Differences in Membrane Properties in Simulated Cases of Demyelinating Neuropathies: Internodal Focal Demyelinations without Conduction Block

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Abstract. The membrane properties (intracellular, extracellular, electrotonic potentials, strength-duration time constants, rheobasic currents and recovery cycles), which can now be measured in healthy subjects and patients with demyelinating neuropathies, are investigated in simulated cases of focal reduction (70%) of the myelin sheath in one, two and three successive internodal segments along the length of human motor fibres. The internodally focally demyelinated cases (termed as IFD1, IFD2 and IFD3, respectively) are simulated using our previous double cable model of the fibres. The results show that the intracellular potentials are with reduced amplitude and slowed conduction velocity in the vicinity of demyelinated segments, however the segmental conduction block is not achieved. The radial decline of the extracellular potential amplitudes slightly increases with the increase of the radial distance and demyelination. In contrast, the electrotonic potentials, strength-duration time constants and rheobases are normal. In the recovery cycles, the refractoriness, supernormality and less late subnormality are close to the normal, showing that the pathology is relatively minor. The obtained abnormalities in the potentials and excitability properties provide new information about the pathophysiology of the demyelinated human motor axons and can be observed *in vivo* in patients with acquired demyelinating neuropathies.

Key words: acquired demyelinating neuropathies, computational neuroscience, potentials, recovery cycles, strength-duration properties

1. Introduction

In some hereditary neuropathies, the demyelination process uniformly affects the nerve throughout its length, delaying saltatory conduction more or less at all the nodes of Ranvier. Charcot-Marie-Tooth disease type 1A (CMT1A) is the most common form of hereditary motor and sensory neuropathy and its hallmark is uniform demyelination [1, 2]. In contrast, segmental conduction slowing/block in certain parts of the nerve characterizes acquired demyelinating neuropathies with non-uniform involvement. The segment of maximal involvement varies from one patient to the next. This helps explain the diversity of clinical findings and of conduction abnormalities in different cases of Guillain-Barré syndrome (GBS), which

consists of a number of subtypes showing different clinical and pathological features. Guillain-Barré syndrome is classified into acute inflammatory demyelinating polyneuropathy (AIDP), acute motor sensory axonal neuropathy (AMSAN) and acute motor axonal neuropathy (AMAN) by pathological and electrodiagnostic criteria [3–5]. Accuracy in establishing the diagnosis in CMT1A and GBS is related, in part, to the appropriate application of electrodiagnostic techniques. Using a non-invasive technique of threshold tracking, the indices of the axonal excitability, such as threshold electrotonus, strength-duration time constants, rheobases and recovery cycles can now be measured in healthy subjects and patients with GBS and CMT1A [6–8]. These indices depend on the biophysical properties of the axonal membrane and myelin sheath. In the recent years, the method of mathematical modeling is also applied as a promising tool for studying the potentials and excitability properties in nerve disorders [9–13]. A previous study [12] investigating these indices in simulated cases of CMT1A shows that the abnormally greater negative electrotonic responses, longer strength-duration time constants, greater refractoriness (the increase in threshold current during the relative refractory period) and supernormality can be used as specific indicators of internodal systematic demyelinations of nerves.

The aim of the present study is to investigate the abnormalities in the potentials and axonal excitability properties of three simulated focally demyelinated cases of human motor nerve fibres applying appropriate stimulated methods for the fibres. The results are consistent with the interpretation that factors causing the focal reduction of the myelin sheath could be responsible for the obtained abnormalities.

2. Methods

The electrogenesis of myelinated human motor axons can be studied successfully using the double cable model of these fibres [14–16]. The interested reader is referred to the first figure of [14] for an electric equivalent circuit representation of the human motor fibre. In the cited paper, Kirchhoff's current law is used to derive a system of partial differential equations for the electric equivalent circuit. The latter is based on a complex extended double cable structure of nodal, paranodal and internodal segments with their corresponding ion (nodal and internodal) channels. The model assumes a high-resistance myelin sheath and a leakage pathway to the internodal axolemma via the paranodal seal resistance and periaxonal space. The same double cable model is used here to simulate the focal demyelinations, which are defined as a reduction (70%) of the myelin lamellae in one (10th), two (9th and 10th) and three (8th, 9th and 10th), respectively, successive internodal segments along the fibre length. The internodally focally demyelinated cases are termed as IFD1, IFD2 and IFD3, respectively. The normal fibre has $N = 150$ myelin lamellae. Its myelin sheath is characterized with C_m (myelin capacitance) 1.5 pF and R_m (myelin resistance) 250 M Ω . Each parameter value for a given spiral differs in arithmetic progression with N . The rule of the arithmetic increasing or decreasing of the value, however, depends on the type of the given parameter. For

70% demyelination, the myelin parameter values are $N = 47$, $C_m = 4.8$ pF and $R_m = 78.3$ M Ω . The other membrane parameter values for the normal motor fibre are the same as described earlier [14, 16], i.e. C_n (nodal capacitance) 1 pF, C_i (internodal axolemmal capacitance) 350 pF, R_{ax} (axoplasmic resistance) 8 M Ω , R_{pa} (periaxonal resistance) 300 M Ω , R_{pn} (paranodal seal resistance) 125 M Ω , V_r (nodal resting potential) -86.7 mV, V_{ra} (internodal axolemmal resting potential) -86 mV, I_{pump}^* (net outward current generated by electrogenic Na^+/K^+ pump) 0.1 nA; channel types and maximum permeabilities ($\text{cm}^3\text{s}^{-1} \times 10^{-9}$): node Na (sodium) 9, K_f^* (fast potassium) 0.07, K_s^* (slow potassium) 0.26; internode, Na^* (sodium) 80, K_f^* (fast potassium) 27, K_s^* (slow potassium) 2, IR^* (inward rectifier) 0.008, L_k^* (leak) 0.0064; ion concentrations (mM): $[\text{Na}^+]_i$ 9, $[\text{Na}^+]_o$ 144.2, $[\text{K}^+]_i$ 155, $[\text{K}^+]_o$ 3. The model fibre comprises 30 nodes of Ranvier and 29 internodes. Each internode is divided into 2 paranodal and 5 internodal segments. All calculations are carried out for fibres with an axon core diameter of 12.5 μm . The lengths of node, paranode and nodal center to nodal center are 1.5, 200 and 1400 μm , respectively. The temperature is 37°C . The normal myelin thickness of 2.4 μm gives an external fibre diameter of 17.3 μm . In the IFD1, IFD2 and IFD3 cases both the myelin thickness and external diameter are reduced.

The stimulation, to produce intracellular potentials is simulated by adding a short (0.1 ms) rectangular current pulse to the center of the first node to correspond to the case of point fibre polarization. This case of intracellular point current application at the center of the node closely approximates the effects of external point stimulation with a needle electrode and realizes a point fibre polarization. The intracellular potentials in the case of adaptation (i.e. in the case of simultaneously intracellular current application at the midpoints of all the available axonal segments) are simulated by adding a long-lasting suprathreshold depolarizing pulse. This case closely approximates the effects of external surface stimulation with a large electrode and realizes a periodic kind of uniform fibre polarization. The model generated intracellular potentials, in the second case of fibre stimulation, are then used as input to a line source model [17], that allows to calculate the corresponding extracellular potentials at various radial distances in the surrounding volume conductor. The electrotonic potentials are simulated by adding a 100 ms subthreshold current to the center of each axonal segment and the case of periodic kind of uniform fibre polarization is also realized. The periodic kind of uniform polarization is first simulated in the human motor electrotonus model [15], since the *in vivo* situation for recording threshold electrotonus in man, where current is applied to the peripheral nerve via its high-resistance sheath and a 1 cm diameter surface electrode [18], is thought to correspond more closely to periodic kind of uniform polarization than point polarization of the fibre. The electrotonic potentials are calculated for hyperpolarizing and depolarizing currents, which correspond to 0.4 times the threshold for a 1 ms current pulse.

The indices of the axonal excitability delivered from single or pairs of threshold stimuli (such as strength-duration and charge-duration curves, strength-duration

time constants, rheobases and recovery cycles) are investigated in the case of adaptation. The strength-duration time constants and rheobasic currents are calculated by the method, which is described earlier [9, 11, 12] and, in brief, the threshold stimulus duration is increased in 0.025 ms steps from 0.025 ms to 1 ms, to obtain the strength-duration curves. A polynomial function of degree 2 (transfer standard parabola), which relates threshold charge (Q) to stimulus duration (t), provides an accurate fit of the data: $Q = a_2[t^2 + (a_1/a_2)t + a_0/a_2]$, where a_0 , a_1 , a_2 are the polynomial coefficients. The strength-duration time constant is defined as the absolute value of the smallest square root of the function (i.e. only one of both direct intercepts of the regression curve on the duration axis has a biophysical sense and only this direct intercept is shown on the below given figures). The rheobasic current is defined as the final decreased threshold value, after which the potential generation is not obtained with the increase of the stimulus duration. When two equal pulses are used in pairs, the response of second (testing) pulse in the refractory period may be greater or less than the response of the first (conditioning) pulse, depending on the conditioning-test intervals. To obtain the time course of recovery of the axonal excitability following a single threshold stimulus (the recovery cycle), test stimuli of 1.0 ms duration are delivered at conditioning-test intervals of 2–100 ms after a threshold conditioning stimulus of 1.0 ms duration. In the recovery cycle, the axon has supernormality when the threshold current of the testing pulse is less than the threshold current of the conditioning pulse and vice versa, the axon has subnormality when the testing pulse necessary to generate second potential is stronger than the conditioning pulse.

3. Results

A comparison between the intracellular and electrotonic potentials for the IFD1, IFD2 and IFD3 cases is presented for the human motor nerve fibres (Figure 1). The temporal intracellular potentials (Figure 1A) are at each node, from the 7th to the 14th. For a given focal demyelination, the intracellular potentials at successive nodes are not of constant amplitude. Although the rate of rise of the potential at the 10th, 9th and 10th, 8th, 9th and 10th nodes is gradually reduced in the IFD1, IFD2 and IFD3 cases, respectively, the potential recovers in subsequent nodes. Increasing the degree of IFD causes a decrease in the potential amplitude. The potentials are propagated with desynchronized amplitudes and velocities. However, the segmental conduction block is not achieved for all investigated demyelinated cases. The conduction velocities, calculated from the times of the potential maxima, for example, at the 9th and 10th nodes are 58, 31, 35 and 40 m/s for the normal, IFD1, IFD2 and IFD3 cases, respectively. The potential amplitudes at the 10th nodes are 38, 28, 24 and 23 mV for the normal, IFD1, IFD2 and IFD3 cases, respectively. (The normal case with uniform amplitudes and velocities of the potentials along the fibre length is not given in Figure 1A). The results show that lengthening the demyelinated zone causes a more rapid decrease of the potential amplitudes and velocities in this zone. However, in the regions distal to the demyelinated zone,

Figure 1. Comparison between the intracellular (A) and electrotonic (B) potentials of human motor fibres in the IFD1, IFD2 and IFD3 (*continued lines*) cases, respectively, as the electrotonic potentials are compared with the normal case (*dotted lines*). The intracellular potentials in response to 0.1 ms threshold current pulses are presented at each node from the 7th to the 14th, respectively. The electrotonic potentials in response to 100 ms depolarizing and hyperpolarizing current pulses ($\pm 40\%$ of threshold) are also presented at each node from the 7th to the 14th, respectively.

for example, at the 14th node, the conduction occurs at its normal velocity and the potential amplitude achieves its normal value. The temporal distributions of the electrotonic potentials (Figure 1B) are also presented at each node, from the 7th to the 14th. There are no differences in the electrotonic potentials during and after 100 ms depolarizing and hyperpolarizing currents ($\pm 40\%$ of threshold) between

the normal and IFD1 cases. For the normal case, each node behaves identically, and an overlap of the potentials at the nodes is obtained. However, when the normal case is compared with the IFD2 and IFD3 cases, the obtained differences are the smaller slow depolarizing and hyperpolarizing phases to the polarizing currents. The depolarizing and hyperpolarizing electrotonic potentials are similar, with a small drop to a minimum amplitude in the 10th node. In the IFD3 case, the depolarizing and hyperpolarizing responses are with a bigger drop to a minimum amplitude again in the 10th node and with a bigger raise to a maximum amplitude in the 14th node than those for the IFD2 case. However, the differences between these two cases are very small and almost invisible.

Figure 2 illustrates the temporal distributions of the intracellular potentials (V) and their corresponding extracellular potentials (P), in the case of adaptation for the normal and demyelinated fibres. For all cases, the potentials are again at each node, from the 7th to the 14th. For the normal case each node behaves identically,

Figure 2. In the case of adaptation, the temporal intracellular potentials (V) and their corresponding extracellular potentials (P) in the normal and three IFD cases are presented for human motor fibres. The intracellular potentials are simulated by adding long-lasting depolarizing pulses, which correspond to 1.1 times the threshold for a 1 ms current pulse. The potentials are at each node from the 7th to the 14th, respectively. The extracellular potentials are given for three radial distances ($r = 0.05$, 1.0 and 5.0 mm).

Figure 3. Comparison between the strength-duration curves, charge-duration curves, strength-duration time constants and rheobasic currents in the normal and three IFD cases of motor human fibres. An overlap of these excitability indices is obtained.

and an overlap of the potentials at the nodes is obtained. For the demyelinated cases, the intracellular and extracellular potentials at the nodes are different and also superimposed. Close to the fibre membrane ($r = 0.05$ mm), the extracellular potentials have a two-phase shape, while further in the volume conductor ($r = 1.0$ mm), the potentials have the usual three-phase shape. With the increase of the radial distance and degree of demyelination, the peak-to-peak amplitude of the extracellular potentials decreases lightly. At large radial distances ($r \geq 5$ mm), the potentials become the same in shape.

The strength-duration and charge-duration curves for the normal and investigated IFD cases are also shown (Figure 3 – first row). To provide a better illustration of the strength-duration time constants and rheobasic currents, histograms are also used (Figure 3 – second row). In the same strength-duration curves, the threshold currents are normal in the IFD1, IFD2 and IFD3 cases. The strength-duration time constants and rheobases are also normal with the increase of the degree of demyelination.

The excitability changes of the human motor axons in the normal and demyelinated cases, during 100 ms recovery cycles, are illustrated in Figure 4. The recovery

Figure 4. Comparison between the recovery cycles in the normal and three IFD cases of motor human fibres. The internodally focally demyelinated fibres show slightly greater threshold changes in supernormal and subnormal periods than normal. The duration of each period for the demyelinated cases is similar. For all investigated cases the y-axis is defined as $100 \times (I_{\text{test}} - I_{\text{cond}})/I_{\text{cond}}$ (%), where I_{test} (nA) and I_{cond} (nA) are the threshold currents of the testing and conditioning pulses, respectively.

cycles show that the excitability changes of the axons in the normal and IFD cases are similar and almost identical. The axonal types are initially unexcitable, then excitable with a raised threshold and after about 2.5 ms they are superexcitable. In the unexcitable case, the axons are absolutely refractory (1–2.3 ms), during which they cannot generate second potential no matter how strong are the testing depolarizing pulses. The axons are then relatively refractory (2.3–2.5 ms), during which a stronger than conditioning stimulus is required to generate second potential. In the superexcitable (2.5–10 ms) case, the testing stimulus necessary to generate second potential is less than the conditioning stimulus. The superexcitability is usually followed by a late subexcitability. In the overlap recovery cycles, all abnormal axons have slightly less refractoriness, greater supernormality and less late subnormality than those for the normal axon.

4. Discussion

The present study shows a number of differences in the potentials and excitability properties between the simulated normal and internodally focally demyelinated

human motor nerve axons. An expecting result is the reduced amplitude, prolonged duration and slowed conduction velocity of the intracellular potentials for the investigated segmental demyelinations. The described earlier kinetics of the ionic currents defining the intracellular potentials [19] shows that the simultaneously decrease of the myelin resistance and increase of the myelin capacitance result in an increase of the external membrane current. (In the paranodal and mid-internodal segments, the external membrane current is equal to the transmyelin current). The given explanation for the reduced conduction obtained is an increase in the transmyelin current generation and the limitation for the externally recorded nodal current to pass longitudinally via the paranodal seal resistance to the periaxonal space.

The electrotonic potentials allow the accommodative responses to polarizing currents to be documented. The results show, that there are no significant differences between the normal and IFS cases in the accommodated responses to prolonged subthreshold depolarizing and hyperpolarizing currents. This suggests that, for the axons studied, the behaviour of internodal accommodative conductances is slightly affected by the demyelinating pathology. The obtained electrotonic potentials are similar to those described in demyelinating neuropathies, namely AIDP.

The current study also shows that the indices of the axonal excitability, such as, strength-duration and charge-duration curves, strength-duration time constants and rheobases are normal in the investigated cases of internodal focal demyelinations. Such excitability properties are also characteristic for AIDP patients [7]. The recovery cycles depend on the regenerative depolarization caused by the first (conditioning) afterpotentials and can be explained by the delay-dependent second (testing) potentials. The pattern of the recovery cycles for the normal and IFD cases are similar and such recovery cycles are obtained by [7] for the median motor nerves in AIDP patients and normal controls. The results confirm that the axonal excitability properties are almost normal when the degree of segmental demyelinations cannot cause conduction block. Moreover, all obtained potentials are quite different from those of our previous report [12], in which the model simulated demyelinations (internodal) without conduction block are systematic (uniform), not focal. Our investigations show that the changes in the electrotonic potentials in the case of mild systematic demyelinations are abnormally greater than those in the case of mild focal demyelinations. This can explain the reason for measured minor changes in the threshold electrotonus in AIDP patients obtained by [7], where focal demyelinated cases without conduction block are also investigated. We plan to show in a later study that simulated cases of internodal focal demyelinations, causing segmental conduction block, produce rather different changes to those described here and can also be observed *in vivo* in patients with AIDP. Our unpublished data show that the transition from conduction slowing to conduction block leads to amplification of the degree of potential and excitability changes, as the direction of these changes is kept the same.

In conclusion, the obtained abnormal potentials and axonal excitability indices can be used as specific indicators of the internodal focal demyelinations without

conduction block and may provide new information about the pathophysiology in acquired demyelinating neuropathies.

References

1. Birouk, N., Gouider, R., Le Guern, E., Gugenheim, M., Tardieu, S., Maisonnobe, T., Le Forestier, N., Agid, Y., Brice, A. and Bouche, P.: Charcot-Marie-Tooth disease type 1A with 17p11.2 duplication. Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases, *Brain* **120** (1997), 813–823.
2. Dyck, P.J., Chance, P., Lebo, R. and Comey, A.J.: Hereditary Motor and Sensory Neuropathies, In: P.J. Dyck, P.K. Thomas, J.W. Griffin, P.A. Low and J.F. Poduslo (eds.) *Peripheral Neuropathy*, 3rd edn., W.B. Saunders, Philadelphia, (1993), pp. 1094–1136.
3. Choudhury, D. and Arora: Axonal Guillain-Barré Syndrome: A Critical Review, *Acta Neurol. Scan.* **103** (2001), 267–277.
4. Feasby, T.E., Gilbert, J.J., Brown, W.F., Bolton, C.F., Hahn, A.F., Koopman, W.F. and Zochodne, D.W.: An Acute Axonal Form of Guillain-Barré Polyneuropathy, *Brain* **109** (1986), 1115–1126.
5. Griffin, J.W., Li, C.Y., Ho, T.W., Xue, P., Macko, C., Gao, C.Y., Yang, C., Tian, M., Mishu, B. and Cornblath, D.R.: Guillain-Barré Syndrome in Northern China. The Spectrum of Neuropathological Changes in Clinically Defined Cases, *Brain* **118** (1995), 575–595.
6. Kuwabara, S., Bostock, H., Ogawara, K., Sung, J.Y., Kanai, K., Mori, M., Hattori, T. and Burke, D.: The Refractory Period of Transmission is Impaired in Axonal Guillain-Barré Syndrome, *Muscle Nerve* **28** (2003), 683–689.
7. Kuwabara, S., Ogawara, K., Sung, J.Y., Mori, M., Kanai, K., Hattori, T., Yuki, N., Lin, C.S., Burke, D. and Bostock, H.: Differences in Membrane Properties of Axonal and Demyelinating Guillain-Barré Syndromes, *Ann. Neurol.* **52** (2002), 180–187.
8. Nodera, H., Bostock, H., Kuwabara, S., Sakamoto, T., Asanuma, K., Sung, J.Y., Ogawara, K., Hattori, N., Hirayama, M., Sobue, G. and Kaji, R.: Nerve Excitability Properties in Charcot-Marie-Tooth Disease Type A1, *Brain* **127** (2004), 203–211.
9. Daskalova, M. and Stephanova, D.I.: Strength-Duration Properties of Human Myelinated Motor and Sensory Axons in Normal Case and in Amyotrophic Lateral Sclerosis, *Acta Physiol. Pharmacol. Bulg.* **26** (2001), 11–14.
10. Stephanova, D.I. and Daskalova, M.: Extracellular Potentials of Human Motor Myelinated Nerve Fibers in Normal Case and in Amyotrophic Lateral Sclerosis, *Electromyogr. Clin. Neurophysiol.* **42** (2002), 443–448.
11. Stephanova, D.I. and Daskalova, M.: Differences in Potentials and Excitability Properties in Simulated cases of Demyelinating Neuropathies. Part II. Paranodal demyelination, *Clin. Neurophysiol.* **116**(5) (2005), 1159–1166.
12. Stephanova, D.I., Daskalova, M. and Alexandrov, A.S.: Differences in Potentials and Excitability Properties in Simulated Cases of Demyelinating Neuropathies. Part I, *Clin. Neurophysiol.* **116**(5) (2005), 1153–1158.
13. Stephanova, D.I., Daskalova, M. and Kristev, I.: Excitability Changes During the Recovery Cycle of Human Myelinated Motor and Sensory Axons in Normal Case and in Amyotrophic Lateral Sclerosis, *Acta Physiol. Pharmacol. Bulg.* **26** (2001), 41–44.
14. Stephanova, D.I. and Bostock, H.: A Distributed-Parameter Model of the Myelinated Human Motor Nerve Fibre: Temporal and Spatial Distributions of Action Potentials and Ionic Currents, *Biol. Cybern.* **73** (1995), 275–280.
15. Stephanova, D.I. and Bostock, H.: A distributed-Parameter Model of the Myelinated Human Motor Nerve Fibre: Temporal and Spatial Distributions of Electrotonic Potentials and Ionic Currents, *Biol. Cybern.* **74** (1996), 543–547.

16. Stephanova, D.I. and Mileva, K.: Different Effects of Blocked Potassium Channels on Action Potentials, Accommodations, Adaptation and Anode Break Excitation in Human Motor and Sensory Myelinated Nerve Fibres: Computer Simulations, *Biol. Cybern.* **83** (2000), 161–167.
17. Stephanova, D.I., Trayanova, N., Gydikov, A. and Kossev, A.: Extracellular Potentials of a Single Myelinated Nerve Fiber in an Unbounded Volume Conductor, *Biol. Cybern.* **61** (1989), 205–210.
18. Bostock, H., Baker, M. and Reid, G.: Changes in Excitability of Human Motor Axons Underlying Post-Ischaemic Fasciculations: Evidence for Two Stable States, *J. Physiol. (Lond.)* **441** (1991), 537–557.
19. Stephanova, D. and Kossev, A.: Action Potentials and Ionic Currents Through Internodally Demyelinated Human Motor Nerve Fibres: I. Computer Simulations, *Comp. Rend. l'Acad. Bulg. Sci.* **50**(3) (1997), 107–110.