



Research Article

Peripheral Nerve Axonal Excitability in Epileptic Patients

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Summary

Introduction: Sodium (Na^+) channels have an important role for action potential formation in both central nervous system (CNS) and peripheral nervous system (PNS). The persistent Na^+ current play a role in repetitive firing of action potentials caused by prolonged depolarization, thus could play a role in epilepsy. Nerve excitability measurements are made to investigate biophysical properties and disturbances of impulse conduction in peripheral axons in human subjects.

Methods: In this study, it was aimed to study strength–duration properties of motor and sensory axons to evaluate whether there is a change in the current through persistent Na^+ channels of sensory and motor axons in peripheral nerves of epileptic patients because of the presence of similiar channels in PNS. The study involved 17 patients newly diagnosed with partial epilepsy and 22 patients with primary generalized epilepsy who were not currently prescribed anticonvulsant therapy, and 15 control subjects.

Results: Rheobase of motor fibres and stimulus strength at 0.1 ms and 1ms of motor fibres of total patients with epilepsy were higher than values of controls.

Conclusions: Our results suggested a decreased axonal excitability and a possible decreased Na^+ conductances in peripheral nerves in patients with epilepsy. While there is hyperexcitability of central neurons, reduced peripheral nerve excitability may be in question in epilepsy.

Key words: Epilepsy; peripheral axonal excitability; voltage-gated Na^+ channels

Epilepsili Hastalarda Periferik Sinir Aksonal Eksitabilitesi

Özet

Giriş: Sodyum (Na^+) kanalları hem santral sinir sistemi hem de periferik sinir sisteminde (PSS) aksiyon potansiyeli oluşumunda önemli bir role sahiptir. Persistan Na^+ akımı, uzamış depolarizasyonun neden olduğu aksiyon potansiyellerinin repetitif ateşlenmesinde rol oynar ki bu epilepsiye neden olabilir. Sinir eksitabilite ölçümleri, insanların periferik aksonlarında impuls iletiminin biyofiziksel özellikleri ve bozukluklarını incelemek için yapılmaktadır.

Metod: Bu çalışmada, PSS'de de benzer kanallar olmasından yola çıkılarak epilepsili hastaların periferik sinir duysal ve motor aksonlarında persistan Na^+ akımında değişiklik olup olmadığını değerlendirmek için motor ve duysal aksonların güç-süre özelliklerini çalışmak amaçlanmıştır. Çalışmaya antikonvülzan tedavi almayan, yeni tanı almış parsiyel epilepsili 17 hasta ile primer jeneralize epilepsili 22 hasta ve 15 kontrol olgu alındı.

Sonuçlar: Epilepsili olgularda motor liflerin reobazı ve 0.1 ms ve 1ms'deki stimulus gücü kontrol grubuna göre yüksek bulundu.

Karar: Sonuçlarımız, epilepsili olguların periferik sinirlerinde aksonal eksitabilitede azalma ve dolayısıyla Na^+ iletiminde olası azalmaya işaret etmektedir. Epilepside santral nöronlarda hipereksitabilite varken, periferik sinir eksitabilitesinde azalma söz konusu olabilir.

Anahtar Kelimeler: Epilepsi; periferik aksonal eksitabilite; voltaj-kapılı Na^+ kanalı

INTRODUCTION

Nerve excitability measurements are made to investigate biophysical properties and disturbances of impulse conduction in peripheral axons in human subjects^(9,10). A classic measure of neural excitability is the strength–duration curve, which is the variation in stimulus intensity needed to achieve the same evoked response at various stimulus durations. The strength–duration properties of an axon are the rheobase and the strength-duration time constant (SDTC). The rheobase is defined as the minimum current intensity needed to obtain excitation with a stimulus of infinite duration. SDTC, defined as the stimulus duration needed to obtain excitation with a current intensity twice the rheobase, is a measure of the rate at which the threshold current for a target potential declines as stimulus duration is increased. In the formulation of Weiss, it equates to chronaxie and essentially reflects the capacitive membrane properties^(2,22). Both variables functionally reflect the nodal membrane and are influenced by changes in membrane potential, impedance, capacitance, and area of axonal membrane devoid of myelin^(3,18). Although, theoretically, several factors can affect the time constant of the strength–duration curve, its measurement provides an indirect estimate of the persistent Na⁺ conductances of the axonal membrane at the node^(16,17). Assessment of the strength–duration properties of motor and sensory axons would therefore represent an interesting new method to test, specifically, the experimental hypothesis of Na⁺ channel dysfunction⁽¹⁸⁾.

Neuronal excitability is determined by the properties of the ion channels in the neuronal membrane. An aberrant excitability associated with an epileptic discharge is mediated by voltage-gated and ligand-gated ion channels, and may be the result of defects in the function of these channels. These ion channels play a

fundamental role in the physiology of all forms of epilepsy, so several of these channels represent the critical sites of action for antiepileptic drugs. Voltage-gated ion channels not only control excitability in the central nervous system (CNS), but also in the peripheral nervous system (PNS)⁽¹⁴⁾. Na⁺ channels have a primary role for the initiation and propagation of action potentials, making them critical determinants of neuronal excitability in both CNS and PNS. Unlike the different classes of K⁺ and Ca²⁺ channels, the functional properties of Na⁺ channels are very similar⁽⁴⁾. Mutations in both α and β subunits of Na⁺ channels have been linked to epilepsy. In this study, it was aimed to study strength–duration properties of motor and sensory axons to evaluate whether there is a change in the current through persistent Na⁺ channels of sensory and motor axons in peripheral nerves of epileptic patients because of the presence of similar channels in PNS.

MATERIAL AND METHODS

Patients:

The study involved 17 patients (10 women and 7 men) with partial epilepsy, 22 patients (13 women and 9 men) with primary generalized epilepsy and 15 control subjects (10 women and 5 men). Three groups of subjects were at similar mean ages (27.5 ± 11.2 , 24.7 ± 10.8 , 28.8 ± 10.8 years, respectively) and similar body mass index. Epilepsy type was determined according to the guidelines of the International League Against Epilepsy and based on detailed histories obtained from the patients and/or witnesses, physical and neurological examinations, electroencephalographies, and neuroradiological findings⁽⁵⁾. The duration of seizures was 51.4 ± 46.3 months, and the frequency of seizures was 2 per month. The reasons of the patients' delayed application to the physician were documented as low socioeconomic status and the patients' and their

families' disaffirmation of the disease because of sociocultural factors.

The study was restricted to the patients who were not currently prescribed anticonvulsant therapy which could have confounding effects on axonal excitability. Thirty-four of the patients had not been treated with anticonvulsant drugs before, and 5 of them had been prescribed anticonvulsant therapy for some period, but not having taken these drugs for at least 2 years. Two patients had a history of febrile convulsion as an etiology of epilepsy. Neurological examination was normal in all subjects and none had intellectual disability. One patient had hippocampal sclerosis on cerebral magnetic resonance imaging, the others had normal findings. Interictal electroencephalography was normal in 22 of 39 patients (10 partial epilepsy, 12 generalized epilepsy). In the remaining, 6 patients had bilateral diffuse spike and wave activity discharges and 4 had bilateral diffuse paroxysmal discharges of theta and sharp waves in generalized epilepsy group. In partial epilepsy group, 2 had secondary generalized discharges of theta and sharp waves originate from right temporo central areas, 4 had 1-3 times nonspecific discharges of theta and sharp waves and 1 had background rhythm irregularity formed by beta and theta waves. All patients and controls had normal routine laboratory serum testing, which included hematocrit, glucose, vitamin B12 and folic acid levels, mean corpuscular volume, and renal, hepatic and thyroid function tests.

All patients were studied during the seizure-free period for at least 24 hours before the recordings, and none of them reported seizures during the recording periods.

The control group included 15 healthy volunteers. The groups were well matched with respect to age, gender, and body mass index. Patients and controls with other systemic or metabolic diseases (diabetes

mellitus, neuropathy, dysparaproteinemia, cancer, AIDS, entrapment syndromes and other neuropathies and causes known to damage to the peripheral nerves) were excluded.

Medical history and physical and neurological examinations were performed by the same neurologist.

Informed consent was obtained from all subjects before enrollment, and the study protocol was approved by the local ethics committee.

Methods:

In all subjects, left ulnar motor and sensory conduction studies were done. Compound muscle action potentials (CMAPs) were recorded from left hypothenar muscles using bipolar surface electrodes over the abductor digiti minimus by stimulating the ulnar nerve at the wrist. The antidromic compound sensory action potentials (CSAPs) were recorded from the fifth finger by ring electrodes set 3 cm apart around the proximal phalanx by stimulating the ulnar nerve at the wrist. Skin temperature was monitored close to the stimulation site and kept $>32^{\circ}\text{C}$ by placing a blanket over the palm and using radiant heat if necessary. The amplitude of the CMAP was measured from peak to peak; CSAP baseline to peak and the target CMAP and CSAP were set to 40% of the maximal response. The 0.2-ms peak response was used to set the target submaximal response (40% of maximum) and the current required to produce the target response was determined for different stimulus durations, 0.05, 0.1, 0.2, 0.3, 0.5 and 1 ms. The adjustment of stimulus intensity to produce the target CMAP and CSAP were performed manually. This generally took 4-5 stimuli before the target CMAP and CSAP (40 % of maximum) were recorded. Stimulus-response curves were then constructed from these data (Fig.1A) and the SDTCs were estimated using Weiss's formula⁽²²⁾. The relation between stimulus duration and stimulus charge (obtained by multiplying

stimulus strength by stimulus duration) was linear (Fig.1B). From the regression equation for this linear relation, SDTC was calculated⁽²⁰⁾. The strength-duration time constant of motor and sensory fibres of ulnar nerve is given by the intercept of the regression line on the X-axis. The rheobasic threshold for axons contributing to the 40% CMAP and CSAP is given by the slope of the regression line.

Statistical analyses: Statistical analyses were performed with SPSS 17.0 packet program. Categorical variables were summarized as number and percent, continuous variables as mean and standard

deviation (if necessary, median and minimum - maximum). Chi-square test was used to compare categorical variables between groups. When continuous variables provided parametric test hypothesis, T-test (Student's t-test) was used to compare patients and control group. When continuous variables didn't provide parametric test hypothesis, Mann-Whitney test was used to compare patient and control groups. Quantitative data were presented as means $\pm$ standard deviation ($\pm$ SD). P values less than 0.05 were considered statistically significant.

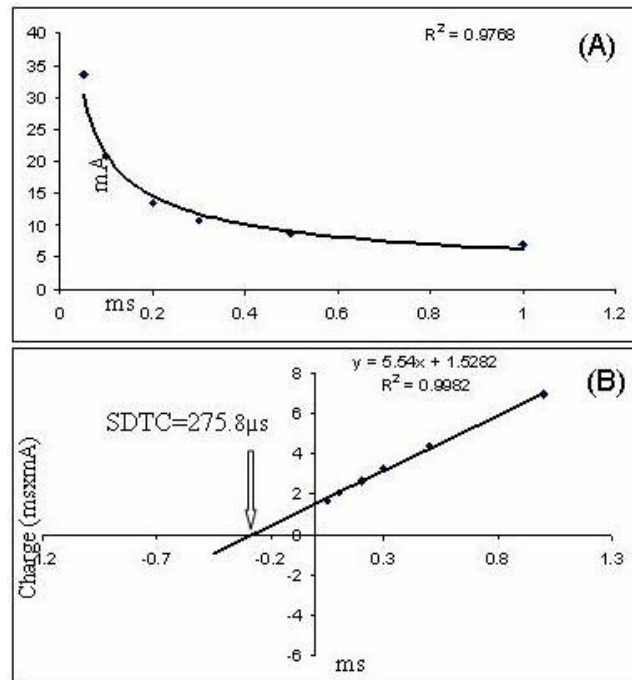


Figure 1: For a single control subject, the measurement of the strength-duration time constant of motor in 2 panels; (A) The relationship between stimulus strength (milliampere) and stimulus duration (millisecond) in an example control subject. “R2” refers to the coefficient determined for the motor fibers. (B) The relationship between stimulus duration and the value achieved by multiplying stimulus duration (charge) for motor fibers. The strength-duration time constant is calculated as follows in this example: When y is accepted as zero, x is found by dividing 1.5282 to 5.54.

RESULTS

All patients and controls had normal results on their motor and sensory conduction velocity studies of ulnar nerve.

CMAPs and CSAPs were similar in study groups.

Rheobase of motor fibres and stimulus strength at 0.1 ms and 1ms of motor fibres

of total patients with epilepsy were higher than values of controls ($p=0.042$, 0.042 , 0.037 , respectively). Stimulus strength at 1ms of sensory fibres of patients with partial epilepsy was higher than control group ($p=0.037$), and the p value of stimulus strength at 0.1ms was 0.053.

Stimulus strength at 0.1 ms of motor fibres of patients with generalized epilepsy was higher than control group. There were not any statistical differences between partial epilepsy group and generalized epilepsy group. SDTC values were similar in all groups (Table 1,2).

Table 1: The values of control group, patients with partial and generalized epilepsy, and total patients with epilepsy

	Control (no:15)	Total patients with epilepsy (no:39)	Patients with partial epilepsy (no:17)	Patients with generalized epilepsy (no:22)
SDTC-M	321±75	324±78	305±58	338±89
SDTC-S	394±102	373±114	363±112	380±118
Rheobase-M	2.5±1.3	4±2.6	3.6±1.9	4.4±3
Rheobase-S	1.9±0.9	2.6±1.9	2.8±2	2.5±1.8
SS at 0.1ms-M	10.6±5.4	16±9.2	14±7	17.5±10.6
SS at 0.1ms-S	9.1±4.3	11.7±6	12.6±5.9	11±6.1
SS at 1ms-M	3.3±1.7	5.2±3.2	4.6±2.4	5.7±3.7
SS at 1ms-S	2.6±1.2	3.6±2.2	3.9±2.3	3.3±2.2

SD, standard deviation; SDTC, strength-duration time constant (ms); M, motor; S, sensory; SS at 0.1ms-M, stimulus strength at 0.1 ms of motor fibres; SS at 0.1ms-S, stimulus strength at 0.1 ms of sensory fibres; SS at 1ms-M, stimulus strength at 1 ms of motor fibres; SS at 1ms-S, stimulus strength at 1 ms of sensory fibres (mA).

Table 2: The significant statistical differences between groups

SS at 0.1ms-S	Control	Partial Epilepsy	P=0.053
SS at 1ms-S	Control	Partial Epilepsy	P=0.037
SS at 0.1ms-M	Control	Generalized Epilepsy	P=0.049
SS at 0.1ms-M	Control	Total Epilepsy	P=0.042
SS at 1ms-M	Control	Total Epilepsy	P=0.037
Rheobase-M	Control	Total Epilepsy	P=0.042

M, motor; S, sensory; SS at 0.1ms-M, stimulus strength at 0.1 ms of motor fibres; SS at 0.1ms-S, stimulus strength at 0.1 ms of sensory fibres; SS at 1ms-M, stimulus strength at 1 ms of motor fibres; SS at 1ms-S, stimulus strength at 1 ms of sensory fibres (mA).

DISCUSSION

In the 1990s, the threshold tracking technique was developed to measure non-invasively several indices of axonal excitability, such as strength–duration properties, threshold electrotonus, and current–threshold relationship, in human subjects^(1,6-10,13). Threshold measures are complementary to conventional electrophysiological tests associated with nerve function. The indices of axonal excitability depend on the biophysical properties of the axonal membrane or the myelin sheath and can provide some information about Na^+ or K^+ channel function. Today, axonal excitability studies have been used in several diseases to investigate the underlying pathophysiology⁽²⁾, but they do not have much value in diagnosis yet. Most of the diseases that have been studied share mechanisms disturbing Na^+/K^+ pump activity, and Na^+ or K^+ conductances.

In a study that we did to evaluate the possible effects of sex on SDTC, we found a difference in SDTC and rheobase between the two sexes, and the mean value of SDTC was smaller in men than it was in women in subjects aged less than 40 years. The sex-related differences shown in this study suggested a possible biochemical or hormonal influence on axonal excitability⁽²⁰⁾. By this study, we could mention to the effects of hormones on PNS, whose effects on CNS are well known. In patients with diabetes, longer or shorter SDTC values (indicating increased or decreased axonal excitability) have been reported, and these changes are thought to develop owing to different mechanisms, such as changes in persistent Na^+ conductance, membrane potential or passive membrane properties^(12,15,19,21). There are many effects of hyperglycaemia on CNS, and peripheral axonal excitability studies may be shown as an example of effects of hyperglycaemia on ion channels of PNS. In another study, we found increased SDTC of motor fibres following

hyperventilation for 20 min in healthy subjects⁽²¹⁾. Hyperventilation have also an effect on CNS and cause a sensation of vertigo. In the light of these studies, we can speculate that different metabolic and physiological conditions affect both CNS and PNS possibly by acting on ion channels. Hence, we wondered whether there is an axonal excitability changes in PNS of epileptic patients whose axonal excitability is changeable in CNS through the ion channels.

We investigated the strength-duration properties of sensory and motor axons to show the possible changes in persistent Na^+ conductances in peripheral nerves of patients with partial and generalized epilepsy. Although they had experienced seizures for some time, most of them had not been prescribed anticonvulsant drugs before, and only 5 of them had been prescribed anticonvulsant therapy for some period, but they had not taken these drugs for at least 2 years. In this group of epileptic patients, SDTC values were not different between patients and control group. However, rheobase and threshold (stimulus strength at 0.1 ms and 1ms) of motor axons in the total patients were higher than in control subjects. When partial and generalized epilepsy subgroups were compared with control group, threshold (stimulus strength at 1ms) of sensory axons in the patients with partial epilepsy was higher than in control subjects, and threshold (stimulus strength at 0.1 ms) of motor axons in the patients with generalized epilepsy was higher. These results suggest a decreased axonal excitability and possible decreased Na^+ conductances in peripheral nerves in patients with epilepsy.

There were not any statistical differences between partial epilepsy group and generalized epilepsy group. We studied parameters which are dependent on persistent Na^+ channels. The similar results in these epilepsy types may result from these channels' playing a role in both of

these epilepsy types. Studying multiple nerve excitability measurements and electrophysiological methods associated with functions of more specific channels (such as Na⁺, K⁺ and Ca²⁺ subtypes) playing a role in different epilepsy types may disclose differences.

Kiernan et al had studied the excitability of sensory and motor axons in five adult generalized epilepsy with febrile seizures plus (GEFS+) patients with established mutations in the beta1 subunit of Na⁺ channels (SCN1B). In that study, multiple nerve excitability measurements (SDTC, refractoriness, relative refractory period, superexcitability) were recorded using threshold tracking and they found higher threshold in patients than in control subjects. The dominant effects of the mutation underlying GEFS+ was a reduction in the number of functioning Na⁺ channels in peripheral nerve axons. The same beta subunit mutation seemed to have a dual effect with hyperexcitability of central neurons but reduced peripheral nerve excitability⁽¹¹⁾.

In this study, we could only measure the parameters which are dependent on persistent Na⁺ channels. Our limited findings suggest a decreased axonal excitability in the PNS of epileptic patients. We believe that there may be found more definite changes in the PNS of epileptic patients by applying both genetic studies on channel pathologies and by measuring multiple parameters of axonal excitability dependent on different types of voltage-gated ion channels in a larger study groups.

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REFERENCES

1. Brigant JL, Mallart A. Presynaptic currents in mouse motor endings. *J. Physiol. (Lond)* 1982; 333:619-636.
2. Burke D, Kiernan MC, Bostock H. Excitability of human axons. *Clin. Neurophysiol.* 2001;112: 1575-1585.
3. Bostock H, Cikurel, K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. *Muscle Nerve.* 1998; 21:137-158.
4. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage gated sodium channels. *Pharmacol. Rev.* 2005; 57: 397-409.
5. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 1989;30: 389-99.
6. Kanai K, Kuwabara S, Arai K, et al. Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment. *Brain.* 2003;126:965-973.
7. Kiernan MC, Bostock H. Effects of membrane polarization and ischemia on the excitability of human motor axons. *Brain.* 2000;123:2542-2551.
8. Kiernan MC, Burke D, Andersen KV, et al. Multiple measures of axonal excitability: a new approach in clinical testing. *Muscle Nerve.* 2000; 23:399-409.

9. Kiernan MC, Hart IK, Bostock H. Excitability properties of motor axons in patients with spontaneous motor unit activity. *J. Neurol. Neurosurg. Psychiatry.* 2001; 70:56- 64.
10. Kiernan MC, Lin CS, Andersen KV, et al. Clinical evaluation of excitability measures in sensory nerve. *Muscle Nerve.* 2001; 24:883-892.
11. Kiernan MC, Krishnan AV, Lin CS, et al. Mutation in the Na⁺ channel subunit SCN1B produces paradoxical changes in peripheral nerve excitability. *Brain.* 2005; 128:1841-1846.
12. Krishnan AV, Kiernan MC. Altered nerve excitability properties in established diabetic neuropathy. *Brain.* 2005; 128: 1178–1187.
13. Kuwabara S, Cappelen-Smith C, Lin CS, et al. Excitability properties of median and peroneal motor axons. *Muscle Nerve.* 2000; 23:1365–1373.
14. Meldrum BS, Rogawski MA. Molecular targets for antiepileptic drug development. *Neurotherapeutics.* 2007; 4:18-61.
15. Misawa S, Kuwabara S, Ogawara K, et al. Strength–duration properties and glycemic control in human diabetic motor nerves. *Clin. Neurophysiol.* 2005; 116: 254–258.
16. Mogyoros I, Kiernan MC, Burke D, et al. Excitability changes in human sensory and motor axons during hyperventilation and ischaemia. *Brain.* 1997; 120: 317–325.
17. Mogyoros I, Kiernan MC, Burke D, et al. Strength–duration properties of sensory and motor axons in amyotrophic lateral sclerosis. *Brain.* 1998; 121: 851–859.
18. Priori A, Cinnante C, Pesenti A, et al. Distinctive abnormalities of motor axonal strength–duration properties in multifocal motor neuropathy and in motor neurone disease. *Brain.* 2002; 125: 2481–2490.
19. Yerdelen D, Koc F, Uysal H, et al. Strength–duration properties in diabetic polyneuropathy. *Diabetes Res. Clin. Pract.* 2007; 78: 56–59.
20. Yerdelen D, Uysal H, Koc F, et al. Effects of sex and age on strength-duration properties. *Clin. Neurophysiol.* 2006; 117: 2069-2072.
21. Yerdelen D., Uysal H, Koc F, et al. The effect of hyperventilation on strength– duration properties in diabetic polyneuropathy. *Clin. Neurophysiol.* 2007;118: 105– 110.
22. Weiss G. Sulla possibilite de rendle comparables entre eux les appareils servant a` l'excitation electrique. *Arch. Ital. Biol.* 1901; 35:413–446.