C. R. Biologies 336 (2013) 142–147

Contents lists available at SciVerse ScienceDirect

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Medical sciences/Sciences médicales

Autonomic nervous system dysfunction: Implication in sickle cell disease

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ARTICLE INFO

Article history:
Available online 16 October 2012

Keywords:
Sickle cell disease
Parasympathetic activity
Hypoxia
Vasoconstriction
Respiratory-mediated reflex

ABSTRACT

Sickle cell disease is an inherited hemoglobinopathy caused by a single amino acid substitution in the β chain of hemoglobin that causes the hemoglobin to polymerize in the deoxy state. The resulting rigid, sickle-shaped red cells obstruct blood flow causing hemolytic anemia, tissue damage, and premature death. Hemolysis is continual. However, acute exacerbations of sickling called vaso-occlusive crises (VOC) resulting in severe pain occur, often requiring hospitalization. Blood rheology, adhesion of cellular elements of blood to vascular endothelium, inflammation, and activation of coagulation decrease microvascular flow and increase likelihood of VOC. What triggers the transition from steady state to VOC is unknown. This review discusses the interaction of blood rheological factors and the role that autonomic nervous system (ANS) induced vasoconstriction may have in triggering crisis as well as the mechanism of ANS dysfunction in SCD.

1. Heart rate variability and autonomic nervous system

The autonomic nervous system (ANS) is responsible for the continual adaptation of cardiac, intestinal, pulmonary and peripheral vascular function. This system is a highly complex network of peripheral and central sensors and specialized neural pathways that differ functionally and anatomically. Interactions between the various components of the autonomic system occur quickly and involve peripheral and central regulation as well as feedback between various parts of the ANS. Heart rate is closely regulated by the ANS with sympathetic inputs increasing heart rate and parasympathetic inputs decreasing heart rate. Thus heart rate variability (HRV) is affected by respiration as well as other factors and can be quantified to study cardiac ANS balance. Peripheral vasoconstriction is controlled by the sympathetic nervous system (SNS). While cardiac sympathetic activity is not directly the same as that regulating peripheral vasoconstriction, a complex network of interactions links the responses [1,2]. Depending on the physiological situation, the parasympathetic and sympathetic nervous systems adjust their activities to respond adequately to an external or endogenous stimulus.

The easiest way to assess the ANS activity in the human is to record heart rate using Holter electrocardiogram and then to quantify the heart rate variability (HRV) [3]. Mathematical analysis of HRV provides reliable information on the parasympathetic and sympathetic activities affecting the heart, as well as the general autonomic balance [1,3].

It is now well accepted that a loss of the HRV is a powerful and independent predictor of an adverse prognosis in patients with heart disease and in several other disorders [4]. ANS imbalance may change electrophysiological properties of the heart and lead to increased mortality [3]. In addition to the well-recognized role of the ANS in regulation of cardiovascular, pulmonary and gastrointestinal function, ANS regulates several aspects of inflammation [1,5,6] and is, in turn, modulated by inflammation detected by vagal afferents [1,7].

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1631-0691/$ – see front matter © 2012 Published by Elsevier Masson SAS on behalf of Académie des sciences.
http://dx.doi.org/10.1016/j.crvi.2012.09.003

Importantly, the ANS and the sympathetic ANS in particular, modulates regional blood flow. With a few exceptions [8], this well-known function of the ANS has not been considered in the context of the pathogenesis of vaso-occlusive crisis in SCD. As we will discuss below, microvascular flow is one of the two main factors that determine vaso-occlusion and decrease of regional flow would favor development of vaso-occlusion in sickle cell patients [9]. VOC pain does not start all over. It starts in one area of the body and may increase to full painful crisis or resolve spontaneously. Emotional stress and change in temperature, factors well known to modulate ANS activity, are well known triggers of crisis.

Standardized mental stress stimulus induces peripheral vasoconstriction and patients classed as "mental stress responders" had parallel myocardial ischemia that was simultaneously detected by peripheral tonometry [10], showing that this biophysical marker of perfusion reflects general ANS vascular responses. Mental stress protocols have been associated with parasympathetic withdrawal in SCD and patients with more withdraw have a higher crisis frequency. Thus, autonomic dysregulation may well be causative in patients with SCD and we suspect the link is through vasoconstriction [11,12].

2. Sickle cell disease

The gene defect of sickle cell disease (SCD) is a mutation of a single nucleotide (A→T) on the β-globin chain, which results in the substitution of valine for glutamic acid in the sixth position of the beta chain of the hemoglobin S (HbS). The hydrophobic residues of valine at position 6 of the β chain in hemoglobin are able to associate with the hydrophobic patch, causing HbS molecules to aggregate and form precipitates under deoxygenated condition. This phenomenon is called "polymerization of HbS" [13,14] and is usually reversible on reoxygenation. However, after many repeated cycles of deoxygenation/reoxygenation, RBCs become and remain irreversibly sickled [15–17].

All of the pathologies of SCD derive from the polymerization of deoxy HbS. However, SCD is a systemic vasculopathy that has features in common with other forms of vascular disease, with the ultimate morbidity being due to tissue ischemia. Inflammation, activation of coagulation, increased adhesion of cells to the vascular endothelium and poor nitric oxide (NO) bioavailability decrease steady state microvascular flow and increase SCD severity [18,19]. These factors are operative at steady state but do not explain what causes the transition to acute VOC in a specific region of the body at a particular moment.

SCD is unique because the vascular pathology stems entirely from the red blood cell (RBC) rheological properties imparted by the abnormal hemoglobin, HbS [20]. RBC must be flexible to deform and pass through the microvasculature. When oxy-HbS looses oxygen in tissues, deoxy HbS polymerization causes the flexible RBC to become rigid. This gives rise to the most basic concept in the physiology of SCD vaso-occlusion: anything that decreases flow in the microvasculature increases the chance that intracellular HbS will polymerize, and the flexible-to-rigid transformation will occur before the RBC reaches a larger vessel, resulting in the cell becoming lodged in the microvasculature. Thus, the likelihood of vaso-occlusion depends solely on two factors:

- the steady state flow through the microvascular segment;
- the delay time between onset of the deoxy-S state and polymerization [21–24].

The rigid RBC cause pathology in several ways:

- the HbS-containing RBC may get stuck if they are not able to traverse the microvasculature before HbS polymerizes;
- circulating abnormal RBC drastically change the viscous properties of blood and significantly alter the forces transmitted to the vessel wall, causing activation and remodeling of the endothelium [25], reducing flow and altering signaling at the endothelium level [26];
- ischemia itself triggers inflammation and reperfusion injury causing significant oxidative stress that alters endothelial function;
- RBC are themselves altered by these processes, leading to adhesion of RBC to endothelium [27,28], activation of leukocytes [29], and activation of the clotting cascade [30,31];
- RBC lysis decreases nitric oxide (NO) bioavailability [32,33] and increases ATP in the circulation [34], thereby altering vessel tone.

The delay time to polymerization of deoxy HbS is a critical factor determining in the likelihood of vaso-occlusion. It is also the factor that has been most effectively modified therapeutically to the benefit of patients with SCD. The delay time, which is normally on the order of 1 second, is proportional to the inverse 30th power of the HbS concentration. Thus, a very small decrease in intracellular HbS concentration results in a great prolongation of the time to polymer formation and makes it more likely that the RBC will escape into a larger vessel before the liquid Hb transforms into a solid and the cell takes on a sickle shape. Hydroxyurea increases the number of hemoglobin F-containing RBC. Increased HbF dilutes the HbS monomer and increases the delay time to 20 or 30 seconds enabling these cells to pass through the microvasculature and back to the lung before sickling [9]. From a clinical standpoint the percentage of HbF measured in patients reflects a greater number "F-cells" and decreases the severity of SCD [35].

The other part of the basic SCD physiology is microvascular flow. If local perfusion is not adequate, RBC will not pass through the microvasculature before HbS polymer forms and occlusion occurs.

However, the pathophysiology of HbS polymerization is insufficient to explain the extremely variable phenotypic expression of SCD and its multiple complications. Although SCD is a monogenic disorder, the pathophysiological mechanisms involved are complex. Most, if not all of the physiological factors purported to alter likelihood of crisis and contribute to the variability of this disorder have an effect on microvascular flow. These include blood viscosity,

nitric oxide depletion, inflammation, activation of coagulation and adhesion of cellular elements to the vascular wall.

Increased hematocrit [36] and blood viscosity [37,38] have been identified as risk factors for painful VOC in SCD patients. Whereas rigid RBCs may fail to enter and negotiate the small capillaries, more deformable SCD RBCs may trigger painful VOC [15,37,39] because of their predilection for adherence to the vascular endothelium. Recently, the greater strength of RBC aggregates previously reported in SCD [40] has been associated with ACS history [37] suggesting that RBC aggregation abnormalities could also play a role in the pathophysiology of SCD complications. Several authors also demonstrated the existence of a pro-inflammatory vascular environment with activated endothelial cells, neutrophils and monocytes [14,41–44]. Sickle RBCs may then adhere to neutrophils causing activation of the neutrophil respiratory burst [29] and increased oxidative stress that in turn may impair sickle RBC rheology and endothelial function [45]. Activated leucocytes secrete pro-inflammatory cytokines that can induce endothelial cells to express ligands for sickle RBCs, leukocyte and platelet adhesion receptors, as well as tissue factor, thereby providing a link between circulating cell-mediated vascular occlusion and activation of blood coagulation [46]. In addition, the circulating-sickle RBCs derived microparticles provide a support for coagulation system involvement [47,48]. Finally, the decreased nitric oxide (NO) bioavailability caused by chronic hemolysis plays a role in endothelial dysfunction in SCD [43,49] and is involved in certain complications such as PHT [50] or glomerulopathy [51].

3. Evidence of autonomic nervous system dysfunction in sickle cell disease

As discussed above, the morbidity and severity of SCD are modulated by several identified factors. There is a growing interest recently in the role of ANS activity in SCD as a modulating and initiating factor [11,12,38,52,53]. The first work investigating ANS activity in this population demonstrated abnormalities in cardiovascular ANS function of SCD patients compared to normal white and normal black controls [53]. Then, Romero-Vecchione et al. [54] demonstrated abnormal responses of SCD patients during a tilt-test in comparison with controls. In baseline condition, SCD patients exhibit autonomic imbalance caused by a reduced parasympathetic activity compared to controls [55]. Findings from Treadwell et al. [56] also provided some evidence that children with SCD exhibit a dampening of parasympathetic responses, particularly to social and sensory challenges, compared with children without chronic physical conditions. More recently, Oguanobi et al. [57] submitted a group of SCD patients to different tests such as valsalva maneuver, heart rate variation during deep breathing, heart rate response to standing, blood pressure response to sustained hand grip, and blood pressure response to standing. Their results strongly suggest cardiovascular autonomic neuropathy, which supports previous data from Sanya et al. [58].

4. Does autonomic nervous system dysfunction participate in the sickle cell disease pathophysiology?

It seems that SCD patients are characterized by ANS dysfunction but it is unknown whether these abnormalities could play a role in the pathophysiology of the disease and/or reflect the clinical severity. Pearson et al. [59] were the first to address this issue but did not offer a mechanism. They submitted SCD children to cognitive and physical challenges, assessed autonomic reactivity and tested the association with a score of severity. The clinical severity was assessed by averaging severity ratings assigned by medical diagnosis over the year prior to study enrollment. A 5-point rating scale was developed by clinicians and scientists at their hospital to categorize and weight medical diagnoses from most severe to least severe [59]. They found that children with greater parasympathetic withdrawal during the challenges had significantly more severe disease. This relationship with clinical severity has been confirmed recently by Nebor et al. [38]. These authors compared nocturnal (i.e. baseline) ANS activity between a group of SCD patients who experienced three or more VOC in the previous year (severe SCD group), a group of SCD patients with no VOC within the two years preceding the study (non-severe SCD group) and a non-SCD control group. While the non-severe SCD and control groups were not significantly different regarding ANS activity, the severe SCD group exhibited autonomic imbalance and reduced parasympathetic activity [38]. In addition, the severe SCD group had higher steady state blood viscosity than non-severe SCD group, strengthening the role of blood viscosity in vaso-occlusion physiopathology [36]. The authors suggested that both blood hyperviscosity and autonomic imbalance might increase the risks for VOC occurrence in SCD by impairing blood flow and increasing the risks for vasoconstriction, respectively [38]. The consequent decrease in local perfusion and increase in transit time mean that the sickle RBCs with deoxygenated hemoglobin could remain longer in the microcirculation where the hemoglobin is more likely to polymerize and the RBCs to sickle [12]. Oguanobi et al. [57] recently reported that leg ulcers, postural dizziness, erectile dysfunction in men and history of recurrent acute chest syndromes (ACS) were more frequent in SCD patients with ANS dysfunction in comparison with SCD patients without. Associations between leg ulcers or history of ACS and ANS dysfunction have been also observed in the study of Mohan et al. [60] and Knight-Madden et al. [61], respectively.

On the whole, it seems that large ANS dysfunction is observed in SCD patients with a severe phenotype. Although these impairments are thought to play a role in the pathophysiology of SCD [11], additional studies are needed to better understand how ANS dysfunction might trigger VOC, ACS or other complication. A decade ago, Tracey [6] reported that parasympathetic activity might regulate inflammation. Decreased parasympathetic activity has been shown to affect the acetylcholine-driven inhibition of cytokines production/release by leukocytes [6]. Indeed, one may hypothesize that the altered baseline ANS activity in SCD, could modulate the pro-inflammatory state observed in this disease, similar to what has been

reported for obstructive sleep apnea syndrome [62], and thus play a role in SCD clinical severity. Altogether, these findings should stimulate further studies in SCD to test whether clinical severity, ANS dysfunction and inflammatory state are related.

5. Mechanisms of ANS dysfunction in sickle cell disease?

Another issue to address in future experiments is the reason of ANS dysfunction in SCD. Repeated nocturnal hypoxemia in patients with obstructive sleep apnea syndrome leads to higher sympathetic and lower parasympathetic tones [63], a finding that has been extensively investigated and confirmed in rodent models [64,65]. Sangkatumvong et al. [8,12,66] showed that transient hypoxic stress elicits parasympathetic withdrawal in SCD patients, but not in controls. One could suggest that hypoxia could be involved in the ANS dysfunction of SCD patients [11,52]. For example, in rats, chronic intermittent hypoxia causes a significant cell loss in the nucleus ambiguous, a structure from which several vagal efferent axons innervate ganglionated plexuses in the dorsal surface of cardiac atria, which in turn may have different functional roles in cardiac regulation [67]. In addition, chronic intermittent hypoxia alters the structure of cardiac ganglia and results in reorganized vagal efferent projections to cardiac ganglia [68].

However, although hypoxia is able to cause loss of parasympathetic activity in SCD patients, Sangkatumvong et al. [12] failed to demonstrate that this ANS hypersensitivity to hypoxia was followed by a decrease in microvascular perfusion. However, they showed that there was marked hypersensitivity to thoracic stretch receptor induced vasoconstriction. The minimal increase in tidal volume that comprises a sigh was able to induce vasoconstriction in SCD subjects but not normal. Although patients with SCD exhibited the same sigh frequency as a control group, the probability of a sigh inducing a peripheral microvascular perfusion drop was greater in SCD patients (78%) than in controls (17%) [12]. These data suggest sigh-induced sympathetic nervous system dominance in patients with SCD but not in control subjects. Since transient hypoxic stress induced ANS dysfunction without affecting peripheral vasoconstriction, the authors concluded that respiratory neural-mediated signals, rather than global hypoxia, could be the primary triggering event of vaso-occlusion [12]. This is in agreement with recent data from L'Esperance et al. [69] who reported that cutaneous vasoconstrictor response to inspiratory breath hold (IBH) maneuver, a much greater change in thoracic volume than a sigh, was greater in SCD children than in controls. In this study, the cutaneous vasoconstrictor response was negatively associated with daytime hemoglobin oxygen saturation, emphasizing a possible role of hypoxemia [69]. Because a more pronounced vasoconstriction to a sigh maneuver has been reported in patients with obstructive sleep apnea, in whom intermittent hypoxia is an important constituent of the disease [70], we believe that both respiratory neural-mediated signals and ANS hypersensitivity to hypoxia could be involved in the pathophysiology of acute events, such as VOC or ACS, in SCD. It is likely that increased vasoconstriction mediated by the ANS decreases blood flow in the microcirculation and triggers sickle vaso-occlusion. Whether the autonomic triggered decrease in regional microvascular flow results in transition from steady state sickling to full vaso-occlusive crisis likely depends on the HbS polymerization delay time and "rheological tone" comprised of RBC aggregation and deformability properties, inflammation, endothelial activation, coagulation status, and cellular adhesion.

6. Conclusion

SCD patients have ANS dysfunction with the level of parasympathetic activity withdrawal being very variable from one patient to another. Although the involvement of the ANS in determining the phenotype of this disease is not yet fully understood, these findings suggest a role of ANS dysfunction in SCD-associated pathophysiological mechanisms. The reduced nitric oxide bioavailability, the marked blood rheological abnormalities, the pro-inflammatory and pro-oxidative states and the important ANS dysfunction could increase the risks for vaso-occlusion in SCD. We suspect that ANS mediated vasoconstriction is the factor that triggers regional transition from steady state to symptomatic crisis and forms the link between many known crisis triggers such as change in temperature and emotional status and events.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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