Mutation in the Na\(^+\) channel subunit SCN1B produces paradoxical changes in peripheral nerve excitability

Matthew C. Kiernan, Arun V. Krishnan, Cindy S.-Y. Lin, David Burke and Samuel F. Berkovic

1Prince of Wales Medical Research Institute and Prince of Wales Clinical School, University of New South Wales, 2Department of Neurology, Prince of Wales Hospital, 3Institute of Clinical Neurosciences, University of Sydney and Royal Prince Alfred Hospital, Sydney and 4Epilepsy Research Centre, University of Melbourne, Austin Health, Victoria, Australia

Correspondence to: Dr Matthew C. Kiernan, Prince of Wales Medical Research Institute, Barker Street, Randwick, Sydney, NSW 2031, Australia
E-mail: M.Kiernan@unsw.edu.au

To determine the effect of an established mutation of the \(\beta 1\) subunit of Na\(^+\) channels on nerve excitability, studies were undertaken in patients diagnosed with generalized epilepsy with febrile seizures plus (GEFS\(^+\)). Multiple nerve excitability measurements were used to investigate the membrane properties of sensory and motor axons in five patients (aged 18–55 years) who were currently experiencing no seizures and were not on anticonvulsants. There was no history of paraesthesiae, fasciculation or cramps to suggest hyperexcitability of peripheral nerve axons. The median nerve was stimulated at the wrist, and compound muscle action potentials (CMAPs) were recorded from abductor pollicis brevis and the antidromic compound sensory nerve action potential (CSAPs) from digit 2. Stimulus–response behaviour, strength–duration time constant, threshold electrotonus, current–threshold relationship and the recovery of excitability following a supramaximal conditioning stimulus were recorded using threshold tracking. Compared with normal controls (\(n = 29\)), the axons of patients were of higher threshold. CMAPs and CSAPs were relatively small, although individual values remained within the normal ranges. Refractoriness and relative refractory period (markers of transient Na\(^+\) channel function) were significantly reduced in GEFS\(^+\) patients with established mutations in SCN1B (\(P < 0.05\)), and strength–duration time constants (dependent on persistent Na\(^+\) conductances) were reduced. It is suggested that, in peripheral nerve axons, the mutation underlying GEFS\(^+\) reduces the number of functioning Na\(^+\) channels at the node of Ranvier and that this rather than any change in gating of individual channels dominates axonal excitability in these patients.

Keywords: Na\(^+\) channel; SCN1B; epilepsy; axonal excitability

Abbreviations: CMAP = compound muscle action potential; CSAP = compound sensory nerve action potential; GEFS\(^+\) = generalized epilepsy with febrile seizures plus; RRP = relative refractory period

Received February 4, 2005. Revised March 21, 2005. Accepted April 1, 2005

Introduction

Sodium (Na\(^+\)) channels are critical for neuronal function in both the CNS and PNS. Na\(^+\) channels comprise a single \(\alpha\) subunit that forms a pore and a number of auxiliary \(\beta\) subunits, the best known being SCN1B and SCN2B. The distinct \(\alpha\) isoforms of skeletal and cardiac muscle are not represented in the CNS or in peripheral nerves. Mutations in skeletal muscle and cardiac muscle genes have been known for some time, but there has been some scepticism whether neuronal sodium channels could be associated with human disease as the excitability function was thought to be so critical to neuronal function that any perturbation might not be compatible with life. However, in the recently recognized epilepsy syndrome of generalized epilepsy with febrile seizures plus (GEFS\(^+\)) (Scheffer and Berkovic, 1997; Singh et al., 1999),

© The Author (2005). Published by Oxford University Press on behalf of the Guarantors of Brain. All rights reserved. For Permissions, please email journals.permissions@oupjournals.org
mutations were first described in SCN1B (Wallace et al., 1998) and subsequently in SCN1A (Escayg et al., 2000; Abou-Khalil et al., 2001; Wallace et al., 2001). Mutations of these genes are the major recognized cause of this childhood epilepsy syndrome (Wallace et al., 1998, 2001; Escayg et al., 2000; Abou-Khalil et al., 2001), with the common phenotypes of febrile seizures and febrile seizures plus. The phenotype febrile seizures plus refers to children who have seizures with fever outside the age of range of 6 months to 6 years or afebrile tonic–clonic seizures (Scheffer and Berkovic, 1997; Singh et al., 1999).

Individuals from families with GEFS+ with established mutations in SCN1B have no clinical features of peripheral nerve dysfunction. However, as the specific Na⁺ channel subunits associated with GEFS+ are present in peripheral nerves, it is conceivable there would be subtle electrophysiological abnormalities in the patients. Such findings would have two major applications. First, whilst Na⁺ channel mutation deficits have been studied in relatively simple systems in vitro (oocytes and mammalian cell culture), their effects on in vivo neuronal systems have been studied only in knockout experiments, which can provide little information about abnormal gene function, as distinct from absent gene function. Secondly, if robust abnormalities could be identified in peripheral nerves, they might provide a neurophysiological mechanism for detecting and monitoring nervous system dysfunction in these human epilepsies.

We therefore performed electrophysiological measurements, designed to be sensitive to Na⁺ channel dysfunction, on peripheral nerve axons of adult patients with a single mutation in SCN1B. In this mutation, a serine is substituted by a phenylalanine, resulting in the breaking of a putative disulfide bridge. In vitro, this mutation has the effect of loss of function of the modulatory β subunit, and this is presumed to lead to increased neuronal excitability (Wallace et al., 1998).

Methods
Measurements of nerve excitability were undertaken in five adult patients (aged 18–55 years; three female, two male) from two previously published families (Singh et al., 1999; Wallace et al., 2002). Each patient had the C121W mutation in SCN1B. Brief case histories are given below. All had a history of febrile convulsions, and a normal neurological examination; none had intellectual disability. The study was restricted to adult patients who were not currently prescribed anticonvulsant therapy (which could have confounding effects on axonal excitability).

Case histories
Patient 1
A 34-year old male (IV-94 in Singh et al., 1999) had febrile seizures plus, as defined above. Onset was at 18 months, and he had numerous febrile and afebrile seizures until the age of 19 years. An episode of status epilepticus occurred at 2 years. He was originally treated with valproate 800 mg daily but had not received medication for some years.

Patient 2
A 55-year old female (III-32 in Singh et al., 1999) had febrile seizures plus. She suffered ~50 seizures with fever between the ages of 2 and 12 years but had never received anti-epileptic medication.

Patient 3
A 50-year old female (V-17 in Wallace et al., 2002) had febrile seizures plus, with onset at 18 months. She had ~20 seizures with fever, ceasing by the age of 14 years. She was treated with phenobarbital in childhood but was not on medication when tested.

Patient 4
An 18-year old male (VI-30 in Wallace et al., 2002) had febrile seizures plus. Febrile convulsions began at the age of 18 months and afebrile generalized tonic–clonic seizures developed at the age of 3 years. Staring spells of uncertain nature began at age 5 years and, when 11 years, he developed partial seizures of both simple and complex type. He was successfully treated with topiramate and vigabatrin, ceased medication at the age of 13 years and has remained seizure free.

Patient 5
A 36-year old female (V-21 in Wallace et al., 2002) had febrile seizures plus. She suffered approximately five seizures with fever between 2 and 8 years of age and was not on medication when tested.

Procedures
Studies were performed using a previously described protocol designed to measure a number of different nerve excitability parameters rapidly (Kiernan et al., 2000, 2001). Compound muscle action potentials (CMAPs) were recorded from thenar muscles using surface electrodes over the abductor pollicis brevi, with the active electrode at the motor point and the reference on the proximal phalanx. Antidromic compound sensory nerve action potentials (CSAPs) were recorded from digit 2. The signals were amplified and digitized by computer (486 PC) with an A/D board (DT2812, Data Translation Inc., Marlboro, MA), using a sampling rate of 10 kHz. Stimulus waveforms generated by the computer were converted to current with a purpose-built isolated linear bipolar constant current source (maximum output ±50 mA). The stimulus currents were applied via non-polarizable electrodes (Red Dot, 3M Health Care, Borken, Germany), with the active electrode over the median nerve at the wrist, and the reference electrode ~10 cm proximal over the muscle. Stimulation and recording were controlled by QTRAC software (version 5.2, Institute of Neurology, London, with the multiple excitability protocol, TRONDXM).

Test current pulses were applied at 0.8 s intervals, combined with supra-threshold conditioning stimuli or subthreshold polarizing currents as required. The amplitude of the CMAP was measured from baseline to negative peak. CSAP amplitudes were measured from negative peak to the subsequent positive peak after baseline subtraction. Skin temperature was monitored close to the stimulation site and kept >32°C.

The sequence of recordings followed that previously described (Kiernan et al., 2000, 2001). Stimulus–response curves were recorded separately for test stimuli of duration 0.2 and 1 ms (motor; Fig. 1A) or 0.1 and 0.5 ms (sensory; Fig. 2A). The stimuli were increased in 6% steps, with two responses averaged for each step, until three averages were considered maximal. The ratio between the
to track the target compound potential (40% of maximal) while the threshold was changed by 100 ms polarizing currents, set to 40% (depolarizing) and −40% (hyperpolarizing) of the control threshold current (Fig. 1F). Each stimulus combination was repeated until three valid threshold estimates were recorded, as judged by the response being within 15% of the target response, or alternate responses being on either side of the target.

The current–threshold relationship (Fig. 1C) was tested at the end of 200 ms polarizing currents, which were altered in 10% steps from +50% (depolarizing) to −100% (hyperpolarizing) of the control threshold. As with the conventional threshold electrotonus protocol, stimuli with conditioning currents were alternated with test stimuli alone, and each stimulus combination was repeated until three valid threshold estimates were obtained.

The final part of the protocol recorded the recovery of excitability following a supramaximal conditioning stimulus (Fig. 1F). These changes were recorded at 18 conditioning–test intervals, decreasing from 200 to 2 ms in an approximately geometric sequence. From the recovery cycle, the following parameters were measured: the relative refractory period (RRP) defined as the interstimulus interval at which threshold recovered to its control value (or, in the absence of superexcitability, the first threshold minimum); refractoriness measured as the threshold increase following a supramaximal stimulus at a conditioning–test interval of 2.5 ms; superexcitability measured as the greatest percentage reduction in threshold; and late subexcitability, measured as the greatest percentage increase in threshold following the superexcitable period (Kiernan et al., 2000, 2001).

Values for each excitability parameter in the current study are expressed as the mean ± SEM and comparison is made with normative data established in previous studies on normal control subjects (Kiernan et al., 2000, 2001a). Data were compared using unpaired two-tailed t tests. Because measurements of refractoriness are very sensitive to skin temperature (Kiernan et al., 2000, 2001a), the individual measurements were all compensated for temperature, using the relationship found in normal control subjects (Kiernan et al., 2001a), before statistical tests were applied.

**Results**

The sequence of excitability measurements described in the Methods was recorded in each patient, and the mean data for the five patients are presented in Figs 1 (motor data) and 2 (sensory data).

Stimulus–response curves for the test stimuli of duration 0.2 and 1 ms are plotted on log–log coordinates (Figs 1A and 2A). The patients had relatively small CMAPs (3.1 ± 0.8 mV; Fig. 1A) and CSAPs (15.8 ± 5.7 μV; Fig. 2A). Axons were of high threshold in the patients, as indicated by a shift to the right of the stimulus–response curve for sensory and motor axons. The threshold for a CMAP 50% of maximum using 1.0 ms stimuli was 5.9 ± 1.0 mA for patients and 4.6 ± 0.2 mA for control subjects \( (P = 0.08) \). Similarly, the threshold for sensory axons using 0.5 ms stimuli was 5.6 ± 0.6 mA for patients and 4.2 ± 0.2 mA for control subjects \( (P < 0.01) \). The two stimulus–response curves in Fig. 1A are replotted in Fig. 1B on linear axes, normalized by plotting the responses as a percentage of maximum and the stimuli as percentages of the stimulus for a response 50% of maximum. The curves for

**Fig. 1** Changes in excitability of motor axons in epilepsy patients with the C121W mutation in the SCN1B subunit, compared with mean data for normal controls (Kiernan et al., 2000). (A) Mean stimulus–response relationships for patients (continuous lines) and controls (dashed lines) for the test stimuli of 1 ms (left of each pair) and 0.2 ms duration (right of each pair). (B) Normalized stimulus–response relationships. (C) Current–threshold relationships. (D) Strength–duration time constants. (E) Threshold electrotonus for 100 ms polarizing currents, ±40% of the resting threshold, i.e. the changes in threshold during and after subthreshold depolarizing and hyperpolarizing currents (±40% of threshold) lasting 100 ms. (F) Recovery of axonal excitability following a single supramaximal conditioning stimulus.
The 0.2 and 1 ms stimuli are very similar and superimpose quite closely on the middle broken line, which represents the mean control data for 1 ms stimuli.

The current–threshold relationships (Fig. 1C) reflect the rectifying properties of the axon (both nodal and internodal axolemma), and the slope of the curve can be used to provide an estimate of the threshold analogue of input conductance. The plots are orientated such that decreases in the threshold occur to the right and increases in threshold occur to the left. The steepening of the curve towards the top right with increasingly strong depolarizing currents results from outward rectification, due to activation of fast and slow K+ channels, while the less prominent steepening towards the bottom left with increasingly strong hyperpolarizing currents indicates inward rectification, due to activation of the hyperpolarization-activated conductance (Ih). The current–threshold relationships were identical for patients and control subjects in the depolarizing direction, as illustrated in Fig. 1C for motor axons. There was a trend for greater changes in threshold for the same currents during hyperpolarization, consistent with the changes in threshold electrotonus (see below and Fig. 1E), but this was not statistically significant.

The strength–duration time constant is a measure of the rate at which the threshold current for a target potential declines as stimulus duration is increased (Mogyoros et al., 1996). Rheobase is the threshold for a stimulus that can be infinitely long, and both are properties of the nodal membrane. The voltage dependence of these properties is determined by persistent Na+ currents that are active near the threshold (Bostock and Rothwell, 1997). Strength–duration time constants were calculated for different fractions of the compound potential (e.g. Figs 1D and 2B). As is usual, the strength–duration time constant (for a CMAP or CSAP 40% of maximal) was significantly less for motor axons than sensory axons (Mogyoros et al., 1996), and both were less in the patients when compared with controls (sensory, 0.41 ± 0.03 ms for patients, 0.53 ± 0.02 ms for controls, P < 0.05; motor axons, 0.36 ± 0.03 ms for patients, 0.43 ± 0.02 ms for controls, not significant; Figs 1D and 2B). Rheobase current for a 50% potential was increased (sensory, 2.9 ± 0.5 mA for patients, 1.9 ± 0.1 mA for controls, P < 0.005; motor, 4.1 ± 0.8 mA for patients, 3.2 ± 0.2 mA for controls, P = 0.06). Taken together, the shorter strength–duration time constant and higher rheobase suggest reduction in persistent Na+ currents (Bostock et al., 1998; Burke et al., 2001).

The mean changes in excitability occurring during and after subthreshold depolarizing and hyperpolarizing currents lasting 100 ms are plotted for motor axons in Fig. 1E. More prominent changes in threshold occurred with hyperpolarizing currents, with a greater increase for motor axons of patients at the 90–100 ms interval (141 ± 4.1%) than controls (117.7 ± 2.7%; P < 0.05). The recovery following hyperpolarizing currents was less in motor axons for the patients (peak overshoot 9.8 ± 1.6% for patients; 16.5 ± 0.7 for controls; P < 0.001) and for sensory axons (11.4 ± 0.8% for patients; 19.0 ± 0.8% for controls; P < 0.01).

The recovery of excitability following a supramaximal conditioning stimulus was flatter in patients: the RRP was shorter

---

**Fig. 2** Changes in sensory nerve excitability properties in patients with the C121W mutation in the SCN1B subunit compared with mean data for normal controls (Kiernan et al., 2001a). (A) Mean stimulus–response relationships for patients (continuous lines) and controls (dashed lines) for the test stimuli of 0.5 ms (left of each pair) and 0.1 ms duration (right of each pair). (B) Strength–duration time constants. (C) Recovery cycles.

**Fig. 3** Comparison of selected axonal excitability parameters for sensory (S) and motor (M) nerves compared with data for normal controls. Results are expressed as mean ± SEM for (A) strength–duration time constant, (B) rheobase current, (C) refractoriness and (D) relative refractory period (RRP). Patient data were compared with normal controls using two-tailed t tests.
Mutation in SCN1B and axonal excitability

Brain (2005) Page 5 of 6

(FIGS 1F AND 2C) AND REFRACTORINESS LESS IN PATIENTS (SENSORY, 11.2 ± 5.1% FOR PATIENTS AND 29.6 ± 2.9% FOR CONTROLS, P < 0.05; MOTOR, 10.8 ± 4.0% FOR PATIENTS AND 27.1 ± 2.9% FOR CONTROLS; P < 0.05). SUPERCITABILITY WAS ALSO REDUCED (SENSORY, 13.9 ± 2.4% FOR PATIENTS AND 17.2 ± 0.8% FOR CONTROLS; MOTOR, 20.2 ± 3.4% FOR PATIENTS AND 25.3 ± 1.0% FOR CONTROLS). AS DISCUSSED BELOW, THE ABNORMALITIES IN EXCITABILITY RECORDED FROM GEFS+ PATIENTS WITH ESTABLISHED MUTATIONS IN SCN1B ARE LIKELY TO BE DUE TO A SMALLER NA+ CURRENT.

DISCUSSION

THE PRESENT STUDY HAS INVESTIGATED THE EXCITABILITY OF SENSORY AND MOTOR AXONS IN VIVO IN FIVE ADULT GEFS+ PATIENTS WITH ESTABLISHED MUTATIONS IN SCN1B. THE PATIENTS HAD NOT EXPERIENCED SEIZURES FOR SOME YEARS, AND WERE NOT ON ANTI-EPILEPTIC MEDICATION. THE DATA DEMONSTRATE COMPLEX ABNORMALITIES IN PARAMETERS DEPENDENT ON NA+ CHANNEL FUNCTION. AXONS IN THE PATIENTS WERE OF HIGHER THRESHOLD THAN IN CONTROL SUBJECTS, AND THE COMPOUND POTENTIALS WERE RELATIVELY SMALL. REFRACTORINESS AND THE RRP ARE MARKERS OF TRANSIENT NA+ FUNCTION, AND THE STRENGTH–DURATION TIME CONSTANT IS DEPENDENT ON PERIODIC NA+ CONDUCTANCES. THESE MEASURES WERE REDUCED IN PATIENTS. IT WILL BE ARGUED THAT THE CHANGES IN PERIPHERAL NERVE EXCITABILITY RESULT FROM ALTERATIONS IN THE NA+ CURRENT, PROBABLY DUE TO A REDUCED NUMBER OF FUNCTIONING CHANNELS, POSSIBLY ASSOCIATED WITH A HYPERPOLARIZING SHIFT IN THE MEMBRANE POTENTIAL.

THE PATIENTS WITH GEFS+ IN THE PRESENT STUDY ALL CARRY A MUTATION IN SCN1B (GNE FOR THE β1 SUBUNIT OF NA+ CHANNELS), WHICH DISRUPTS A PUTATIVE DISULFIDE BRIDGE THAT NORMALLY STABILIZES AN EXTRACELLULAR DOMAIN OF THE β1 SUBUNIT. PREVIOUS IN VITRO STUDIES HAVE SUGGESTED THAT THIS MUTATION RESULTS IN LOSS OF FUNCTION OF THE MODULARITY B SUBUNIT (WALLACE ET AL., 1998), LEADING TO INCREASED OPENING TIME OF THE CHANNEL AND AN INCREASE IN NEURONAL EXCITABILITY. HOWEVER, OTHER FACTORS COULD MODIFY NA+ CHANNEL BEHAVIOUR IN VIVO. VOLTAGE-DEPENDENT NA+ CHANNELS UNDERLIE ACTION POTENTIAL GENERATION AND ARE THE CHIEF DETERMINANTS OF AXONAL EXCITABILITY IN HUMAN NERVE (SCHWARZ ET AL., 1995; BURKE ET AL., 2001). TWO FUNCTIONALLY DISTINCT SODIUM CURRENTS CAN BE DISTINGUISHED IN HUMAN PERIPHERAL NERVES (BOSTOCK AND ROTHWELL, 1997): ‘TRANSIENT’ NA+ CURRENT, THROUGH CHANNELS THAT ACTIVATE RAPIDLY WITH MEMBRANE DEPOLARIZATION AND THEN INACTIVATE, SO THAT FURTHER NA+ IONS CANNOT PASS THROUGH THE CHANNEL NO MATTER HOW MUCH THE MEMBRANE IS DEPOLARIZED; AND ‘PERSISTENT’ NA+ CURRENT, THROUGH CHANNELS THAT ACTIVATE EQUALLY RAPIDLY BUT UNDERGO MINIMAL INACTIVATION.

PATIENTS IN THE PRESENT STUDY WITH GEFS+ AND ESTABLISHED MUTATIONS IN SCN1B HAD CLEAR ABNORMALITIES IN TRANSIENT NA+ CHANNEL FUNCTION, SPECIFICALLY REDUCTIONS IN THE LEVEL OF REFRACTORINESS AND THE DURATION OF THE RRP. SUCH CHANGES COULD SUGGEST LESS INACTIVATION OF NA+ CHANNELS IN GEFS+ PATIENTS AT REST. THIS CHANGE IN CHANNEL GATING IN GEFS+ WOULD INCREASE THE ABILITY OF NERVES TO FIRE ACTION POTENTIALS SPONTANEOUSLY AND AT HIGH FREQUENCY, AS DOCUMENTED BY IN VITRO STUDIES. DISRUPTION OF THE EXTRACELLULAR DOMAIN IN SCN1B IS EXPECTED TO RESULT IN REDUCED NA+ CHANNEL EXPRESSION, IN ADDITION TO SLOWER INACTIVATION AND SLOWER RECOVERY FROM INACTIVATION (McCORMICK ET AL., 1998). REFRACTORINESS AND THE DURATION OF THE REFRACTORY PERIOD ARE DETERMINED BY INACTIVATION OF TRANSIENT NA+ CHANNELS BY PRIOR DEPOLARIZATION. THE FINDING OF LOWER REFRACTORINESS AND SHORTER REFRACTORY PERIODS IN GEFS+ PATIENTS WITH ESTABLISHED MUTATIONS IN SCN1B, ENABLING A FASTER RECOVERY FROM INACTIVATION, COULD RESULT FROM THE EFFECTS OF A REDUCED NA+ CURRENT AND/OR A HYPERPOLARIZING SHIFT IN MEMBRANE POTENTIAL.

a hyperpolarizing shift in membrane potential and, thereby, secondary changes in measures of excitability, and these would probably be more prominent for sensory axons.

In intact humans, interpreting ion channel behaviour at the molecular level must be speculative, and is fraught with difficulty because of cascading secondary effects of the primary change in gating behaviour, any one of which could dominate the physiological findings. As argued above, a parsimonious explanation for many of the present findings is that, in peripheral nerve axons, the dominant effect of the mutation underlying GEFS+ is a reduction in the number of functioning Na\(^+\) channels, and that this rather than the changes in gating behaviour of individual channels determines the nodal Na\(^+\) current. Such a change would not result in hyperexcitability. Our patients had outgrown their seizures but, in any case, it should be noted that GEFS+ patients with established mutations in SCN1B do not complain of paraesthesiae, fasciculation and cramps, as might be expected with hyperexcitability of peripheral nerve axons. It is possible that the different effects of the \(\beta\) subunit mutation in peripheral axons compared with central neurons may reflect the expression of a different ensemble of Na\(^+\) channel \(\alpha\) subunits in the two regions (Cummins et al., 2004). Nevertheless, a dual effect of the mutation could explain why the same \(\beta\) subunit mutation is associated with hyperexcitability of central neurons but reduced peripheral nerve excitability. The issue of why patients with a fixed genetic defect have an age-specific epilepsy is not well understood, but an intriguing possibility is that the tendency of seizures to abate as patients mature results from a change in the balance between these differing effects on neuronal excitability. We conclude that peripheral nerve changes documented here could represent an accessible marker of the underlying mutation. It would be of interest to carry out similar studies on unmedicated patients currently experiencing seizures, although it remains possible that peripheral axons may not demonstrate hyperexcitability even through the patients are experiencing seizures.

Acknowledgements

We wish to thank Dr Mark Baker for helpful comments on the manuscript, and Dr Ingrid Scheffer for assistance in recruiting subjects. M.K. was supported by the Australian Physicians Independent Neuroscience Award (Pfizer Neuro Science Research Grant), Grant support from the Australian Research Council and the National Health & Medical Research Council of Australia is acknowledged.

References


Bharucha VA, Chen C, Chen Y, et al. Mice lacking sodium channel \(\beta\)2 subunits have reduced sodium channel densities and negative shifts in the voltage dependence of channel inactivation. Soc Neurosci Abstr 1999; 26: 1109.


Ogata N, Ohishi Y. Molecular diversity of structure and function of the human node of Ranvier. Pflugers Arch 1995; 430: 283–92.


