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Effect of fampridine on axonal excitability in multiple sclerosis

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HIGHLIGHTS

- Fampridine, a sustained-release form of 4-aminopyridine has been demonstrated in clinical trials to be effective in improving disability and fatigue in multiple sclerosis.
- An *in vivo* study of fampridine effects on axonal function.
- Fampridine administered at recommended therapeutic doses, has clear effects on nerve excitability in MS patients which are likely governed by modulation of fast K^+ channels.

ABSTRACT

Objective: To investigate the effects of fampridine on nerve excitability, the present study utilized peripheral axonal excitability techniques in 18 MS patients receiving treatment with fampridine.
Methods: Studies were performed at baseline and repeated 3 months after institution of fampridine at standard dosing.
Results: Following treatment with fampridine there were significant changes in axonal excitability for those parameters associated with fast K^+ channels that shifted towards normal control values. Specifically, increases were noted in the peak superexcitability of recovery cycle (fampridine, −25.6 ± 1.6%; baseline −22.8 ± 1.7%; $p < 0.004$), peak depolarizing threshold electrotonus (fampridine, 69.1 ± 1.0%; baseline 67.0 ± 1.4%; $p < 0.004$), and depolarizing threshold electrotonus between 40 and 60 ms after onset of depolarization (fampridine, 52.8 ± 1.3%; baseline 49.9 ± 1.4%; $p = 0.02$).
Conclusion: The present study has established that fampridine at standard doses exerts effects on peripheral nerve function that may be mediated by reduction of fast K^+ conductances.
Significance: Modulation of fast K^+ conductances by fampridine may contribute to the improvement observed in MS symptoms including motor fatigue.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disorder of the human central nervous system and is the most common cause of non-traumatic chronic neurological disability among the young adult population (Hauser and Oksenberg, 2006; Dutta and Trapp, 2011). Eighty-five percent of new-onset MS is typically characterized by a relapsing-remitting (RRMS) course (Ransohoff et al., 2015). With the advent of novel disease modifying drugs that effectively expands the neurologist's therapeutic armamentarium, the frequency of clinical and/or radiological relapses are reduced. However, such therapies do not have effects on sustained disability that may be a result of poor recovery from a previous relapse or a reflection of conversion to progressive MS. In this regard, treatment options are required that specifically target the significant functional impairment associated with both relapsing and progressive forms of MS. Recent insights into the role of ion channel and synaptic transmission dysfunction in MS have uncovered opportunities to develop therapeutic strategies aimed

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at improving disability and impairment by modulating ion channel function and neurotransmission. Of particular relevance, fampridine, a sustained-release form of 4-aminopyridine (4-AP) has been demonstrated in clinical trials to be effective in improving disability (Goodman et al., 2009, 2010; Hobart et al., 2013; Rabadi et al., 2013; Ruck et al., 2014; Arnold et al., 2015).

There is a correlation between development of clinical deficits and the acute inflammation in MS with subsequent impairment of neural conduction (Waxman, 2006; Krishnan and Kiernan, 2013) and the underlying mechanism of conduction block and neurotransmission failure in demyelinated axons in MS may be related to ion channel dysfunction (Ng et al., 2008; Vucic et al., 2010, 2012). Whilst previous animal models have demonstrated a K^+ channel blocking effect of 4-AP and related compounds (Bostock et al., 1981), the precise mechanisms that underlie fampridine's therapeutic effects in human subjects with MS remain to be elucidated. Previous reports have demonstrated changes in peripheral axonal function in processes affecting the central nervous system, that may reflect similar changes in ion channel properties occurring more centrally (Krishnan et al., 2008; Ng et al., 2008, 2013; Tomlinson et al., 2010). Of relevance, clear changes in K^+ conductance have been shown in peripheral nerve excitability studies in patients with multiple sclerosis (Ng et al., 2008, 2013). In an attempt to study the mechanisms by which fampridine exerts its modulatory effects on nerve excitability in MS, the current study utilized threshold-tracking peripheral nerve excitability techniques combined with longitudinal clinical assessments to establish the *in vivo* effects of fampridine in MS.

2. Materials and methods

A total of 18 MS patients were consecutively recruited from the Prince of Wales MS Clinic (Table 1). The mean age was 54.9 years (range 33–67) and there were 14 females and 4 males. Nine patients had relapsing-remitting MS whilst seven had secondary progressive and two had primary progressive MS. The study was approved by South East Sydney Area Health Service and the University of New South Wales. All participants gave written informed consent in accordance with the Declaration of Helsinki. All patients underwent median motor nerve excitability studies. Excitability studies were performed at baseline and following 3 months of fampridine treatment at the standard dose of 10 mg twice daily.

Table 1
Baseline clinical characteristics of MS patients. RRMS, relapsing-remitting MS; SPMS, secondary progressive MS; PPMS, primary progressive MS; EDSS, Expanded Disability Status Scale.

Age	Sex	Type of MS	EDSS score	Treatment type
56	F	SPMS	3.5	Nil
52	M	SPMS	6.5	Nil
22	F	RRMS	2.5	Fingolimod
56	F	RRMS	1	Interferon Beta 1A
61	F	SPMS	3	Interferon Beta 1A
58	M	RRMS	1	Fingolimod
49	F	RRMS	5	Natalizumab
67	M	SPMS	6	Nil
61	M	SPMS	3	Nil
67	F	SPMS	2.5	Nil
61	F	PPMS	1	Nil
56	F	RRMS	3.5	Fingolimod
33	F	RRMS	3.5	Natalizumab
56	F	RRMS	3	Fingolimod
59	F	PPMS	4.5	Nil
58	F	RRMS	1	Nil
50	F	SPMS	6	Nil
67	F	RRMS	5.5	Interferon Beta 1A

2.1. Peripheral axonal excitability

Nerve excitability was performed by recording compound muscle action potential (CMAP) from abductor pollicus brevis following stimulation of the median nerve at the wrist using surface electrodes (Unomedical, Bikerod, Denmark). Skin temperature was monitored close to the site of stimulation and maintained >32 °C for the duration of the study with the use of warmed towels around the limb if required (Kiernan et al., 2001). Nerve excitability was assessed using the TRONDNF protocol of the QTRAC automated software (Digitimer, London, UK) (Kiernan et al., 2000). Multiple nerve excitability parameters were recorded from the five testing paradigms of the protocol: stimulus response (SR) curve, strength–duration-time-constant (SDTC), threshold electrotonus (TE), current threshold relationship (*I*/*V*) and the recovery cycle (RC) (see glossary for explanation for terms in Appendix).

The SR curve was generated by manually increasing the stimulus of 1 ms duration current to obtain maximal CMAP amplitude. In the remaining tests, the target potential of ~40% of maximal CAMP was utilized and the stimulus necessary to produce this was termed the "threshold". SDTC was determined using Weiss' Law (Mogyoros et al., 1996) and shows the relationship between the strength and the duration of a stimulus using four different stimulus durations (0.2, 0.4, 0.8 and 1 ms). This parameter displays persistent Na^+ conductances at the node (Krishnan et al., 2009). TE was determined by delivering prolonged subthreshold conditioning currents and plotting the percentage change in threshold when 1 ms test impulses were applied during and after these currents of +40% (depolarising) and −40% (hyperpolarising) of the control threshold. TE provides information about the rectifying properties of the nodal and internodal axolemma (Burke et al., 2001). The current–voltage relationship was determined by the change in threshold when 1 ms test pulses were delivered after 200 ms depolarising and hyperpolarising conditioning current (+50 to −100 ms). The *I*/*V* relationship provides information on the rectifying properties of the internodal membrane of the axon (Kiernan et al., 2000). The RC assesses changes in threshold following a supramaximal conditioning stimulus. This provides information regarding changes in Na^+ and K^+ channel function and membrane potential (Kiernan et al., 2000).

2.2. Statistical analysis

Statistical analysis was performed using SPSS v22 (IBM Corp, USA). Group data are expressed as mean ± SEM and compared using either Student's *t*-test or Mann–Whitney *U*-test depending on normality of data distribution, and applied when analyzing patient against control data. Likewise, baseline and follow-up fampridine paired data for each considered electrophysiological or functional parameter were compared using either paired *t*-test or Wilcoxon test. Correlations between electrophysiological parameters and clinical scores were assessed by Spearman rank test. A *P*-value of <0.05 was regarded as statistically significant.

3. Results

Peripheral nerve excitability tests were undertaken in all patients before and after commencement of fampridine therapy (Table 2, Figs. 1A, B, 2 and 3). Baseline studies in MS patients demonstrated no significant differences compared to age-matched controls aside from a trend towards reduction in depolarizing threshold electrotonus at 90–100 ms, TEd (90–100 ms) (MS patients 42.56 ± 0.98; controls 45.13 ± 0.86; $p = 0.05$). There was a non-significant trend towards increased S2 accommodation in the MS cohort (MS patients 24.48 ± 1.02; controls 22.27 ± 0.94, $p = 0.12$).