Fatigability of rat hindlimb muscles after acute irreversible acetylcholinesterase inhibition

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Panenic, Robert, Victor Gisiger, and Phillip F. Gardiner. Fatigability of rat hindlimb muscles after acute irreversible acetylcholinesterase inhibition. J. Appl. Physiol. 87(4): 1455–1462, 1999.—The purpose of this study was to investigate the functional impact of acute irreversible inhibition of acetylcholinesterase (AChE) on the fatigability of medial gastrocnemius and plantaris muscles of Sprague-Dawley rats. After treatment with methanesulfonyl fluoride (a lipid-soluble anticholinesterase), which reduced their AChE activity by >90%, these muscles were subjected to an in situ indirect stimulation protocol, including a series of isolated twitch and tetanic contractions preceding a 3-min fatigue regimen (100-ms trains at 75 Hz applied every 1.5 s). During the first minute of the fatigue regimen, the effects of AChE inhibition were already near maximal, including marked reductions in peak tension and the force-time integral (area), as well as a decrement of compound muscle action potential amplitudes within a stimulus train. Neuromuscular transmission failure was the major contributor of the force decreases in the AChE-inhibited muscles. However, despite this neuromuscular transmission failure, muscles of which all AChE molecular forms were nearly completely inhibited were still able to function, although abnormally, during 3 min of intermittent high-frequency nerve stimulation.

Acetylcholinesterase (AChE; EC 3.1.1.7) is an enzyme highly concentrated at the neuromuscular junction (NMJ), where it plays an important role in cholinergic transmission by terminating the action of ACh on the postsynaptic nicotinic ACh receptors (nAChRs) (21). A common approach to study the role of AChE on neuromuscular transmission (NMT) is through the use of anticholinesterase agents, which inhibit this enzyme's activity (17). It is well documented in the literature that the inhibition of AChE results in the potentiation of indirectly evoked muscle twitches and the inability of muscle to maintain a tetanic contraction in response to repetitive nerve stimulation, the latter known as tetanic fade (3, 5, 17). Consistent with these alterations, anticholinesterases have been shown to also affect the amplitude and decay times of the miniature end plate potentials and currents, as well as the end plate potentials and currents (22, 27–29, 36).

However, AChE may have additional functions such as the prevention of postsynaptic desensitization during high-frequency activity, thus preserving NMT efficacy during rapid muscle movement. During prolonged intermittent indirect stimulation of incubated muscle with intact AChE, a portion of the decrease in muscle force that occurs has been attributed to NMT failure (NMTF), as determined by comparing forces evoked by direct stimulation with forces evoked by indirect stimulation during the progression of fatigue (19, 24). This failure can occur at frequencies of stimulation that are well within the physiological range (16). The source of this failure has yet to be firmly established, but some evidence points toward alterations in ACh release (23), as well as desensitization of postsynaptic nAChRs (28, 32). With reference to the latter phenomenon, results emanating from our laboratory and others (9, 11, 12, 18) have shown that muscle AChE increases, in particular its mainly perijunctional G4 molecular form, in response to enhanced physical activity. This may indicate an adaptive strategy to reduce the desensitization of the nAChRs during repetitive activation and, thus, attenuate this fatigue component.

The purpose of the present study was to ameliorate our understanding of the functional role of AChE during intermittent repetitive contractions simulating rhythmic exercise leading to muscle fatigue. This was accomplished by subjecting an in situ muscle preparation to a fatigue protocol after the acute inhibition of AChE with a lipid-soluble irreversible anticholinesterase, methanesulfonyl fluoride (MSF), at a dose producing a >90% inhibition of AChE. Medial gastrocnemius (MG) and plantaris (PL) muscles were chosen for their high G4 AChE concentrations (18) and diversity in fiber type populations (2). The main finding of our study was that the MSF-treated muscles were still able to function, although abnormally, in response to short intermittent high-frequency bursts of nerve stimulation under conditions where they were deprived of >90% of their AChE activity.

METHODS

Care and Anesthesia of Animals

Adult female Sprague-Dawley rats (n = 41) weighing 250–350 g were obtained from Charles River (St. Constant, PQ, Canada). The rats were housed individually in grid cages, maintained on a 12:12-h light-dark cycle, and provided commercially available laboratory rat chow and water ad libitum from the time of reception until the day of the experiment. The care and treatment of the animals were in accordance with the principles of the Guide to the Care and Use of Experimental Animals (Canadian Council of Animal Care). The rats were anesthetized with pentobarbital sodium (45 mg/kg ip; Somnotol, MTC Pharmaceuticals) before each acute experiment. Supplemental doses of pentobarbital so-

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dium were given intraperitoneally as required to maintain deep anesthesia during each experiment.

Administration of MSF or Saline

In the AChE inhibition experiments, a waterproof reservoir was made around the muscle under study, the MG or the PL of the left leg, with use of plastic wrap (Saran Wrap, Dow-Brands, Paris, ON, Canada) and agar (Sigma Chemical, St. Louis, MO). The plastic wrap was cut in a square, slid gently under the muscle, and folded in a conical shape around the muscle's proximal end near the popliteal fossa. The lumen of the cone-shaped plastic wrap covering was closed with liquid agar (2.0%). A solution of 1 mg/ml MSF (Aldrich, Milwaukee, WI) was pipetted into the reservoir so that the muscle was completely submerged. After 80 min, the fluid was removed, and the muscle was thoroughly rinsed with saline before the reservoir was removed. Control rats were treated similarly, except saline alone was applied to the muscle reservoir of the left leg. Then the animal was moved to a stereotaxic table, where it was stabilized and prepared for nerve-evoked isometric contractions, as described by Vanden Noven et al. (33). Briefly, the head, vertebral column, left knee, and left foot were rigidly fixed with the rat in a prone position. The knee was stabilized with a drill inserted transversely through the distal femur and clamped to an upright post, and the foot was stabilized using a rigid clamp. The hindlimb skin flaps were used to prepare a pool that was filled with heated mineral oil (liquid paraffin) kept at 36°C by recirculation through a thermostatically regulated bath. The distal tendon of the muscle was attached to a force transducer (Statham Gould UC3:UL4-20) with a 2-0 silk ligature.

In Situ Isometric Contractile Properties

Indirect electrical stimulation. In the first set of experiments, after the temperature of the rat and hindlimb oil bath had stabilized at 36°C, indirect contractions of the MG or PL muscle were evoked by direct-current square-wave pulses of 50-µs duration via a bipolar stimulating electrode placed around the sciatic nerve with use of a Grass S88 stimulator (Quincy, MA). The length of the muscle was adjusted to give maximum twitch tension at the shortest muscle length in response to supramaximal stimulation by using a force transducer mounted on a rack-and-pinion apparatus. The hindlimb skin flaps were used to prepare a pool that was filled with heated mineral oil (liquid paraffin) kept at 36°C by recirculation through a thermostatically regulated bath. The distal tendon of the muscle was attached to a force transducer (Statham Gould UC3:UL4-20) with a 2-0 silk ligature.

For the force, EMG responses to twitches repeated every 10 s (0.1 Hz) for 2 min were recorded 40 min after removal of the MSF or saline solution. Two minutes later, contractile and EMG responses were recorded for one series of 800-ms trains at 75-Hz trains lasting 100 ms applied every 1.5 s for 3 min. Force and EMG responses (4-Hz high pass, 2.5-kHz low pass) were displayed and simultaneously displayed on an oscilloscope (Hewlett-Packard, Colorado Springs, CO) and recorded on a PC-based 80486 microcomputer and FM tape (Vetter, Rebersberg, PA). Fatigue and recovery records were collected on FM tape only. In turn, these records were digitized and stored off-line onto the hard disk of a PC-based 80486 microcomputer. Custom-made software allowed for storage and analysis of all the recorded data. At the end of each experiment, the MG or PL muscles of the left and right legs were rapidly excised, trimmed of superficial fat and connective tissue, and frozen in melting isopentane precooled in liquid nitrogen. The muscles were stored in a freezer at −80°C until AChE analysis. The animals were then killed with an overdose of pentobarbital sodium.

Assessment of NMTF and muscle failure. In a second set of experiments, the MG muscles of female Sprague-Dawley (250–325 g) rats were treated with the AChE inhibitor (MSF) or saline (C1). These muscles were stimulated indirectly and directly by 100-ms trains at 200 Hz (separated by 10 s) before and after the 3-min fatigue regimen described previously. For direct stimulation, two 33-gauge wires (Cooner Wire, Chatsworth, CA) with 10-mm desensitized tips were inserted into the extremities of the muscle with a 20-gauge hypodermic needle. The electrodes were held in place by ligatures at their point of entry and exit of the muscle. In succession, maximal twitch force was determined in response to supramaximal stimulation of the sciatic nerve (indirect) and direct stimulation of the muscle (80–120 V) with a pulse duration of 50 µs.

The relative contributions of NMTF and muscle failure to the reduction in force induced by the fatigue regimen were calculated according to equations of Pagala et al. (30): NMT (%) = ((I − D/I) × 100, and muscle failure (%) = (D/I) × 100, where I (indirect) is the percent reduction in the force-time integral (g·s) with nerve stimulation (total failure) and D (direct) is the percent reduction in the force-time integral with muscle stimulation.

AChE Analysis

Whole frozen muscles were weighed and homogenized in 2.5 ml (PL) or 5.0 ml (MG) of the following high-salt detergent buffer containing antiproteolytic agents: 10 mM Tris-HCl, pH 7.0, 10 mM EDTA, 1 M NaCl, 1% Triton X-100, and 1 mg/ml bacitracin (Sigma Chemical) (25). Muscles were homogenized on ice with a Polytron three times for 15 s at a setting of 6. Supernatants of low-speed centrifugation (20,000 g for 15 min at 4°C) were used to measure AChE activity by the spectrophotometric method of Ellman et al. (8), as modified by Gisiger and Stephens (13) in the presence of the nonspecific cholinesterase inhibitor tetraisopropylpyrophosphoramide (Sigma Chemical).

The possibility that free MSF remaining in the muscle might further inhibit AChE activity during the homogenization procedure was verified by mixing equal volumes of MSF-treated and contralateral muscle supernatants of the same animal after low-speed centrifugation and comparing the AChE activity of this 1:1 dilution with the theoretically expected value. Our analysis indicates that there was no difference between the measured and the theoretical AChE activity values, demonstrating that the AChE measurements obtained in the MSF-treated muscles corresponded to their AChE activities at the moment they were frozen at the end of the stimulation protocol.

Analysis of AChE Molecular Forms

Velocity sedimentation analyses of the AChE molecular forms were performed as described in detail earlier (13, 18). Aliquots (50 µl) of the muscle extracts were loaded on 5–20% sucrose gradients and centrifuged in a Beckman SW41 rotor at 40,000 rpm for 19 h at 3°C. The sucrose solutions were made up in a buffer similar to that used for homogenization, except it contained 0.05 M MgCl₂ instead of EDTA, and aprotonin was omitted. Approximately 45 fractions were collected from each gradient and assayed for AChE activity, as described above.
In the first set of experiments, a two-way ANOVA was employed to analyze the data for the main effects of muscle and treatment, and a Newman-Keuls a posteriori test was used to determine differences between individual means when significant interactions were evident. In the second set of experiments (indirect/direct stimulation), a two-tailed independent t-test was employed to determine differences between group means. Values are means ± SD; n is the number of muscles. Differences were considered significant at P < 0.05.

RESULTS

AChE Activity

The specific AChE activity (nmol·min⁻¹·g muscle wt⁻¹) of the hindlimb plantar flexor muscles, MG and PL, was greatly reduced by MSF treatment. Compared with a pool of control muscles (left and right leg), these decreases in specific AChE activity corresponded to 99% in the MG muscles (Ctl: 648.5 ± 149.1, n = 18; MSF: 3.6 ± 5.6, n = 6; P < 0.05) and 92% in the PL muscles (Ctl: 760.5 ± 115.3, n = 12; MSF: 62.4 ± 38.3, n = 7; P < 0.05). In addition, AChE was inhibited 50% in the MG (323.69 ± 175.17, n = 6, P < 0.05) and 44% in the PL (427.2 ± 83.2, n = 7, P < 0.05) of the untreated contralateral right leg compared with the same pool of control muscles, demonstrating that MSF was transported via the circulation.

AChE Molecular Forms

The effects of the MSF treatment on the individual AChE molecular forms were investigated in four MG muscles exhibiting a total AChE activity equivalent to 4–8% of the controls. All MSF-treated muscles yielded essentially identical AChE distributions, which were clearly distinct from those displayed by the control or the contralateral muscles. Characteristically, the AChE profile of the MSF-treated muscles showed a predominant G₁ peak containing about one-half the total AChE profile of the MSF-treated muscles showed a predominant G₁ peak containing about one-half the total AChE activity, together with low amounts of the heavy forms, G₄, A₈, and A₁₂ (Fig. 1). This AChE profile, obtained from muscles taken 2–3 h after MSF treatment, is consistent with the AChE distributions displayed by skeletal muscles and nervous structures taken a few hours after irreversible AChE inhibition. These previous studies have established that the predominant G₁ peak results from the active postinhibition AChE neosynthesis that starts immediately after removal of the inhibitory agents (14, 15, 35). The AChE molecular form profiles displayed by the contralateral MG muscles were indistinguishable from those of the control, untreated muscles (data not shown) (18).

Effects of AChE Inhibition on Nerve-Evoked Twitch and Tetanic Contractions and EMG

Consistent with previous reports, twitch peak tension in response to 0.1-Hz stimulation was significantly potentiated by MSF treatment in both muscles compared with controls. Twitch peak tension was increased by a factor of 2 (MG, P < 0.05) to 3.5 (PL, P < 0.05) in these AChE-inhibited muscles. In addition, the contractile responses of the MSF-treated muscles to repetitive nerve stimulation showed the classical inability of muscle exposed to anticholinesterases to maintain tetanic tension, known as tetanic fade. This tetanic fade was frequency dependent, with the peak and plateau contractile tensions developed in the two muscles being affected differently. Compared with controls, peak tension of the MSF-treated muscles was significantly increased at 25 Hz (MG: 54%; PL: 104%; P < 0.05), unchanged at 50 Hz, and markedly reduced at 200 Hz (MG: 46%; PL: 21%; P < 0.05). Plateau tension (i.e., the tension at the end of the tetanic train) was diminished by MSF treatment at all stimulation frequencies in both muscles compared with their controls (P < 0.05), except at 25 Hz in the MSF-treated PL muscles. Differences in plateau tension between the two AChE-inhibited muscles were observed at 50 and 200 Hz, where MSF-treated PL muscles generated greater tension than the treated MG muscles (P < 0.05).
The compound muscle action potentials (CMAPs) of MSF-treated muscles exhibited repetitive activity that was evident in the twitch (0.1 Hz), as well as the initial CMAP of contractions elicited at 25, 50, and 200 Hz. Unlike the saline-treated muscles, the MSF-treated muscles were unable to maintain the amplitude of their CMAPs throughout the 800-ms trains evoke at 25, 50, and 200 Hz. As the frequency of stimulation increased, the decrement in CMAP amplitude within a train became more pronounced and occurred earlier in both MSF-treated muscles.

Altogether these results show that the near-complete inhibition of AChE by MSF with use of an in situ muscle preparation reproduced essentially all the effects of AChE inhibition that have previously been observed only in vitro, namely, a potentiation of the twitch peak tension, a repetitive activity in the CMAPs, and a tetanic fade accompanied by a CMAP decrement that was frequency dependent (3–5, 17, 29). These results confirmed that our in situ experimental model was adequate to evaluate the impact of AChE inhibition on the fatigue induced by intermittent high-frequency nerve stimulation simulating rhythmic exercise.

Effects of AChE Inhibition on the Fatigability of Muscle

Peak tension. The initial (0-min) peak force responses of the MSF-treated and control muscles to 75-Hz stimulation were quite similar (Figs. 2A and 3). Thereafter, the MSF-treated muscles generated significantly less peak tension than their controls (P < 0.05). Already after the 1st min of the fatigue regimen, the effects of MSF treatment on peak tension were almost maximal. At this time the AChE-inhibited MG muscles produced 47% less peak tension than their controls, whereas the inhibited PL muscles generated 24% less tension. Thereafter, these reductions in peak tension remained basically identical up to the completion of the fatigue regimen.

Force-time integral. Force-time integral measurements of the 75-Hz, 100-ms-long trains demonstrated significant decreases in area (37–70%) for both MSF-treated muscles during the fatigue regimen (Fig. 2B). The decrease appeared earlier and was slightly more pronounced in the MG than in the PL muscles. The reduction in area compared with controls became statistically significant by the 1st min of fatigue in the AChE-inhibited MG muscles (68%) and by the 2nd min in the inhibited PL muscles (37%, P < 0.05).

CMAP. In control muscles the amplitude of CMAPs only slightly varied during a given burst, whereas it decreased progressively with successive bursts, as illustrated in Figs. 4A and 5. In contrast, in the MSF-treated muscles, CMAP amplitude sharply decreased during each burst but recovered during the rest interval between trains, so that the amplitude of the first CMAP of each burst only slightly decreased with successive bursts (Figs. 4B and 5). Compared with the control muscles, a significant decrement in CMAP amplitude was already observed in the MSF-treated MG and PL muscles as of the fourth stimulus in the initial burst. In subsequent bursts, this decrement occurred earlier, for example, at the 3rd stimulus of the 40th and 80th bursts and at the 2nd stimulus of the last (120th) burst.

Relative Contribution of NMTF and Muscle Failure

In a second set of experiments, indirect and direct test stimulations (100-ms trains at 200 Hz) were applied before and after the fatigue regimen to determine the origin (NMT vs. muscle) of the failure in AChE-inhibited and control MG muscles (Fig. 6, Table 1). Before the fatigue regimen the force profiles produced by direct stimulation in the AChE-inhibited muscles (Fig. 6B) and control muscles (Fig. 6A) were similar, whereas indirect stimulation produced the tetanic fade in the former. After the fatigue regimen the force-time integral (g·s) of the inhibited muscles was reduced by 31.5% with indirect stimulation but only by 11.6% with direct stimulation (Table 1). In contrast, in the control muscles, indirect and direct stimulations reduced the force-time integral in almost identical proportions, i.e.,
40.9 and 35.5%, respectively. It should also be pointed out that the force-time integral generated by indirect stimulation was decreased by similar proportions in both groups of muscles; however, the inhibited muscles displayed a marked tetanic fade (Fig. 6B), i.e., a distinct indication that the decreases were due to different causes. Processing these data according to Pagala et al. (30) (see METHODS) showed that the contribution of NMTF to fatigue was significantly greater in the AChE-inhibited muscles (59.7%) than in the control muscles (17.1%); the reverse was true for muscle failure (Table 1). Interestingly enough, however, a small degree of NMTF (17.1%) was definitely noticeable in the control muscles.

**DISCUSSION**

Increasing evidence (7) suggests that, in addition to terminating the action of ACh at the NMJ to allow for the next stimulus, AChE also prevents the desensitization of the postsynaptic nAChRs that may develop during repetitive high-frequency activity (28, 32), thereby preserving NMT efficacy during rapid movements. Thus, to examine this additional function more
closely, rat hindlimb flexor muscles, MG and PL, were subjected to a fatigue stimulation protocol simulating rhythmic exercise after acute irreversible inhibition (>90%) of their AChE. Sedimentation analysis of the AChE molecular forms confirmed that MSF acted like other irreversible anticholinesterases, in that it affected all forms uniformly and triggered an early neosynthesis of G1 (15, 35).

The results of this study demonstrated for the first time that skeletal muscle almost completely deprived of functional AChE was still able to sustain 3 min of intermittent activity in response to a fatigue protocol.

Fig. 5. A 3-dimensional representation of effects of AChE inhibition on peak-to-peak amplitude of CMAPs of nerve-evoked 100-ms-long, 75-Hz trains used to induce fatigue in MG (A) and PL (B) muscles. In control (Ctl) muscles, amplitude of 8 CMAPs of a given burst progressively declined, whereas a marked decrement in CMAP amplitude was observed in MSF-treated muscles during every burst. Absolute CMAP amplitude values (mV) were normalized by dividing each CMAP amplitude value by initial CMAP amplitude of 1st burst (0 min) and expressed as percent initial CMAP. Ctl MG, n = 10; MSF MG, n = 6; Ctl PL, n = 6; MSF PL, n = 7.

Fig. 6. Effects of AChE inhibition on force evoked by 100-ms trains of 200-Hz direct (dashed lines) and indirect (solid lines) stimulations of an MG muscle before and after fatigue. Control MG muscle (A), before fatigue, shows a similar contractile profile whether it is stimulated directly or indirectly. In MSF-treated MG muscle (B), plateau force is maintained during direct stimulation, which is not the case during indirect stimulation, as shown by presence of tetanic fade.
entailing short high-frequency trains. Although this was an unexpected result, especially considering the large NMTF exhibited by the AChE-inhibited muscles (i.e., 59.7% in MG), several facts suggest the soundness of this observation. First, in our in situ experimental model, control muscles exhibited the expected muscle contractile failure in response to the fatigue protocol (10). Second, the responses of the MSF-treated muscles to isolated stimuli (0.1 Hz), as well as to high-frequency trains (800 ms, 25–200 Hz), reproduced the twitch potentiation, tetanic fade, and decrement of CMAP amplitude classically displayed in vitro by AChE-inhibited muscles (3–5, 17, 29). Third, the contractile and EMG responses to the fatigue regimen were similar in MG and PL muscles; the small differences exhibited by the two muscles paralleled their differences in the degree of AChE inhibition, 99 vs. 92%.

The preservation of contractile activity for several minutes during the fatigue protocol in the AChE-inhibited muscles, despite an important NMTF, may appear paradoxical. Actually, the NMTF was already present during the initial train, and it did not significantly increase as the fatigue trial progressed. During the initial 75-Hz burst, there is already a tetanic fade (Fig. 3B) and a sharp drop in amplitude of the CMAPs in the blocked muscles (Figs. 4B and 5). The important element here is that the rest period that followed and separated the stimulation trains allowed the NMJ to recover. As a result, the tetanic fade had diminished to some extent, whereas the CMAP decrement had almost disappeared at the start of the subsequent stimulation train (Figs. 3B and 4B). In our fatigue protocol the AChE-inhibited muscles were activated only shortly during the beginning of the train, after which an important proportion of muscle fibers became inactive because of the large NMTF. Thus what happened during each train of the fatigue protocol was essentially the same as in the initial test train. Consequently, the AChE-inhibited muscles actually escaped most of the fatigue trial.

Anticholinesterase-induced tetanic fade and CMAP decrement in response to a nerve-evoked train have been classically ascribed to the accumulation of ACh in the synaptic cleft. Indeed, in the absence of active AChE, ACh can be removed only by diffusion, a process that in the NMJ is much slower than hydrolysis (21). Lingering of ACh at the NMJ causes the NMTF by desensitization of the postsynaptic nicotinic receptors (1, 21, 28), as well as a reduction in nerve-evoked transmitter release, as a consequence of presynaptic receptor desensitization and/or reduced ACh synthesis (5, 6, 31, 34, 36). The same explanation holds true for the tetanic fade and CMAP decrement observed during each burst of the fatigue trial. It suggests that under our conditions the intertrain rest period provided enough time to allow diffusion to sufficiently reduce the synaptic ACh concentration.

Consistent with this interpretation, the results of our study demonstrate that skeletal muscle deprived of almost all of its AChE activity is still able to respond to rhythmic nerve activation, although abnormally, as long as the stimulation trains are of short duration and separated by adequately long rest periods. Accordingly, one would expect that as the rest period becomes shorter with more rapid activation, increasing amounts of functional AChE are required to maintain NMT. In this respect, the NMTF displayed by the control muscles in response to the fatigue trial is especially relevant.

Unlike the AChE-inhibited muscles, the control muscles did not exhibit a decrement in CMAP amplitude within a train; rather they displayed a progressive decrease in CMAP amplitude that was paralleled by a reduction in force. This force reduction is known to be caused by muscle contractile failure induced by fatigue as a consequence of the buildup of metabolites within the muscles (26) and the extracellular accumulation of potassium (20). Still, the control muscles showed some degree of NMTF in response to our fatigue protocol. Indeed, in control MG muscles, ~17% of the fatigue induced by intermittent high-frequency nerve stimulation was contributed by NMTF (Table 1, Fig. 6). This observation significantly strengthens the earlier proposal (18) that, even without any AChE inhibition, muscles may exhibit a degree of desensitization in response to high-frequency rhythmic activity. From this perspective, the NMTF shown by control muscles taken from cage-confined rats is consistent with the large G4 AChE increases exhibited by muscles of highly active animals, such as those performing intense voluntary wheel running (11). In these latter animals, increased G4 AChE most likely helps reduce receptor desensitization and this NMTF during rhythmic neuromuscular activation.

In summary, the main finding of our study was that the MSF-treated muscles were still able to function, although abnormally, in response to short intermittent high-frequency bursts of nerve stimulation under conditions where they were deprived of ~90% of their AChE activity, an inhibition affecting all molecular forms. Contrary to noninhibited muscles, in which the main contributor to fatigue was contractile failure, the fatigue observed in the AChE-inhibited muscles was due primarily to NMTF. Our results demonstrate that some degree of NMTF is also possible during intermittent high-frequency bursts of short duration in the absence of AChE inhibition, suggesting that AChE plays an important role in these muscles by preventing a large-

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**Table 1. Effects of AChE inhibition in MG muscles on NMTF vs. MF**

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<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Indirect</th>
<th>Direct</th>
<th>MF</th>
<th>NMTF</th>
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<tr>
<td>Ctl</td>
<td>6</td>
<td>40.9 ± 20.8</td>
<td>35.5 ± 18.3</td>
<td>82.9 ± 18.4</td>
<td>17.1 ± 18.4</td>
</tr>
<tr>
<td>MSF</td>
<td>6</td>
<td>31.5 ± 8.5</td>
<td>11.6 ± 7.6*</td>
<td>40.3 ± 25.0*</td>
<td>59.7 ± 25.0*</td>
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Values are means ± SD; n, number of muscles. Indirect and direct correspond to percent decrease in force-time integral of muscles stimulated indirectly and directly with 200-Hz 100-ms trains. NMTF and MF correspond to relative contribution (%) of neuromuscular transmission failure and muscle failure to reduction in force induced by 3-min fatigue regimen calculated according to equations of Pagala et al. (30); AChE, acetylcholinesterase; MG, medial gastrocnemius; Ctl, control. *Significantly different from Ctl (P < 0.05, by independent t-test).
scale NMTF, as seen in the inhibited muscles, thereby allowing muscle to be fatigued by processes within the muscle.

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