Methocarbamol blocks muscular Na\(_v\)1.4 channels and decreases isometric force of mouse muscles

Yaxin Zhang PhD\(^1\) | Philipp Otto BSc\(^1\) | Lu Qin BSc\(^1\) | Nane Eiber MSc\(^2\) | Said Hashemolhosseini PhD\(^2\) | Stephan Kröger PhD\(^3\) | Heinrich Brinkmeier PhD\(^1\)

Abstract

**Background:** The muscle relaxant methocarbamol is widely used for the treatment of muscle spasms and pain syndromes. To elucidate molecular mechanisms of its action, we studied its influence on neuromuscular transmission, on isometric muscle force, and on voltage-gated Na\(^+\) channels.

**Methods:** Neuromuscular transmission was investigated in murine diaphragm-phrenic nerve preparations and muscle force studied on mouse soleus muscles. Nav1.4 channels and Nav1.7 channels were functionally expressed in eukaryotic cell lines.

**Results:** Methocarbamol, at 2 mM, decreased the decay of endplate currents, slowed the decay of endplate potentials and reduced tetanic force of soleus muscles. The drug reversibly inhibited current flow through muscular Nav1.4 channels, while neuronal Nav1.7 channels were unaffected.

**Conclusions:** The study provides evidence for peripheral actions of methocarbamol on skeletal muscle. Muscular Na\(^+\) channels are a molecular target of methocarbamol. Since Nav1.7 currents were unaffected, methocarbamol is unlikely to exert its analgesic effect by directly blocking Nav1.7 channels.

**KEYWORDS**
methocarbamol, muscle force, muscle relaxant, neuromuscular transmission, voltage-gated Na\(^+\) channel

1 INTRODUCTION

Muscle relaxants are a heterogeneous group of drugs with the capability to reduce skeletal muscle tone and control muscle spasms.\(^1,2\)

**Abbreviations:** AChR, acetylcholine receptor; CHO, Chinese hamster ovary (cells); CMAP, compound muscle action potential; CNS, central nervous system; CSA, cross-sectional area; EPC, endplate current; EPP, endplate potential; FCS, fetal calf serum; GABA, 𝛾-aminobutyric acid; HEK 293, human embryonic kidney (cells); LBP, lower back pain; NMJ, Neuromuscular junction; μ-CTX, μ-conotoxin.

**Presentation on a meeting:** Part of this work has been presented at the Europhysiology 2018 organized by The Physiological Society, 14.-16. September 2018 in London.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. Muscle & Nerve published by Wiley Periodicals LLC.
LBP, methocarbamol can reduce pain intensity and pain-related disability and improve lumbar mobility.14

Although methocarbamol has been used clinically for more than 50 y,16 its mechanism of action is still unclear. Some studies suggested a central mode of action of methocarbamol on spinal interneurons.17,18 The reduction of reflex contractions and the mono- and polysynaptic components of ventral root potentials by methocarbamol are consistent with an effect of the drug at higher centers concerned with motor function.19 However, some early reports raise doubts about this view. Crankshaw and Raper reported that methocarbamol caused a prolongation of the refractory period of cat tibialis muscles20 and showed a suppression of polysynaptic reflex contractions in this preparation. They discussed their results in terms of depressant actions of the drug on α-motor neurons and muscle fibers. Since the refractory period of muscle fibers is mainly determined by voltage-gated Na⁺ channels,21 Na⁺,1.4 channels are potential molecular targets of methocarbamol. In contrast to the Na⁺,1.4 that is almost exclusively expressed by extrafusal muscle fibers, peripheral nociceptive neurons express Na⁺,1.7 channels, which are essential for pain perception. Thus, the pain-relieving effect of methocarbamol could also be explained by interference with Na⁺,1.7 channels of nociceptive nerve fibers.

The current study was designed to investigate effects of methocarbamol on neuromuscular transmission, muscle force, and voltage-gated sodium channels.

2 | METHODS

2.1 | Animals, nerve-muscle preparation, and soleus muscle

Experiments were performed with isolated diaphragm-phrenic nerve preparations and soleus muscles from male C57BL/6J mice aged between 83 and 125 days. Animals were killed by cervical dislocation during brief ether anaesthesia. All tissue dissections occurred within 5 min post-mortem. The procedures were carried out in accordance with the German guidelines for animal protection. The use of animals for experiments was approved by the District Veterinary Office in Anklam, Germany.

2.2 | Solutions, drugs, and toxins

Isolated diaphragm-phrenic nerve preparations were maintained in Liley’s solution.22 The Na⁺ channel blocker μ-conotoxin GIIIB (μ-CTX) was obtained from the Peptide Institute (Osaka, Japan) and rhodamine-α-bungarotoxin from Life Technologies (Darmstadt, Germany). Force measurements were performed with isolated soleus muscles bathed in Krebs–Henseleit solution containing (in mM): 137 NaCl, 24 NaHCO₃, 2.5 KCl, 2 CaCl₂, 1 NaH₂PO₄, 1 MgSO₄, and 11 D-glucose. The solution was continuously bubbled with carbogen at pH 7.4. The standard external solution for whole cell recordings of Na⁺ currents contained (in mM): 140 NaCl, 3.5 KCl, 1 CaCl₂, 1 MgCl₂, 5 HEPES-NaOH. The standard internal solution contained (in mM): 140 CsCl, 1.4 MgCl₂, 10 EGTA, and 10 HEPES-CsOH. The pH of both solutions was adjusted to 7.4. Methocarbamol was obtained as Ortoton® solution (Recordati Pharma GmbH, Ulm, Germany) containing 100 mg/mL methocarbamol. For experimentation, the stock solution was appropriately diluted in Liley’s solution. Krebs–Henseleit solution or the standard external solution for cell physiology.

2.3 | Analysis of neuromuscular transmission by extracellular recordings

Electrophysiological recordings were essentially done as previously described.23,24 Isolated diaphragm-phrenic nerve preparations were maintained in Liley’s solution, gassed with carbogen, and kept at room temperature. The recording chamber had a volume of approximately 1 mL and was perfused at a rate of 1 mL/min. The nerve was drawn up into a suction electrode for stimulation with pulses of 0.1 ms duration. The preparation was placed on the stage of a Zeiss Axios Examiner 21 microscope fitted with incident light fluorescence illumination with filters for red (Zeiss filter set 20) fluorescing fluorophore (Carl Zeiss Micro-Imaging, Göttingen, Germany). At the beginning of the experiment, the compound muscle action potential (CMAP) was recorded using a micro-pipette with a tip diameter of ~10 μm, filled with bathing solution. The electrode was positioned so that the latency of the major negative peak was minimized. The electrode was then positioned 100 μm above the surface of the muscle and CMAP was recorded. Unless stated otherwise, a concentration of 2 mM methocarbamol was used in all experiments, since in pilot experiments this concentration appeared to result in maximal effects. Muscles were incubated for 20 min with methocarbamol before CMAPs were measured.

2.4 | Intracellular recordings of muscle fibers and data analysis

To block muscle action potentials, so that endplate potentials (EPPs) and endplate currents (EPCs) could be recorded25,26 2 μM μ-conotoxin GIIIB were added to Liley’s solution. At the same time acetylcholine receptors (AChRs) were labeled by adding 50 nM of rhodamine-labeled α-bungarotoxin to the solution. In some experiments, after 1–2 h, the preparations had to be exposed a second time to the same concentration of the conotoxin. Two intracellular electrodes (resistance 10–15 MΩ) were inserted within 50 μm from the neuromuscular junctions (NMJ) under visual inspection.26 Current was passed through one electrode to maintain the membrane potential within −75 ± 1 mV while voltage transients were recorded with the other electrode. Signals were amplified by an Axoclamp 900A and digitized at 40 kHz by a Digidata 1440A under the control of pCLAMP 10 (Molecular Devices, Sunnyvale, California). Voltage records were filtered at 3 kHz and current records at 1 kHz (8-pole Bessel filter). Current transients were recorded using the two-electrode voltage-clamp facility of
the Axoclamp 900A. Clamp gains were usually 300–1000, reducing the voltage transients to <3% of their unclamped amplitudes.

To study the effects of methocarbamol on EPPs and EPCs, the muscle was incubated after the μ-conotoxin GIIIB solution for 20 min with 2 mM methocarbamol. Afterward the recording was started with freshly gassed Liley’s solution containing 2 mM methocarbamol, which was renewed every 20 min. Records were analyzed using pCLAMP 10. Spontaneous events were extracted using the “template search” facility and edited by eye to remove obvious artifacts. Events recorded from each NMJ were averaged and the amplitude, rise time and single exponential decay time determined.

2.5 Force measurements on soleus muscles

After dissection, muscles were transferred into a recording chamber and fixed as described previously.27 Experiments were performed at 25°C. Supramaximal electrical stimuli of 40 V and 0.2 ms duration were applied through two platinum electrodes. Stimulation and data acquisition were performed with a special software (MyoMat, MyoTronic UG, Heidelberg, Germany) and data evaluated and plotted with SigmaPlot. Before recordings were started, optimal muscle length was determined as described.27 Isometric forces in response to single stimuli (twitches) and to 500-ms tetanic stimuli of 10, 50, and 120 Hz were recorded (“1st run”). Then the control group of muscles remained in Krebs–Henseleit solution and the methocarbamol group was exposed to a solution containing 2 mM methocarbamol. A second series of experiments was performed after 10 min (“2nd run”). Time to peak (time from stimulation start to the time point of maximum force) and half relaxation time (time from maximum force to 50% decline of twitch force, or, for tetani, time from end of stimulation to 50% decline of force) were calculated. Finally, a so-called fatigue protocol was applied consisting of 500-ms tetani at 50 Hz repeated every 2 s for a total time of 400 s. The half fatigue time was taken, that is, the time between stimulation start and decline of force to 50% of the initial force. After the experiments, muscles were weighed and optimal length and weight were used to calculate muscle cross-sectional area (CSA).28,29 Specific muscle force was defined as force/CSA.

2.6 Cell lines and cell culture

HEK 293 cells stably expressing the α-subunit of the Na+,1.430 were grown in a medium composed of 90% MEM and 10% fetal calf serum (FCS; both from Gibco/Thermo Fisher Scientific, Darmstadt, Germany). Chinese hamster ovary (CHO) cells stably expressing the α-subunit of the human Na+,1.7 were obtained from Genionics AG (Zürich, Switzerland). They were grown in a medium composed of 90% DMEM/F12 (1:1, v:v, Gibco/Thermo Fisher Scientific) an 10% FCS. To positively select for cells expressing the Na+ channels, the media contained 800 μg/mL of the antibiotic gentamicin (G418, Gibco/Thermo Fisher Scientific) or 500 μg/mL hygromycin (InvivoGen, Toulouse, France).

2.7 Whole cell recording of Na+ currents

Whole-cell Na+ currents were recorded at room temperature using an EPC 10 patch-clamp amplifier (HEKA Elektronik GmbH, Lambrecht, Germany). Pipettes were filled with the standard internal solution and had resistances of 2–3 MΩ. To show the inhibitory effect of methocarbamol, the maximum current amplitude was determined in

| Parameter                          | Untreated          | 2 mM Methocarbamol |
|------------------------------------|--------------------|--------------------|
| Miniature EPP amplitude (mV)       | 0.46 ± 0.05 (n = 17) | 0.37 ± 0.03 (n = 12) |
| Miniature EPP rise time (ms)       | 0.35 ± 0.03 (n = 17) | 0.50 ± 0.12 (n = 12) |
| Miniature EPP decay time (ms)      | 2.02 ± 0.11 (n = 17) | 2.94 ± 0.31 (n = 12) |
| Input resistance (MΩ)              | 0.44 ± 0.03 (n = 19) | 0.42 ± 0.02 (n = 17) |
| EPP amplitude (mV)                 | 16.06 ± 1.85 (n = 19) | 13.97 ± 1.46 (n = 14) |
| EPP rise time (ms)                 | 0.29 ± 0.01 (n = 19) | 0.33 ± 0.03 (n = 14) |
| EPP decay time (ms)                | 1.95 ± 0.11 (n = 19) | 4.55 ± 0.15 (n = 14) |
| EPP 5 Hz decrement (%)             | 79.87 ± 0.04 (n = 19) | 81.01 ± 0.03 (n = 14) |
| Miniature EPC amplitude (nA)       | 2.82 ± 0.36 (n = 20) | 2.33 ± 0.46 (n = 14) |
| Miniature EPC rise time (ms)       | 0.27 ± 0.01 (n = 20) | 0.28 ± 0.01 (n = 14) |
| Miniature EPC decay time (ms)      | 1.03 ± 0.05 (n = 20) | 1.17 ± 0.02 (n = 14) |
| EPC amplitude (nA)                 | 94.30 ± 22.84 (n = 17) | 91.45 ± 16.62 (n = 12) |
| EPC rise time (ms)                 | 0.22 ± 0.03 (n = 17) | 0.34 ± 0.05 (n = 12) |
| EPC decay time (ms)                | 1.21 ± 0.06 (n = 17) | 3.59 ± 0.14 (n = 12) |

Note: The values represent the mean ± SEM of three mice; n represents the number of measured fibers. Significance was determined by unpaired t-test.
responses to 0.5-Hz repetitive 10-ms pulses before, during and after application of the test solution.

To determine the voltage-dependence of activation of the currents, a cyclic pulse program was applied, each cycle consisting of a constant 100-ms prepulse to $-135$ mV and a 10-ms test pulse, varied from $-80$ mV to $+40$ mV in 5-mV steps. The peak currents were plotted against the test potential (current/voltage curves). The voltage dependence of steady-state activation of the Na$^+$ channels was derived from these plots and Boltzmann equations were fitted to the data points. To study the voltage dependence of steady-state inactivation of the Na$^+$ channels, another cyclic pulse program was applied with each cycle consisting of a 100-ms prepulse that was varied between $-120$ mV and $-10$ mV in 5-mV steps followed by test pulses to $-10$ mV (10 ms). The obtained current peaks were normalized and plotted against the prepulse potential. Finally, Boltzmann curves were fitted to the data points. To investigate the recovery of the Na$^+$ channels from inactivation, a cyclic pulse program was used with each cycle consisting of a 100-ms voltage pulse to $-140$ mV, followed by an inactivating pulse to $-10$ mV for 10 ms. Then a prepulse of $-105$ mV was applied, the duration ranging from 1 ms to 3.2 s. Finally, a 10-ms test pulse to $-10$ mV was given to test for the fraction of channels that had recovered from inactivation. Current amplitudes were plotted against the prepulse duration and the data points were fitted by the equation $f(t) = C - A \cdot \exp(-t/\tau_1) - D \cdot \exp(-t/\tau_2)$ with $t$ being the prepulse duration, $f(t)$ the Na$^+$ current amplitude, $C$ a constant, $A$ and $D$ the

FIGURE 1  A, Effect of methocarbamol on amplitude of CMAP at 1 Hz. Representative recordings of CMAP of diaphragm muscle in control solution and methocarbamol (2 or 4 mM) solution. B,C, decrement of CMAP amplitude (25th/1st) after repetitive stimulation at 5 or 50 Hz in untreated and 2 mM B, or 4 mM C, methocarbamol treated muscles; note aggravated decrement when incubated with methocarbamol ($^*P < .05$, $^{**}P < .01$; unpaired two-tailed Student’s $t$ test; $n = 3$ recorded measurements)

TABLE 2  Methocarbamol effects on the CMAP amplitude and decrement

| Parameter                  | Untreated          | 2 mM Methocarbamol | 4 mM Methocarbamol |
|----------------------------|-------------------|--------------------|--------------------|
| CMAP amplitude, 1 Hz (mV)  | 1.05 ± 0.30 (n = 3) | 0.47 ± 0.10 (n = 3) | $P = .0336^a$      |
| CMAP decrement, 5 Hz (%)   | 102.38 ± 6.49 (n = 3) | 84.05 ± 2.61 (n = 3) | $P = .0105^b$      |
| CMAP decrement, 50 Hz (%)  | 75.71 ± 8.04 (n = 3) | 41.69 ± 8.92 (n = 3) | $P = .0080^b$      |

Note: Values represent the mean ± SEM of three animals; $n$: number of mice. Significance was determined by unpaired $t$-test;

$^a$Compared with untreated group.

$^b$Compared with untreated CMAP decrement at 5 Hz and 50 Hz, respectively.
coefficients, and $\tau_1$ and $\tau_2$ the time constants of fast and slow recovery, respectively.32

3 | RESULTS

3.1 | Methocarbamol modulates neuromuscular transmission

We studied neuromuscular transmission in phrenic nerve-diaphragm explants. While most parameters of miniature EPPs were unaffected, the decay times of the EPCs and those of the EPPs induced by the phrenic nerve stimulation were significantly increased at 2 mM methocarbamol (Table 1, Supporting Information Figure S1, which is available online). At 5-Hz stimulation, the EPP decrement was not affected by methocarbamol (Table 1).

In contrast, the CMAP amplitude of the diaphragm-phrenic nerve preparation was markedly reduced by methocarbamol (Figure 1A). Furthermore, the decrement of the CMAP was larger in the presence of the drug, both at 5 and 50-Hz stimulation (Figure 1, Table 2).

3.2 | Methocarbamol affects muscle force

Application of methocarbamol had no effect on amplitudes of twitches (Figure 2A,B). Kinetics of twitches was slightly faster in the presence of the muscle relaxant, but the same effect occurred in the control group (Table 3, 2nd run). However, during high frequency tetanic stimulation, methocarbamol decreased tetanic force moderately, but significantly (Figure 2D,E,F). At 50-Hz stimulation, the reduction was about 4% and at 120 Hz about 9%, while in the control group both parameters appeared identical (Table 3, results from first and second run). Since twitch amplitudes were unaffected, the tetanus/twitch ratio was reduced by methocarbamol by about 9% (Figure 2C, Table 3).

(Figure 2) Effect of methocarbamol on muscle force. A,B, Representative recordings of isometric twitch contractions of soleus muscles. To induce twitches, supramaximal direct electrical stimuli were given at $t = 0$ s. C,F, To show the reproducibility of force production in two subsequent series of experiments, twitches and tetani were recorded two times in succession under control conditions (CTL CTL). In case of the methocarbamol group, twitches and tetani were first recorded in control solution and then, in a second series of experiments, in the presence of methocarbamol (CTL Metho). C, Ratio of maximal tetanic forces and twitches of soleus muscles in control solution and at 2 mM methocarbamol ($n = 14$ vs. $n = 15$). D,E, Representative traces of tetanic contractions in the presence and absence of methocarbamol. Electrical stimulation at 120 Hz was applied between $t = 0$ ms and $t = 500$ ms. F, Tetanic forces are given for the control and the methocarbamol group ($n = 14$ vs. $n = 15$). C,F, Boxplots showing the effects of methocarbamol on tetanic force (F) and the tetanus/twitch ratio; stars indicate significant difference between indicated columns, $P < .001$, paired t-test.
Decline of force during sustained repetitive tetanic stimulation (fatigue protocol) was slightly, but not significantly aggravated in the presence of methocarbamol (Supporting Information Figure S2, open circles, Table 3) compared with the decline under control conditions. The time to reach 50% of initial force was reduced from 181.4 ± 25.4 s in standard solution to 157.7 ± 40.5 s in the presence of methocarbamol. Thus, the most striking effect of methocarbamol on muscle mechanics was the reduction of tetanic force at high frequency stimulation.

### TABLE 3

Effects of methocarbamol on isometric force parameters of mouse soleus muscles

|                     | Control group N = 14 | Methocarbamol group N = 15 |
|---------------------|-----------------------|-----------------------------|
|                     | CTL1st run            | CTL2nd run                  | Metho1st run | Metho2nd run |
| **Twitches**        |                       |                            |
| Absolute force (mN) | 28.6 ± 3.5            | 28.3 ± 4.1                  | 28.9 ± 4.8   | 28.0 ± 4.4   |
| Specific force (mN/mm²) | 22.8 ± 4.0           | 22.6 ± 4.3                  | 23.6 ± 4.9   | 22.9 ± 4.7   |
| Time to peak (s)    | 0.041 ± 0.003         | 0.038 ± 0.002, P = .0116    | 0.041 ± 0.003| 0.039 ± 0.003, P = .0327 |
| Half relaxation Time (s) | 0.042 ± 0.004       | 0.037 ± 0.005, P = .0025    | 0.043 ± 0.005| 0.037 ± 0.004, P < .0001 |
| **50 Hz tetani**    |                       |                            |
| Absolute force (mN) | 173.8 ± 21.5          | 173.6 ± 23.6                | 170.4 ± 20.3 | 164.5 ± 20.1, P = .0005 |
| Specific force (mN/mm²) | 138.5 ± 24.5         | 138.4 ± 24.6                | 138.4 ± 21.4 | 133.7 ± 21.5, P = 0.0003 |
| Half relaxation Time (s) | 0.064 ± 0.003       | 0.061 ± 0.003, P < .0001    | 0.065 ± 0.007| 0.065 ± 0.004 |
| **120 Hz tetani**   |                       |                            |
| Absolute force (mN) | 199.1 ± 25.6          | 198.4 ± 27.5                | 195.0 ± 26.6 | 171.2 ± 24.4, P < .0001 |
| Specific force (mN/mm²) | 158.6 ± 27.8         | 158.1 ± 28.3                | 158.3 ± 25.8 | 139.1 ± 24.4 P < .0001 |
| Half relaxation time (s) | 0.080 ± 0.003        | 0.077 ± 0.006               | 0.080 ± 0.003| 0.078 ± 0.003 P = .006 |
| Derived parameters  |                       |                            |
| 120 Hz tetanus/ twitch | 7.0 ± 0.5            | 7.0 ± 0.5                   | 6.8 ± 1.0    | 6.2 ± 0.7 P = .0002 |
| Half fatigue Time (s) | –                    | 181.4 ± 25.4*              | –            | 157.7 ± 40.5* P = .07 |

Note: Means ± SD are given in all cases, x: not applicable, paired t-test was applied between data of first and second run within each group. Abbreviations: CTL, control solution; Metho, 2 mM methocarbamol.

*Unpaired t-test applied, since experiments performed only once after second run.

**FIGURE 3**  Effect of methocarbamol on muscular of Na⁺ channels recorded from HEK 293 cells. Recordings were performed in the whole cell configuration from cells stably expressing the α-subunit of the human Na₁.4. A, Exemplary current transients in response to depolarizing voltage pulses going from −85 to −10 mV for 10 ms in standard external solution (black line) and in the presence 2 mM methocarbamol (gray line). B, Time course of the methocarbamol effect. Voltage pulses as applied in A, were given every 2 s and peak current maxima plotted every 4 s. Current maxima (circles) were normalized for each cell to the means of current maxima obtained between 0 and 40 s. Then, 2 mM methocarbamol were applied. Means ± SD are given for all current maxima plotted (n = 16 cells tested).
3.3 Methocarbamol affects muscular sodium channels

When HEK 293 cells, stably expressing the Na\textsubscript{v}1.4, were stimulated with square voltage pulses increasing from −85 to −10 mV, they responded with transient inward currents that were stable over time. Upon application of methocarbamol, the Na\textsuperscript{+} currents were inhibited on average by 37 ± 8% (Figure 3). The inhibition occurred within about 10 s and was reversible upon washout (Supporting Information Figure S3A). Complete solution change was achieved within less than 1 s. Na\textsuperscript{+} current kinetics remained apparently unchanged in the presence of methocarbamol (Figure 3A and Supporting Information Figure S4).

To investigate whether the methocarbamol effect on Na\textsubscript{v}1.4 channels is voltage-dependent, we determined current/voltage relations. These curves were only marginally affected by methocarbamol (Figure 4A). Voltage dependence of activation of the Na\textsubscript{v}1.4 channels was completely unaffected (Figure 4B). The small decrease of the I/V curve over the whole voltage range (Figure 4A) can only partly explain the observed Na\textsuperscript{+} current inhibition shown in Figure 3. Most of the inhibitory effect of methocarbamol on Na\textsubscript{v}1.4 currents is owing to a shift of the steady-state inactivation curve (Figure 4C). The shift (average of fits) amounted to 8.4 mV toward negative potentials (from −71.6 to −80.0 mV) and was widely reversible upon re-application of the standard external solution. The calculated shift was statistically significant (P < .001, paired t-test, N = 16) indicating that methocarbamol stabilized Na\textsubscript{v}1.4 channels in a state of inactivation. Therefore, we next studied the effect of methocarbamol on the recovery from inactivation of the Na\textsubscript{v}1.4 channels. The Na\textsubscript{v}1.4 currents almost completely inactivated within 5 ms at a test potential.
of $-10$ mV (Figure 3A). To allow recovery from inactivation we applied $-105$ mV for variable time periods. Recovery of Na$^+$ channels from inactivation occurred within a few milliseconds and reached nearly 100% at 10 ms (Figure 4D). The data points could be fitted by an exponential curve (Figure 4D, black line), resulting in a recovery time constant ($\tau_1$) of $3.2 \pm 0.7$ ms. In the presence of methocarbamol recovery from inactivation was slowed and interestingly split into a fast ($\tau_1$) and a much slower phase ($\tau_2$, Figure 4D, red line). In agreement with this observation, the data points could not be appropriately fitted with a single exponential curve, but by an equation with two time constants ($\tau_1 = 4.0 \pm 0.9$ ms and $\tau_2 = 55.3 \pm 20.1$ ms). In summary, these results demonstrate that methocarbamol reversibly affects voltage dependence of inactivation of Na$_{1.4}$ channels.

3.4 Methocarbamol has no effect on Na$_{1.7}$ currents

Methocarbamol (at 2 mM) did not change amplitude and kinetics of Na$_{1.7}$ currents (Supporting Information Figure S3B). Neither I/V-curves nor inactivation curves and recovery from inactivation of the Na$_{1.7}$ currents were influenced by the relaxant.

4 DISCUSSION

Our study supports the view that methocarbamol has peripheral relaxing effects directly interacting with the neuromuscular system, since we observed a marked reduction of the CMAP amplitude and an aggravation of the CMAP decrement in the diaphragm-phrenic nerve model. This indicates a relaxing effect of the drug and is in agreement with the clinical observation that methocarbamol exacerbates symptoms of myasthenia gravis.$^2$

In the diaphragm-phrenic nerve preparation, methocarbamol prolonged both EPP and EPC decay times. Prolongation of these two parameters could be caused by altered transmitter release, transmitter degradation, or by altered AChR function.$^{33}$ However, the underlying effect would not explain the aggravation of the CMAP decrement discussed above.

In addition to its effects on neuromuscular transmission, methocarbamol attenuated isometric force of isolated mouse soleus muscles. The results are in line with the observed inhibition of the CMAP amplitudes by methocarbamol. However, the marked effects on CMAP amplitude and the moderate inhibition of tetanic force (4%-9%) do not indicate a linear relationship between the two parameters. Probably, the 40% block of Na$^+$ channels is not sufficient to suppress action potentials in muscle fibers, but merely reduces action potential amplitudes$^{34}$ and CMAP amplitudes without much influence on excitation/contraction coupling. Furthermore, direct supramaximal stimulation of muscles in the presence of methocarbamol may compensate for the weakened indirect stimulation via motoneurons.

At very high frequency stimulation (400 Hz) Crankshaw and Rape$^{20}$ showed a collapse of tetanic force of cat muscle in the presence of methocarbamol. The authors concluded that the mechanism by which the drug caused its effect was a prolongation of the refractory period of muscle.$^{20}$ Our results support this view, since the inhibition of isometric force by methocarbamol was most prominent at 120 Hz stimulation frequency. As the time course of twitches and tetani were unaffected we have no evidence that methocarbamol influenced the intracellular Ca$^{2+}$ cycle during muscle performance.

A key finding of this study is the inhibition of Na$^+$ currents by methocarbamol. The underlying electrophysiological mechanisms of Na$_{1.4}$ current inhibition were a shift of the steady-state inactivation curve to more negative potentials and a prolonged recovery from inactivation of the channels. These results are compatible with the view that binding of methocarbamol to Na$_{1.4}$ channels slows their recovery from inactivation substantially. This effect could indeed prolong the refractory period of muscle fibers and is in agreement with the observed attenuation of isometric forces at high stimulation frequencies.$^{20}$ Surprisingly, the methocarbamol effect on Na$_{1.4}$ channels could not be reproduced with Na$_{1.7}$ channels, indicating that impulse propagation of nociceptive axons is probably not modified by 2 mM methocarbamol, at least not via modulation of Na$^+$ currents. However, this result does not exclude influences of the muscle relaxant on the Na$_{1.7}$ at higher concentrations and on other neuronal sodium channels.

Restrictions on dosing of methocarbamol are important to observe to minimize the risk of potential adverse effects at the injection sites and on the CNS. It is unclear if the concentrations of methocarbamol used in our study are achieved in the patients’ plasma with commonly accepted dosing levels. In a study evaluating the abuse potential of methocarbamol, adult male volunteers were administered up to 12g per day orally.$^{35}$ Although plasma levels were not presented in the latter investigation, a concentration of up to 2 mM may have been reached. However, this dose exceeds the intake of methocarbamol recommended by the FDA and international prescriptions, suggesting that plasma levels in the millimolar range are normally not likely to be achieved. In a pharmacokinetic study with rats, the plasma level reached 0.8 mM after IV injection of 150 mg/mL methocarbamol (highest dose)$^{36}$ On the other hand, intramuscular injection of methocarbamol at a concentration of 100 mg/mL$^{37}$ would locally cause much higher concentrations than the 2 mM used in the present work.

We conclude that the muscle relaxant methocarbamol, in addition to its effects on the CNS, has the potential to modulate neuromuscular transmission, force production of muscles and voltage-gated Na$^+$ channels. This study shows that the Na$_{1.4}$ channel is a molecular target of methocarbamol, which explains some of its peripheral effects.

Funding

This work was supported by the German Research Council DFG to SH (HA 3309/3-1, HA3309/6-1, HA3309/7-1). The laboratory of S.K. obtained financial support from Recordati Pharma GmbH, Ulm, Germany.

ACKNOWLEDGEMENTS

The authors thank Ms Stephanie Tesenvitz and Heidrun Kenk for expert technical assistance. Said Hashemolhosseini obtained support from the German Research Council DFG. Open access funding enabled and organized by Projekt DEAL.
REFERENCES

1. Chou R, Peterson K, Helfand M. Comparative efficacy and safety of skeletal muscle relaxants for spasticity and musculoskeletal conditions: a systematic review. J Pain Symptom Manage. 2004;28:140-175.

2. See S, Ginzburg R. Skeletal muscle relaxants. Pharmacotherapy. 2008;28:207-213.

3. Waldman HJ. Centrally acting skeletal muscle relaxants and associated drugs. J Pain Symptom Manage. 1994;9:434-441.

4. Krause T, Gerbershagen MU, Fiege M, Weishom R, Wappler F. Dantrolene—a review of its pharmacology, therapeutic use and new developments. Anesthesia. 2004;59:364-373.

5. Boon M, Martini C, Dahan A. Recent advances in neuromuscular block during anaesthesia. F1000Res. 2018;7:167.

6. Aljuhani O, Kopp BJ, Patanwala AE. Effect of methocarbamol on acute pain after traumatic injury. Am J Ther. 2017;24:e202-e206.

7. Brand RA. 50 Years ago in CORR: a clinical study of 46 males with low-back disorders treated with Methocarbamol. Andres Grisolia MD and J.E.M. Thomson CORR 1959;13:299-304. Clin Orthop Relat Res. 2009;467:2752-2754.

8. Dent RW, Ervin DK. Relief of acute musculoskeletal symptoms with intravenous methocarbamol (robaxin injectable): a placebo-controlled study. Curr Ther Res Clin Exp. 1976;20:661-665.

9. Witenko C, Moorman-Li R, Motycka C, et al. Considerations for the appropriate use of skeletal muscle relaxants for the management of acute low back pain. P T. 2014;39:427-435.

10. Schneider MS. Pain reduction in breast augmentation using methocarbamol. Aesthetic Plast Surg. 1997;21:23-24.

11. Valtonen EJ. A double-blind trial of methocarbamol versus placebo in painful muscle spasm. Curr Med Res Opin. 1975;3:382-385.

12. Voci JM, al-Hakim M, Dokko Y, Katiiri MB. Intravenous methocarbamol in the treatment of stiff-man syndrome. Muscle Nerve. 1993;16:434-435.

13. Emrich OM, Milachowski KA, Strohmeier M. Methocarbamol in acute low back pain. A randomized double-blind controlled study. MMW Fortschr Med. 2015;157(Suppl S5):9-16.

14. Uberall MA, Emrich OMD, Muller-Schwefe GH. Real-life efficacy and tolerability of methocarbamol in patients suffering from refractory muscle-related low/back pain - results of a health care research project based on data from the German pain practice registry. MMW Fortschr Med. 2017;159:6-17.

15. Abdel Shaheed C, Maher CG, Williams KA, McLachlan AJ. Efficacy and tolerability of muscle relaxants for low back pain: systematic review and meta-analysis. Eur J Pain. 2017;21:228-237.

16. Lewis WB. Use of methocarbamol in orthopedics. Calif Med. 1959;90:26-28.

17. Cymbalist MA, Shapero M. A comparative study of the effect of some centrally acting skeletal muscle relaxants in mice. J Pharm Pharmacol. 1974;26:109-112.
intradmuscular injection pain: a randomized clinical trial. J Educ Health Promot. 2018;7:172.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Zhang Y, Otto P, Qin L, et al. Methocarbamol blocks muscular Na\textsubscript{1.4} channels and decreases isometric force of mouse muscles. Muscle & Nerve. 2021;63:141–150. https://doi.org/10.1002/mus.27087