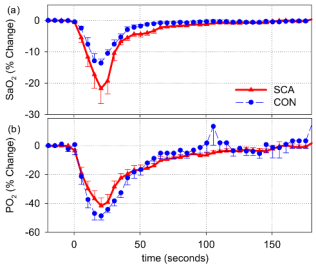

Figure 3.

Changes in SaO_2 (a) and PO_2 (b) from their own baselines following hypoxia stimulus. Time $t = 0$ indicates the onset of the oxygen saturation drop after nitrogen breathing for each subject.

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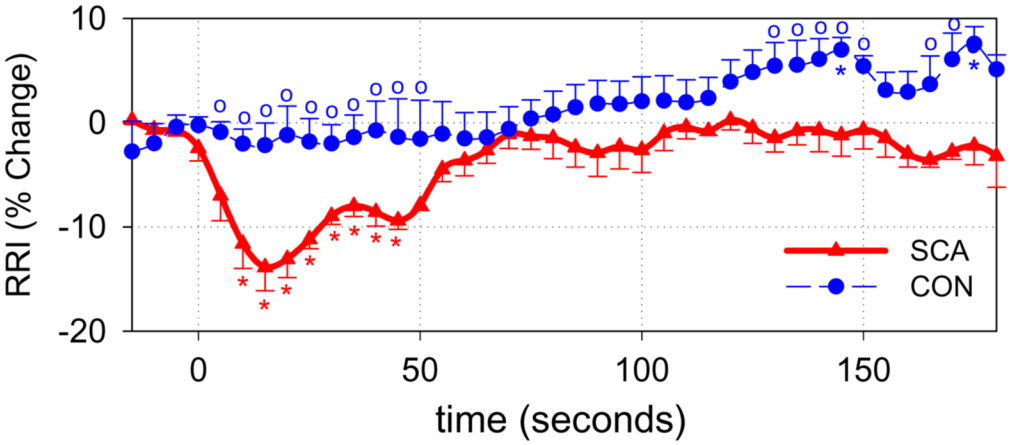

Figure 4.

Time course of the *R*-wave to *R*-wave interval (RRI). Time $t = 0$ indicates the onset of the oxygen saturation drop after nitrogen breathing for each subject. ° indicates a significant difference between control and sickle cell subjects ($P < 0.05$). * indicates a significant difference from the baseline of the same time course ($P < 0.05$). The unit is displayed in percentage (%) change of the parameter from its baseline value.

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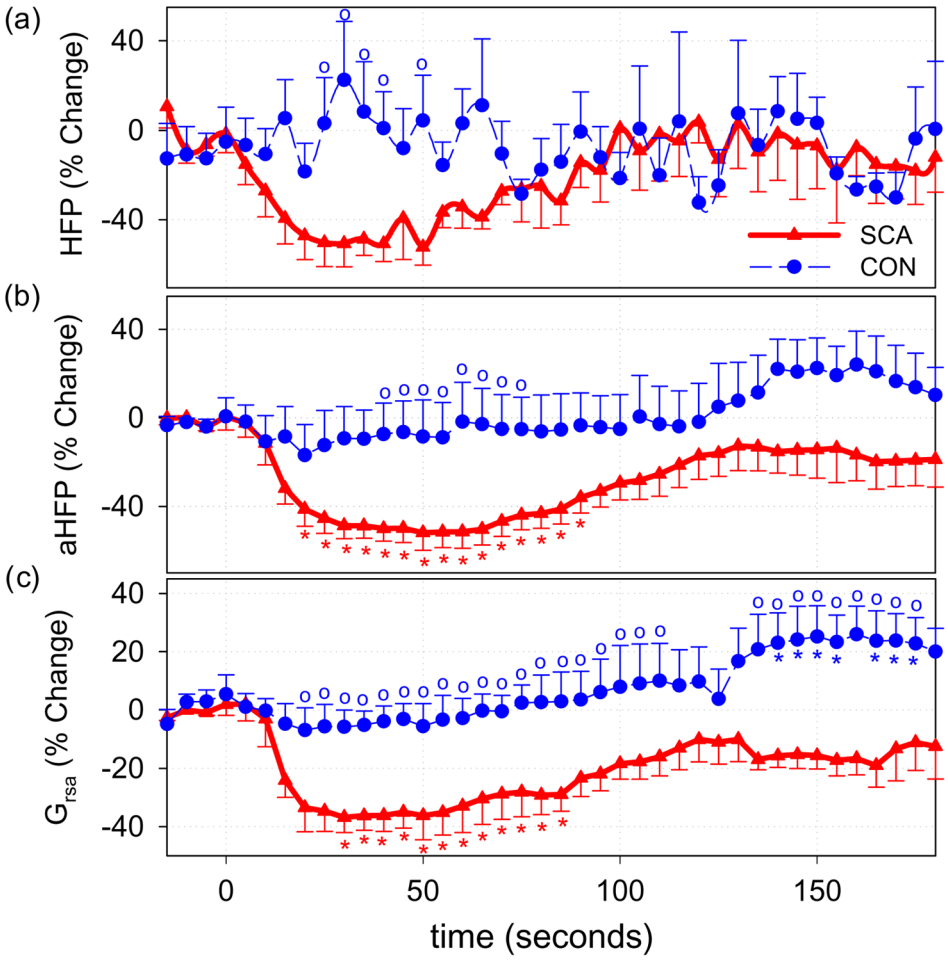

Figure 5.

Time courses of the parasympathetic HRV indices. Time $t = 0$ indicates the onset of the oxygen saturation drop after nitrogen breathing for each subject. ° indicates a significant difference between control and sickle cell subjects ($P < 0.05$). * indicates a significant difference from the baseline of the same time course ($P < 0.05$). (a) High frequency power of HRV (HFP), (b) adjusted high frequency power (aHFP), (c) respiratory sinus arrhythmia gain (G_{rsa}). All parameters are displayed in percentage (%) change of the parameters from their baseline values.

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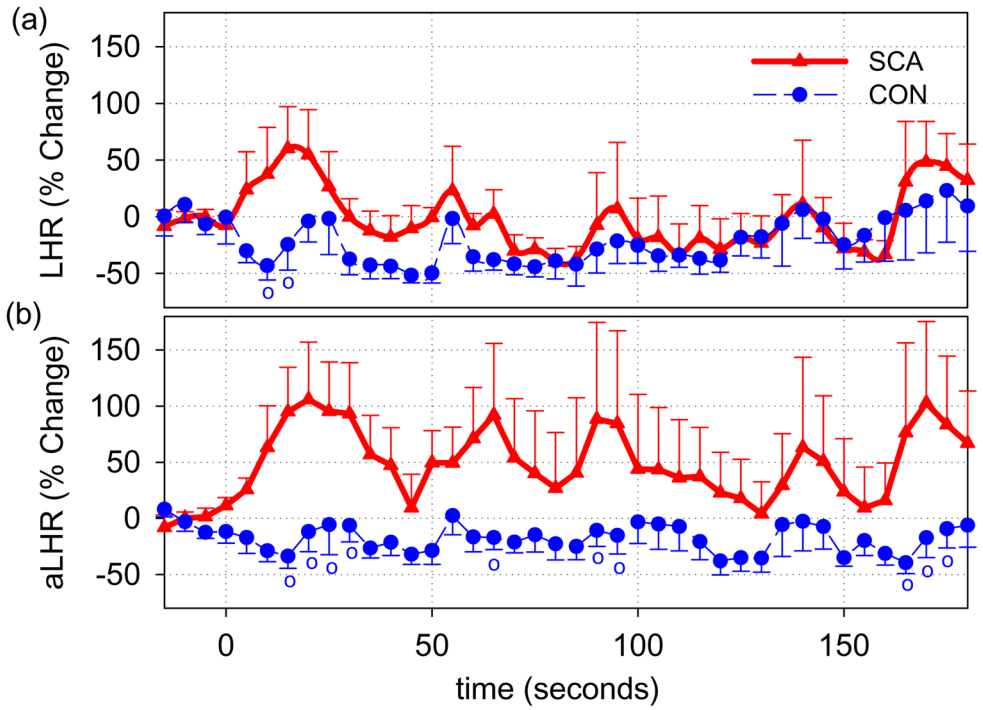

Figure 6.

Time courses of the sympathovagal balance indices. Time $t = 0$ indicates the onset of the oxygen saturation drop after nitrogen breathing for each subject. ° indicates a significant difference between control and sickle cell subjects ($P < 0.05$). (a) Ratio between high-frequency and low-frequency powers (LHR), (b) adjusted ratio between high-frequency and low-frequency powers (aLHR). All parameters are displayed in percentage (%) change of the parameters from their baseline values.

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Table 1

Subject characteristics.

Subject	Gender	Age (yr)	Hydroxyurea
Sickle cell anemia patients: *N* = 5			
SCA1	F	20	Y
SCA2	F	16	Y
SCA3	F	19	Y
SCA4	M	16	Y
SCA5	M	18	N
Mean ± SD	–	17.75 ± 1.67	–
Control subjects: *N* = 7			
CON1	F	28	–
CON2	M	21	–
CON3	F	35	–
CON4	F	32	–
CON5	F	21	–
CON6	F	26	–
CON7	F	37	–
Mean ± SD	–	28.57 ± 6.40	–

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Table 2

Comparison of (A) SaO_2 and PO_2 drops after hypoxia exposure and (B) SaO_2 and PO_2 values.

Parameter	SCA	CON	*P*-value
(A)			
SaO_2 value	71.61 (9.07)	82.69 (4.11)	0.038
SaO_2 (% baseline)	−25.31 (9.43)	−15.98 (4.61)	0.082
PO_2 value	42.21 (7.33)	46.84 (6.63)	0.325
PO_2 (% baseline)	−42.66 (9.73)	−51.10 (8.32)	0.370
(B)			
Baseline SaO_2 (%)	95.875 (1.595)	98.431 (0.804)	0.013
Baseline PO_2 (mmHg)	77.966 (7.538)	96.908 (11.359)	0.015

The values are shown in mean (SD).

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Table 3

Summary of *P*-value from statistical analysis on HRV indices (2W RMANOVA).

Index		Time	Group	Time × group interaction
Conventional	RRI	0.014[a]	<0.001[a]	0.013[a]
	HFP	0.352	0.793	0.055
	LHR	0.332	0.003[a]	0.960
Respiratory adjusted	G_{rsa}	0.017[a]	<0.001[a]	0.002[a]
	aHFP	0.094	<0.001[a]	0.065
	aLHR	0.061	0.279	0.786

Significant difference ($P < 0.05$).