Burning pain: axonal dysfunction in erythromelalgia

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Abstract
Erythromelalgia (EM) is a rare neurovascular disorder characterized by intermittent severe burning pain, erythema, and warmth in the extremities on heat stimuli. To investigate the underlying pathophysiology, peripheral axonal excitability studies were performed and changes with heating and therapy explored. Multiple excitability indices (stimulus–response curve, strength–duration time constant (SDTC), threshold electrotonus, and recovery cycle) were investigated in 23 (9 EMSCN9A+ and 14 EMSCN9A−) genetically characterized patients with EM stimulating median motor and sensory axons at the wrist. At rest, patients with EM showed a higher threshold and rheobase ($P < 0.001$) compared with controls. Threshold electrotonus and current–voltage relationships demonstrated greater changes of thresholds in both depolarizing and hyperpolarizing preconditioning electrotonus in both EM cohorts compared with controls in sensory axons ($P < 0.05$). When average temperature was raised from 31.5˚C to 36.3˚C in EMSCN9A+ patients, excitability changes showed depolarization, specifically SDTC significantly increased, in contrast to the effects of temperature previously established in healthy subjects ($P < 0.05$). With treatment, 4 EMSCN9A+ patients (4/9) reported improvement with mexiletine, associated with reduction in SDTC in motor and sensory axons. This is the first study of primary EM using threshold tracking techniques to demonstrate alterations in peripheral axonal membrane function. Taken together, these changes may be attributed to systemic neurovascular abnormalities in EM, with chronic postischaemic resting membrane potential hyperpolarization due to Na+/K+ pump overactivity. With heating, a trigger of acute symptoms, axonal depolarization developed, corresponding to acute axonal ischaemia. This study has provided novel insights into EM pathophysiology.

Keywords: Erythromelalgia, Pain, SCN9A, Sodium channel, Excitability, Mexiletine

1. Introduction
Erythromelalgia (EM) is a rare neurovascular disorder characterized by recurrent severe pain associated with erythema, swelling, and warmth in the feet or hands. Symptoms are induced by warmth or exercise and eased by cooling. Mutations in the SCN9A gene, encoding the α-subunit of Na+, result in a gain of function and are responsible for primary EM.

Functional characterization of mutant Na+, channels using patch clamp techniques has established hyperpolarizing shifts in activation curve, increases in ramp current, slowed deactivation, resurgent currents during repolarization, and increased firing frequency with stimulation. Na+, channels are predominantly expressed on dorsal root ganglia and sympathetic ganglion (DRG) neurons hyperexcitable. By contrast, sympathetic ganglion depolarization produces resting inactivation of all the voltage gated sodium channel (VGSC) isoforms and hypoexcitability. These findings provide a molecular basis for clinical manifestations in EM. The hyperexcitability of DRG results in neuropathic pain while dysfunction of the sympathetic tone disturbs vasomotor regulation and skin perfusion.

In terms of understanding the disturbed sympathetic control of vascular tone in EM, previous clinical studies have established the coexistence of marked increases in skin blood flow associated with decreased oxygenation during attacks presenting with sudden swelling, redness, severe pain, and increased temperature. It has been postulated that microvascular arteriovenous shunting may induce a maldistribution of blood flow, resulting in hypoxia, ischaemia, and pain, similar to diabetic neuropathy. The warmer temperature may be expected to increase cellular metabolism and modulate VGSC properties, thereby establishing a vicious cycle to sustain attacks. Despite these clinical and pathophysiological insights, there has not been an effective therapeutic armamentarium for EM, with up to 84 medications prescribed, including sodium channel and sympathetic nerve blockers. Patients may go to extreme measures to alleviate their symptoms, such as avoiding footwear, using fans, and immersing affected areas with cold water and ice, highlighting the profound impact of temperature on clinical manifestations.

Of relevance to gaining insight into EM pathophysiology, protocols for testing of axonal membrane function have recently been developed for clinical assessment. These techniques...
have been applied to various genetic channelopathies and have demonstrated that nerve impulse conduction along the peripheral axons may be altered, even when the principal channel is not expressed at the site of assessment. The purpose of this study was to evaluate whether EM has changes in axonal excitability and the influence of temperature and mexiletine on axonal excitability.

2. Methods

Clinical manifestations were combined with conventional and specialized neurophysiological assessments in patients with primary EM. The coding region of the human SCN9A gene was sequenced in 23 patients. Authentication of a pathological mutation was performed by screening 200 controls and family segregation analysis. Patients with EM were divided into 2 subgroups according to the presence or absence of a pathogenic mutation in SCN9A: SCN9A-mutated EM (EMSCN9A+) or EM not related to the mutation of SCN9A (EMSCN9A−), respectively. In the latter group secondary causes of EM resulting from an underlying organic disease were excluded, including Fabry disease, diabetes mellitus, medications, toxins, alcoholism, HIV infection, and Lyme disease. All the patients provided informed consent or parental consent to the procedures, which were approved by the South Eastern Sydney and Illawarra Area Health Service Human Research Ethics Committee or the National Taiwan University Hospital Ethics Committee.

Axonal excitability studies used the threshold tracking technique, which measures changes in the strength of a test stimulus needed to produce a target potential. This is set to 40% of the maximum (located in the steepest slope of the stimulus response curve). Different conditioning-test paradigms, including altering the stimulus duration and polarization, were applied to produce changes in excitability and provide indirect information about axonal ion channel function, resting membrane potential, and passive membrane properties. In vitro experiments and modelling studies have provided evidence for the relationship of specific ion conductances with cohesive changes in multiple excitability parameters.

Axonal excitability studies were undertaken stimulating the median nerve at the wrist and recording the compound muscle action potentials (CMAPs) from the abductor pollicis brevis muscle or sensory nerve action potentials (SNAPs) from index finger using the rapid automated threshold tracking protocol known as “TROND” (named after a 3-day training symposium held in 1999 at Trondheim, Norway) within QTRAC software (Institute of Neurology, University College London). Skin temperature was measured at the site of stimulation and varied by less than 1°C during the recordings. A number of assessments were incorporated in excitability testing that measured the nodal and internodal properties for analysis.

Initially, a stimulus–response curve was obtained. This curve was used to determine the threshold for a CMAP or SNAP around 40% of maximum; this was the target response and threshold was the stimulus required to obtain this potential. The strength–duration time constant (SDTC) of the nerve was then determined by adjusting the intensity of stimuli of different durations so that the target response was maintained. To measure threshold electrotonus (TE), prolonged 100-millisecond subthreshold currents were delivered and their strength was set to be +40% (depolarizing, TED) and −40% (hyperpolarizing, TEH) of the control threshold current. Test pulses were delivered at varying intervals during and after the conditioning currents, and the change in threshold required to produce the target 40% response was recorded. Subsequently the current–threshold relationship (I/V) was measured, reflecting the rectifying properties of the axons. The I/V relationship was assessed by measuring the change in threshold at the end of polarizing currents of 200-millisecond duration, the strength of which was altered in 10% steps from +50% (depolarizing) to −100% (hyperpolarizing) of the control threshold. Finally, the recovery cycle (RC) was tested, which determines the change in threshold following a supramaximal stimulus at 18 conditioning-test intervals from 2 to 200 milliseconds. The RC consists of a relative refractory period at short interstimulus intervals, a superexcitable period, when a smaller stimulus is necessary to produce the test response, and a late subexcitable period, during which a greater stimulus than control is required.

Because warmth can trigger and exacerbate symptoms in otherwise asymptomatic areas, the influence of temperature on axonal excitability was examined in 6 SCN9A+ mutated patients with EM. The arm was covered with warm towels and heated gradually up to where the subject can tolerate (average skin surface temperature 5°C higher than at rest).

Measurements of excitability indices have previously been used for noninvasive assessment and monitoring of the effects of mexiletine, which nonselectively partially blocks VGSCs. These studies included patients with neuropathic pain or muscle cramp or fasciculation and demonstrated with mexiletine therapy suppression of persistent Na⁺ currents in human motor axons associated with improvements in clinical phenotype. To identify an effective treatment, we assessed the alterations in axonal excitability before and 3 months after mexiletine at a dose of 300 mg daily, a selective sodium channel blocker in 4 SCN9A+ mutated patients with EM.

2.1. Data analysis

Peripheral nerve excitability parameters in EMSCN9A+ patients were compared to EMSCN9A− patients and 28 age- and temperature-matched normal controls (17 men; mean age 33.1 ± 1.6 years, range 7–67 years; temperature 33.2 ± 0.2°C). Excitability parameters were tested for normality of distribution before performing statistical tests. The one-way analysis of variance was used to compare mean differences between groups for excitability indices that showed the Gaussian distribution. The Kruskal–Wallis (nonparametric one-way analysis of variance) was used for parameters that did not show the normative distribution. All results were expressed as mean ± SEM. A P value of <0.05 was considered statistically significant. The paired t test was used to compare patients’ parameters before and after heating and treatment for statistical analysis.

2.2. Modelling

To assess the possible underlying physiological mechanisms of the nerve excitability changes in patients with EM, a model of the human sensory axon was used to undertake mathematical simulations using MEMFIT software within QtracP. Initially, the model was adjusted to closely match the normal sensory control group data. Subsequently, the mechanisms underlying the differences between the excitability of EM and normal control were explored. MEMFIT software uses an iterative approach to minimize the difference between the simulated and the target group (EM) data. The difference between the simulated and group data was calculated using the sum of the squares at each data point with calculations made during
the various excitability components (TE, RC, current–threshold relationship, and SDTC) given equal weighting.

3. Results

3.1. Clinical features

The clinical and genetic features for 23 patients with EM are summarized in Table 1. The study population included 9 EMSCN9A+ patients (mean age 23.8 ± 4.5, range 6-54 years, 5 men) and 14 EMSCN9A− patients (mean age 53.6 ± 4.6 years, range 18-77 years, 7 men). By definition, all patients with EM experienced intermittent bouts of heat, erythema, and severe burning pain, with symptoms located in the extremities of 22 patients and isolated to the face in 1 patient. Physical exertion, hot weather, tight shoes, and stress were frequent exacerbating factors. Patients preferred not to wear socks or closed shoes, even in winter. Physical examination between episodes frequently demonstrated erythematous feet, cool limbs with a reticular cutaneous pattern, and excoriated skin. To relieve pain, patients frequently immersed their hands and feet in ice buckets with cold water. Some medications had been prescribed with variable effect, including aspirin, mexiletine, carbamazepine, clonidine, propranolol, gabapentin, and imipramine. Conventional neurophysiological assessments demonstrated no significant changes in distal latencies, conduction velocities, and amplitudes for CMAP and SNAP (median CMAP 11.0 ± 1.1 mV, distal motor latency 3.6 ± 0.1 ms, motor conduction velocity 56.7 ± 1.1 m/s, SNAP 32.6 ± 6.4 μV, and sensory conduction velocity 59.5 ± 1.7 m/s). The patients did not demonstrate any abnormal quantitative skin response such as temperature or vibration sensations.

To provide an understanding of the therapeutic approach and challenges in EM, the case of patient 1 is described in detail. Symptoms of severe EM began at 2 years of age. Sequencing of the SCN9A gene identified a heterozygous de novo missense variant c.2623C>G which results in a p.Q875E mutation. The biophysical properties of this mutation predict an early-onset severe phenotype, with a large hyperpolarizing shift in voltage dependence of activation of Na\textsubscript{1.7}, slowed deactivation, and shortened time to peak current. She has severe, persistent pain and itch in her feet, exacerbated by heat and relieved by cooling with ice packs. She has badly excoriated feet (Fig. 1) and markedly disturbed sleep, as do her parents. Multiple treatments were used including Na\textsuperscript{+} channel blockers, modulators of vascular tone, anaesthetic agents, antihistamines, sedatives, and physical therapy. The effects were evaluated by Faces pain score, parental report, and observations of skin.

| Patient number | Current age, y | Age at symptom onset, y | Other clinical conditions | Current medications | SCN9A testing |
|----------------|---------------|-------------------------|--------------------------|---------------------|--------------|
| 1              | 6             | 2                       | None                     | Mexiletine          | Q875E        |
| 2              | 14            | 8                       | None                     | None                | I136V        |
| 3              | 22            | 10                      | None                     | Mexiletine          | I136V        |
| 4              | 23            | 12                      | Peptic ulcer disease     | None                | I136V        |
| 5              | 54            | 14                      | None                     | None                | I136V        |
| 6              | 58            | 16                      | None                     | None                | I136V        |
| 7              | 24            | 13                      | None                     | None                | L245V        |
| 8              | 30            | 18                      | Pituitary adenoma        | Mexiletine, clonazepam, carbamazepine | I848T |
| 9              | 18            | 11                      | None                     | Mexiletine, misoprostol, clonazepam, carbamazepine | V1316A |
| 10             | 18            | 10                      | None                     | None                | Nil          |
| 11             | 22            | 30                      | None                     | None                | Nil          |
| 12             | 28            | 11                      | None                     | None                | Nil          |
| 13             | 35            | 10                      | Tension headache         | None                | Nil          |
| 14             | 54            | 54                      | Epilepsy                 | None                | Nil          |
| 15             | 58            | 52                      | None                     | Imipramine, carbamazepine | Nil          |
| 16             | 60            | 53                      | Sjögren syndrome         | None                | Nil          |
| 17             | 62            | 42                      | Lumbar spondylosis       | None                | Nil          |
| 18             | 62            | 58                      | Hypertension, stroke, asthma | Imipramine          | Nil          |
| 19             | 63            | 54                      | None                     | None                | Nil          |
| 20             | 64            | 62                      | Diabetes mellitus        | None                | Nil          |
| 21             | 65            | 56                      | Chronic obstructive pulmonary disease, colon cancer, basal cell carcinoma, hepatitis B carrier, depression | None                | Nil          |
| 22             | 67            | 66                      | Sjögren syndrome         | None                | Nil          |
| 23             | 76            | 72                      | None                     | None                | Nil          |
Carbamazepine, gabapentin, pregabalin, amitriptyline, clonidine, various antihistamines, and low-dose aspirin provided no benefit. Mexiletine and phenoxymethylamine were partially helpful before treatment. Faces pain rating scale [visual analogue scale] is 10; on mexiletine, is 8; with both mexiletine and phenoxymethylamine, is 7. At severe attacks, ketamine infusion was partly beneficial. Because medical modulation of vascular tone had a positive effect, a transient percutaneous lumbar sympathectomy was performed with some utility. Subsequently, radioablation of lumbar sympathectomy was undertaken; however, pain persistently worsened. Subsequently, topical 0.2% midodrine ointment transiently ameliorated the symptoms following sympathectomy. Topical and environmental cooling with air-conditioning to modify chronic pain has partly alleviated the symptoms.

3.2. Excitability differences between SCN9A mutations, without SCN9A mutations, and healthy controls

The threshold currents required to elicit a 50% response were greater in both EM patient cohorts compared with controls (motor: EMSCN9A+, 6.9 ± 1.2 mA; EMSCN9A−, 5.5 ± 1.1 mA; controls, 4.0 ± 1.1 mA, F = 20.5, P < 0.001. Sensory: EMSCN9A+, 4.9 ± 1.5 mA; EMSCN9A−, 10.2 ± 1.2 mA; controls, 3.0 ± 1.1 mA, F = 26.8, P < 0.001, Fig. 2A, B and Table 2). In agreement with this, rheobase measurements were significantly greater in patients with EM than in controls (motor: EMSCN9A+, 4.3 ± 1.3 mA; EMSCN9A−, 6.4 ± 1.2 mA; controls, 2.7 ± 1.1 mA, F = 17.9, P < 0.001. Sensory: EMSCN9A+, 2.0 ± 1.5 mA; EMSCN9A−, 4.8 ± 1.2 mA; controls, 1.3 ± 1.1 mA, F = 24.9, P < 0.001, Table 2).

There were significant alterations in the strength–duration properties in patients with EM compared with healthy controls in both motor and sensory axons, which provide an indirect measurement of nodal persistent Na+ conductances. Strength–duration time constant was significantly prolonged in EMSCN9A+ patients compared with EMSCN9A− patients and healthy controls (motor: SCN9A+, 0.53 ± 0.04 ms; SCN9A−, 0.42 ± 0.02 ms; controls, 0.43 ± 0.02 ms, F = 4.7, P = 0.01; sensory: SCN9A+, 0.63 ± 0.05 ms; SCN9A−, 0.46 ± 0.02 ms; controls, 0.58 ± 0.02 ms, F = 3.6, P < 0.05, Fig. 2C, D).

Threshold electrotonus demonstrated greater changes in response to depolarizing and hyperpolarizing currents in both EM cohorts compared with controls in sensory axons. This was accompanied by similar changes in the current–voltage relationship. Threshold electrotonus and current voltage relationships were similar between patients with EM and controls in motor fibres (Fig. 2E, F).

The RC showed variable changes in subexcitability in EM SCN9A+ and SCN9A− patients compared with controls, predominantly in sensory fibres (motor: EMSCN9A+, 14.5 ± 1.6%; EMSCN9A−, 19.3 ± 2.4%; controls, 14.1 ± 0.9%, F = 3.7, P < 0.05. Sensory: EMSCN9A+, 6.8 ± 1.4%; EMSCN9A−, 18.6 ± 4.2%; controls, 11.4 ± 0.6%, F = 7.3, P < 0.05, Fig. 2G, H).

3.3. Nerve excitability changes with temperature

The pathophysiological mechanisms leading to the clinical attacks with increasing temperature in EM raise the importance of investigating their effects on axonal dysfunction. As such, the changes in nerve excitability parameters with different temperature were investigated in 6 EMSCN9A+ patients. Figure 3 demonstrates the changes in a SCN9A+ patient with heating where both motor and sensory excitability were recorded simultaneously to document the gradual changes as the temperature increases. Similar to the effects of temperature previously established in healthy subjects over the range of 29˚C to 35˚C, heating reduced the relative...
refractory period ($P < 0.05$) and quickened the accommodation to depolarising currents ($P = 0.01$) in both motor and sensory fibres. By contrast, SDTC in EMSCN9A+ patients increased with increased temperature (motor: preheating, $0.49 \pm 0.07$ ms vs postheating, $0.54 \pm 0.07$ ms, $P < 0.05$; preheating temperature, $31.5 \pm 1.2^\circ$C, postheating temperature, $36.3 \pm 0.5^\circ$C, $P < 0.005$. Sensory: preheating, $0.62 \pm 0.05$ ms, postheating, $0.75 \pm 0.04$ ms, $P < 0.05$; preheating temperature, $29.7 \pm 1.3^\circ$C, postheating temperature, $35.2 \pm 2.1^\circ$C, $P < 0.05$; Fig. 4A, B).

Figure 2. Comparison of multiple measurements of axonal excitability in motor (top panel) and sensory axons (bottom panel) in patients with erythromelalgia (EMSCN9A+, open; EMSCN9A−, hatched) and normal controls (filled) plotted as mean and SEM of motor and sensory fibres. (A, B) Stimulus for 50% maximum response. (C, D) Strength-duration time constant. (E, F) TE90 (90-100 ms). (G, H) Subexcitability.
3.4. Nerve excitability changes with mexiletine

Among our SCN9A+ patient cohorts, only 4 patients reported clinical improvement with mexiletine, accompanied by a 1- or 2-point reduction in Faces pain rating scale. These patients were studied using nerve excitability tests before or after medication, and the results are shown in Figures 4C and D: there is a reduction in SDTC in both motor and sensory axons while other excitability measures were not altered (motor SDTC: pre-mexiletine, 0.46 ± 0.03 ms vs on mexiletine, 0.42 ± 0.04 ms; sensory SDTC: pre-mexiletine, 0.61 ± 0.00 ms vs on mexiletine, 0.51 ± 0.08 ms). These results are in agreement with previous reports on the effects of mexiletine on excitability parameters.15,40,41,50,73

3.5. Mathematical modeling of abnormal excitability properties

A mathematical model of the human sensory axon was used to assist in the interpretation of the changes measured in clinical nerve excitability in patients with EM. Membrane hyperpolarization best explained the differences between the EM SCN9A+ and EMSCN9A− patients and the sensory controls. Specifically, hyperpolarization of the resting membrane potential by 2.8 mV accounted for 89.4% of the discrepancy between the EMSCN9A+ and sensory control data. Similarly, a 1.6-mV hyperpolarization of resting membrane potential accounted for 83.6% of the discrepancy between the EMSCN9A− patients and sensory controls. Internodal shortening was also included in the modelling and reduced the discrepancy by 25%. It was not considered the most plausible explanation for the findings. The model did not show any further improvement with additional changes in Na⁺ channel expression or the fraction of Na⁺ channels operating in a persistent mode. The effect of temperature on nerve excitability changes in EM is shown in Figure 5. The 5°C warming in 1 subject was modelled well by a 2-mV depolarization of resting membrane potential from the EM model.

4. Discussion

This is the first study of primary EM using threshold tracking techniques to evaluate peripheral motor and sensory axonal function and the excitability changes related to temperature and mexiletine treatment. The techniques applied in this study allowed noninvasive in vivo assessment of the biophysical properties of axonal membranes, although Na⁺,1.7 is not known to be expressed on large alpha-myelinated motor fibres and may only be expressed in some of these sensory fibres.3,24 Accordingly, this approach differs from previous electrophysiological studies of EM, which are focused on the short-term effects on single cells by voltage and current clamp directly or microneurography at the site of abnormal Na⁺,1.7 expression.50 Notably, there were significant differences in sensory and motor excitability parameters between EM patients with and without SCN9A pathogenic variants compared with the healthy controls in this study. These findings reveal alterations in peripheral axonal membrane potential and conductances and reflect systemic abnormalities in EM. A paradoxical pattern of changes in Na⁺ channel-dependent parameters with heating was evident in SCN9A mutant patients in comparison to normal controls. There was variable and partial clinical benefit or change in neurophysiological parameters with pain medications, revealing challenges in therapy.

4.1. Peripheral axonal function in patients with erythromelalgia: alterations in membrane potential and conductances

Significant reductions in sensory axonal excitability were seen in both groups of EM patients, with a “fanning out” of responses
Figure 3. Nerve excitability changes in motor (top panel) and sensory axons (bottom panel) with increase temperature in EMSCN9A+ patient. (A, E) Stimulus response curve. (B, F) Strength–duration time constant. (C, G) Threshold electrotonus waveforms. (D, H) Recordings were made at wrist temperatures of 32°C (blue), 33.5°C (green), and 37.8°C (red) by heating the arm proximal to the wrist.
during TE, increases in threshold and rheobase, similar to the effects established with membrane hyperpolarization. A similar but lesser pattern of changes occurred in motor nerves of patients with EM. While these changes are subclinical, in part related to Nav1.7 not being expressed in these neurons, they are important in comprehensively understanding EM pathophysiology and systemic effects. In the same way, subclinical changes in axonal membrane function have been demonstrated with temperature changes or maturation paralleling alterations in conduction velocity and in many neuropathies. Associated with greater membrane hyperpolarization in EMSCN9A+ patients, there was also evidence of reduction in slow K currents, with decreased subexcitability, S2 accommodation, TE undershoot, and greater superexcitability. The mathematical modelling suggests that these changes are secondary to the changes in membrane potential, as approximately 35% of slow K channels are open at resting membrane potential. 

Na,1.7-mediated disturbances in vascular regulation may underlie the distinct electrophysiological changes observed in myelinated motor and cutaneous afferents in this study, leading to membrane hyperpolarization following nerve ischemia. This contrasts with previously established hyperexcitability and membrane depolarisation in smaller diameter nociceptive fibers directly associated mutant Na,1.7 activity. While the effects of Na,1.7 signaling are cell background dependent, intriguingly in each cell type activation of Na,1.7 promotes vasodilatation and ensuing abnormal arteriole–venule shunting with decreased oxygenation may establish a vicious cycle of pain, redness, swelling, and warmth or chronic mild ischaemia. Similar clinical vascular changes are observed in EMSCN9A+ patients, although the pathophysiological mechanisms are unclear, further supporting the secondary effects of EM on peripheral axons demonstrated in this study. Previous cellular models of nerve hypoxia and in vivo models of nerve ischaemia have demonstrated increases in persistent Na currents. The model was unable to resolve a small increase in persistent Na currents in this case, but this may be due in part to the fact that the measurements were made interictally. A small increase in resting persistent Na currents could lead to increased intracellular Na concentration, thereby stimulating the Na/K pump. Consequently, in the postischaemic state,
the Na\(^+\)/K\(^+\) pump is overactive to maintain electrochemical gradients, exporting 3 Na\(^+\) ions in exchange for 2 K\(^+\) ions, producing a chronic state of axonal hyperpolarization. Furthermore, axonal ischaemic vulnerability differs between types of nerve fibres, with sensory more susceptible than motor axons and greater vulnerability in large myelinated fibres compared with small unmyelinated fibres.\(^{31,35,37,55,62}\) This is consistent with the pattern of changes in this study. Separately, increased levels of intracellular Na\(^+\) have been observed in dorsal root ganglion neurons expressing Na\(_{v}1.7\) gain-of-function mutations.\(^{25}\) Interestingly, sodium channel blockers have provided an in vitro protective benefit against the adverse long-term effects of anoxia on axons.\(^{30,80}\)

Altered ion channel expression or gating properties may provide an alternative explanation for alterations in membrane potential.\(^{67}\) While this was not supported by changes in multiple excitability parameters or mathematical modelling, this may be complicated by interneuronal heterogeneity in the dynamics of Na\(^+\) conductances that reflect differences in the structure of the underlying channel molecule in the neuronal membrane.\(^{65}\) Alterations in passive cable properties (myelin thickness and internodal length) may also change membrane potential or ion channel conductances, for example, shorter internodes in regenerated nerve and an increased number of nodes provide a greater intra-axonal Na\(^+\) load to drive the Na\(^+\)/K\(^+\) pump.\(^{58}\) This concept is unlikely as latency and conduction velocities were maintained and mathematical modeling did not support this as the most plausible explanation.

We deliberately chose to test the median nerve at the wrist, remote from the ongoing clinical symptomatology, to avoid possible secondary changes associated with EM (eg, cutaneous thickening or swelling) that may have influenced nerve excitability testing and thereby ensuring changes were constitutional.

4.2. Heat hyperalgesia: pathophysiological mechanisms

The most striking result was the significant increases in SDTC in EMSCN9A\(^+\) patients, an indirect measure of the activity of nodal persistent Na\(^+\) current with heating,\(^9\) the opposite of controls.\(^{96}\) Similar to controls, increases of skin temperature reduced superexcitability and shortened accommodation to depolarizing current, related to activation of slow K\(^+\) channels during hyperthermia. Heating typically triggers acute EM symptoms, such that the excitability changes with increased temperature are likely to measure acute modulations in membrane potential and conductances. Mathematical modelling indicated that the acute excitability changes with heating in patients with EM correspond to axonal ischaemic depolarisation and supporting the concept of vascular dysregulation.\(^{42}\) This will produce an intra-axonal Na\(^+\) load to chronically drive the Na\(^+\)/K\(^+\) pump. Recent in vitro studies have demonstrated that heating selectively increases excitability in gain-of-function mutant Na\(_{v}1.7\) channels, in

![Mathematical model of axonal excitability changes with heating in patients with erythromelalgia.](image-url)

**Figure 5.** Mathematical model of axonal excitability changes with heating in patients with erythromelalgia. (A) Threshold electrotonus in a single subject during heating: 33.4\(^\circ\)C, red; 35.1\(^\circ\)C, green; 37, blue; 38.4\(^\circ\)C, grey. (B) Modelled depolarization of resting membrane potential in threshold electrotonus, with resting membrane potentials [RMPs]: −79.5 mV, red; −78.8 mV green, −78.2 mV, blue; −77.5 mV, grey. C. Recovery cycles during heating in the same subject (temperatures and colours as in A). D. Modeled depolarization of recovery cycle during heating (RMPs and colours are the same as in B).
comparison to wild-type Na\textsubscript{v}1.7 channels.\textsuperscript{12,82} Together with increases in skin metabolism and oxygen consumption, the resultant neurovascular changes would exacerbate tissue hypoxia and worsen cutaneous arteriovenous shunting in EM, creating a vicious cycle to evoke the heat hyperalgesia.\textsuperscript{59} It is possible that these paradoxical changes may also be caused by structural membrane changes as a function of temperature, disturbing the temperature dependence of Na\textsuperscript{+} channel conductance and gating kinetics.\textsuperscript{58} Tissue oedema from skin hyperaemia and release of inflammatory peptides may also influence the membrane structure and Na\textsuperscript{+} channel activity. Importantly, increases in persistent Na\textsuperscript{+} conductances in peripheral axons have been linked to a number of common painful neuropathies,\textsuperscript{17,52,53} participating in the generation of ectopic neuropathic symptoms.

4.3. Challenges in therapy

Concerning the episodic excruciating pain in patients with EM, a frequently used treatment of this disorder are the sodium channel blockers such as lidocaine and mexiletine. This nonselective partial block of voltage-gated Na\textsuperscript{+} channels targets segment 6 of domain IV of the alpha subunit.\textsuperscript{63} Clinical response varies and suggests that genotyope may be important.\textsuperscript{12,81} In agreement with the previous study by Kuwabara et al.,\textsuperscript{50} the effect of mexiletine on axonal excitability in EMSCN9A+ patients demonstrates small reductions in SDTC. Even so, SDTC remained increased such that it may be expected to have an ongoing part in neuropathic symptoms. There is an urgent clinical need to develop more effective Na\textsuperscript{+} channel blockers for the treatment of neuropathic pain, particularly the selective and potent Na\textsubscript{v}1.7 channel blockers, with initial results showing promise in EM.\textsuperscript{12}

The variability of pain between and within subjects poses challenges in assessing response to treatment and highlights the need to develop sensitive and relevant biomarkers to facilitate establishing novel disease modifying therapies. Recently, molecular modelling, thermodynamic analysis, and functional profiling were able to predict the responsiveness to treatment and may provide a platform for personalized medicine in the future with a preclinical assessment of potential efficacy.\textsuperscript{32}

5. Conclusions

Excitability studies are now being used widely in the clinical investigation of patients with neurological disease to provide functional information about peripheral nerves, with changes in measurements of excitability being interpreted in terms of direct and/or remote effects of altered ion channel function.\textsuperscript{77} These have provided novel insights into disease mechanisms and treatment strategies.\textsuperscript{26,27,29,49,54,57,73} This study advances this possibility by exploring the variations in excitability properties along with temperature in patients with EM. Specific indices of nerve excitability responded very differently to increases in temperature and these effects can be related to the indirect behaviour of mutant Na\textsubscript{v}1.7 channels systemically, producing a vicious cycle of pain. These preliminary findings support the use of excitability techniques as a biomarker to assess abnormalities of axonal function and monitor changes with the development of novel therapies in vivo.

Conflict of interest statement

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