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Nerve Conduction and Neuromuscular Transmission in C57Bl/6 Mice with Genetically Determined Peripheral Neuropathy

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Charcot–Marie–Tooth disease is one of the most widespread forms of hereditary peripheral neuropathy in humans. C57Bl/6 mice are considered the most appropriate animal model for studies of this disease. We measured the conduction velocity in the sciatic nerve (NCV) and amplitude of the M-wave in mice of strains C57Bl/6 and C57Bl. It was found that the mean conduction velocity in the right and left sciatic nerves of C57Bl/6 mice was about 3.5-fold lower than that in C57Bl animals. In the former mice, the mean amplitude of compound nerve action potentials (CNAPs) and that of the M-wave in the *m. gastrocnemius-soleus* after stimulation of the sciatic nerve were 5- and 4-fold, respectively, lower, than those in the control (C57B1). Thus, the data obtained show that the genetically determined pathology of the peripheral nervous system caused by a mutation of the *PMP22* gene results in dramatic negative shifts of the characteristics of conduction via the peripheral nerves and of neuromuscular transmission.

Keywords: congenital peripheral neuropathy, C57Bl/6 mice, sciatic nerve, combined action potential, conduction velocity, M-wave.

INTRODUCTION

Functional disorders in the peripheral nervous system (PNS) can result from various traumas, aging, and a number of diseases, including genetically determined hereditary ones [1]. Pathologies of the PNS are quite common, especially in aged subjects [2]. Depending on the degree of PNS damage, dysfunction of this system can lead to atrophy of skeletal muscles and changes in the perception of peripheral stimuli, nociceptive and thermal in particular [3, 4]. In humans, electrophysiological methods are extensively used in the diagnostics of neuromuscular disorders and neuropathies. Measurements of the conduction velocity via peripheral nerves are routine procedures in this case. Changes in this parameter may provide physicians with objective information on the intensity of pathological changes in these nerves, in particular on the degree of demyelinating processes [5]. Recording of compound nerve action potentials (CNAPs), whose amplitude correlates with a number of functional excited nerve fibers, and of the so-called M-wave reflecting the number of excited motor fibers and the state of neuromuscular transmission, is rather valuable for objective diagnostics of different neuropathies [1, 6].

One of the most common forms of hereditary peripheral neuropathy in humans is Charcot–Marie–Tooth disease (CMT). This is an autosomal dominant peripheral neuropathy of the 1A type (CMT1A) associated with duplication of DNA. Such duplication may occur at the site of the gene for a peripheral myelin protein 22 (*PMP22*) located on chromosome 17p11.2. PMP22 is an integral membrane protein expressed in Schwann cells and localized in the peripheral myelin sheath. [7, 8]. Mice of the C57Bl/6 strain have an identical leucine–proline mutation in the transmembrane region of the PMP22 protein. Mice of this strain are recognized as the most adequate animal model for research of CMT disease. At the same time,

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objective data on functional changes in the PNS of such animals are rather limited.

Thus, we tried to estimate the differences between the conduction velocities via a large peripheral nerve and characteristics of neuromuscular transmission in mice of strain C57B1/6 with hereditary peripheral neuropathy and of strain C57B1 without such disease.

METHODS

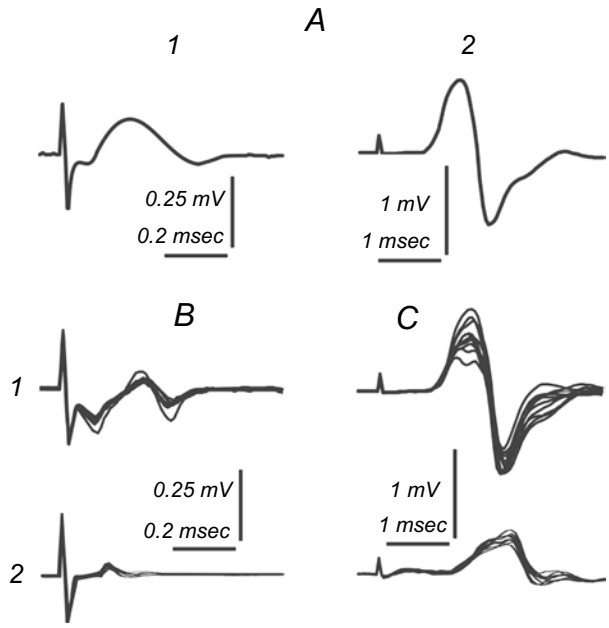
Experimental groups. Adult mice with body mass 23–28 g were used in the experiments. Healthy animals of the C57B1 strain (n = 6) formed the first control group, while the second group included mice of the C57B1/6 strain with hereditary peripheral neuropathy (n = 6). Animals of these two groups were obtained from The Jackson Laboratory (USA). Animals of the second (C57B1/6) group were characterized by noticeable tremor of the hindlimbs.

Surgery and electrical stimulation. The mice were anesthetized with sodium pentobarbital (Sigma, USA, 75 mg/kg, i.p.) and fixed in a specialized stereotaxic framework with a system of fixation of the head, pelvis, and limbs. Sciatic nerves in both hindlimbs were opened, separated from surrounding tissues, and transected proximally. On the incisions, pools were formed and filled with paraffin oil. Bipolar Ag–AgCl wire electrodes with the interelectrode distance of about 2.5 mm were used for electrical stimulation of the nerve (rectangular 0.2-msec-long pulses) and recording of the propagated wave. The stimulation intensity providing minimum contractions of the hindlimb muscles was considered the threshold one. Subsequent stimulation was provided with an intensity of 1.3 threshold at a frequency 3 sec^{-1}; stimulus trains were 30 sec long. Usually, three stimulation series separated by 60-sec-long intervals were used.

During stimulation of the nerve, we also recorded the M wave from the *m. gastrocnemius-soleus* (GS) using two Ag–AgCl electrodes (diameter 0.15 mm) inserted via injection needles to the depth of 2–3 mm. The interelectrode distance during M-wave recording was 4–4.5 mm.

During the entire experiment, the heart rate, ECG amplitude and temperature of the body and in the oil pool were monitored. A 12-bit AD/DA converter, CED Power 1401 (Cambridge Electronic Design, Great Britain), was used for recording of the signals. For stimulation of the nerve, a DS2A stimulator with isolated outputs (Digitimer, Great Britain) was used. The recorded signals were amplified by a Model 440 amplifier (Brownlee Precision, USA) and digitized at a 10^{-4} sec frequency. The conduction distance between the active stimulating electrode and proximal recording electrode was measured using a calipers.

Data processing. Data analysis was performed using Spike 2 (Cambridge Electronic Design, Great Britain) and Origin 7.0 (OriginLab Corporation, USA). Mean values of the conduction velocity via the nerve, CNAP amplitude, and M wave amplitude upon stimulation of proximal parts of the right and left sciatic nerves in animals of two examined groups were compared using two-way ANOVA. In the case of significant intergroup differences ($P < 0.05$), the *a posteriori* Bonferroni criterion was applied. Numerical data are shown below as means ± ± s.e.m.



F i g. 1. Potentials evoked by electrical stimulation of the sciatic nerve in experimental mice. A) Examples of the compound nerve action potential (CNAP) recorded from the distal region of the above nerve (1) and of the M-wave recorded from the *m. gastrocnemius-soleus* (*m*. GS) of a mouse of the control group (C57Bl). B) Examples of superposed CNAPs recorded from a control mouse of strain C57Bl (1) and from a mouse of strain C57Bl/6 (2). C) Examples of superposed M-waves recorded form mice of the two above groups (1 and 2, respectively).

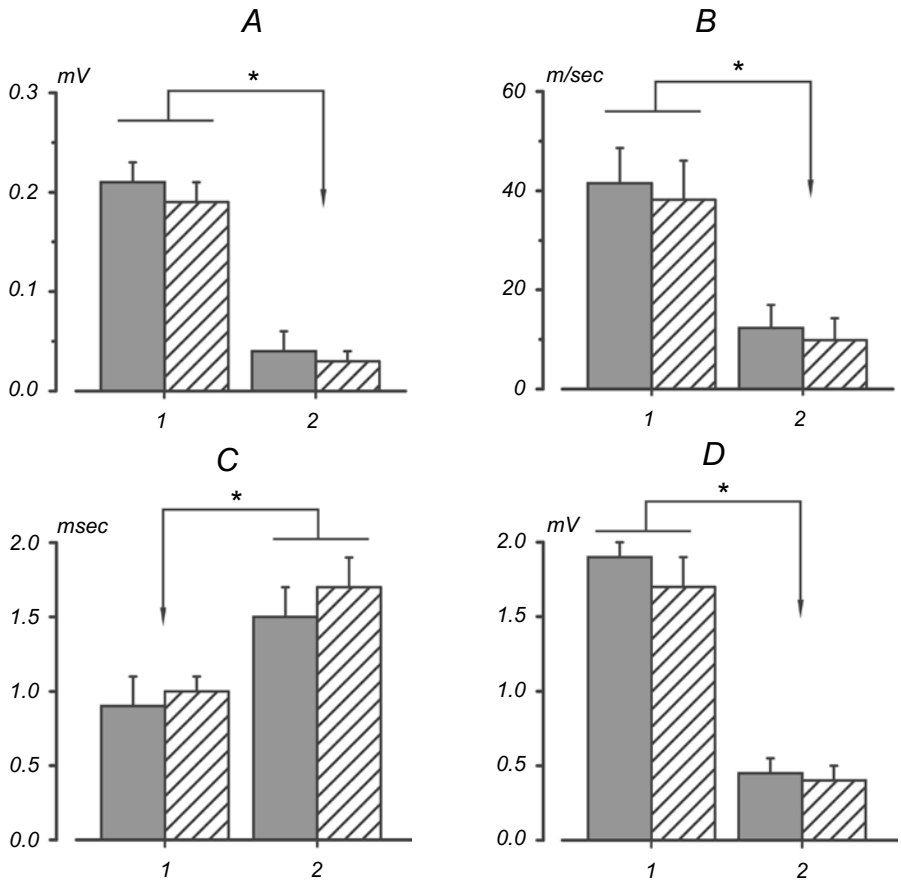
RESULTS

Electrical stimulation of proximal parts of the sciatic nerves of mice belonging to both experimental groups evoked clear CNAPs in more distal parts of these nerves and M-waves in the GS of both hindlimbs (Fig. 1 A). This allowed us to measure the conduction velocity via the above nerves and amplitude/time characteristics of the evoked M-waves.

The CNAPs recorded in all mice of group C57Bl/6 clearly differed from the respective potentials observed in mice of group C57Bl by lower amplitudes, greater durations, and longer delays (Fig. 1 B). The mean amplitude of CNAPs in mice of the latter (control) group with no pathology of nerve fibers was 0.21 ± 0.02 and 0.19 ± 0.02 mV in the right and left sciatic nerves, respectively. The mean conduction velocity in the above nerves of C57Bl mice was equal to 41.5 ± 6.9 and 38.2 ± ± 7.1 m/sec, respectively. In both cases, there were no significant differences between the mentioned values ($P > 0.05$).

The mean latency of the M-wave in the right and left *m. GS* of the control C57B1 mice was 0.9 ± 0.2 and 1.0 ± 0.1 msec; the mean amplitude of this wave was equal to 1.9 ± 0.1 and 1.7 ± 0.2 mV for the right and left limbs, respectively, and all these values did not show statistically significant differences (Fig. 2 A, B).

The respective measurements in mice of group C57Bl/6 showed that all these parameters were characterized by highly significant differences from the analogous values in mice of the control C57Bl group. The mean values of the CNAP amplitude for the right and left sciatic nerves in animals of this group were only 0.04 ± 0.02 and 0.03 ± 0.01 mV, i.e., these figures were nearly five times smaller than those in the control group ($P < 0.05$). The mean



F i g. 2. Mean values (M ± s.e.m.) of the parameters of potentials evoked by electrical stimulations of the sciatic nerve in mice of the experimental groups. A) Amplitude of the compound nerve action potentials (CNAPs) in the distal part of the sciatic nerve; B) conduction velocity via this nerve; C) latency of the M-wave in the *m*. GS, and D) amplitude of the above wave. 1 and 2 are groups of the mice C57Bl and C57Bl/6, respectively; gray and dashed columns correspond to the data obtained at stimulation of the left and right sciatic nerves, respectively. Asterisks show cases of significant differences between values for animals of groups 1 and 2 ($P < 0.05$).

conduction velocities via the above nerves in mice with hereditary neuropathy were 12.3 ± 5.1 and 9.9 ± 4.8 m/sec, i.e., the difference in comparison with the control was about 3.5-fold ($P < 0.05$).

The differences between the parameters of M-waves in mice of the control and "neuropathic" groups were rather similar to the above described. The mean latencies of the M-wave in C57Bl/6 mice were 1.5 ± 0.2 and 1.7 ± 0.2 msec, respectively ($P > 0.05$ in comparison with the control). At the same time, the mean amplitude of the above wave in C57Bl/6 mice was 0.45 ± 0.01 and 0.40 ± 0.1 mV in the right and left limbs, respectively. In other words, the mean amplitude of this wave was about four times lower than in the control.

DISCUSSION

Thus, electrical stimulation of the sciatic nerves of control animals (strain C57Bl) and mice with hereditary neuropathy (C57Bl/6) showed that the measured electrophysiological indices typical of these groups demonstrate significant differences. First of all, CNAPs evoked by stimulation of the above nerves in the "neuropathy animals" were characterized by much (severalfold) smaller amplitudes and longer latencies (i.e., much smaller conduction velocities), as compared with the respective indices in control mice.

The CNAPs recorded under the experimental conditions used are formed due to summation of action potentials evoked by electrical stimulation in efferent and afferent fibers of the sciatic nerve (orthodromic impulses in the former case and antidromic ones in the latter case). It should be taken into account that, under our experimental conditions, the obtained data are related mostly to relatively thick (fast-conducting) nerve fibers. One of the crucial factors affecting the velocity of propagation of signals via nerve fibers can be structural changes in these axons leading to a decrease in the diameter of these axions as compared to the control. Such changes can be related to disorders in the process of formation of cytoskeletal structures and slowing down of transportation of neurofilaments. These effects are most probably determined by metabolic disorders and violations of phosphorylation of structural proteins. Disorders in the transport of neurofilaments may result, to a significant extent, in a decreased level of the nerve growth factor in nerve tissues [9, 10]. A myelinopathy state in the peripheral nerves can also be a significant reason for disturbed propagation of spike signals via the nerve fibers [1]. As was shown, the gene that codes protein PMP22 is a member of the gene family specific for delays of growth processes (growth arrest-specific). When such genes undergo mutations, this can result in disorders in the axonal growth and subsequent disorders of the process of propagation. Under conditions of a mutation of *PMP22*, the number of Schwann cells increases because of excessive mitotic activity [11]. Robertson et al. [11] described a threefold increase in the number of these cells in the sciatic nerves of Trembler-J mice. Such modifications may be associated with injuries of the Cajal bands in Schwann cells, and this can lead to changes in the distance between Ranvier nodes and significant slowing down of conduction via the nerve [12, 13].

We also observed that the amplitude of the M-wave in the *m*. GS C57Bl/6 mice is severalfold lower than that in control animals. The M-wave is a signal determined by the number of action potentials coming via motor nerve fibers to the neuromuscular junctions and by synchronization of these potentials. This muscle evoked potential is frequently used in studies of muscle fatigue and ion flows and activity of the Na^+-K^+ pump in the respective synapses. The latter factors determine the parameters of neuromuscular transmission in skeletal muscles. The M-wave can be interpreted as a predictor of the muscle force [14]. Measurements of the M-wave in mammalian muscles showed that high-frequency voluntary or stimulation-induced muscle contractions result in decreases in the amplitude of this wave and, finally, in decreases in the contraction force [15]. Recording of the M-wave is widely used in the analysis of the effects of pharmacological agents on muscle activity. Recording of the M-wave and measurement of its parameters is a necessary diagnostic component in the search for means preventing neurodegeneration phenomena [1].

Thus, we found that the conduction velocity via the sciatic nerve of mice C57Bl/6 is dramatically lower than that in the control, and such changes are bilateral. The process of neuromuscular synaptic transmission in this mice strain also demonstrates significant negative disorders. Such changes in the nerve and neuromuscular processes in mice C57Bl/6 confirm the statement that such mice can be considered a rather adequate animal model of Charcot–Marie–Tooth disease (of course, with certain reservations).

All experimental procedures on animals were approved by the Ethics Committee of the Bogomolets Institute of Physiology and performed in accordance with the Council of Europe convention, November 24, 1986 (86/609/EEC), as well as in compliance with the Law of Ukraine "On Protection of Animals from Inhumane Treatment," February 21, 2006, No. 3447-IV.

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