Vasculopathy in sickle cell disease

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Chapter 3

Dynamic cerebral autoregulation in homozygous sickle cell disease

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Abstract

Background and Purpose — Sickle cell disease (SCD) is associated with cerebral hyperperfusion and an increased risk of stroke. Also, both recurrent microvascular obstruction and chronic hemolysis affect endothelial function, potentially interfering with systemic and cerebral blood flow control. We addressed the question whether cerebrovascular control in SCD patients is affected and related to hemolysis.

Methods — Systemic- and cerebrovascular control were studied in 18 patients with SCD and 10 healthy subjects (CTRL). Dynamic cerebral autoregulation was evaluated by transfer function analysis assessing the relationship between mean cerebral blood flow velocity (V_{mean}) and mean arterial pressure (MAP).

Results — Normal baroreflex sensitivity and postural cardiovascular reflex responses indicated integrity of systemic cardiovascular control. In the low (0.07-0.15 Hz) frequency region, MAP variability was comparable for both groups, but a larger V_{mean} variability in SCD (6.1 (4.6-7.0) vs. 4.2 (2.6-5.2) $(cm \cdot s^{-1})^2 \cdot Hz^{-1}$, $P<0.05$) indicated a reduced capacity to buffer the transfer of blood pressure surges to the cerebral tissue. Impairment of dynamic cerebrovascular control was confirmed by a reduced MAP-to-V_{mean} transfer function phase lead in SCD vs. CTRL (32±18° vs. 50±19°, $P<0.05$) that was unrelated to the severity of hemolysis.

Conclusions — In SCD patients dynamic cerebral autoregulation is impaired but appears unrelated to hemolysis.

Introduction

Sickle cell disease (SCD) exhibits coexistence of contrasting perfusion profiles with microcirculatory hypoperfusion and systemic circulatory hyperperfusion with increased regional blood flow.[1] SCD patients are proposed to have a lower systemic vascular resistance[2] and blood pressure (BP)[3] compared to healthy subjects, but yet cerebral infarction with acute neurological deficits affects 5% to 17% of SCD patients by 15 years of age.[4-6] Silent cerebral infarctions are present in about one third of homozygous SCD patients without clinically apparent neurologic events, but whether control of cerebral blood flow (CBF) functions normally is unknown.

Generally, BP is a determinant of the risk of ischemic stroke. Although in SCD patients BP is within the so-called cerebrovascular autoregulatory range, where constancy of CBF is maintained for a wide range of BP, patients may be nevertheless exposed to cerebral hyperperfusion,[7] reflected by an increased CBF and cerebral blood flow velocity (CBFV).[8-10] Cerebral hyperemia is assumed to be a consequence of the anemic state and a relationship with a higher incidence of stroke in SCD patients has been proposed.[4] This questions the efficacy of cerebrovascular autoregulatory capacity to protect the brain against hyperperfusion.

Furthermore, SCD is characterized by recurrent microvascular obstruction and chronic hemolysis. Both affect endothelial function, and are associated with a reduced nitric oxide (NO) bioavailability as the result of reduced formation of NO and increased scavenging of NO by cell-free circulating hemoglobin (Hb), released due to chronic hemolysis. Cerebrovascular endothelium plays an important role in the regulation of CBF,[11] and endothelial dysfunction may interfere with cerebral autoregulation.

We questioned whether cerebrovascular control in SCD patients is affected and related to hemolysis, and therefore set out to evaluate systemic cardiovascular and cerebral blood flow control in patients with SCD in relation to the degree of hemolysis.

Subjects and methods

Subjects

Afro-Caribbean black subjects with SCD and age- and ethnicity-matched healthy subjects (CTRL) were consecutively recruited from the outpatient clinic at the Academic Medical Center. Group characteristics are presented in Table 1. All subjects gave their written informed consent as approved by the AMC Medical Ethical Committee and experiments were performed in accordance with the Declaration of Helsinki. Only SCD patients with genotype HbSS or HbSβ^0 thalassemia, confirmed by high performance liquid chromatography, were included in the study. Exclusion criteria consisted of: a sickle cell crisis in the preceding four weeks, history of symptomatic cerebrovascular disease, clinical manifestation of heart failure or other cardiovascular diseases, uncontrolled hypertension (BP >160/100 mmHg), orthostatic hypotension, use of medication with

potential influence on autonomic cardiovascular function, or a blood transfusion in the preceding four months. The studies were performed in morning sessions in a room at 22 ºC. Subjects were requested to abstain from caffeinated beverages for at least 12 hours prior to measurements.

Blood samples

Degree of hemolysis was represented by Hb, reticulocyte count, lactate dehydrogenase (LDH) and haptoglobin plasma levels. Venous blood samples were drawn from all subjects and centrifuged immediately at 3000 g for 15 minutes and then stored at -80ºC. Complete blood counts (Hb, hematocrit (Ht), leucocyte, reticulocyte percentage) were determined (Cell-Dyn 4000, Abbott, Illinois, USA) and LDH was analyzed using spectrophotometry (Roche Hitachi Modular P800, Basel, Switzerland).

Hemodynamic parameters

Continuous BP was measured non-invasively by a servo-controlled finger photoplethysmograph (Portapres, FMS, Amsterdam, The Netherlands) with the cuff placed on the middle phalanx of the left middle finger kept at heart level. Changes in BP measured by photoplethysmography are not different from intra-arterial BP measurements both at rest and during orthostatic stress.[12] An automated non-invasive BP measuring device (HEM-705CP, Omron, Kyoto, Japan) was used to calibrate the finger BP measurements. Stroke volume (SV) was determined by pulse wave analysis using the Modelflow method (BeatScope 1.0 software, BMEye, Amsterdam, The Netherlands).[12] Heart rate (HR) was the inverse of the interbeat interval (IBI). Cardiac output (CO) was the product of HR and SV, and systemic vascular resistance (SVR) was mean arterial pressure (MAP) divided by CO. The transcranial Doppler (TCD, DWL Multidop X4, Sipplingen, Germany) derived CBFV was measured in the proximal segment of the right middle cerebral artery (MCA) and the MCA was insonated through the posterior temporal window. Once the optimal signal-to-noise ratio was obtained, the probe was secured with a headband (Marc 600, Spencer Technologies, Seattle, USA). Arterial CO_2 tension influences CBF independently of cerebral autoregulation.[13] To account for the cerebrovascular effects of CO_2, end-tidal CO_2 tension ($P_{ET}CO_2$) was measured by a sampling infrared capnograph (Tonocap, Datex-Ohmeda, Madison, USA). Transcutaneous O_2 saturation (SpO_2) was measured using a pulse oximeter (Novametrix 515A, Wallingford, Connecticut, USA). The signals of BP, spectral envelope of MCA velocity, and $P_{ET}CO_2$ were analog/digital converted at 100 Hz and stored on a hard disk for off-line analysis.

Systemic cardiovascular control

The MAP, HR and SVR responses to orthostatic stress assessed efferent sympathetic vasomotor function.[14] Afferent, central and vagal efferent baroreceptor reflex pathways were evaluated by quantifying baroreflex sensitivity (BRS) using the sequential method.[15] Beat-to-beat values of systolic BP and IBI were interpolated and resampled at 1 s. Cross-correlations were calculated using a 10 s window containing systolic BP for delays in the IBI window of 0-5 s. The highest coefficient of correlation was selected and accepted if $P<0.01$. BRS was the slope of the regression line between changes in IBI vs. systolic BP, expressed as ms·mmHg^{-1}.

Table 1. Group Characteristics

Characteristic	CTRL (n=10)	SCD (n=18)
M/F	3/7	5/13
Age, y	35 (9)	33 (12)
BMI, kg/m^2	25.6 (4.6)	22.1 (3.0)†
History of hypertension*	0	0
Blood pressure, mmHg		
Systolic	120 (9)	120 (11)
Diastolic	78 (6)	72 (7)†
Mean	92 (6)	88 (8)
SpO_2, %	97.4 (0.8)	96.3 (1.3)†
Hb, mmol/L	8.0 (7.6-8.3)	5.7 (5.3-6.1)‡
Ht, L/L	0.39 (0.03)	0.26 (0.04)‡
LDH, U/L	154 (24)	392 (328-454)‡
Reticulocyte, %	1.4 (0.8-1.7)	7.1 (6.4-9.5)‡
Organ damage		
Microalbuminuria	0	7
Pulmonary hypertension	0	5
Retinopathy	0	2
Medication		
Hydroxyurea	0	6
Folic acid	0	18

* defined as blood pressure >140/90 mmHg; † $P<0.05$ and ‡ $P<0.01$ versus CTRL. Data are presented as means (SD) or medians (interquartile range).

Cerebral blood flow control

The steady-state response of mean MCA velocity (MCA V_{mean}) to postural change was assessed from steady-state arterial pressure and CBFV sampled from 1 min before standing up to 5 min upright. With standing, the positioning of the head ca. 30 cm above heart level within a few seconds results in an abrupt reduction in cerebral perfusion pressure of approximately 20 mmHg,[16] with a decrease in cerebral tissue oxygenation[16, 17] and CBF[18]

reflected by the TCD determined MCA V_{mean}.[13] Such steady-state reductions in cerebral perfusion take place even though the cerebral perfusion pressure remains within, what is considered to be, its autoregulatory range. Static cerebral autoregulation limits the steady-state postural reduction in MCA V_{mean} to ~ 15%.[16, 17] Beat-to-beat values for MCA V_{mean} and MAP were derived as the integral over one beat divided by the corresponding beat interval. MAP at brain level (MAP_{brain}) was calculated from MAP measured at heart level and the vertical finger-to-TCD probe distance.[17] Cerebrovascular resistance (CVR) was the ratio of MAP_{brain} and MCA V_{mean}. The Gosling pulsatility index of the MCA was taken as an index of cerebral microangiopathy expressed as the amplitude of CBFV divided by time-averaged CBFV.[19]

Frequency domain analysis quantified the counter-regulatory capacity of dynamic cerebral autoregulation from spontaneous BP oscillations in the upright position.[20, 21] A 4 min tracing of beat-to-beat data of MAP and MCA V_{mean} was spline interpolated and resampled at 4 Hz. To quantify the variability of BP and CBFV, the power spectra of the two variables were estimated by transforming the time series of BP and CBFV with discrete Fourier transformation to the frequency domain. From the cross spectrum, transfer function phase shift and gain were derived. According to the high-pass filter model of cerebral autoregulation, autoregulatory capacity is reflected by the positive phase relation between oscillations of BP (input function) and CBFV (output function).[20] At high frequencies, less cerebral attenuation of MAP surges to MCA V_{mean} implies that the cerebral autoregulation cannot respond fast enough to rapid changes in MAP.[20] Results were expressed as the integrated area in the low frequency (LF) range (0.07 to 0.15 Hz). The gain as the ratio of the amplitudes of MCA V_{mean} and MAP was taken to reflect the effective amplitude dampening of BP fluctuations. To examine the strength of the relationship between MAP and MCA V_{mean}, coherence was used to signify that the two cardiovascular signals co-vary significantly in the LF area. Like a correlation coefficient, it varies between 0 and 1, and a coherence above 0.5 was considered to provide a reliable estimate of the transfer function variables. Data with a coherence lower than 0.5 were excluded from further analysis. Phase shift was defined positive where MCA V_{mean} leads MAP. In healthy subjects, MCA V_{mean} leads MAP with 50°~60° in the LF range.[20, 21] To account for the intersubject variability, the gain was normalized for MCA V_{mean}, and expressed as the percentage change in $cm \cdot s^{-1}$ per mmHg.

Statistical analysis

Data are presented as means (SD) or medians (interquartile range). Differences between groups were identified by unpaired Student t-test when data fitted a normal distribution, and a Mann-Whitney rank sum test was applied when data were not normally distributed. Two-way ANOVA was used to identify differences across condition (SCD vs. CTRL) and body position. $P<0.05$ was considered to indicate a statistically significant difference. To investigate the relationship between hemolysis and dynamic cerebral autoregulatory capacity, a multivariate stepwise regression model was constructed with MAP-to-MCA V_{mean} transfer function phase as the dependent variable and Hb and LDH as the independent variables, as well as age, systolic and diastolic BP, Ht, reticulocyte count, presence of chronic organ failure (pulmonary hypertension, retinopathy, microalbuminuria), use of hydroxyurea or folic acid, and body mass index (BMI) with forward entry and removal.

Results

Group characteristics and baseline measurements

Eighteen Afro-Caribbean black subjects (5 male and 13 female) with SCD (17 patients with HbSS and 1 with HbSβ^0 thalassemia) and 10 age- and ethnicity-matched healthy subjects (3 male and 7 female, CTRL) were included. Among the SCD vs. CTRL groups, there were no differences with regard to gender ratio and age, whereas BMI, SpO_2, diastolic BP ($P<0.05$) and SVR ($P=0.012$) were lower in SCD (Table 1). In the SCD group plasma LDH and reticulocyte count were higher, and Hb lower ($P<0.01$) whereas haptoglobin was undetectably low. A limited (<15%) variation of these values in the preceding 2 years conformed to a relatively stable level of hemolysis. Baseline MAP, HR, SV, CO, $P_{ET}CO_2$ and pulsatility index were comparable between groups (Table 2). MCA V_{mean} was higher (87±16 vs. 64±13 $cm \cdot s^{-1}$, $P<0.01$) and CVR lower (0.94±0.16 vs. 1.35±0.23 $mmHg \cdot cm^{-1} \cdot s^{-1}$, $P<0.01$) in SCD.

Systemic cardiovascular control

A normal orthostatic increase in SVR confirmed intact efferent sympathetic vasomotor function in SCD (Figure 1). The postural reduction in SV and CO did not differ between groups. A normal orthostatic HR response together with normal BRS indicated intact parasympathetic HR control in SCD.

Cerebral blood flow control

The postural decline in MAP_{brain} (-15%), MCA V_{mean} (-13% vs. -14%) and $P_{ET}CO_2$ (-4% vs. -6%) was comparable. In 1 subject from the CTRL group and in 2 subjects from the SCD group, coherence was below 0.5 and these data were excluded from further analysis. MAP LF power was comparable for both groups (Table 3), whereas MCA V_{mean} LF power was higher in the SCD group (6.1 (4.6-7.0) vs. 4.2 (2.6-5.2) $(cm \cdot s^{-1})^2 \cdot Hz^{-1}$, $P=0.043$). Representative examples of individual recordings are given in Figure 2. In the SCD group the transfer function phase lead between MCA V_{mean} and MAP was lower (32 (19-39) ° vs. 50 (44-60) °, $P<0.05$, Figure 3), with phase lead lower than 40° in 83% of the patients. MAP-to-MCA V_{mean} transfer function phase did not relate to Hb, LDH, Ht, reticulocyte count, age, systolic and diastolic BP, BMI or presence of chronic organ damage. No relationship was found between Hb levels and MCA V_{mean}.

Table 2. Cerebrovascular and Cardiovascular Response to Postural Change

	Groups	Supine	Standing t = 5 min	Δ	P
MAP_{heart}, mmHg	CTRL	83 (10)	93 (10)	+13 %**	0.28
	SCD	80 (7)	91 (11)	+14 %**	
MAP_{brain}, mmHg	CTRL	83 (10)	71 (9)	-15 %**	0.15
	SCD	80 (7)	68 (11)	-15 %**	
MCA V_{mean}, $cm \cdot s^{-1}$	CTRL	64 (13)	56 (11)	-14 %**	<0.001
	SCD	87 (16)	76 (14)	-13 %**	
CVR, $mmHg \cdot cm^{-1} \cdot s^{-1}$	CTRL	1.35 (0.23)	1.30 (0.25)	-4 %	<0.001
	SCD	0.94 (0.16)	0.90 (0.16)	-5 %	
HR, bpm	CTRL	72 (13)	81 (4)	+13 %**	0.34
	SCD	75 (8)	86 (12)	+14 %**	
SV, ml	CTRL	96 (14)	68 (9)	-29%**	0.57
	SCD	96 (16)	73 (12)	-25%**	
CO, $l \cdot min^{-1}$	CTRL	6.8 (1.2)	5.5 (0.8)	-19%**	0.13
	SCD	7.1 (1.1)	6.0 (0.9)	-15%**	
SVR, $dyn \cdot s \cdot m^{-5}$	CTRL	731 (182)	1022 (229)	+40%**	0.012
	SCD	638 (78)	871 (135)	+37%**	
$P_{ET}CO_2$, mmHg	CTRL	39.8 (2.7)	37.3 (2.3)	-6 %**	0.72
	SCD	39.3 (2.6)	37.5 (2.4)	-4 %**	
Pulsatility Index	CTRL	0.78 (0.15)	0.75 (0.14)	-4 %	0.90
	SCD	0.78 (0.10)	0.78 (0.10)	-1 %	
BRS, $ms \cdot mmHg^{-1}$	CTRL	19 (8)	10 (5)	-49%**	0.93
	SCD	19 (12)	10 (4)	-47%**	

** P<0.01 versus supine. P: Probability value represents overall difference between CTRL and SCD groups. Data are given in means (SD) for n=10 (CTRL) vs. n=18 (SCD).

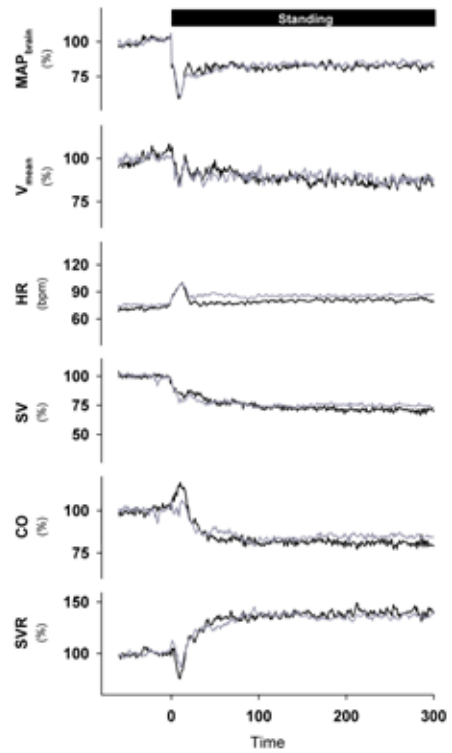

Figure 1. Cerebro- and cardiovascular response to postural change. The steady-state hemodynamic response to standing was comparable between the SCD group (n=18, grey line) and CTRL group (n=10, black line). Bar indicates standing

Table 3. Transfer function gain, phase and coherence function during standing

Low frequency (0.07 - 0.15 Hz)	CTRL (n=9)	SCD (n=16)
MAP power, $mmHg^2 \cdot Hz^{-1}$	4.0 (1.3)	4.4 (3.7)
MCA V_{mean} power, $(cm \cdot s^{-1})^2 \cdot Hz^{-1}$	4.2 (2.6-5.2)	6.1 (4.6-7.0)†
Coherence, k^2	0.86 (0.08)	0.85 (0.08)
Phase, degrees	50 (44-60)	32 (19-39) †
Normalized gain, $\%/mmHg^{-1}$	1.65 (0.36)	1.69 (0.28)

† *P*<0.05 vs. CTRL; Data are presented as means (SD) or medians (interquartile range).

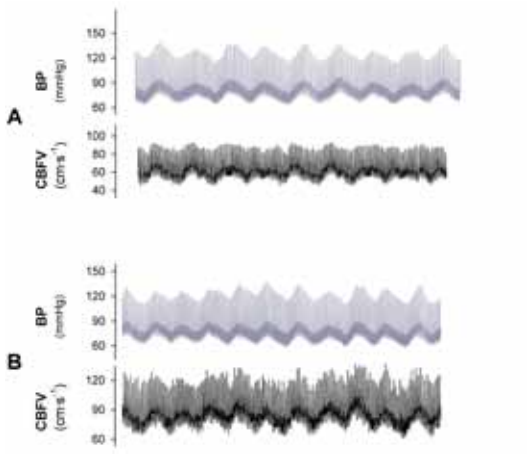

Figure 2. Representative continuous recordings of BP and CBFV. A) healthy control subject and B) SCD patient. BP variability is comparable whereas CBFV variability is enhanced in the SCD patient indicating a reduced capacity to buffer the transfer of BP fluctuation to the cerebral circulation.

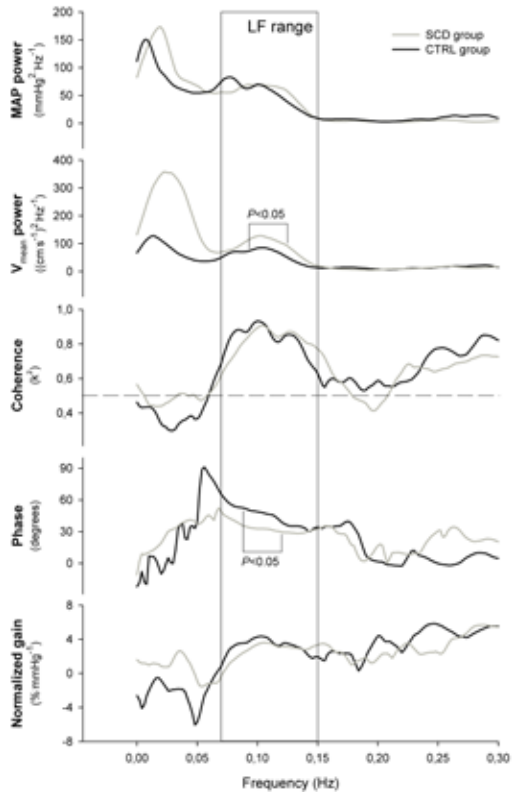

Figure 3. Cross-spectral analysis of the entire spectrum from 0 to 0.30 Hz. Group averaged MAP and V_{mean} variability, coherence, phase and normalized gain between MAP and V_{mean} are shown for SCD (n=16, grey line) vs. CTRL groups (n=9, black line). Lines indicate low frequency (0.07-0.15 Hz) range.

Discussion

The present study provides novel information regarding the control of cerebral blood flow in patients with SCD. We found evidence for impairment of dynamic cerebral autoregulation in SCD that appeared unrelated to the degree of hemolysis, whereas systemic cardiovascular control was unaffected.

Impaired cerebral autoregulation has been linked to an increased risk for stroke,[22] particularly in severe obstructive carotid artery disease.[23] Silent cerebral infarctions are present in about one third of homozygous SCD patients.[5] The observation in the present study that cerebrovascular autoregulatory capacity was affected in the majority of consecutively recruited SCD patients with asymptomatic cerebrovascular disease supports that impairment of dynamic cerebral blood flow control precedes cerebral ischemic events. Our data represent the findings in adult patients and may therefore not be applicable in young children with homozygous SCD. However, although the clinical presentation of stroke appears to differ between children and adult SCD patients, it seems likely that these clinical forms represent the same pathophysiological process. Both homozygous SCD and low hemoglobin are major risk factors for both ischemic and hemorrhagic stroke. In addition, many SCD patients present with secondary hemorrhagic stroke after a previous ischemic stroke.[4] Future long-term follow-up studies in both young and adult SCD patients are needed to strengthen these assumptions.

Impairment of dynamic cerebral autoregulation in SCD patients puts forward a potential linkage to cerebral small vessel disease. This notion is supported by the finding that cerebral autoregulation is equally impaired in type 2 diabetic patients.[24, 25] The higher variability in cerebral blood flow velocity in the SCD group indicated a reduced capacity to buffer the transfer of blood pressure surges to the cerebral tissue. Impairment of dynamic cerebrovascular control was confirmed by a reduced phase lead of the blood pressure -to- cerebral blood flow velocity transfer function in SCD. In a previous study, the cerebral arterial pulsatility index has been proposed as an indicator of cerebral microangiopathy in diabetic patients.[19] However this was not substantiated in the present study questioning its applicability in SCD patients.

SCD is characterized by an ongoing state of vascular inflammation with endothelial activation,[26] resulting in microvascular damage. This process is enhanced by the elevated oxidative stress associated with chronic hemolysis, added to ischemia-reperfusion injury due to transient vaso-occlusive events.[27] Both result in reduced NO bioavailability due to NO scavenging by cell-free hemoglobin, and reduced NO formation due to increased arginase levels.[28] We did not measure NO concentrations but consider that the relationship between hemolysis and NO bioavailability is well established.[29] Controversies exist regarding the role of NO in dynamic cerebral autoregulation in humans.[30, 31] In the present study, no relationship was found between dynamic cerebral autoregulatory capacity and severity of hemolysis. However larger studies are needed to establish the functional significance of this observation.

An inverse relationship between cerebral blood flow and hematocrit level has been

reported under physiological conditions both in animal models[32-34] and in humans.[35, 36] In the present study no relationship was found between either cerebral blood velocity or dynamic cerebral autoregulatory capacity and hemoglobin, rendering a role for anemia as an independent factor in the impairment of dynamic cerebral autoregulation unlikely. When considering that in healthy subjects cerebral blood velocity changes occur independently of cerebral autoregulatory capacity,[37] the finding of an elevated cerebral blood velocity in the SCD group does not in itself imply affected dynamic cerebral autoregulation.

Hydroxyurea has been demonstrated to reduce cerebral blood velocities in children with SCD.[38] Although hydroxyurea may reduce cellular adhesion of leukocyte and erytrocytes to endothelial cells[39] there are no data indicating an effect of folic acid or hydroxyurea on dynamic cerebral autoregulation. In the present study all patients were treated with folic acid and 6 patients received hydroxyurea.whereas no correlation was found between the use of hydroxyurea and transfer function phase.

The finding of comparable cardiac output but lower systemic vascular resistance and diastolic blood pressure in SCD vs. CTRL is consistent with earlier observations.[3, 40, 41] In contrast to an earlier report,[42] we found intact baroreflex cardiovascular control and no signs of cardiovascular autonomic dysfunction in the SCD group. Thus, integrity of cardiovascular control contrasts to impairment of dynamic cerebrovascular control in SCD patients.

Sickle cell disease is associated with an increased cerebral blood velocity assessed by TCD ultrasonography.[43] Blood velocity rather than blood flow was monitored and it could be considered that changes in the diameter of the insonated vessel by enhanced sympathetic activity modulate blood velocity independently of flow. However, the large cerebral arteries are conductance rather than resistance vessels and moderate sympathetic activation does not modify the luminar diameter of a systemic conduit artery.[16] Thus, cerebral blood flow increases in proportion to changes in middle cerebral artery mean blood velocity[44-46] or in internal carotid flow,[47] and constancy of the diameter links changes in cerebral blood velocity to changes in flow.[48] An increased blood velocity may reflect intracranial stenosis in the middle cerebral artery at the background of an unchanged global cerebral blood flow, but it also may indicate cerebral hyperperfusion as a compensatory response to a SCD associated reduced O_2 binding capacity. Recently an increased cerebral blood velocity in SCD patients has been attributed to hyperperfusion rather than intracranial stenosis,[7] which conforms to earlier studies reporting an increased cerebral blood flow as determined by ^{133}Xe [9] and MRI.[10]

In conclusion, the capacity to buffer the transfer of BP surges to the cerebral tissue is reduced in patients with SCD but appears unrelated to hemolysis. Whether a hampered dynamic cerebral autoregulation plays a role in the high incidence of cerebrovascular complications in SCD remains to be elucidated.

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