Peripheral Vasoconstriction and Abnormal Parasympathetic Response to Sighs and Transient Hypoxia in Sickle Cell Disease

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Rationale: Sickle cell disease is an inherited blood disorder characterized by vasoocclusive crises. Although hypoxia and pulmonary disease are known risk factors for these crises, the mechanisms that initiate vasoocclusive events are not well known.

Objectives: To study the relationship between transient hypoxia, respiration, and microvascular blood flow in patients with sickle cell.

Methods: We established a protocol that mimics nighttime hypoxic episodes and measured microvascular blood flow to determine if transient hypoxia causes a decrease in microvascular blood flow. Significant desaturations were induced safely by five breaths of 100% nitrogen.

Measurements and Main Results: Desaturation did not induce change in microvascular perfusion; however, it induced substantial transient parasympathetic activity withdrawal in patients with sickle cell disease, but not controls subjects. Marked periodic drops in peripheral microvascular perfusion, unrelated to hypoxia, were triggered by sighs in 11 of 11 patients with sickle cell and 8 of 11 control subjects. Although the sigh frequency was the same in both groups, the probability of a sigh inducing a perfusion drop was 78% in patients with sickle cell and 17% in control subjects ($P < 0.001$). Evidence for sigh-induced sympathetic nervous system dominance was seen in patients with sickle cell ($P < 0.05$), but was not significant in control subjects.

Conclusions: These data demonstrate significant disruption of autonomic nervous system balance, with marked parasympathetic withdrawal in response to transient hypoxia. They draw attention to an enhanced autonomic nervous system–mediated sigh–vasoconstrictor response in patients with sickle cell that could increase red cell retention in the microvasculature, promoting vasoocclusion.

Keywords: hypoxia; autonomic nervous system; respiration; vasoconstriction; sickle cell disease

Sickle cell disease (SCD) is an inherited blood disorder that results from an amino acid substitution in the β globin chain of hemoglobin, producing sickle hemoglobin (HbS) (1, 2). HbS

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Vasoocclusive crisis is a leading cause of death and hospitalization for patients with sickle cell. Nonetheless, the mechanism that initiates the crisis is not yet fully understood. The possible contribution from the pulmonary triggered neural signals has not been considered.

What This Study Adds to the Field

Our results demonstrate that patients with sickle cell have a hypersensitive autonomic nervous system response to transient hypoxia and that they have a high rate of vasoconstriction induced by sighs. We speculate that respiration-initiated neural-based vasoconstriction events may trigger sickle cell crisis.

polymerizes when it releases oxygen to tissues, causing the normally flexible red blood cells to become rigid and to obstruct blood flow (3). The subsequent ischemia results in chronic organ damage and ultimately premature death (3–5). Although SCD is caused by a single amino acid substitution, significant variability in the frequency of overt sickling episodes has been reported among subjects with the same genotype, suggesting that other factors cause the transition from steady-state sickling to full vasoocclusive crisis (VOC).

The specific factors that affect this transition are incompletely understood. Flexible, oxygenated, HbS-containing red blood cells (SRBC) traverse capillaries and release their oxygen. After HbS releases its oxygen, it polymerizes after a short delay and the SRBC become rigid (6). If the SRBC fail to escape the microvasculature within the delay time period, the SRBC become lodged. Thus, any factor that increases the SRBC transit time or shortens this delay time to polymerization increases the likelihood that the local microvascular bed will become obstructed. Adhesion of SRBC to endothelium, inflammation, up-regulation of endothelial adhesion molecules, activation of coagulation, depletion of nitric oxide, increased blood viscosity, and decreased SRBC deformability all act to decrease blood flow, increase transit time, and make it more likely that occlusion in a regional microvascular bed cascades to others and culminates in a symptomatic VOC.

Hypoxia is considered to trigger VOC and is often thought, incorrectly, to directly induce formation of deoxy-HbS. These two events, although time-related, are not the same. Nonetheless, low steady-state daytime Sa_{O_2} is associated with high transcranial Doppler velocities, which predict stroke (7). Furthermore, nocturnal hypoxia is associated with an increased frequency of VOC (8) and predicts central nervous system events, such as stroke or seizure, in subjects with SCD (9).

To study the relationship between transient hypoxia and microvascular blood flow in subjects with SCD, we established a protocol to mimic transient nighttime hypoxia and measured microvascular blood flow to determine if transient hypoxia could cause a decrease in microvascular blood flow. Although we did not find a direct relationship between hypoxia and decreased blood flow, we did find that the autonomic nervous system (ANS) response to hypoxia was significantly abnormal in subjects with SCD, and that sighs induced decreases in perfusion through neural signaling from a pulmonary reflex mechanism. These data demonstrate a neural link between respiration and transient drops in perfusion that would increase microvascular transit time and thereby promote VOC. Some of the results of these studies have been previously reported in the form of abstracts (10–13).

METHODS

All experiments were performed at Children's Hospital Los Angeles, Los Angeles, California, under the auspices of a protocol that was approved by the Committee on Clinical Investigation. The experimental protocol was designed to measure the physiologic responses to transient hypoxia such as may occur naturally during sleep. To accomplish this, single episodes of hypoxia were induced by breathing five breaths of 100% nitrogen (N_2).

The subject was placed comfortably in a supine position with the head of the bed adjusted at a 30-degree angle. A standard fitted anesthesia mask was held over the nose and mouth, sealed in place by elastic bands. The subject was connected to devices that allow switching of the breathing air content from room air to 100% N_2 without the subject's knowledge. After the subject had rested quietly for at least 15 minutes until all vital signs had stabilized, the inspired gas was switched to 100% N_2 at the beginning of inspiration and back to room air after the subject had taken five tidal volume breaths of N_2.

Respiration, Sa_{O_2} in the right index finger, and electrocardiogram were recorded continuously using the LifeShirt physiologic monitoring system (VivoMetrics, Ventura, CA). Microvascular perfusion was recorded at the nail capillary bed of the right index finger using the PeriFlux laser Doppler monitoring system (Perimed, Jarfalla, Sweden). The electrocardiogram r-wave to r-wave interval (RRI), respiratory rate, and tidal volume (V_T) were derived from the basic data using VivoLogic software (VivoMetrics).

Safety Protocol

The research protocol was accepted by the local ethics committee as "minimal risk." Nonetheless, because of concerns that inducing hypoxia might lead to a VOC, each subject was interviewed about his or her symptoms before and at 1 hour, 12 hours, 24 hours, and 1 week after the experiment. We used the same five breaths of N_2 and safety monitoring protocol for separate experiments using magnetic resonance imaging. The magnetic resonance imaging data are not reported in this paper; however, we do report safety data from all 52 experimental episodes (19 control [CTL] subjects and 33 patients with SCD) from both studies.

Assessment of the ANS Responses

ANS status was evaluated noninvasively by frequency domain analysis of cardiac beat-to-beat variability, also known as heart rate variability (HRV). Parasympathetic activity can be represented by high-frequency power (HFP; 0.15–0.4 Hz) of HRV. Because the low-frequency components of HRV (0.04–0.15 Hz) may be caused by both parasympathetic and sympathetic activity (14), the ratio of low-frequency to high-frequency spectral power (low-to-high ratio, LHR) was used as a broad index of "sympathovagal balance" (15, 16). Note that both HFP and LHR represent degrees of autonomic regulation rather than absolute levels of autonomic tone.

Moreover, because respiration causes sinusoidal variations in heart rate (17), we compensated for the effects of changes in breathing patterns on HRV by using the respiratory waveform (18). Consequently, the respiratory-adjusted ANS data presented here represent all autonomic inputs other than respiration. We denoted respiratory-adjusted HFP and LHR as HFP_{ra} and LHR_{ra}. We have published the details of these computations elsewhere (18–20). For additional information of the experimental method and data analysis technique, see the online supplement.

RESULTS

The characteristics of the subjects are presented in Table 1. All subjects were African American. Five of the 11 subjects with SCD were treated with hydroxyurea. Comparisons performed with the hemoglobin SS (HbSS) group alone were not significantly different from when the three hemoglobin SC (HbSC) subjects were included. Thus, all subjects with SCD were analyzed as one group. Two of the controls had sickle cell trait. Excluding these two subjects did not alter the final analysis results; thus, their results were combined with the nontrait CTL subjects.

Although CTL subjects were older than the subjects with SCD, none of the baseline HRV indices were different between the two groups (HFP and LHR). In the general population, both LFP and HFP of HRV are expected to decrease with age (21, 22). Although our baselines were measured for only 5 minutes rather than overnight as in the standard baseline assessment, the baselines are lower in the older CTL group. Thus, even with a possible age-related effect, the differences between SCD and CTL are still not statistically different. Only baseline LHR was positively correlated with age ($R^2 = 0.23$; $P = 0.016$). The increase of LHR with age has been reported in the general population but is controversial (21, 22). Despite this effect of age, we found that LHR responses to autonomic stimuli were higher in the SCD group.

Safety of the Hypoxia Protocol

The symptoms that were observed in the N_2 challenge experiments are noted in Table 2. Time zero is the time of the first N_2 exposure. The symptoms reported at 1 hour represent symptoms that occurred during the hypoxic period itself. Only 16% of all subjects were able to tell when they were hypoxic because they experienced symptoms, such as headache, lightheadedness, or shortness of breath. In all cases, the symptoms lasted less than 2 minutes and resolved when hypoxia ended. No patient had any symptoms after their Sa_{O_2} returned to normal. Nevertheless, most of the subjects were not able to tell when the hypoxia occurred despite significant drops in Sa_{O_2}.

No subject reported pain within 12 hours of the N_2 exposure. By the attribution rules, any event that occurred within 12 hours was considered to be related to the hypoxia. One subject was admitted to the emergency room 12.5 hours after the procedure for what was ultimately determined to be a hyperventilation episode. This was reported as a serious adverse event related to the protocol, even though it was technically outside the 12-hour window. This subject volunteered to participate again and

TABLE 1. CHARACTERISTICS OF THE SUBJECTS

	SCD (*n = 11*)	CTL (*n = 14*)	P Value
Female/male	5/6	8/6	0.6951
Age, yr	21.5 ± 4.4	30.9 ± 7.3	0.0007
Genotype	8 SS, 3 SC	12 AA, 2 AS	n/a
Hemoglobin, g/dl	10 ± 1.5	13.6 ± 1.5	<0.0001
Reticulocyte count, %	8.2 ± 5.4	1.1 ± 0.3	0.0006

Definition of abbreviations: AA = homozygous hemoglobin A; AS = sickle trait; CTL = control subjects; SC = hemoglobin SC; SCD = sickle cell disease; SS = homozygous hemoglobin S.

TABLE 2. SAFETY DATA FROM 19 CTL EXPERIMENTS AND 33 SCD EXPERIMENTS

		Number of Subjects Who Experienced the Symptom							
		1 h		12 h		24 h		1 wk	
Time elapsed after experiment Subject groups		CTL	SCD	CTL	SCD	CTL	SCD	CTL	SCD
Adverse event									
VOC	Mild	0	0	0	0	0	4	0	4
	Moderate	0	0	0	0	0	0	0	1
HA	Mild	3	0	0	1	1	0	0	5
Lightheaded	Mild	3	6	0	3	0	1	0	2
SOB	Mild	1	5	1	0	0	0	0	1
ACS		0	0	0	0	0	0	0	0
TIA		0	0	0	0	0	0	0	0
Stroke		0	0	0	0	0	0	0	0
Cough	Mild	0	0	0	0	0	0	1	3
Fever	Mild	0	0	0	0	0	0	1	0
	Moderate	0	0	0	0	0	0	0	1
Nausea and vomiting	Mild	0	0	0	0	0	0	0	1
Diarrhea		0	0	0	0	0	0	0	0

Definition of abbreviations: ACS = acute chest syndrome; CTL = control subjects; HA = headache; SCD = sickle cell disease; SOB = shortness of breath; TIA = transient ischemic attack; VOC = vasoocclusive crisis.

Symptoms reported at 1 hour occurred during the 30–45 seconds of hypoxia.

had no problems subsequently. Four subjects with SCD reported pain, which they identified as mild VOC pain, within 24 hours after the experiment; none of them visited the clinic or the emergency room for the pain. One subject was not able to complete the study after several attempts because she became claustrophobic as soon she had to breathe through the mask. Thus, these data show that in this group of subjects, the five-breath N_2 protocol is well-tolerated.

Baseline Measurement of Vital Signs and HRV Parameters

Five-minute baseline measurements of heart rate, respiration, and laser Doppler parameters are shown in Table 3. There was no difference between the SCD and CTL groups in any of the parameters, except Sa_{O_2}. This difference is caused in part by the left shift in the oxyhemoglobin saturation curve for HbS (23). Note that the Pa_{O_2} derived from the Sa_{O_2} using the oxyhemoglobin saturation curves was not significantly different, indicating that the differences in Sa_{O_2} are primarily the result of

TABLE 3. PHYSIOLOGIC MEASUREMENTS DURING 5-MINUTE BASELINE

	SCD	CTL	P Value
RR, Br/M	16.5 (14.6–17.6)	16.9 (13.7–18.9)	0.969
$V_{T,inspired}$, ml	602.7 (494.2–902.1)	503.5 (332.2–623.4)	0.298
RRI, seconds	0.83 (0.77–0.91)	0.83 (0.79–0.91)	0.681
Sa_{O_2}, %	95.9 (94.5–97.2)	97.9 (97.8–98)	0.017*
Pa_{O_2}, mm Hg	81.6 (72.2–88.3)	88.4 (85.6–95.2)	0.222
PU, au	20.1 (18–27.9)	22.5 (19.9–30.9)	0.431
Vel, au	41 (33.7–58.7)	59.9 (41.9–95.2)	0.076
LFP, ms²	971 (304.9–1,421.5)	828.2 (591.8–949.1)	0.681
HFP, ms²	1,914.5 (1,038.7–3,777.3)	1498.2 (226.5–2,325)	0.311
LHR	0.306 (0.168–0.738)	0.812 (0.342–1.702)	0.095

Definition of abbreviations: CTL = control subjects; HFP = high-frequency power of heart rate variability; LFP = low-frequency power of heart rate variability; LHR = low-frequency to high-frequency ratio of heart rate variability; Pa_{O_2} = partial pressure of O_2 derived from Sa_{O_2} measurement; PU = arbitrary perfusion unit by laser Doppler; RR = respiratory rate; RRI = electrocardiogram r-wave to r-wave interval; Sa_{O_2} = O_2 saturation by pulse oximetry; SCD = sickle cell disease; Vel = red-cell velocity; $V_{T,inspired}$ = inspired tidal volume.

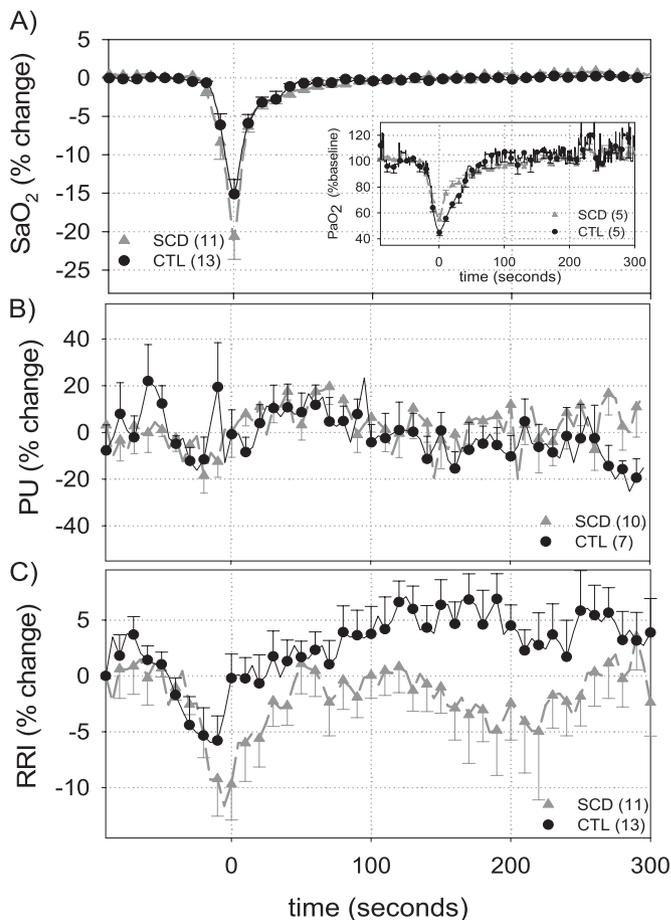Data show median (25 percentile to 75 percentile).

* $P < 0.05$.

Figure 1. Response of perfusion (PU) and r-wave to r-wave interval (RRI) to hypoxia. (*A*) Changes in Sa_{O_2} and derived Pa_{O_2} (*inset*) after five breaths of 100% N_2. Changes in PU (*B*) and RRI (inverse of heart rate) (*C*). Time t = 0 indicates nadir of Sa_{O_2}. Each plot shows mean ± standard error at 10-second segments. CTL = control subjects; SCD = sickle cell disease.

the differences in the oxygen binding properties of HbS and hemoglobin A (HbA).

Similarly, baseline ANS balance, as indicated by the HRV indices, did not differ between the two groups (Table 3). In contrast to published studies on HRV in SCD that were done over many hours (24, 25), the baseline HRV indices in this study were computed from a 5-minute baseline RRI segment for each subject, just before the N_2 exposure. This shorter time frame is more relevant to the duration of the hypoxic challenge used in these studies. Nonetheless, to obtain a "true" baseline HRV measurement for each subject, and in particular for proper assessment of lower-frequency components, a standard longer duration recording is needed.

Physiologic Responses to Transient Hypoxia

The time-course of change in Sa_{O_2} and derived Pa_{O_2} (*inset*) as a percent of baseline for 11 subjects with SCD and 13 control subjects is shown in Figure 1A. The magnitude and pattern of the hypoxic response was very similar in subjects with SCD and CTL subjects. One of the 14 CTL subjects was excluded from this analysis because he did not have a decrease in Sa_{O_2}. Other than this one outlier, a clear and very predictable hypoxia was observed in both groups of subjects with this protocol.

Contrary to our initial hypothesis that hypoperfusion would be seen in subjects with SCD in response to hypoxia, we did not
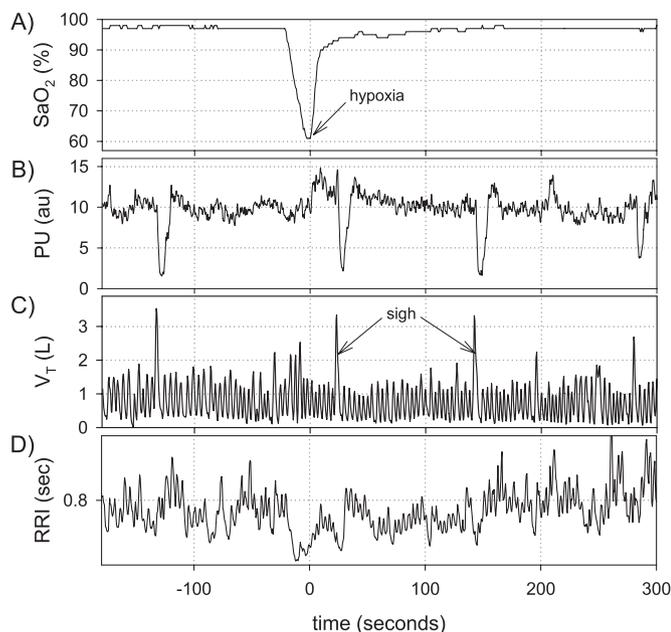
Figure 2. An example of measurements recorded during a hypoxia episode in a patient with sickle cell disease. (*A*) Sa_{O_2} measured by a pulse oximeter. (*B*) Microvascular perfusion as measured by laser Doppler; perfusion unit (PU) is represented as arbitrary unit (au). (*C*) Tidal volume (V_T). (*D*) r-Wave to r-wave interval (RRI).

Figure 3. Heart rate variability responses during hypoxia. (*A*) Time-course of % change from baseline of HFP indication of change in parasympathetic modulation in response to hypoxia (*shaded*), t = 0 is the nadir of Sa_{O_2}. (*B*). Each bar shows the mean ± SE. HFP = high frequency power, LHR = low-frequency to high-frequency ratio, HFP_{ra} = respiratory-adjusted HFP, LHR_{ra} = respiratory-adjusted LHR, and G_{rsa} = respiratory sinus arrhythmia gain. *Difference from baseline, $P < 0.05$. +Difference between groups, $P < 0.05$.

observe any change in microvascular perfusion in the subjects with SCD, nor in the CTL subjects as expected (Figure 1B). Although no change in perfusion occurred, there was a clear decrease in RRI in response to hypoxia, indicating an increase in heart rate. This tachycardia response to hypoxia was more pronounced and lasted longer in subjects with SCD than in CTL subjects (Figure 1C).

Because changes in heart rate and perfusion are controlled by the sympathetic and parasympathetic nervous system, we measured the sympathetic–parasympathetic balance using frequency domain analysis of HRV. By using a novel methodology that permits quantitation of ANS components over short time periods, we were able to study ANS changes in response to hypoxia while removing contributions to HRV caused by normal respiration (18, 26, 27). An example of the change in RRI (panel D) with respect to hypoxia (panel A) is seen in Figure 2. The timecourse of the HFP in response to hypoxia for subjects with SCD and CTL subjects is shown in Figure 3A. A reduction in HFP after hypoxia was seen in subjects with SCD but not in CTL subjects. This indicates a significant loss of parasympathetic activity, resulting in tachycardia.

To statistically compare the changes in HRV indices between groups, we calculated the areas under the time-course curves from t = −15 to +30 seconds (*shaded region* in Figure 3A) with time t = 0 defined as the nadir of Sa_{O_2} after the hypoxia stimulus. These values were then used to calculate means and standard errors for each group (Figure 3B). The drop in HFP was not eliminated after adjustment for respiration (HFP_{ra}). The respiratory sinus arrhythmia gain, G_{rsa}, which represents the magnitude of the effect of breathing on heart rate, decreased in both groups after hypoxia (Figure 3B), with the effect greater in subjects with SCD. Decreases in both HFP_{ra} and G_{rsa} in the subjects with SCD suggest that the loss in parasympathetic activity after hypoxia is a direct effect of hypoxia on HRV, regardless of changes in respiratory patterns. Sympathetic modulation, which is represented by LHR, seemed to be unaffected by hypoxia in either group. After adjustment for the effect of respiration,
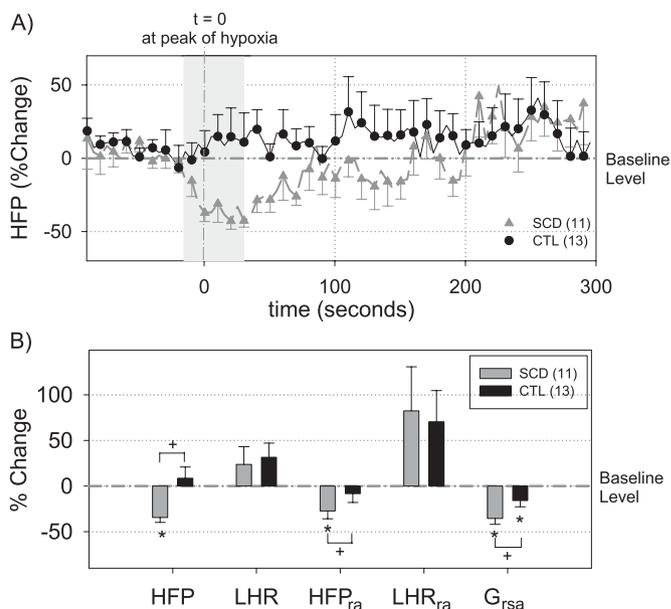
increases in LHR_{ra} were more apparent in both groups but still were not statistically different from baseline.

These data indicate a significantly abnormal ANS regulation in subjects with SCD in response to transient hypoxia. In particular, there is a significant loss of the parasympathetic nervous system activity in response to hypoxia that was not present in CTL subjects.

Physiologic Responses to Spontaneous Sighs

Although there was no apparent change in microvascular perfusion directly associated with the drop in Sa_{O_2}, we observed regular episodes of hypoperfusion that were associated with spontaneous sighs (an example is shown in Figure 2), with a nadir at 3.85 ± 1.06 seconds after the peak of the sigh. There was no difference in the latency between sigh and hypoperfusion in the SCD and CTL groups. To statistically identify the association between sighs and perfusion drops, we analyzed the occurrences of spontaneous sigh-induced perfusion drops in 11 subjects with SCD and 11 CTL subjects. Statistical analysis for the relationship between sigh and perfusion drop was performed for each individual subject, and for each subject each breath was considered an event. A significant relationship ($P < 0.001$ for 18 subjects and < 0.03 for one subject) between perfusion drops and sighs was observed in 19 out of 22 subjects (11 of 11 SCD and 8 of 11 CTL). No sighs were detected in two control subjects; therefore, the chi-square statistical analysis could not be performed in these two subjects.

The frequencies of sighs (percentage of breaths quantified as sighs from all breaths) were not statistically different between the two groups ($P = 0.694$; median = 2.10% vs. 2.05% in SCD vs. CTL) (Figure 4A). However, the median probability of a sigh being immediately followed by a perfusion drop was much higher in the SCD group than in the CTL group (77.8% vs. 16.7%; $P < 0.001$) (Figure 4B). Thus, a spontaneous sigh is
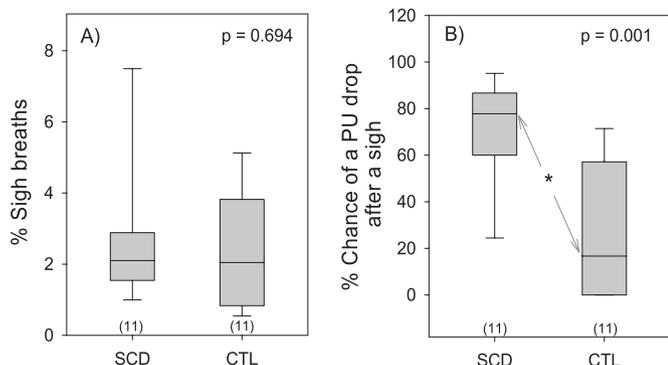
Figure 4. Comparison of sigh frequencies and probabilities of perfusion (PU) drop for each sigh. (*A*) Frequency of breaths classified as sighs and (*B*) probability of PU drop for each spontaneous sigh in 11 patients with sickle cell disease (SCD) and 11 control subjects (CTL). Box plots show median, 10th, 25th, 75th, and 90th percentiles. *P* values were calculated from rank-sum test.

much more likely to result in transient hypoperfusion in patients with SCD than in CTL subjects.

A decrease in RRI (i.e., an increase in heart rate) was observed during sighs. The levels of RRI drops from baseline were not different between the two groups (Figure 5). These RRI drops were observed after sighs whether or not the sighs caused a perfusion drop. Consequently, we consolidated all spontaneous sighs from each subject in all subsequent computations of RRI, regardless of the perfusion drop responses.

To understand the ANS modulation that resulted in this tachycardia response to sigh, we performed HRV analysis on the RRI signal during the time immediately after a sigh. For each subject, the HRV indices were represented with the area under the time-course curves of the indices from t = 5–20 seconds, with time t = 0 indicating the beginning of a sigh inspiration (Figure 6). Two-way repeated-measures analysis of variance of the HRV indices showed an increase in parasympathetic activity (HFP, Figure 6) after a sigh for the CTL group (*P* < 0.05); this increase was less, and nonsignificant for subjects with SCD (*P* < 0.05). We suspected that this discrepancy could be caused by their variations in breathing pattern during sigh. Therefore we adjusted for respiration (HFP$_{ra}$); after the adjustment, the parasympathetic activity for both groups was greater than baseline and the SCD group was no longer different from the CTL group.
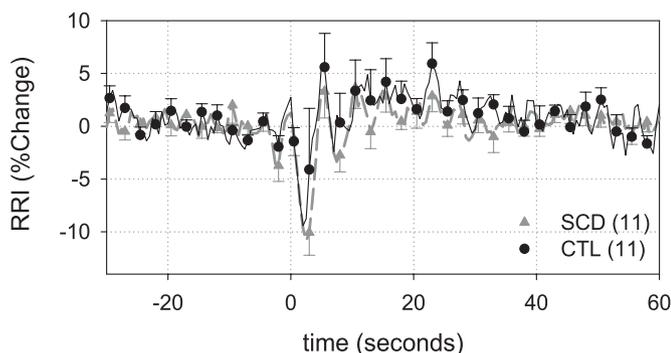
LHR, which reflects sympathetic activity, was significantly increased after a sigh (*P* < 0.05 for the SCD group, and nonsignificant for the CTL group) and was more pronounced in subjects with SCD than in CTL subjects (*P* < 0.05). However, this difference was no longer statistically significant after adjustment for the effects of respiration, although subjects with SCD still had an increase in LHR over baseline (LHR$_{ra}$, Figure 6). Thus, sympathetic activity (LHR$_{ra}$) increased more and parasympathetic activity (HFP$_{ra}$) increased less after a sigh in the SCD groups than in the CTL groups.

Taken together, these data suggest that a sigh stimulates sympathetic activity and that the magnitude of this effect is greater in subjects with SCD than in CTL subjects. This finding is consistent with the observation that a sigh is more likely to stimulate a peripheral microvascular perfusion drop in SCD than in normal subjects.

DISCUSSION

Hypoxia is widely thought to be a trigger of VOC in patients with SCD. Consequently, we expected to see a transient drop in microvascular perfusion after the hypoxia stimulus. Interestingly, the assumed change in perfusion was never observed in this study. Instead, we observed quasiperiodic episodes of hypoperfusion that were prominent in subjects with SCD and much less evident in control subjects. Episodes of hypoperfusion in subjects with SCD have been reported by others, but no mechanism was considered (28). From our recordings of the respiratory waveform, the relationship between perfusion drops and sighs was clear.

After spontaneous sighs, subjects with SCD have exaggerated sympathetic and suppressed parasympathetic responses compared with control subjects (Figure 6). After adjusting for the effect of respiration, we observed no significant difference in both parasympathetic and sympathetic indices between the two groups, suggesting that parts of these differences were attributable to respiration. Nonetheless, the significantly higher probability of postsigh perfusion drops in subjects with SCD suggests that their peripheral sympathetic responses to sighs are increased, regardless of their normal cardiac sympathetic response to sigh.

Post–deep-inspiration vasoconstriction was observed in subjects without SCD as early as 1895 by Binet and Sollier (29). Later, in 1936, Bolton and coworkers (30) reported a loss of this reflex in two patients with a sympathectomized limb and in one
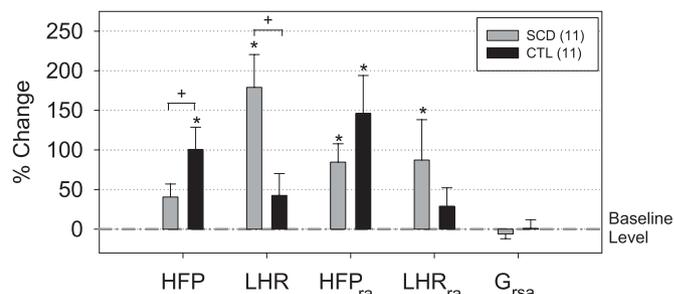

Figure 5. Changes in r-wave to r-wave interval (RRI) after a sigh. Time t = 0 indicates peak of sigh inspiration for each subject. Graphs show mean ± standard error. CTL = control subjects; SCD = sickle cell disease.

Figure 6. Heart rate variability responses to sigh. Bars are mean ± standard error of area under the curve of each parameter between t = 5 and 20 seconds, when t = 0 indicates the beginning of a sigh. CTL = control subjects; G$_{rsa}$ = respiratory sinus arrhythmia gain; HFP = high-frequency power; HFP$_{ra}$ = respiratory-adjusted HFP; LHR = low-frequency to high-frequency ratio; LHR$_{ra}$ = respiratory-adjusted LHR; SCD = sickle cell disease. * Difference from baseline, *P* < 0.05. + Difference between groups, *P* < 0.05.

patient who had an accidentally denervated limb, whereas the reflex was still intact in the nonaffected limbs. Although various mechanisms, such as impaired hemodynamic or lung functions of patients with SCD, may affect this vasoconstriction response, these earlier data are in agreement with our findings that the observed perfusion drops are caused by autonomic neural signals triggered by sensors in the lung and not by hypoxia or by transmitted intrathoracic pressure changes.

Our result shows that subjects with SCD were much more likely to trigger a perfusion drop after a sigh than CTL subjects, although there were three control subjects who had high sigh–vasoconstriction coupling. The fact that frequent perfusion drops occur in some control subjects does not negate in any way the possible importance of lung–vasculature coupling in the genesis of VOC. Lung–peripheral vascular coupling seems to be a general phenomenon and may play a role in other types of vascular disease in patients without SCD. Obstructive sleep apnea, for example, is associated with myocardial infarction and with stroke (31–34), and with sympathetic overflow and decreased parasympathetic modulation (35). The mechanisms in these disorders may well be related to episodic hypoperfusion induced by the pulmonary stretch signals. The consequences are just more severe for patients who have HbS. Only one of the subjects with SCD had low sigh–vasoconstriction coupling, and so on average most subjects with SCD had high coupling and most CTL subjects did not. If these episodes of vasoconstriction are important in triggering VOC, these differences in coupling may be yet another source of variability in severity of disease in SCD.

Although we did not observe a perfusion response to hypoxia, a significant parasympathetic withdrawal that persisted after correction for the respiration (HFP$_{ra}$, Figure 3B,) was seen in subjects with SCD and not in CTL subjects (Figure 3A). Hypoxia also seemed to generate sympathetic surges in both subject groups (LHR$_{ra}$, Figure 3B), although they did not reach statistical significance. This combination of sympathetic surge together with parasympathetic withdrawal would be expected to rapidly increase heart rate and to stress the patient's cardiovascular system. Acute hypoxia has been shown by Buchheit and coworkers (36) and Iwasaki and coworkers (37) to increase sympathetic modulation while decreasing parasympathetic modulation in healthy subjects. However, much longer periods of hypoxia were used in their experiments, which would raise some safety concerns in patients with SCD. The five breaths of N$_2$ protocol used here were insufficient to evoke a parasympathetic response in CTL subjects but were clearly able to generate significant response in the subjects with SCD, indicating that they have a much more sensitive ANS response to hypoxia.

These observations point to an alternative mechanism regarding the relation of hypoxia to VOC. The data suggest that the neural-mediated signal, and not global hypoxia, is the primary triggering event. Deoxy-HbS can form in the absence of systemic hypoxia by simply decreasing local perfusion. Although the distinction between hypoxia and formation of deoxy-HbS is well known and certainly systemic hypoxia would increase the risk of crisis, the existence of a low global Sa$_{O_2}$ should not be equated with local formation of deoxy-HbS. We suspect that nighttime hypoxia increases the likelihood of stroke and crisis through a neurovascular mechanism rather than a direct effect of systemic hypoxia on HbS. Particularly, we suspect that hypoxia activates the chemoreceptors causing increased ventilation. This hyperventilation causes a mechanically triggered neural signal to decrease perfusion. Such neurally mediated lung-to-vasculature mechanisms would also explain the connection between asthma (38, 39) and other lung diseases with VOC frequency.

Although subclinical vascular occlusions are frequent and ubiquitous, VOC does not occur over all the body simultaneously, but rather starts as painful ischemic symptoms in a particular region and spreads to either contiguous or noncontiguous areas. This pattern of presentation is consistent with a neural-mediated alteration of regional blood flow. A global background of inflammation, nitric oxide depletion, dehydration, or hypoxia would increase the chance that such regional triggers would cascade into clinically evident VOC (40, 41). Consistent with our finding of interindividual differences in sigh–vasoconstriction coupling, the variability in crisis frequency among patients with SCD could be in part caused by such differences in autonomic sensitivity among subjects with SCD. Pearson and coworkers (42) showed that children with SCD who had greater parasympathetic withdrawal during emotional challenges showed more clinical disease severity, suggesting a connection between autonomic dysfunction and SCD severity.

Autonomic dysfunction has been observed in patients with SCD and individuals with sickle cell trait (24, 25, 42–44). Loss of HRV is also a significant risk factor for cardiovascular adverse events, including sudden death, in the general population (45). Interestingly, up to 23% of deaths in adults with SCD are so-called "sudden death" events with no detectable cause found at autopsy (4, 46, 47). The present study demonstrates significant withdrawal of parasympathetic activity in response to transient hypoxia in subjects with SCD that was not present in non-SCD control subjects. Furthermore, approximately 78% of sighs in subjects with SCD cause transient microvascular hypoperfusion events, which are related to increased or unopposed sympathetic nervous system activity.

These data demonstrate autonomic dysregulation in subjects with SCD that enhances normal vasoconstriction reflexes. The cause of this regulation is still a topic of investigation. Cerebral injury, which is known to be common in SCD, is likely very important in this process. For instance, a recent study showed that 45% of patients with sickle cell have cerebral infarcts even though they received transfusions regularly (48) and another study showed that about 37% of patients have had silent stroke by the age of 18 (49). Moreover, cerebral thinning has been observed in patients with SCD (50) and may contribute to abnormalities of autonomic functions. We suspect that a hypersensitivity of the ANS responses to hypoxia may also be caused by irregularities of their brain function.

Inflammation, nitric oxide depletion, red cell adhesion, and percent hemoblobin F among other factors result in an increased probability of crisis (40, 41), but none are likely to be the trigger that brings a patient from steady-state to crisis at a particular moment. We suggest that the ANS is likely to play an important role in the pathophysiology of SCD. Although a clinical study needs to be done to test whether patients with a high degree of lung–hypoperfusion coupling have more frequent VOC, the present data support a direct lung-induced neural mechanism that results in hypoperfusion and thus retention of SRBC in the microvasculature that could trigger the VOC cascade.

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