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The effect of methocarbamol and mexiletine on murine muscle spindle function

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Abstract

Introduction/Aims: The muscle relaxant methocarbamol and the antimyotonic drug mexiletine are widely used for the treatment of muscle spasms, myotonia, and pain syndromes. To determine whether these drugs affect muscle spindle function, we studied their effect on the resting discharge and on stretch-induced action potential frequencies of proprioceptive afferent neurons.

Methods: Single unit action potential frequencies of proprioceptive afferents from muscle spindles in the murine extensor digitorum longus muscle of adult C57BL/6J mice were recorded under resting conditions and during ramp-and-hold stretches. Maximal tetanic force of the same muscle after direct stimulation was determined. High-resolution confocal microscopy analysis was performed to determine the distribution of $Na_v1.4$ channels, a potential target for both drugs.

Results: Methocarbamol and mexiletine inhibited the muscle spindle resting discharge in a dose-dependent manner with IC_{50} values around 300 μM and 6 μM, respectively. With increasing concentrations of both drugs, the response to stretch was also affected, with the static sensitivity first followed by the dynamic sensitivity. At high concentrations, both drugs completely blocked muscle spindle afferent output. Both drugs also reversibly reduced the specific force of the extensor digitorum longus muscle after tetanic stimulation. Finally, we present evidence for the presence and specific localization of the voltage-gated sodium channel $Na_v1.4$ in intrafusal fibers.

Discussion: In this study we demonstrate that both muscle relaxants affect muscle spindle function, suggesting impaired proprioception as a potential side effect of both drugs. Moreover, our results provide additional evidence of a peripheral activity of methocarbamol and mexiletine.

Abbreviations: AU, arbitrary units; CNS, central nervous system; EDL, extensor digitorum longus; Na_v, voltage-gated sodium channel; Lo, baseline length; ACSF, artificial cerebrospinal fluid; DP, dynamic peak; SR, static response.

Bridgette Watkins and Hedwig M. Schuster have contributed equally to this work

KEYWORDS
intrafusal fiber, mechanosensation, muscle relaxant, proprioception, stretch response, voltage-gated sodium channel

1 | INTRODUCTION

Skeletal muscle relaxants can be classified as antispasticity or antispasmodic agents and they act in the central nervous system (CNS) or peripherally.[1-4] For many of these drugs, it is not known whether tissues other than skeletal muscle are also affected.

Muscle spindles are encapsulated sensory structures located within almost every skeletal muscle.[5] They provide the CNS with proprioceptive information, the processing of which is required for all coordinated movements.[5-7] Muscle spindles consist of specialized muscle fibers (intrafusal fibers) that are innervated by two types of neurons: in the central (equatorial) region, the terminals of group Ia and group II proprioceptive sensory neurons encircle intrafusal muscle fibers with annulospiral endings. These sensory nerve terminals are mechanosensitive structures and detect how much and how fast a muscle is lengthened. In addition, efferent gamma-motoneurons innervate the peripheral (polar) regions of intrafusal fibers (fusimotor innervation), where they form cholinergic synapses that are functionally and developmentally similar to the neuromuscular junctions formed by alpha-motoneurons on extrafusal fibers.[8,9]

Although the effects of peripherally acting muscle relaxants on extrafusal muscle fibers have been rather well characterized, their effect on muscle spindles is less well understood. Patients experience an increased risk of falls and bone fractures after the initiation of a skeletal muscle relaxant,[10] suggesting that these drugs in addition to skeletal muscle fibers may also affect proprioception. Therefore, data on the action of muscle relaxants on muscle spindles are essential to understand the mode of action of the drugs in patients.

In our study, we investigated the effect of a muscle relaxant (methocarbamol) and the antimyotonic drug mexiletine on proprioceptive sensory output of adult murine muscle spindles. Methocarbamol, mexiletine, and their derivatives belong to the most widely prescribed antispasmodic, non-benzodiazepine group of muscle relaxants.[3,11] Methocarbamol is used for the treatment of lower back pain, as an adjunct to physical therapy for the relief of acute musculoskeletal pain, such as after acute traumatic injury,[4,12-15] treatment of stiff-man syndrome,[16] and painful muscle spasm.[12,13,17-20] For many years, methocarbamol was considered a centrally acting relaxant[4,21,22]; however, recently the specific inhibition of the voltage-gated sodium channel 1.4 ($Na_v1.4$), but not of $Na_v1.7$, was reported.[23] Because $Na_v1.4$ is the primary voltage-gated sodium channel responsible for the initiation of action potentials in skeletal muscle fibers, a peripheral action of methocarbamol is likely to contribute to its muscle-relaxing activity. Accordingly, Crankshaw and Raper reported that methocarbamol caused a prolongation of the refractory period of cat tibialis muscle and a suppression of polysynaptic reflex contractions without an effect on spinal interneurons.[24,25]

Mexiletine is an orally active class IB antiarrhythmic, which is clinically used to treat cardiac arrhythmia, muscle cramps, and skeletal muscle channelopathies, including dystrophic and nondystrophic myotonias and myotonic syndromes.[26-34] Similar to methocarbamol, mexiletine's primary mechanism of action is blocking fast sodium channels, including $Na_v1.4$.[26,35] Like methocarbamol, mexiletine prolongs the refractory period of sodium channels by delaying their recovery from the inactivated state.[36]

In this study we tested the hypothesis that methocarbamol and mexiletine, in addition to their effect on extrafusal fibers, also influence muscle spindle function. Toward this end, we determined single unit sensory neuron afferent resting discharges and changes in the frequency of firing in responses to ramp-and-hold stretches. Moreover, because both drugs bind to the $Na_v1.4$ channel, we investigated its distribution in intrafusal fibers using high-resolution confocal microscopy.

2 | METHODS

2.1 | Animals and muscle preparation

Experiments were performed on muscles from 43 C57BL/6J mice of both sexes aged 10 to 15 weeks and weighing 22 to 28 grams. Fourteen animals were used for the electrophysiological analysis of methocarbamol, 16 for mexiletine, 9 were used to analyze vehicle (dimethysulfoxide or artificial cerebrospinal fluid), and 4 were used for immunocytochemistry. Animal procedures were performed according to guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. All experiments were approved by the local authorities of the State of Bavaria, Germany (ROB-55.2-2532.Vet 02-17-82).

2.2 | Electrophysiology

Afferent sensory neuron responses to stretch were assayed using an isolated muscle-nerve preparation, as described elsewhere.[37-40] In brief, the extensor digitorum longus (EDL) muscle from adult C57BL/6J mice was dissected and the sensory activity was determined using extracellular recording.[37] A detailed description of the electrophysiological analysis can be found in the Supplementary Information online.

The effect of the drugs on the resting discharge was quantified by determining the number of action potentials over a 30-second period at 15-minute intervals after addition of the drug. The results are shown as percent of the action potentials of the same muscle spindle before addition of drug. The effect of the drugs selectively on the dynamic and static response to ramp-and-hold stretches was analyzed by determining the dynamic peak

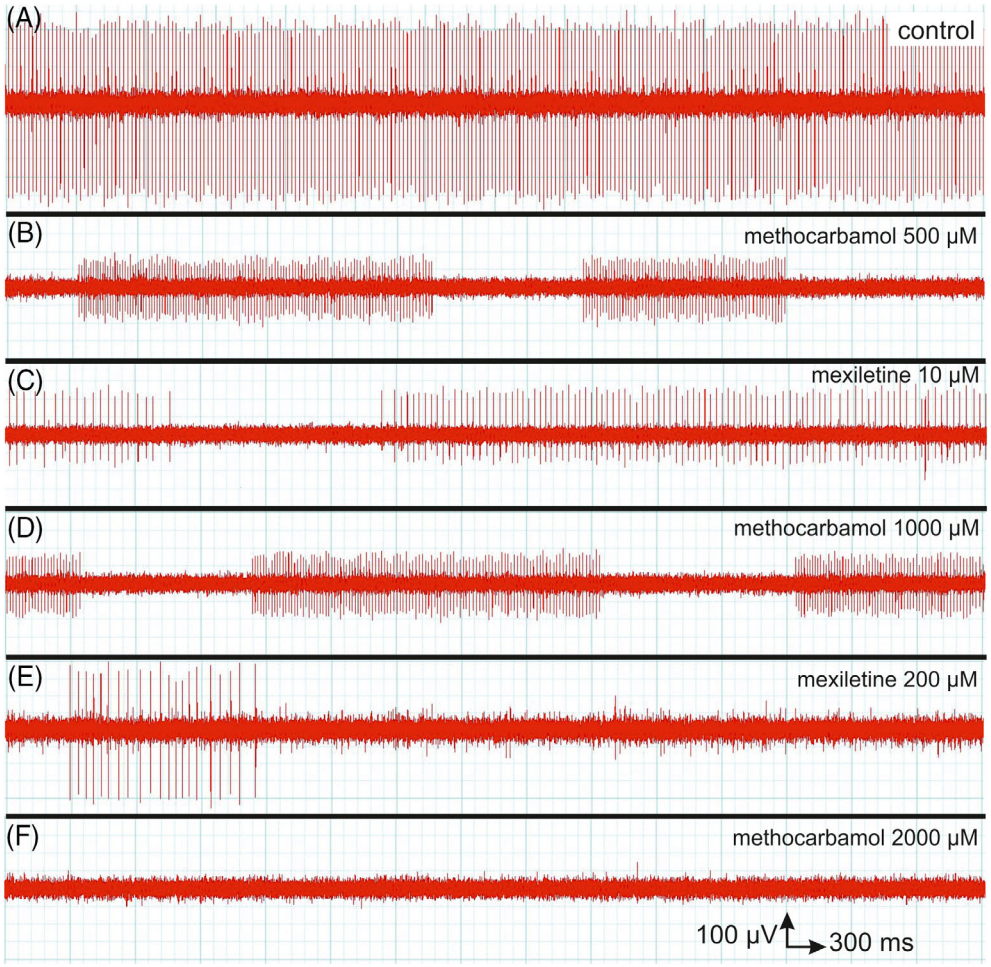

FIGURE 1 Mexiletine and methocarbamol reduce muscle spindle afferent firing in resting muscle spindles. Representative recordings of the resting discharge are shown in the absence (A) and presence (B-F) of various concentrations of mexiletine or methocarbamol. Each panel represents approximately 15 seconds of recording taken 60 minutes after addition of drug. Without any drug, muscle spindle afferents show a constant resting discharge frequency (A). In contrast, with increasing concentrations of either drug, the time in which the muscle spindle afferent did not fire action potentials ("silent periods") also increased. At concentrations of 2000 μM (methocarbamol) or 500 μM (mexiletine), the muscle spindle afferents were completely silenced (F, and data not shown). Different action potential amplitudes in the various recordings are the result of different resistance levels between suction electrode and nerve during the extracellular recordings and do not represent an effect of the drug. Scale bars for time and membrane potential are shown in F

(highest instantaneous frequency during ramp − baseline firing rate) and the static response (firing rate 3.25 to 3.75 seconds into stretch − baseline firing rate[39,40]) using LabChart version 8.1.5 (ADInstruments, Sydney, Australia). Dose-response curves were calculated using:

$$y = \frac{100}{1 = 10^{((logIC50 - x) \times hillslope)}}$$

where x = concentration (μM) ($\log_{10}$). The mean passive mechanical tension generated at maximum length (Lo) under resting conditions as well as 2 seconds after the start of the ramp-and-hold stretch were determined in triplicate and the values of the same muscle spindle were compared before and after addition of drug. The values are expressed as mean ± standard error of the mean (SEM), and the statistical significance of differences before and after addition of drug was determined using the unpaired *t* test.

2.3 | Maximal tetanic force

At the end of each recording, the maximal contractile force during a direct tetanic stimulation of the muscle via paddle electrodes in the tissue bath (500-millisecond train at 120 Hz and approximately 1-millisecond square pulse length, supramaximal voltage [Grass SD9 stimulator; Natus, Pleasanton, California]) was determined, as described elsewhere.[37,39,40] The specific force (force / physiological cross-sectional area: a measure of the general health status of the muscle) of the EDL muscle at Lo was determined in the presence and absence of

the drug as well as after a 1-hour washout. Toward this end, the EDL muscle was weighted at the end of the experiment (average weight was between 8 and 11 mg) and the maximal tetanic force was calculated using the equation:

$$\text{maximal tetanic force } \left[\text{N/cm}^2\right] = \frac{\text{Tension } [N]/1000}{\frac{\text{muscle weight } [g]}{Lo\ [cm] * 1.06\ [g/cm^3]}}.$$

The values were then compared with the previously reported peak force of the healthy EDL of 23.466 ± 6 N/cm^2.[37,41,42]

2.4 | Statistical analysis

The number of action potentials in a 30-second period before addition of the drug was counted and set as 100%. After addition of drug, the number of action potentials was counted again and expressed as percentage of the initial frequency before addition of drug. The means of the overall changes in firing rate of all groups were compared statistically vs the no-drug control group using one-way analysis of variance with Dunnett post hoc test. The Wilcoxon test was used to calculate the significance of effects of drug application on dynamic peak and static response during ramp-and-hold stimulations. The IC_{50} values were determined by plotting the log of the drug concentration against the normalized response (expressed as percent of control, which was set to 100%). All statistical analyses were performed using GraphPad Prism (GraphPad, Inc, La Jolla, California). The levels of significance (*P* values) for the statistical tests were set at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

2.5 | Immunocytochemistry

Immunofluorescence labeling was performed as described elsewhere.[39,40,43] Refer to the Supporting Information online for a more detailed description of the staining protocol.

Sensory nerve terminals were identified using antibodies from guinea pig against vGluT1 (AB5905; Millipore, Darmstadt, Germany [1:1000]).[9,39] The $Na_v1.4$ distribution was determined by staining with a polyclonal rabbit antibody (SCN4A; #ASC-020; Alomone Labs, Jerusalem, Israel [1:500]). This antibody is specific for the alpha-subunit of the $Na_v1.4$ channel and shows no cross-reactivity with other voltage-gated sodium channels. The S46 monoclonal antibody against the slow tonic myosin heavy chain 6, developed by Miller et al[44] (diluted 1:50), was obtained from the Developmental Studies Hybridoma Bank, created by the National Institute of Child Health of the National Institutes of Health and maintained at the University of Iowa (Department of Biology, Iowa City, Iowa).[45-47]

Primary antibodies were detected using the appropriate Alexa 488-, Alexa 594-, and Alexa 647-conjugated goat anti-rabbit (A11034; Thermo Fisher Scientific-Invitrogen, Waltham, Massachusetts [1:1000]), goat anti-guinea pig (A11076; Thermo Fisher-Invitrogen [1:1000]), or goat anti-mouse (A32723; Thermo Fisher-Invitrogen [1:1000]) secondary antibody. Actin filaments were labeled using Alexa 488-conjugated phalloidin (A123379; Thermo Fisher-Invitrogen [1:500]).

After immunofluorescence labeling, the sections were embedded in Mowiol mounting medium (Carl Roth, Karlsruhe, Germany) and analyzed using a laser scanning confocal microscope (LSM 710; Carl Zeiss AG, Oberkochen, Germany), as described elsewhere.[39,40] We observed no difference in the structure of muscle spindles from male and female mice.[48]

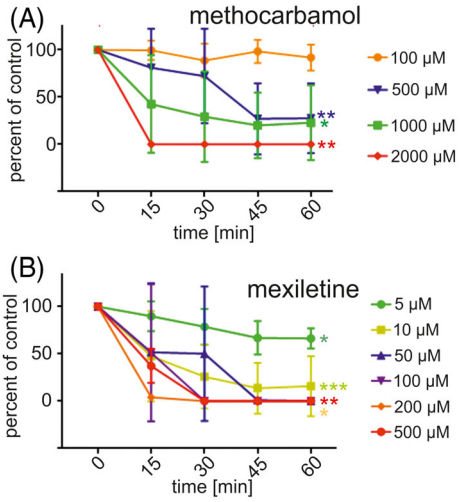

FIGURE 2 Dose- and time-dependent inhibition of muscle spindle resting discharge by mexiletine and methocarbamol. Different concentrations of methocarbamol (A) and mexiletine (B) were added to the bathing solution and the effect on the resting discharge over time was monitored every 15 minutes. The inhibitory effect of both muscle relaxants on the action potential frequency is expressed as percent of control, that is, the resting discharge before addition of drug. There was no significant difference between the 45-minute values and the 60-minute values for any drug or concentration, demonstrating that an equilibrium concentration was reached after 45 minutes. The values represent the mean ± standard error of the mean (SEM) of triplicate recordings of a single muscle spindle per extensor digitorum longus muscle from each of the 30 mice. For clarity, significant differences vs control (before addition of drug) are indicated by asterisks only for the 60-minute values. See Table S1 for values of the mean and SEM and the statistical significance of the other data points

3 | RESULTS

3.1 | Effect of methocarbamol and mexiletine on resting discharge of muscle spindle afferents

We first determined the action potential frequency in resting muscle spindles in the presence and absence of either relaxant. We did not observe an effect of either drug on the kinetics of individual action potentials or a gradual decline of the frequency of the resting

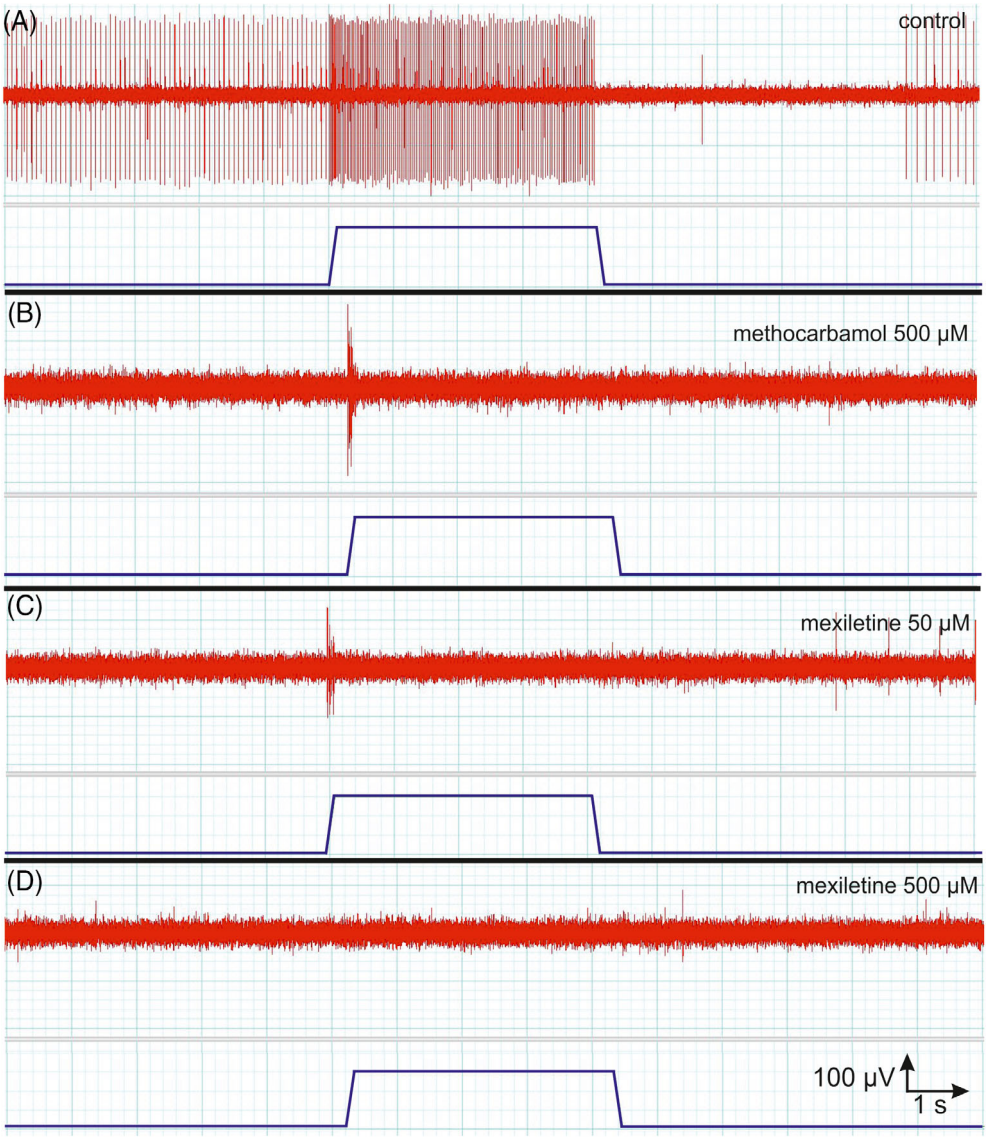

FIGURE 3 Mexiletine and methocarbamol progressively abolish firing of muscle spindle afferents in response to ramp-and-hold stretches. Representative muscle spindle afferent responses to ramp-and-hold stretches were recorded in the absence (A) and presence (B-D) of different concentrations of methocarbamol (B) or mexiletine (C,D). Although under control conditions muscle spindle afferents fire action potentials with frequencies that are determined by the length change and to the speed of stretching (A), this response is impaired by increasing concentrations of either muscle relaxant. In the presence of 500 μM methocarbamol (B) or 50 μM mexiletine (C), only a dynamic response during the ramp phase could be recorded. Higher concentrations of either muscle relaxant completely inhibited any response to ramp-and-hold stretches (D, and data not shown). The length changes (as indicated by the blue line below the action potential recordings) and the speed of stretching are identical in A-D. Scale bars for the extracellular recordings and the time are shown in D

discharge. Instead, while under control conditions (before addition of drug), the resting discharge frequency was constant over time (Figure 1A), and increasing concentrations of either muscle relaxant resulted in prolonged periods in which the muscle spindle afferent was silent (Figure 1B-F). The frequency of the resting discharge outside the silent periods was not altered by either drug (with the exception of the last action potentials before a silent period, which sometimes appeared after a small delay; Figure 1C,E) and was similar to the instantaneous frequency before addition of drug. The silent periods were first observed at a concentration of 100 μM methocarbamol or 5 μM mexiletine, respectively (Figure 1). At concentrations of 2000 μM (methocarbamol) and 500 μM (mexiletine), resting muscle spindles completely ceased action potential firing (Figure 1F).

We next determined the number of action potentials over a period of 30 seconds at different time-points after addition of drug and expressed this value as a percent of control (action potentials in 30 seconds before addition of drug; Figure 2). We observed no statistically significant difference between results after 45 and after 60 minutes, demonstrating that an incubation time of 60 minutes is sufficient for maximal effect of methocarbamol and mexiletine activity. All data points in Figure 2, including SEM and statistical significance, are summarized in Table S1. The IC_{50} value for the blockade of the resting discharge was calculated as 298 μM (mean with 95% confidence interval between 208.3 and 428.5 μM; n = 14) for methocarbamol and 5.86 μM (mean with 95% confidence interval between 4.6 and 7.4 μM; n = 16) for mexiletine. These results demonstrate that methocarbamol and mexiletine influence muscle spindle discharge frequencies at rest, albeit at different concentrations.

3.2 | Effect of methocarbamol and mexiletine on response of muscle spindle afferents to ramp-and-hold stretches

We observed a progressively reduced response to ramp-and-hold stretches in the presence of increasing concentrations of mexiletine

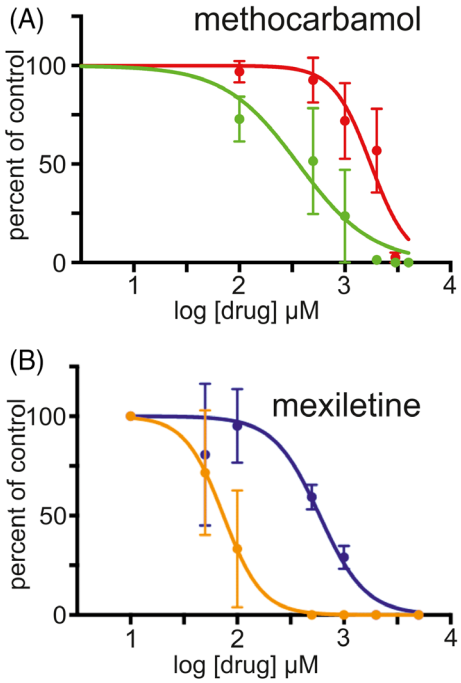

FIGURE 4 Dose-dependent inhibition of the dynamic and static response to stretch. The effects of methocarbamol (A) and mexiletine (B) on the dynamic (red line in A, blue line in B) and static (green line in A, orange line in B) responses to stretch 60 minutes after addition of drug are indicated. Results are expressed as percent of control, that is, the dynamic peak and the static response before addition of drug. Data points represent the mean of triplicate recordings ± standard error of the mean (n = 30). Note that both muscle relaxants inhibit the static response at lower concentrations compared with the dynamic response

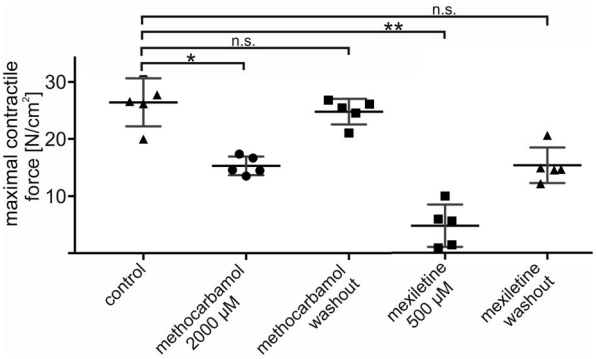

FIGURE 5 Reversible inhibition of the specific contractile force after tetanic stimulation by methocarbamol and mexiletine. Dot plot shows the maximal specific contractile force (in N/cm^2) after tetanic stimulation of muscle spindles in the presence or absence and after washout of methocarbamol (2 mM) or mexiletine (0.5 mM). Both drugs reduced the specific force to approximately 50% (methocarbamol) or 20% (mexiletine). After a 60-minute washout of the drug, the specific contractile force returned to almost control values. Each dot represents an independent experiment. Error bars represent the mean ± standard error of the mean (n = 5)

or methocarbamol compared with control conditions (Figure 3A). At low concentrations of either drug, the resting discharge and static response were absent and the muscle spindle fired only during the ramp phase (Figure 3B,C). The response to ramp-and-hold stretches was completely abolished in the presence of 4 mM methocarbamol or 500 µM mexiletine (Figure 3D, and data not shown).

We next determined the dynamic peak and the static response in the presence of increasing concentrations of either drug (Figure 4). From these dose-response curves, the IC_{50} values for methocarbamol were calculated as 1756 (mean with 95% confidence intervals of 1265 to 2331; hillslope coefficient: −2.44; n = 11) for the dynamic response and 362 (mean with 95% confidence intervals of 160.4 to 676.2; hillslope coefficient: −1.24, n = 16) for the static response. Likewise, the IC_{50} values for mexiletine were 59.2 µM (mean with 95% confidence intervals of 32.42 to 88.11 µM; hillslope coefficient: −2.01) and 7.42 µM (mean with 95% confidence intervals of 5.27 to 10.86 µM; hillslope coefficient: −2.40) for static and dynamic responses, respectively. These results demonstrate that the response of muscle spindles to ramp-and-hold stretches is impaired by methocarbamol and mexiletine in a dose-dependent manner and that the dynamic response is less sensitive to either drug compared with the static response.

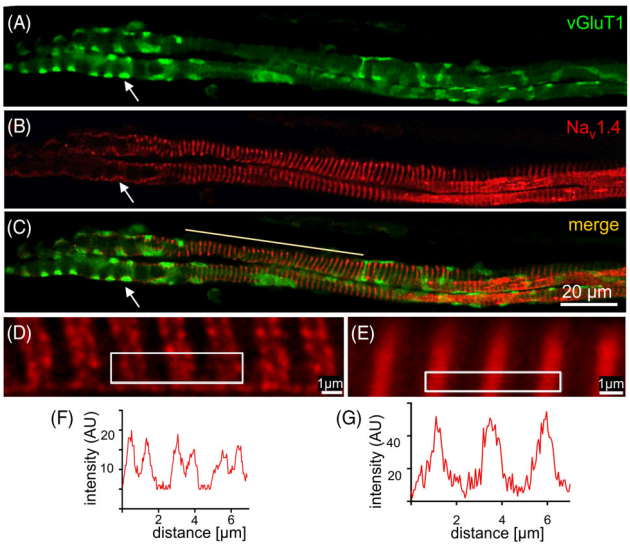

FIGURE 6 Distribution of the $Na_v1.4$ alpha-subunit in muscle spindles. Single confocal longitudinal cryostat sections of adult soleus (A-D) muscles were stained with antibodies against vGluT1 (A) to label the sensory nerve endings and against $Na_v1.4$ (B). The merged picture is shown in C. Note that $Na_v1.4$ immunoreactivity is distributed in a striated pattern localized primarily in the polar regions of intrafusal fibers (indicated by a yellow line in C). Considerably less immunoreactivity was observed in the equatorial region of intrafusal fibers. There appears to be no specific accumulation of $Na_v1.4$ immunoreactivity at the contact site between the sensory nerve ending and the intrafusal fiber in the equatorial region (arrows). High magnifications of the distribution of the $Na_v1.4$ immunoreactivity in extrafusal (D) and intrafusal (E) muscle fibers, respectively. Note the double stripes in extrafusal and the single stripes in intrafusal fibers. Optical intensity measurements of the $Na_v1.4$ immunoreactivity distribution in the areas marked by the rectangles in D and E are shown in F and G, respectively. Scale bars: C, 20 µm; D and E, 1 µm

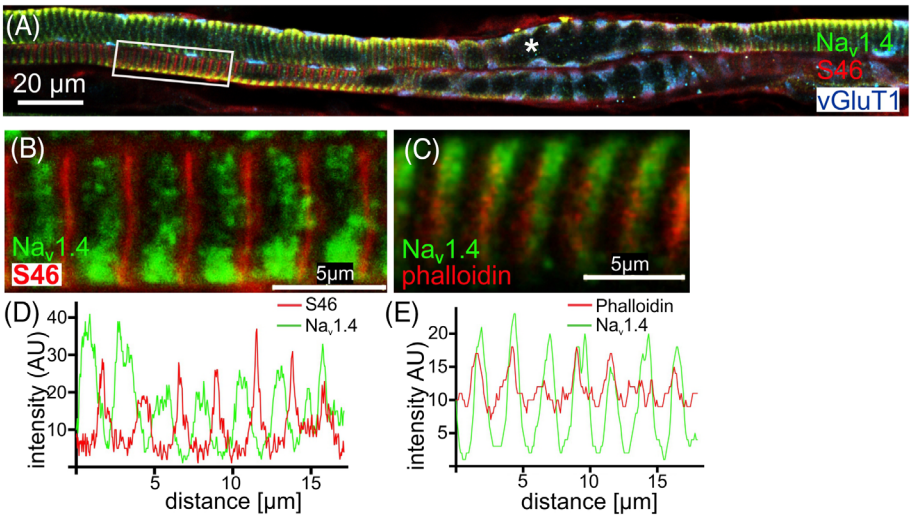

FIGURE 7 $Na_v1.4$ codistributes with actin but not with myosin filaments. Intrafusal fibers from adult extensor digitorum longus (EDL) muscles labeled with antibodies against vGluT1, $Na_v1.4$ and against slow tonic myosin heavy chain (MYH6; antibody S46) are shown (A). Note the similarly striped distribution of $Na_v1.4$ in the predominantly fast-twitch EDL muscle (A-C) and in the slow-twitch soleus muscle (see Figure 6B). The area marked by a rectangle (A) is also shown at higher magnification (B) to compare the distribution of both proteins. C, Comparable area in the polar region of an intrafusal fiber stained for actin filaments with fluorescently labeled phalloidin (red channel) and antibodies against $Na_v1.4$ (green channel). The stripes labeled by anti-$Na_v1.4$ antibodies were strictly non-overlapping with the distribution of the slow myosin heavy chain labeled by the S46 antibody (B), as indicated by the corresponding optical intensity measurement (D). In contrast, $Na_v1.4$ immunofluorescence codistributed with the distribution of actin filaments labeled by fluorescent phalloidin (C), as indicated by the corresponding optical intensity measurement (E). Scale bar: A, 20 μm; B and C, 5 μm

We did not observe a statistically significant difference in the tension displayed at Lo before (set to 100%) compared with after the addition of either drug (data not shown; 500 μM mexiletine: 85.1 ± 7.9% [mean ± SEM], n = 3, $P = .09$; 4 mM methocarbamol: 93.1 ± 8.4%, with n = 3, $P = .28$). Likewise, the passive tension during a stretch was similar before (set to 100%) compared with after the addition of either drug (500 μM mexiletine: 75.7 ± 12.6%, with n = 3, $P = .10$; 4 mM methocarbamol: 100.3 ± 10.2%, with n = 3, $P = .99$). This indicates that both drugs had no apparent effect on the passive viscoelastic properties of the EDL muscle under resting conditions and in response to stretch.

3.3 | Methocarbamol and mexiletine reduce maximal tetanic force

The physiological specific force (in N/cm^2) during high-frequency tetanic stimulation was reduced by approximately 40% at a methocarbamol concentration of 2 mM and by about 80% at a concentration of 500 μM mexiletine (Figure 5A). After a 1-hour washout, however, the specific maximal force returned to almost normal values, demonstrating that the interaction of both drugs with their target protein is reversible and that both drugs do not apparently induce long-lasting effects in extrafusal muscle fibers.

3.4 | $Na_v1.4$ expression in intrafusal fibers

Particularly in the polar region of intrafusal fibers, antibodies against the alpha-subunit of $Na_v1.4$ specifically labeled transverse oriented structures, which appeared as individual stripes oriented perpendicular to the longitudinal axis of intrafusal fibers (Figures 6A-C and 7A). In the equatorial region of intrafusal fibers, $Na_v1.4$ immunoreactivity was mostly absent, consistent with the small number of sarcomeres in this region. Likewise, anti-$Na_v1.4$ immunoreactivity was not particularly concentrated at the contact sites between the sensory nerve terminal (labeled by antibodies against vGluT1; see arrows in Figure 6A-C) and the intrafusal fiber. In extrafusal fibers, anti-$Na_v1.4$ antibodies labeled a double band (Figure 6D). Optical intensity measurements showed that the distance between the single bands was ~1 μm and the distance between the double bands was ~2 μm, consistent with them being T tubules. In contrast, the same antibodies labeled single bands in the polar region of intrafusal fibers (Figures 6E and 7A). These bands exhibited a spacing of ~2.3 μm, as determined by optical intensity measurements (Figure 6G). The distribution of $Na_v1.4$ bands in intrafusal fibers did not overlap with the slow myosin heavy chain (labeled by the S46 antibody; Figure 7B,D). In contrast, actin filaments (detected with fluorescently labeled phalloidin) and $Na_v1.4$ immunoreactivity were codistributed (Figure 7C,E). These results demonstrate that $Na_v1.4$ is present in intrafusal fibers, particularly in their polar

regions, albeit with a different distribution compared with extrafusal fibers. In addition, these results are consistent with the possibility that both drugs may carry out their effects on muscle spindle function by affecting this channel.

4 | DISCUSSION

In this study we have shown that methocarbamol and mexiletine inhibit generation of action potentials in muscle spindle afferents at rest as well as in response to stretch, but they do not affect muscle tension. Mexiletine and methocarbamol both bind to several voltage-gated sodium channels, including $Na_v1.4$, and both drugs preferentially affect the inactivated state.[23,33] Several sodium channels have been localized in muscle spindles by immunocytochemistry.[49] $Na_v1.6$ immunoreactivity is concentrated in the first heminodes, as well as in the sensory terminals of group Ia afferents. Likewise, $Na_v1.1$ is concentrated in sensory terminals together with $Na_v1.6$, whereas $Na_v1.7$ is mainly expressed in the axons of the sensory neuron. Consistently, single nucleus transcriptome analyses revealed that proprioceptive sensory neurons express $Na_v1.1$, $Na_v1.2$, $Na_v1.6$, and $Na_v1.7$, but little if any $Na_v1.3$, $Na_v1.4$, $Na_v1.5$, $Na_v1.8$, or $Na_v1.9$.[50] It remains to be solved, however, which of these sodium channels is the target of mexiletine and/or methocarbamol in muscle spindles. Both drugs are promiscuous with respect to the sodium channels they interact with, but the strong similarity of the effect of both drugs on the muscle spindle resting discharge and the stretch-induced action potentials suggest that both drugs affect muscle spindles via the same sodium channel.

We have provided evidence that $Na_v1.4$ is present in intrafusal fibers, particularly in their polar regions, which is consistent with the accumulation of sarcomeres in this region. We did not observe any $Na_v1.4$ immunoreactivity associated with the sensory neuron and there was no concentration of $Na_v1.4$ in the contact region between the sensory nerve terminal and intrafusal fiber. Interestingly, the subcellular distribution of $Na_v1.4$ was different in extrafusal fibers (double band) when compared with intrafusal fibers (single bands). The double bands in extrafusal fibers have been shown to correspond to the T-tubule system.[51,52] It will therefore be of considerable interest to determine whether the single bands observed in intrafusal fibers similarly correspond to T tubules, which would suggest a different three-dimensional distribution of the T-tubule system in intrafusal fibers. In principle, T tubules have been detected in intrafusal fibers by electron microscopy,[53,54] but their exact spatial distribution has not been analyzed by light microscopy. Therefore, the subcellular structure with which $Na_v1.4$ is associated in intrafusal fibers remains to be determined.

We did not observe a gradual decline of the muscle spindle resting discharge frequency in response to increasing concentrations of either drug. In contrast, the periods in which the muscle spindle was silent became longer, suggesting an "all-or-none" effect of the drugs on action potentials. Therefore, we consider an effect of both drugs on the initial generation of action potentials or on their conduction along the proprioceptive afferents more likely than an effect on the generation or modulation of the mechanically gated change of the membrane potential. It remains to be determined whether both drugs affect proprioceptive sensory neuron activity by inhibiting intrafusal fiber–associated $Na_v1.4$. The absence of an effect of both drugs on the passive muscle tension at rest and during ramp-and-hold stretches would suggest a different target. Moreover, the preferential effect of both drugs on the static compared with dynamic sensitivity also supports our idea that action potential generation is affected and not a mechanosensation.

The US Food and Drug Administration recommendation for the treatment of muscle spasms with methocarbamol is an initial dose of 1500 mg orally four times per day for the first 48 to 72 hours, up to a maximum dose of 8 g/day for severe symptoms. Peak levels of the plasma concentration are 20 μg/mL (corresponding to ~83 μM) about 1 hour after oral application of 1500 mg methocarbamol.[55] The concentration of methocarbamol used in our study (IC_{50} at ~300 μM for the resting discharge) is approximately fourfold higher than the concentration achieved in the plasma of patients with commonly accepted dosing levels, suggesting that the concentration of methocarbamol needed to affect muscle spindles may not be reached in patients. On the other hand, intramuscular injections could cause much higher local concentrations.

Mexiletine is used at a typical dose of between 100 and 200 mg three times per day, but the frequency of muscle cramps in amyotrophic lateral sclerosis patients can be reduced with doses as low as 150 mg twice daily.[32] The mean mexiletine serum level at the end of a 4-week treatment period at 600 mg/day was approximately 1 μg/mL (corresponding to ~5.6 μM).[33] The concentration of mexiletine used in our study is approximately equivalent to the concentration reached in vivo (IC_{50} = 5.8 μM for resting discharge), suggesting that an effect of this drug on proprioception is clinically relevant.

The consequences of the inhibitory activity of methocarbamol and mexiletine on muscle spindle proprioceptive afferents in humans would include coordination difficulties, unstable gait, and frequent falls. An increased risk of injury after administration of skeletal muscle relaxants, including methocarbamol, has been consistently reported, particularly in the elderly.[10,56,57] Our results suggest that the effects of mexiletine and methocarbamol on muscle spindles may contribute to these symptoms. Accordingly, the Beers Criteria for Potentially Inappropriate Medication Use in Older Adults, a collection of recommendations for health-care providers on medications with potential adverse side effects, includes methocarbamol, due to the increased risk for falls and fractures.[58] Moreover, because muscle relaxants are often used in general anesthesia, the recovery of proprioceptive function in the postanesthetic period should be monitored. In general, cautionary use of these medications, particularly in elderly patients, continues to be advisable.

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CONFLICT OF INTEREST

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ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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