

Pharmacologic Intervention in Axonal Excitability: *In Vivo* Assessment of Nodal Persistent Sodium Currents in Human Neuropathies

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Abstract: Axonal excitability testing can provide new insights into the ionic mechanisms underlying the pathophysiology of hyperexcitability of motor and sensory axons in human neuropathies. Threshold tracking was developed in the 1990's to non-invasively measure a number of axonal excitability indices that depend on sodium and potassium channel function, and this makes it possible to monitor the effects of pharmacologic intervention with ion channel modulators. This paper reviews recent advances in ionic-pathophysiological studies in humans. (1) Neuropathic pain or muscle cramp/fasciculation is partly caused by hyperexcitability of the injured axons. The enhanced excitability can result from altered ion channel function; such as an increase in persistent sodium currents. Persistent sodium currents can be reliably estimated using threshold tracking. In peripheral neuropathy, persistent sodium currents usually increase possibly due to over-expression of sodium channels associated with axonal regeneration, and could be responsible for ectopic firings. Administration of sodium channel blockers such as mexiletine, results in marked alleviation of muscle cramping in parallel with a decrease in nodal persistent sodium currents. (2) In diabetic neuropathy, the activation of the polyol pathway mediated by an enzyme, aldose reductase, leads to reduced Na^+/K^+ pump activity, and intra-axonal sodium accumulation; sodium currents are reduced presumably due to decreased trans-axonal sodium gradient. Aldose reductase inhibitors improve nodal sodium currents, as well as nerve conduction, and this can be objectively assessed by threshold tracking. Studies of ion-channel pathophysiology in human subjects have recently begun. Investigating ionic mechanisms by monitoring the corresponding ionic currents is of clinical relevance, because once a specific ionic conductance is identified, pharmacologic blocking or modulation could provide a new therapeutic option.

Keywords: Neuropathic pain, muscle cramp, sodium channel, persistent sodium channel, sodium channel blocker.

INTRODUCTION

After peripheral nerve injury, changes in ion channel expression and passive membrane properties can lead to changes in axonal excitability. The altered excitability can result in abnormal ectopic activity that leads to positive symptoms of sensory axons (pain and paresthesiae) and motor axons (muscle cramps and fasciculations) [1-3]. Patients with peripheral neuropathy frequently suffer from these positive symptoms even in the recovery phase of the disease, suggesting persistently increased axonal excitability. Various agents have been used to alleviate neuropathic pain, such as anticonvulsant, antidepressant, non-steroidal anti-inflammatory drugs, opioid, and ion channel blockers [4]. However, the precise mechanisms of positive neuropathic symptoms such as pain and muscle cramps have not yet been sufficiently elucidated, and until development of the threshold tracking technique, there were no appropriate tools to monitor the effects of pharmacological ion channel modulators on axonal excitability in human subjects.

Testing the excitability of axons can provide insights into the ionic mechanism responsible for the pathophysiology of axonal dysfunction in human neuropathies. The technique of threshold tracking was developed in the 1990s to non-invasively measure a number of axonal excitability indices that depend on axonal sodium and potassium channel function, and this makes it possible to monitor the effects of

pharmacologic intervention with ion channel modulators on axonal excitability [1, 5]. This paper reviews recent advances in ionic-pathophysiological studies in human axons, focusing on changes in excitability indices mediated by pharmacologic intervention.

AXONAL SODIUM CHANNELS, AND *IN VIVO* ASSESSMENT OF NODAL PERSISTENT SODIUM CURRENTS

Nodal and Paranodal Ion Channels in Myelinated Axons

Ion channels are not evenly distributed along a myelinated axon. The action potential of myelinated axons can be modeled using only the properties of transient sodium channels concentrated at the nodes of Ranvier [6]. Other channels are activated during and after the generation of action potentials. Potassium channels contribute to resting membrane potential, and thereby determine the threshold for generating the action potential. The density of sodium channels at the nodes of Ranvier is high (30 times the density on the internodal axonal membrane), and the internodal density is too low to propagate the action potential.

Recent studies suggest that 9 types of sodium channel are expressed in the human nervous system, and muscle [7], but only one (Nav 1.6) is found at the nodes of Ranvier [8]. However, two functionally distinct types of sodium current can be distinguished, classical transient channels and persistent channels. The molecular structure of the two types of channels is identical but the magnitude of inactivation at the resting membrane potential is different. Whereas the classical transient sodium channel is rapidly activated by mem-

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brane depolarization and then inactivated, the “persistent sodium channel” is activated equally rapidly but inactivated at a membrane potential that is 10-20 mV more negative than the resting membrane potential. Its inactivation is therefore minimal, giving rise to a persistent inward leak of sodium ions at the resting membrane potential. Recent studies indicate that “persistent” sodium currents are present in human peripheral nerve, and the currents constitute 2.5% of the total sodium currents on sensory axons, and 1.0% on motor axons [9]. The persistent sodium current is one of the major determinants of axonal excitability, and increasing the fraction of persistent currents will produce hyperexcitability of axons [2, 3, 10].

Estimation of Nodal Persistent Sodium Currents in Human Axons

The excitability of human axons can now be studied reliably using the technique of threshold tracking, which allows the intensity of a test stimulus to be adjusted by computer to activate a defined fraction of the maximal nerve or muscle action potential (i.e., 40% of the maximal response), and this can be achieved by the use of appropriate software (QTRAC, copyright, Prof. Hugh Bostock, Institute of Neurology, London, UK) [1]. Following delivery of conditioning stimuli, which alter membrane potential or activate specific ion channels, the current required to produce a target potential (threshold) will change. Tracking the threshold change makes it possible to estimate ionic conductances indirectly. For a detailed discussion of the technique, please refer to previous reviews [1, 2]. Persistent sodium conductances are estimated by threshold tracking using the strength-duration time constant and latent addition.

Strength-duration time constant (SDTC) is a classical measure of axonal excitability [11-13], and equates to chronaxie. Chronaxie is defined as the stimulus duration for which the threshold current is twice the rheobase (the threshold current for a stimulus of infinite duration) [1]. SDTC partly depends on persistent sodium conductance. When persistent sodium conductance increases, the strength-duration time constant becomes larger. However, the SDTC is also affected by passive membrane properties at the nodes of Ranvier, and the two factors (persistent sodium conductance, and passive membrane properties) cannot be analyzed separately.

Latent addition using automatic threshold tracking is a new technique, and is currently considered the best way to non-invasively estimate nodal persistent sodium currents *in vivo* [1, 9]. The major advantage of the latent addition method is to separately evaluate passive membrane properties and persistent sodium currents. The threshold current required to produce the target response, set to 40% of the maximal compound muscle action potential, is determined using a test stimulus of 0.06 ms duration. A brief hyperpolarizing conditioning current delivered to axons results in transient closure of persistent sodium channels, and thereby a slight increase in threshold current. This is the principle of latent addition. The conditioning-test interval is systematically increased from 0.02 to 0.5 ms. The decay of the threshold increase produced by the hyperpolarizing stimulus is the sum of two exponential components (Fig. 1) [9, 10, 14]. The

fast component (the conditioning-test interval, 0.02 ms - 0.2 ms) is determined by the passive membrane constant, and the slow component (0.2 ms - 0.5 ms) depends on a persistent sodium current active at the resting membrane potential. The increase in threshold current measured at the 0.2 ms-interval (a time when the first exponential has decayed almost to zero) can be used as an indicator of nodal persistent sodium currents [9, 10].

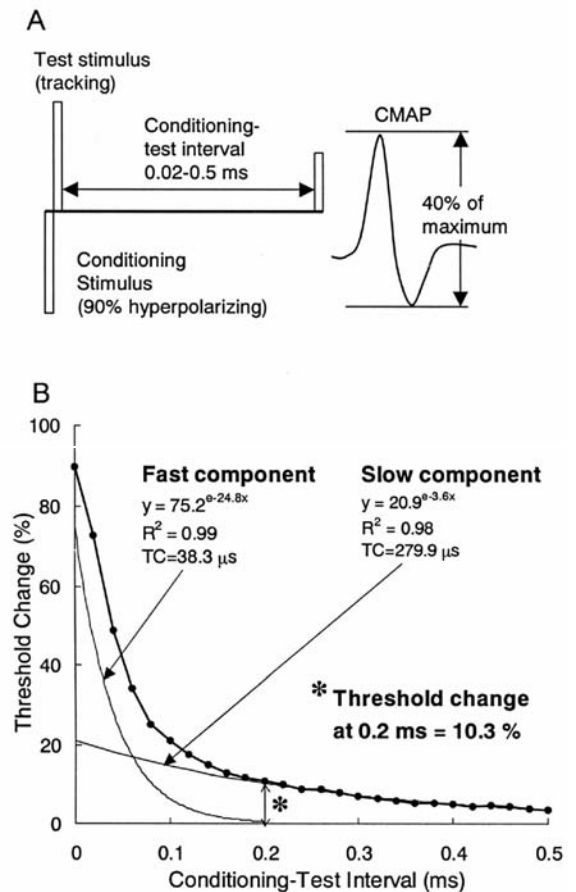


Fig. (1). Latent addition: the test stimulus was conditioned by a hyperpolarizing stimulus fixed at -90% of the current required to produce the test response. The conditioning-test interval was systematically increased from 0.02 to 0.5 ms (A). At the wrist of median motor axons, the decay of the threshold increases as fitted accurately by two exponential curves (B). The fast component (0.02-0.2 ms; $R^2=0.99$) is dependent on a passive input membrane constant, and the slow component (0.2-0.5 ms; $R^2=0.98$) is associated with persistent sodium currents. The increase in threshold measured at the 0.2 ms-interval (a time when the first exponential has decayed almost to zero) can be used to estimate persistent sodium currents (Bostock and Rothwell, 1997). From Misawa, *et al.* (2006) [37], with permission.

PHARMACOLOGIC INTERVENTION IN AXONAL EXCITABILITY

Sodium Channel Blockers for Treatment of Muscle Cramp

In disorders involving lower motor neurons/axons such as peripheral neuropathies, amyotrophic lateral sclerosis (ALS), and spinal muscular atrophy, a loss of motor axons is

usually accompanied by collateral innervation of denervated muscles by the remaining motor neurons, or by regeneration of the injured axons. Experimental studies have shown that sodium channels are over-expressed on the axolemma when nerves are growing and sprouting, and that local remodeling of sodium channels results in altered axonal excitability [15-17]. Previous studies have suggested the increased excitability of motor axons in ALS, spinal muscular atrophy, and peripheral neuropathies, presumably due to an increase in persistent sodium currents estimated by SDTC measurements [13, 18, 19] and latent addition [10]. Fig. (2) shows SDTC and 0.2 ms threshold changes in latent addition in patients with peripheral neuropathy, spinal muscular atrophy, or ALS. These indices are commonly increased in peripheral neuropathies and motor neuron diseases, suggesting an increase in persistent sodium currents in these disorders. Clinical implications of these findings includes that patients with neuropathy, spinal muscular atrophy, or ALS frequently suffer disabling muscle cramping that substantially disturbs sleep, working, and daily activities [18]. Muscle cramp is caused by spontaneous or voluntary contraction-induced abnormal firing of motor axons, and generally originates from the motor nerve terminals [20].

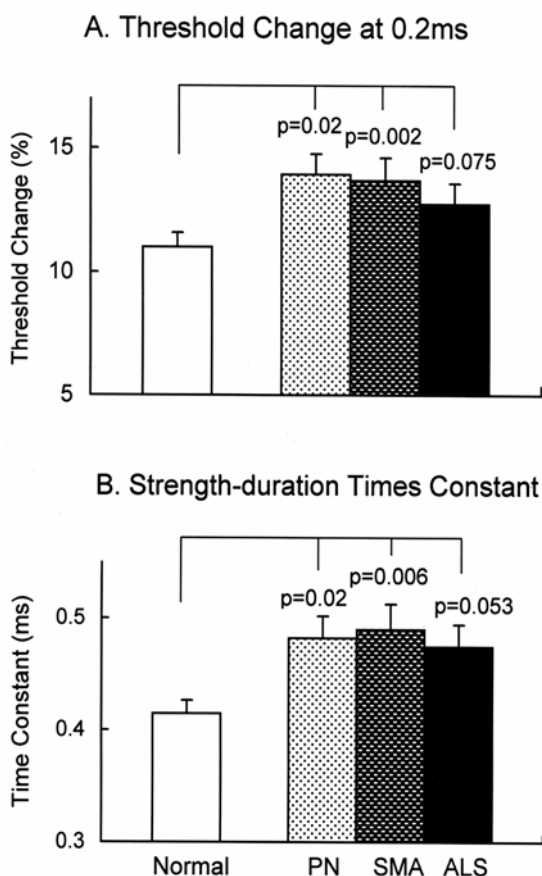


Fig. (2). Threshold change at the 0.2-ms interval in latent addition (A) and the strength-duration time constant (B) in age-matched normal subjects (n=27), and patients with peripheral neuropathy (PN; n=38), lower motor neuron disorders (LMND; Kugelberg-Welander disease and bulbo-spinal muscular atrophy; n=19), or amyotrophic lateral sclerosis (ALS; n=36). From Tamura *et al.* (2006) [10] with permission.

Mexiletine is an orally active local anesthetic agent, which is structurally related to lidocaine, and has been used for alleviating neuropathic pain [21-23], and occasionally for muscle cramp (Kanai *et al.*, 2003). Neuropathic pain and muscle cramping partly arise from axonal hyperexcitability, which leads to abnormal spontaneous firing associated with increased sodium channel expression [24]. The mechanism of action of mexiletine is a blockage of sodium channels [25], and this agent decreases axonal excitability by reducing nodal sodium currents. Although this has rarely been demonstrated in human axons until 2000 because of the lack of appropriate tools to assess axonal ionic conductances in human subjects, a recent study has shown that mexiletine treatment results in dramatic reduction of abnormal muscle cramps, associated with a decrease in SDTC [26]; 14 patients with disabling muscle cramping involving trunk and hand muscles, as well as lower limb muscles, were treated with oral 300 mg mexiletine per day, and muscle cramps were nearly abolished in all the patients after treatment. The mean SDTC was 0.50 ms before and 0.46 ms after mexiletine therapy (p=0.01), whereas the mean SDTC in normal subjects was 0.40 ms. Fig. (3) shows changes in SDTC in individual patients; mexiletine administration results in decreased SDTC that was more prominent in patients with longer SDTC before treatment. These findings suggest that increased nodal persistent sodium currents are responsible for abnormal muscle cramping, and nerve excitability testing can be used for monitoring the effects of sodium channel blockers in individual patients. Mexiletine appears to affect SDTC especially when the time constant is abnormally long, and this is consistent with a previous experimental study, which showed that mexiletine at a low dose blocks inactivation-deficient mutant muscle sodium currents in the resting-state [27]. There are only a few studies investigating the effects of mexiletine on axonal sodium currents, but mexiletine is an analogue of lidocaine, which at low concentrations has a selective action on persistent sodium currents in primary sensory neurons [28, 29]. Similar trials of sodium channel blockers for neuropathic pain involving monitoring persistent sodium currents are on-going.

Aldose Reductase Inhibitors for Diabetic Neuropathy

Reduced Nodal Sodium Currents in Hyperglycemia

Diabetic polyneuropathy results from a complex interplay between metabolic factors directly related to hyperglycemia and structural changes such as axonal degeneration and demyelination [30-32]. The mechanisms underlying nerve dysfunction are not well understood, but it is generally assumed that the metabolic factors mediate acutely reversible functional impairment. The major metabolic factors include activation of the polyol pathway and decreased Na⁺-K⁺ AT-Pase activity. The conversion of excess glucose to sorbitol, and the resulting depletion of myo-inositol that leads to inactivation of Na⁺-K⁺ ATPase activity then results in intraxonal sodium accumulation, and leads to a decrease in the sodium gradient across the axolemma [33, 34]. An altered membrane sodium gradient could affect axonal sodium conductance, which can be restored by glycemic control. Rigorous insulin replacement can result in rapid reversal of

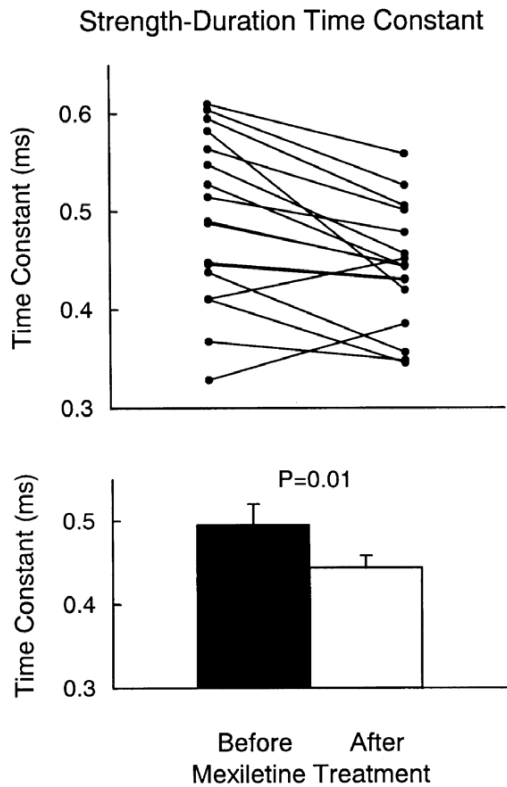


Fig. (3). Strength-duration time constant (SDTC) of median motor axons before and after mexiletine treatment in patients with disabling muscle cramping (n=14). There was a significant decrease in SDTC after treatment (p=0.01). From Kuwabara *et al.* (2005) [26], with permission.

the Na⁺-K⁺ ATPase activity, and there is evidence that the slowing of nerve conduction is caused, in part, by metabolic abnormalities in human diabetics [35, 36]. Fig. (4) shows

axonal edema in diabetic rat nerves [33], and the possible cascade from activation of the polyol pathway to impaired nerve conduction.

A recent study has shown that there is an inverse relationship between the state of glycemic control (serum hemoglobin A1c levels) and SDTC or threshold changes at 0.2 ms in latent addition [37] (Fig. 5). These findings suggest the view that hyperglycemia suppresses nodal persistent sodium currents. Furthermore, glycemic control by intensive insulin treatment results in a rapid increase in SDTC or threshold changes at 0.2 ms in latent addition (Fig. 6), again suggestive of suppressed persistent sodium currents under hyperglycemia [38-40].

The Effects of an Aldose Reductase Inhibitor, Epalrestat

The restoration of nodal sodium current after insulin treatment can be mediated by multiple factors such as improvement in tissue acidosis, hyperosmolarity, impaired microcirculation, and other factors. However, a recent report has shown the effects of aldose reductase inhibition on persistent sodium currents [40]. Because the polyol pathway is mediated by an enzyme, aldose reductase, inhibition of this enzyme could improve sodium currents. Fig. (7) shows an improvement in nodal persistent current and nerve conduction after treatment with eparlestat, an aldose reductase inhibitor. One month after administration of eparlestat, there was a significant improvement in nerve conduction (distal latency and F-wave latency) associated with increased persistent sodium currents estimated by SDTC and latent addition. After 6 months, nerve conduction still improved, but the persistent currents decreased. The changes in persistent sodium currents might result from down-regulation of persistent channels due to increased currents. These findings support the view that activation of the polyol pathway and resulting reduction of nodal sodium currents play an important

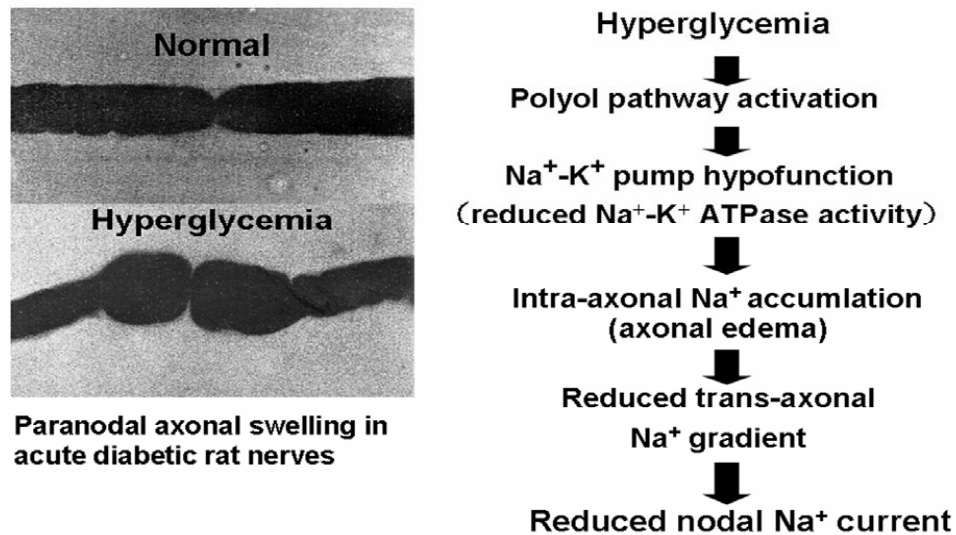


Fig. (4). Paranodal axonal swelling in acute diabetic rat (Modified from Brismar *et al.* (1987) [33], with permission), and a possible cascade from activation of the polyol pathway to impaired nerve conduction

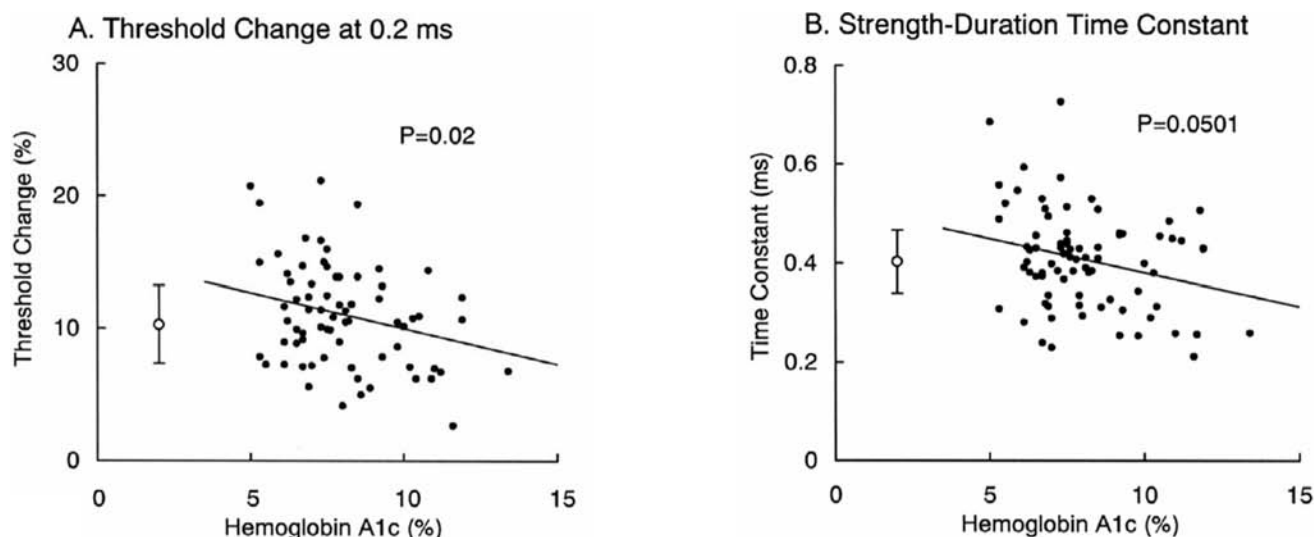


Fig. (5). Plotting of threshold change at the 0.2 ms-interval (A) and strength-duration time constant (B) against hemoglobin A1c levels in diabetic patients. There was a reverse linear correlation between threshold change at 0.2 ms and hemoglobin A1c levels ($p=0.02$). Strength-duration time constant had a similar relationship with hemoglobin A1c levels ($p=0.0501$). Open symbol and error bars indicate the normal mean value and SD, respectively. From Misawa *et al.* (2006) [37], with permission.

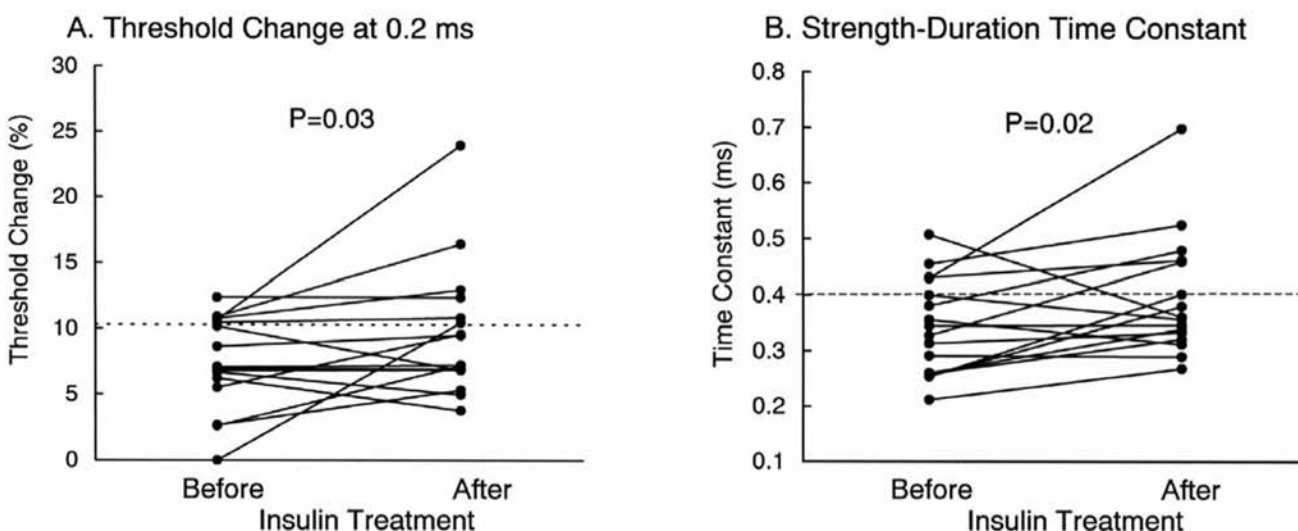


Fig. (6). Changes in the threshold change at 0.2 ms (A) and strength-duration time constant (B) in 17 diabetic patients before and 4-8 weeks after intensive insulin treatment. Both indices increased significantly after treatment. Dotted lines indicate the normal mean values. From Misawa *et al.* (2006) [37], with permission.

role in the pathophysiology in human diabetic neuropathy. Aldose reductase inhibition can partly reverse nerve conduction abnormalities due to reduced persistent sodium currents, and this can be monitored by latent addition and SDTC measurements. A recent randomized control study has shown the positive effects of epalrestat on nerve function in patients with diabetic neuropathy [41].

CONCLUSIONS

Studies of ion channel pathophysiology in human subjects have recently begun. Investigating ionic mechanisms for each symptom/sign or disease process could be of great

clinical relevance, because once a specific ionic conductance is identified, pharmacologic blocking or modulation could provide a new therapeutic option. Successful treatment of muscle cramping with sodium channel blockers, and of diabetic neuropathy with aldose reductase inhibitors are examples. Further studies will be required to elucidate ionic mechanisms of positive and negative symptoms in a variety of neuromuscular disorders. Pharmacologic intervention in axonal excitability while monitoring the responsible ionic currents could be a new strategy for treatment of positive neuropathic symptoms or disease processes caused by altered axonal excitability.

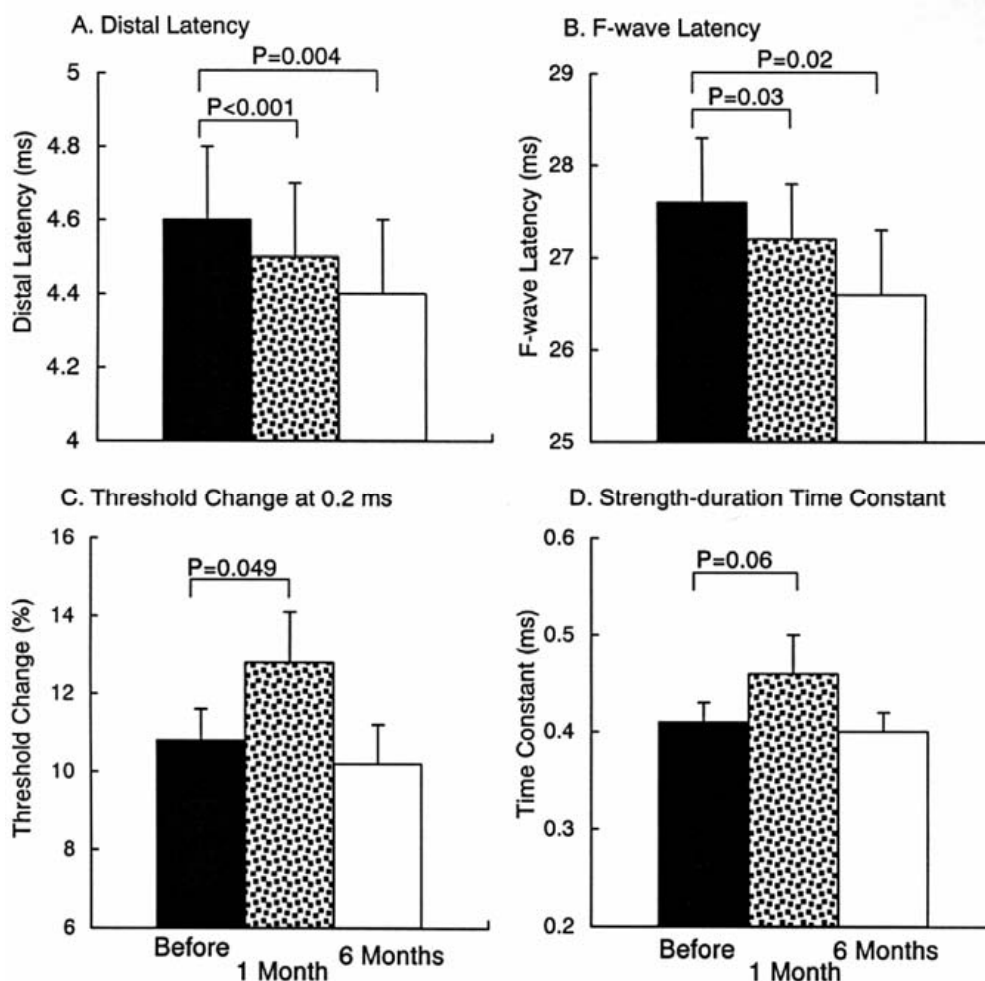


Fig. (7). Changes in distal latency (A) and minimal F-wave latency (B) in median motor nerve conduction studies, and indicators of nodal persistent Na⁺ currents (threshold changes at 0.2 ms in latent addition, C; and strength-duration time constant, D) before, and 1 and 6 months after the start of treatment with epalrestat. From Misawa *et al.* (2006) [40], with permission.

ABBREVIATIONS

ALS = Amyotrophic lateral sclerosis
SDTC = Strength-duration time constant

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