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Sensitization of Nociceptors and Dorsal Horn Neurons Contributes to Pain in Sickle Cell Disease

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Abstract

Sickle cell disease (SCD) describes a group of disorders associated with a point mutation in the beta chain of hemoglobin. The mutation leads to the creation of sickle hemoglobin (HbS) and causes distortion of erythrocytes through polymerization under low oxygen, resulting in characteristic sickle red blood cells. Vaso-occlusion episodes caused by accumulation of sRBCs results in ischemia-reperfusion injury, reduced oxygen supply to organs, oxidative stress, organ damage and severe pain that often requires hospitalization and opioid treatment. Further, many patients suffer from chronic pain, including hypersensitivity to heat and cold stimuli. Progress towards the development of novel strategies for both acute and chronic pain in patients with SCD has been impeded by a lack of understanding the mechanisms underlying pain in SCD. The purpose of this review is to highlight evidence for the contribution of peripheral and central sensitization that leads to widespread, chronic pain and hyperalgesia. Targeting the mechanisms that initiate and maintain sensitization in SCD might offer effective approaches to manage the severe and debilitating pain associated with this condition.

Keywords

Sickle cell disease; hyperalgesia; nociceptor sensitization; central sensitization

1. Introduction

Sickle cell disease (SCD), the world's most common inherited hemoglobinopathy, is characterized by impaired β^S globin chains that lead to the polymerization of hemoglobin S which gives red blood cells (RBCs) their sickle shape [1]. Sickle RBCs occlude blood vessels, leading to impaired oxygen supply and ischemia/reperfusion-induced vasculopathy, inflammation, oxidative stress, organ damage and pain [2–7]. Indeed, pain is a hallmark of SCD, beginning early in life and worsening with age [8–9]. SCD patients reported

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Declarations of Interest
None.

experiencing pain on more than half of the days during a 6-month period, with over a quarter of the patients experiencing pain on nearly all days [10]. Patients also suffer from unpredictable, devastating acute pain episodes due to vaso-occlusive crises (VOC) believed to be the result of sickling RBCs and vascular occlusion and often require hospitalization. Interestingly, mortality rates were increased in patients who reported more than three crises per year [11]. Many SCD patients also experience chronic pain [12] resulting from tissue damage and persistent inflammation. When describing their pain, patients used a variety of descriptors of nociceptive and neuropathic pain [13–16]. Pain intensity varies considerably among patients, with one study in adolescents showing that while mean visual analog scale (VAS) ratings at the time of the study were low (1.15 ± 3.00 cm), scores ranged from 0 (no pain) to 10 [17]. Patients also reported a high mean VAS score for the worst level of pain that was managed at home (8.79 ± 1.99 cm) and in hospital (7.70 ± 3.27 cm). In adult patients hospitalized for acute sickle crisis, pain intensity was high at admission (mean VAS: 9.5 ± 0.63 cm) and remained elevated at the time of discharge (mean VAS: 4.8 ± 0.97 cm) [18]. An outpatient study in which adult patients were asked to rate their pain during acute crises on a scale of 0 (no pain) to 10 (pain as intense as it can be) also found high pain intensity during these periods (7.5 ± 2.7) [19]. A study comparing the pain experiences of children and young adults with SCD using the Adolescent Pediatric Pain Tool reported similar pain intensity levels among those being treated in the clinical setting (5.92 ± 2.62), during short-term hospitalization (6.99 ± 1.56), and during long-term hospitalization (6.88 ± 2.05) [15]. Studies using quantitative sensory testing revealed that patients with SCD had ongoing pain and altered cutaneous sensitivity to mechanical, heat and cold stimuli [20–23]. Thus, pain in SCD is complex and includes ischemic, inflammatory, and neuropathic components [24], making it difficult to manage. Opioids, particularly morphine, are the primary treatment for managing pain in SCD [25,26], often require high doses [27–30], and are problematic due to serious side effects..

Transgenic mice expressing exclusively human sickle hemoglobin have been used to study the pathophysiology of SCD and have many features that are consistent with humans with SCD. For example, homozygous HbSS-BERK sickle mice [31] exhibit major hematologic changes, inflammation, and organ damage as seen in patients [31–33]. Behavioral studies showed that mice with SCD have cutaneous and deep tissue hyperalgesia [32,34–38], consistent with psychophysical studies in patients [20–23]. Also consistent with pain in SCD patients [39], hyperalgesia in sickle mice increased with age and following hypoxia/reoxygenation [34,40]. Interestingly, the mouse models that exhibited the greatest magnitude of hyperalgesia were those that had human sickle hemoglobin [34]. Thus, humanized transgenic mouse models are valuable tools to investigate mechanisms underlying pain in SCD and to develop novel therapeutic approaches.

2. Peripheral mechanisms of pain in SCD

A variety of morphological and biochemical changes have been shown in the skin of sickle mice that contributes to hyperalgesia in sickle mice. For example, the epidermis and dermis of skin isolated from sickle mice were narrow and had decreased neural innervation, indicating peripheral neuropathy [32]. This is consistent with results from quantitative sensory testing in patients, the descriptors used by SCD patients to describe their pain, and

neuropathic pain questionnaires that suggested approximately 30% of patients have neuropathic pain [13,14]. The skin of mice expressing sickle hemoglobin showed enhanced immunoreactivity to substance P (SP) and calcitonin gene-related peptide (CGRP) [32], two neuropeptides known to play a role in the transmission of pain. In addition, release of these neuropeptides produces vasodilatation and plasma extravasation [41], termed neurogenic inflammation [42], and degranulation of mast cells, both of which have been described in sickle mice [43]. Mast cells release a variety of inflammatory mediators [44], including proteases such as tryptase, and pro-inflammatory mediators such as prostaglandins, cytokines, interleukins, and nerve growth factor that can sensitize primary afferent nociceptors [45–47] and were found to be elevated in the plasma of SCD patients [48–50]. Pharmacological inhibition of mast cells in sickle mice decreased inflammatory mediators and hyperalgesia [43]. Consistent with these studies, treatment targeting mast cell activation in SCD patients led to significant improvement, suggesting a role for mast cell activation in the severity of pain in human sickle cell phenotypes [51].

Substances released from endothelial cells also contribute to the inflammatory state of SCD [52,53]. In SCD, hemolysis increases levels of circulating heme, which activates endothelial cells through toll-like receptor 4 (TLR4) and leads to vaso-occlusion [54]. Activated endothelial cells release pro-inflammatory proteins, such as endothelin-1 (ET-1) and ET-3. Cultured monocytes treated with ET-1 released pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, TNF- α , and granulocyte-macrophage colony-stimulating factor (GM-CSF) [55,56]. Plasma levels of endothelin-1 (ET-1) are increased in patients with SCD and further increased during acute sickle crises [57,58]. Levels of ET-3, another vasoactive factor which induces the release of the pro-inflammatory cytokine interleukin-6 (IL-6), were also increased in the plasma of patients with SCD [59]. Administration of ET-1 evoked nocifensive behaviors [60,61] and sensitized C-fiber nociceptors [62,63]. It is unknown whether endothelin receptor antagonism decreases pain in SCD; however, endothelial vasoactive factors may be a target as part of a multifaceted approach to the treatment of pain in SCD.

Electrophysiological studies have compared response properties of nociceptors between sickle HbSS-BERK mice and control HbAA-BERK mice. Recordings from single primary afferent nerve fibers in sickle mice revealed that a large proportion of both A δ - and C-fiber nociceptors exhibited spontaneous activity and sensitization to mechanical, heat and cold stimuli applied to the skin as compared to control mice [36, 64]. Nociceptor sensitization was characterized by lower evoked response thresholds and/or increased responses to suprathreshold stimuli (Figure 1). Spontaneous activity likely contributes to ongoing pain and elevated responses to mechanical, heat, and cold stimuli may contribute to mechanical and thermal hyperalgesia that are observed in sickle mice [32,34–38]. Little is known about the precise mechanisms by which nociceptors become sensitized in SCD, however, it has been shown that TRPV1 channels contribute to sensitization to mechanical stimuli and mechanical hyperalgesia [36].

Interestingly, increased responses to mechanical stimuli were also observed in non-nociceptive, rapidly adapting A β mechanoreceptors and A δ D-hair afferent fibers in sickle mice and might contribute to mechanical allodynia observed in these mice [65]. Although

activation of low threshold mechanoreceptors normally does not evoke pain, enhanced activity in these afferent fibers might provide additional input to sensitized nociceptive dorsal horn neurons to produce tactile allodynia. Collectively, these studies show that ongoing pain and hyperalgesia in SCD involves the sensitization of multiple types of primary afferent fibers. Importantly, ongoing activity in nociceptors might drive central sensitization in sickle mice.

3. Central sensitization contributes to pain in SCD

In early human psychophysical studies of cutaneous secondary hyperalgesia, the spread of hyperalgesia from a site of injury into non-injured skin, Hardy and colleagues [66] first proposed that changes in the central nervous system contributed to this enlarged area of hypersensitivity. In subsequent psychophysical studies of mechanical hyperalgesia produced by intradermal injection of capsaicin in humans, LaMotte and colleagues [67,68] demonstrated a role for the central nervous system. These studies included the finding that intraneural electrical stimulation of A β low threshold mechanoreceptive primary afferent fibers, which produced only tactile sensations under normal conditions, produced pain after capsaicin. However, direct evidence that the central nervous system plays a role in the development of hypersensitivity after tissue injury was first provided by Woolf in 1983 [69] who found that increased excitability of the flexor reflex following tissue injury resulted from changes in the spinal cord. Central sensitization refers to increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input [70]. It includes synaptic plasticity and increased synaptic efficacy in nociceptive neurons in the dorsal horn of the spinal cord following noxious stimulation [71,72] resulting in elevated spontaneous activity, enlarged receptive fields, lowered response thresholds, and increased responses to suprathreshold stimuli. Studies of secondary hyperalgesia produced by intradermal injection of capsaicin demonstrated that sensitization of identified spinothalamic tract neurons in monkeys correlated with the magnitude and spatial extent of mechanical and heat hyperalgesia in humans [73], demonstrating the relation between sensitization of spinal cord nociceptive neurons and secondary hyperalgesia. It is now accepted that central sensitization contributes to many inflammatory and neuropathic pain syndromes [72,74].

It has been proposed that certain characteristics of pain are likely to be associated with central sensitization [74]. These include enhanced temporal summation of pain, aftersensations following removal of a stimulus, secondary hyperalgesia when there is a localized injury, and the presence of tactile allodynia mediated by activity in low threshold A β nerve fibers. Using some of these criteria, it was reported that approximately 60% of patients with SCD had evidence of central sensitization [14], and central sensitization in adult patients was associated with low levels of fetal hemoglobin [75]. Interestingly, enhanced temporal summation of pain (increase in pain with repeated stimulation) and lower pain tolerance were associated with more intense and frequent episodes of pain in non-SCD patients [76–78]. In this regard, Campbell and colleagues [79] divided patients with SCD into subgroups with high central sensitization or low/no central sensitization subgroups based on results from quantitative sensory testing of aftersensations and temporal summation produced by repeated heat stimuli. It was found that patients in the high central

sensitization group reported more pain at the time of testing and at follow-up visits in the absence of crisis, a greater percentage of crisis days, and worse sleep. The fact that patients with a high likelihood of central sensitization had disturbances in sleep is particularly interesting because there is a relationship between sleep deprivation and pain [80,81].

3.1. Central sensitization in sickle mice

Only one study has examined central sensitization directly through electrophysiological recording from nociceptive dorsal horn neurons in anesthetized sickle HbSS-BERK and control HbAA-BERK mice [82]. Nociceptive dorsal horn neurons were recorded from the lumbar enlargement and were characterized functionally as wide dynamic range (WDR) and high threshold (HT) according to their responses evoked by innocuous and noxious mechanical stimuli applied to their receptive fields [83]. Several differences in response properties were found between sickle and control mice, and between WDR and HT neurons in sickle mice. First, receptive field areas identified by mechanical stimulation for both WDR and HT neurons were approximately 2-fold greater in sickle mice as compared to control mice. Second, mechanical response thresholds of WDR neurons, determined using calibrated von Frey monofilaments, were lower in sickle mice whereas there were no differences in thresholds of HT neurons. Third, responses to suprathreshold mechanical stimuli (von Frey monofilaments) increased dramatically in both WDR and HT neurons (Figure 2). These changes might be related to mechanical hyperalgesia in SCD mice and might contribute to hyperalgesia in SCD patients. Fourth, in addition to greater responses evoked by mechanical stimuli, approximately half of the WDR and HT neurons in sickle mice exhibited prolonged afterdischarges following removal of the von Frey monofilament. In control mice, afterdischarges might last for a few seconds if they occur. Although the durations of afterdischarge were variable in sickle mice, at times they persisted for over 1 minute. These afterdischarges might be related to aftersensations reported in patients with SCD that suggest the presence of central sensitization. Our findings show that nociceptive spinal dorsal horn neurons are sensitized in sickle mice and are consistent with psychophysical measures of central sensitization in patients with SCD.

The mechanisms that contribute to sensitization of nociceptive dorsal horn neurons in HbSS-BERK mice are largely unknown. However, it was reported that phosphorylation of the MAPK family of kinases, including extracellular signal-regulated kinase (ERK) and p38, was increased in the spinal cord of HbSS-BERK mice as compared to HbAA-BERK mice [82]. MAPKs can be activated in primary sensory neurons, nociceptive dorsal horn neurons, astrocytes, and microglia following noxious stimulation and play a major role in neuronal sensitization [84–86]. For example, activation of ERK produces hyperexcitability of dorsal horn neurons [87] in part by phosphorylation of the NR1 subunit of N-methyl-D-aspartate (NMDA) receptors, which are essential for the induction of central sensitization [88]. Nociceptive inputs from the periphery rapidly activate ERK in spinal dorsal horn neurons [89]. ERK also contributes to transcriptional regulation, particularly via phosphorylation of cAMP-response element binding protein (CREB), which can maintain long-term neuronal plasticity by inducing gene transcription (including *c-fos*, BDNF, and dynorphin) and the formation of new synapses [90,91]. For neuronal populations expressing *c-fos*, ERK-dependent *c-fos* transcription may be assessed as an indirect marker of neuronal activation

that can be induced by noxious input [92–94]. Transcription of *c-fos* is at best an incomplete marker of neuronal activity, as it does not occur in all neurons following noxious input, and it should be noted that some neurons express *c-fos* even in the absence of noxious stimuli [93]. Activation of p38 in the spinal cord increases expression of enzymes such as COX-2 [95] and iNOS [96] associated with synthesis of proinflammatory mediators which contribute to central sensitization. In addition to COX-2 and iNOS, increases in TLR4 and IL-6 in the spinal cord of HbSS sickle mice have been reported and, in addition to pro-inflammatory mediators released from activated microglia and astrocytes, can contribute to central sensitization through glial-neuronal interactions [97–99]. The activation of MAPKs in spinal glia is essential in the development of neuropathic pain [100,101]. Peripheral nerve and spinal cord injuries lead to increased activation of p38 and ERK in spinal microglia [100, 102]. Activation of ERK occurs in microglia within days of nerve injury, then shifts to astrocytes in the following weeks [103]. Nerve injury also activates c-Jun N-terminal kinase (JNK) in astrocytes [104].

It has also been found that Ca^{2+} /calmodulin-dependent protein kinase IIa (CaMKIIa), an important component of intracellular Ca^{2+} signaling pathways that also contributes to central sensitization [105], was activated in the spinal cord (and DRG) of sickle mice and inhibition of spinal CaMKIIa attenuated hyperalgesia in sickle mice [106].

In addition to increased primary nociceptive afferent activity in initiating and maintaining central sensitization, a decrease in inhibitory transmission in the spinal cord might also contribute to central sensitization in sickle mice. Decreasing inhibitory transmission in the spinal cord produced hyperalgesia and increased excitability of nociceptive dorsal horn neurons, and could be involved in chronic pain conditions [107–108]. It is not known whether inhibitory transmission is altered in the spinal cord of sickle mice.

Changes in activity of descending pathways from the brain stem that modulate nociceptive transmission in the spinal cord might also contribute to hyperalgesia and central sensitization in sickle mice. Although early studies focused on descending inhibition of pain, parallel descending pathways facilitate nociceptive transmission and contribute to the development of chronic pain and hyperalgesia [109,110]. Studies are needed to determine whether changes in descending modulation of nociceptive transmission, which may include enhanced facilitation or decreased inhibition of nociceptive transmission, contribute to central sensitization in sickle mice.

4. Conclusions

Mouse models of SCD are providing new information on the complex mechanisms underlying chronic pain in this condition. It appears that peripheral and central sensitization plays a major role, but the underlying mechanisms remain largely unknown. An important question is whether targeting those mechanisms that contribute to the development and maintenance of sensitization will be beneficial for managing pain in SCD. Activation of NMDA receptors in the spinal cord is an essential step in initiating and maintaining central sensitization [111] and it was reported that subanesthetic doses of ketamine, a non-competitive NMDA receptor antagonist, given as an adjunct to opioids for vaso-occlusive

episodes, reduced pain and opioid consumption [112]. Targeting mediators of neuroinflammation, such as prostaglandins, or administration of cytokine inhibitors or endogenous mediators that play a role in the resolution of inflammation, such as Resolvins which have been shown to reduce inflammation and organ damage in sickle mice [113], may be effective in managing pain in SCD.

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Highlights

- Pain in sickle cell disease (SCD) can be severe and difficult to treat.
- Sensitization of both nociceptors and dorsal horn neurons contribute to pain in SCD.
- Targeting the processes the underlie sensitization in SCD may identify novel approaches for effective pain management in patients with SCD.

Figure 1.

A: Examples of C-fiber nociceptors recorded from anesthetized HbAA-BERK control (top row) and HbSS-BERK (bottom row) mice. Left column: overlapping traces of conduction latency. Left arrow indicates onset of the stimulus and the right arrow indicates the evoked action potential. Middle column: examples of spontaneous activity for a period of 45 s. Right column: example of a response evoked by 147 mN applied for 5 s. Time of stimulation is illustrated below the evoked response. **B:** Mean spontaneous discharge rates and responses to 147 mN were higher in C-fibers isolated from HbSS-BERK sickle mice in comparison to HbAA-BERK control mice. *** $p < .001$ vs HbAA-BERK mice. From Uhelski et al., 2017 *Pain* 158(9):1711-1722.

Figure 2.

Receptive field (RF) areas and responses of nociceptive dorsal horn neurons evoked by mechanical stimuli are greater in sickle mice. Upper panels: Location of the RF, and peristimulus time histograms showing discharge rates evoked by stimuli used for functional characterization (brush, pressure pinch applied to the RF) and responses evoked by von Frey monofilaments of controlled force for a single WDR neuron from a control (top panels A, B, and C) and from a sickle (lower panels D, E, and F) mouse. Solid horizontal lines represent the time of application of the stimuli (5 s). Discriminated output pulses from a window

discriminator are provided below the histograms. Lower panels: Same format as above but RF areas and evoked responses are shown for single HT neurons from a control (A-C) and sickle (D-F) mouse. Bin width = 100 ms. From Cataldo et al., 2015 *Pain* 156:722-730, 2015.

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