Measurement of force and both surface and deep M wave properties in isolated rat soleus muscles

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Harrison, A. P., and J. A. Flatman. Measurement of force and both surface and deep M wave properties in isolated rat soleus muscles. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1646–R1653, 1999.—In isolated soleus muscles of 4-wk-old rats, M wave parameters were recorded with surface and deep recording electrodes and examined in relation to both twitch and tetanic force. Addition of ouabain (10−5 M for 16 min) to isolated muscles caused an −40% decrease in twitch amplitude and area (P < 0.01) that was associated with a 98% decrease in surface M wave amplitude, a 78% decrease in deep M wave amplitude (both P < 0.001), a 98% decrease in surface M wave area (P < 0.01), 48% of which occurred within 60 s of addition of ouabain (P < 0.05), and a 55% decrease in deep M wave area (P < 0.05). The decrease in twitch parameters on addition of ouabain was most closely correlated with deep M wave area (r = 0.92). Direct tetanic stimulation at a frequency of 30 Hz resulted in an initial potentiation of M waves, which was not seen at a frequency of 90 Hz. Instead, 90 Hz stimulation resulted in a prompt decrease in tetanic force that was correlated with a decrease in both deep M wave amplitude (r = 0.94; P < 0.01) and deep M wave area (r = 0.96; P < 0.01). It is concluded that simultaneous surface and deep recordings involving area and amplitude are fundamental to analysis of the effects of pharmacological agents on muscle performance and the use of M waves as predictors of muscle excitability.

skeletal muscle; compound action potential; electromyogram; ouabain; Na+-K+ pump

M WAVES, WHICH REFLECT THE CURRENT developed during the action potential of many muscle fibers, have often been used in fatigue experiments to provide information about the net effects of ion fluxes, Na+-K+ pump activity, and neuromuscular transmission in skeletal muscles. Indeed, a recent review discussed the potential of M wave analysis as a noninvasive predictor of muscle force (13).

M wave measurements in mammalian muscle indicate that repetitive voluntary or stimulated contractions lead to a decline in M wave amplitude, ultimately causing a decline in contractile force (4, 28). This agrees with observations that stimulation of muscles leads to an increase in intracellular Na+ (34, 35), a substantial loss of intracellular K+ (11, 25), and an ensuing reduction in excitability (7, 23, 26).

Whereas the ultimate fate of M waves recorded in muscles exposed to repetitive stimulation is a decrease in M wave amplitude, there are conflicting reports on the initial response of M waves. Some authors report a transient increase in M wave amplitude associated with contraction (2, 15, 23), whereas others find no change in M wave amplitude (5) or a decline (3, 18). The transient potentiation of M wave amplitude has been attributed to either an increased synchronization of fibers (6) or an electrogenic effect of increased Na+ –K+ pumping. The latter is based on observations that, after short periods of contractions of muscle, Na+ –K+ pumping causes hyperpolarization of muscle fibers facilitating excitation of Na+ channels (23). These studies, along with others (28, 29), focused on changes in M wave amplitude and obtained M wave recordings percutaneously or from the surface fibers of exposed muscles. It is well known, however, that fiber-type proportions differ between the superficial and deep layers of a muscle (24) and that differences in electromyogram signals exist between different fiber types (28). Thus M wave recordings from surface fibers may not be representative of the whole muscle.

This study has therefore addressed the following questions. 1) What benefits, if any, are there to be gained from measuring both surface and deep M waves? 2) Does inhibition of Na+-K+ pumping by addition of ouabain affect the excitability of isolated muscles? 3) Do deep muscle fibers exhibit a temporal increase in M wave amplitude during stimulation, and, if so, can it be observed under both low- and high-frequency stimulation? 4) Should M wave amplitude be used as an index of change in excitability in isolated muscles?

METHODS

Equipment

As described in part (8), isolated rat muscles were mounted vertically in thermostatically controlled chambers, stimulated directly with supramaximal pulses, and force development and M wave properties were recorded. The thermostatically controlled chambers had an internal depth and diameter of 5.5 and 3.2 cm, respectively, holding ~44 ml of Krebs-Ringer (KR) bicarbonate buffer (Fig. 1). The mounting/stimulation block, made of perspex, was 8 cm long, 1.5 cm wide, and 1 cm thick. Into this perspex block were inserted two steel pins to hold the isolated muscle, two silver stimulating electrodes (0.88 mm diameter), and two silver recording electrodes (1.0 mm diameter) fashioned out of jewelry-grade silver (Dansk Hollands Ædelmetal A/S, Copenhagen, Denmark). The silver electrodes were covered with heat-shrink plastic coating and set in the perspex block in such a way to allow movement toward or away from the suspended muscle.

The stimulator used (Fig. 1), was a Master 8 Programmable Pulse Generator (AMPI, Jerusalem, Israel) linked to a modified stimulus isolator (isolator-10, Axon Instruments, Foster City, CA). Tetani were elicited by stimulation through two silver electrodes on the mounting/stimulation block by supramaximal field stimulation at either 30 or 90 Hz, 100 mA, and 1-ms pulse duration. Twitches were evoked by...
supramaximal constant current field stimulation at 2 Hz, 100 mA, and 0.2-ms pulse duration. Deep M waves were collected from stimulated muscles by a microelectrode that was linked to a home-built high impedance differential amplifier. Surface M waves were collected from muscles by one of two flat silver recording electrodes placed adjacent to the muscle that sent signals to a DAM 70 Differential Amplifier mounted with a low noise headstage (World Precision Instruments, Sarasota, FL). Twitch and tetanic contractions were measured using a FTO3 force displacement transducer (Grass Instrument, West Warwick, RI) connected to a CP122 AC/DC strain gauge amplifier (Grass Instrument) via a home-built bridge amplifier. Contractile force measurements were sent 1) to a chart recorder (Serevolgen Pen DriveREA 310, Radiometer, Copenhagen, Denmark), 2) via an analog-to-digital converter (TL-125 DMA Interface, Axon Instruments) to a Compaq 386 computer hard disk running pClamp software (Axon Instruments), and 3) via a MacLab 4S (AD Instruments, Hastings, UK) 8200/120 computer hard disk.

Animals, Muscles, and Force Development

Wistar rats (own breed), typically between 60 and 70 g (4 wk of age), were used. They were fed a standard rat pellet (Altromin Nr. 1314 Spezialfutterwerke, Lage, Germany) ad libitum. Water was freely available, environmental temperature was maintained at 21°C, and lighting was on a 12:12-h light-dark cycle. Rats were killed by a blow to the head, followed by cervical dislocation in accordance with local and national guidelines and with permission of the Animal Welfare Officer, University of Aarhus, Denmark.

Soleus muscles were dissected intact with both the tendons attached to a portion of the fibula, to facilitate anchoring, and a portion of the Achilles tendon at the opposite end of the muscle. The portion of fibula was placed between two metal pins and secured in place by a piece of thread. The section of Achilles tendon was impaled on a steel rod that was subsequently fastened to a force transducer, with the result that muscles lay both between two silver stimulating electrodes and on top of two silver recording electrodes. Suspended isolated muscles were placed into the thermostatically controlled chambers that were filled with a standard incubation medium, KR bicarbonate buffer, containing (in mM): 120 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.3 CaCl2, and 5.0 D-glucose (8). The buffer was kept at 30°C and equilibrated continuously with a mixture of 95% O2 and 5% CO2 (pH 7.3). Soleus muscles of 4 wk-old rats weigh 20.7 ± 0.6 mg wet wt and have an isometric force of 16.2 ± 0.4 N/g wet wt and a twitch-to-tetanus ratio of 1.5 (21, 22). Adjustments to muscle length were made to ensure that maximal isometric force was achieved, after which muscles were tested with twitch and tetanic stimulation.

In experiments involving 2-Hz stimulation (trains of 4 twitches), suspended muscles received a number of control stimuli to establish a stable baseline prior to the addition of ouabain (10⁻⁵ M). On addition of ouabain, muscles were stimulated at regular intervals to assess the time course of the effects of ouabain on both twitch and M wave parameters. The recovery of both twitch and M wave parameters on addition of drug-free KR buffer were then followed using 2-Hz twitches at regular intervals. In experiments involving 90-Hz stimulation with ouabain, isometrically suspended muscles were incubated with ouabain (1.5 × 10⁻⁶ M) for 30 min, during which test twitches were recorded regularly at 1-min intervals for the first 10 min and then again, individually, at 15 min, just before muscles were stimulated continuously (90 Hz) for 40 s. The recovery of tetanic force and deep M wave parameters 30 and 60 s after a period of continuous high-frequency stimulation were assessed by a 1.5-s tetanus at 90-Hz stimulation. Incubation of muscles with 10⁻⁵ M ouabain for a period of 15 min and 10⁻⁶ M ouabain for a period of 30 min has previously been shown to result in occupancy of ~40% and ~20% of the ouabain binding sites, respectively (10).

M Wave Recordings

Surface M wave recordings were made via one of two silver field electrodes (0.8-mm² recording area) placed adjacent to the muscle surface, whereas intramuscular M wave recordings (deep M waves) were made via a glass microelectrode (5-µm tip) filled with 170 mM NaCl, positioned in the center of the muscle but directly over the surface recording electrode. Conduction velocity, expressed in meters per second, was calculated by measuring the difference in the time to peak of M waves recorded individually from the two silver field electrodes, which were positioned 3.5 mm apart. To prevent distortion of M wave recordings, which results from movement of the electrode tip within contracting muscles, a device capable of absorbing movement was designed (Patent Pending: PA 1998 01020). In this way, compound action potentials could be transferred from the microelectrode positioned deep within the muscle to the differential amplifier while at the same time preventing any contraction-associated movement of the microelectrode tip. M wave area and amplitude were measured as shown in Fig. 2. Analysis of muscles inserted with glass microelectrodes filled with the stain tolue ne blue showed that the tip of deep recording electrodes was indeed located in the central region of muscles (results not presented). A chlorided silver reference electrode, positioned in the KR incubation buffer, was used for both surface and deep M wave recordings.

RESULTS

M Wave Conduction Velocity

M waves associated with twitches had a conduction velocity of 2.6 ± 0.1 m/s (n = 7) for soleus muscles. Preincubation of muscles with ouabain at a concentra-
tion of $1.5 \times 10^{-6} \text{M}$ for 30 min caused a 19% decrease in the average conduction velocity of M waves ($2.1 \pm 0.1 \text{m/s; } n = 5; P < 0.001$), compared with controls.

**Ouabain, Twitches, and M Wave Parameters**

In Fig. 3, a clear difference was observed between the M wave area measured using a surface recording electrode and that measured using a deep recording electrode. The surface recordings showed a rapid and almost total decrease in M wave area on addition of $10^{-5} \text{M}$ ouabain and a very slow and incomplete recovery on replacement of the incubation buffer with drug-free buffer. In contrast, the deep recording electrode showed an initial increase in M wave area on addition of ouabain followed by a decrease in deep M wave area that closely matched the changes in the twitch parameter. Deep M wave area recordings returned to the control level after ~35 min of washout, at about the time that the twitch returned to the control level. It became apparent from this one experiment alone that there was an advantage to be gained from simultaneously recording M waves both from a surface and a deep recording electrode.

Figure 4, A and B, presents the results of further experiments with ouabain. After only 1 min of incubation of muscles with $10^{-5} \text{M}$ ouabain, a significant 36% decrease in surface M wave amplitude and a 48% decrease in surface M wave area were measured (both $P < 0.05$). Sixteen minutes of incubation with $10^{-5} \text{M}$ ouabain caused a significant decrease in twitch amplitude (42%) and twitch area (47%; both $P < 0.01$) compared with control values. These changes in twitch parameters were associated with a 98% ($P < 0.001$) decrease in twitch amplitude.
decrease in M wave amplitude recorded from the surface of the muscle, a 98% \((P < 0.01)\) decrease in M wave area recorded from the surface of the muscle, a 78% \((P < 0.001)\) decrease in M wave amplitude recorded from deep within the muscle, and a 55% \((P < 0.05)\) decrease in M wave area recorded from deep within the muscle.

Approximately 3 min after washout, twitch amplitude began to increase linearly, reaching 98% of the control level after 20 min. Likewise, twitch area began to increase linearly 3 min after washout, reaching 95% of the control level after 20 min. This recovery of twitch force was associated with recovery of 32% of surface M wave amplitude, recovery of 66% of surface M wave area, recovery of 36% of deep M wave amplitude, and a 60% recovery of deep M wave area compared with control values.

Ouabain, Tetani, and M Wave Parameters

Muscles incubated for 30 min with ouabain at a concentration of \(1.5 \times 10^{-6}\) M and stimulated at a frequency of 90 Hz for 40 s showed a rapid decrease in force and deep M wave parameters (Fig. 5). A 9% greater decrease in tetanic force was noted after 5 s of continuous stimulation at 90 Hz for muscles incubated with ouabain (Fig. 5) compared with control muscles (Fig. 6B); 88.7 ± 1.6% and 97.8 ± 2.1% of initial isometric force, respectively \((n = 6; P < 0.01)\). After 20 s of stimulation, however, no difference in terms of tetanic force existed between ouabain-incubated and control muscles. Neither deep M wave area nor deep M wave amplitude was significantly affected by incubation with ouabain compared with controls.

Within 30 s of cessation of continuous 90-Hz stimulation, there was a significant 41\% \((P < 0.001)\) increase in tetanic force that was matched by a 57\% \((P < 0.001)\) increase in deep M wave amplitude and a 45\% \((P < 0.05)\) increase in deep M wave area compared with values after 40 s of stimulation.

Effects of Low- and High-Frequency Stimulation

M wave parameters and tetanic force. Continuous direct stimulation (30 Hz) was applied for 25 s to isolated muscles (Fig. 6A). Tetanic force remained constant over this period of time. There was, however, a rapid and significant potentiation of both deep M wave amplitude and area that occurred over the first 5–25 s of stimulation.

At a higher stimulation frequency (90 Hz), a different pattern was observed (Fig. 6B). A significant decrease in contractile force, compared with the initial value, was measured after only 15 s of continuous direct
stirulation (an ~20% decrease). Deep M wave amplitude also decreased rapidly on continuous direct stimulation of isolated muscles, being significantly different from the initial value after only 10 s of stimulation. The rapid decrease in deep M wave amplitude and contractile force was closely correlated (r = 0.98; P < 0.01). Measurement of deep M wave area, however, revealed no significant effect until muscles had been stimulated for a period of 25 s.

Correlations of force with M wave amplitude and area

In Figs. 4, A and B, twitch amplitude, with addition of ouabain (10\(^{-5}\) M), correlated most closely with deep M wave amplitude (r = 0.92) followed in rank order by deep M wave area (r = 0.85), surface M wave area (r = 0.72), and surface M wave amplitude (r = 0.68). During the washout period, however, when twitch amplitude recovered almost completely over 20 min, the closest correlation between force and M wave parameters was for surface M wave area (r = 0.94) followed by deep M wave area (both r = 0.83) and surface M wave amplitude (r = 0.81). In terms of tetanic force (Fig. 5), both deep M wave parameters were closely correlated with the decrease in force and subsequent recovery, although the best correlation was found to be that for deep M wave area (r = 0.96, P < 0.01) followed by deep M wave amplitude (r = 0.94, P < 0.01).

**DISCUSSION**

The results of this paper, in which contractions have been related to both surface and deep M wave parameters of isolated skeletal muscles, show that excitability, defined as electrical activity originating from the transmembrane passage of current during an action potential, is closely related to M wave properties. Results highlight 1) the importance of the simultaneous measurement of surface and deep M wave recordings when investigating the effects of pharmacological agents on muscle function in vitro, 2) that potentiation of deep M wave properties occurs initially during low rather than high-frequency stimulation, and 3) that measurement of M wave area is a good index of excitability during both twitch and tetanic contractions.

M Wave Conduction Velocity and Electrode Recordings

Measurement of M wave conduction velocity was made to assess the condition of the isolated muscles used. In the present study, M wave conduction velocities for soleus muscles of 4-wk-old rats (2.6 ± 0.1 m/s) compare favorably with those reported by others: soleus muscles of the mouse (2.9 ± 0.4 m/s) (26) and of adult rats (2.7 ± 0.4 m/s; 35°C) (27). This finding indicates that the technique of isolation used in this study does not result in appreciable damage of muscle fibers.

Analysis of M waves recorded by surface electrodes is prone to movement artifacts and depends on both the distribution of the motor units that are active and the geometry of the recording electrodes. Small recording electrodes give a clear signal provided the amplifier used has a high impedance and a low capacitance, but the sampling area of the muscle is restricted. This problem is well illustrated in the present study by Figs. 3 and 4, which show that a surface recording electrode positioned adjacent to a small isolated muscle (~20 mg wet wt and 1 cm diameter) cannot record the action potentials of all fibers within the muscle. Conversely, large recording electrodes record from a greater number of fibers, but a shunting effect can be created by the metallic surface of the electrode in conjunction with adjacent fibers, such that the recorded amplitude of individual action potentials of fibers is reduced (36). Similarly, recordings from deep recording electrodes will be influenced by the geometry of the electrode, the distribution of fiber types within the muscle, and the area of muscle sampled (30, 36), and, unless a device capable of absorbing movement is used, movement artifacts arising from rapid or forceful contractions will distort the recorded signals. Thus in the present study, simultaneous recordings made with both superficial and deep electrodes were found to give greater information about the state of excitability of the muscle as a whole.

Ouabain Effects on M Waves

After incubation of muscles with 10\(^{-5}\) M ouabain for 16 min (inhibiting ~40% of functional Na\(^+\)-K\(^+\) pumps), there was a very rapid decrease in M wave amplitude and M wave area that was restricted to surface fibers. The fact that within 60 s of addition of ouabain to the incubation buffer, the M wave area of surface fibers had decreased by 48% is indicative of the potency and toxicity of this compound. The decrease in M wave amplitude could, to some extent, be the result of decreased synchronization of contracting fibers due to variable reduction of conduction velocity in different fibers; however, the decrease in M wave area on addition of ouabain is indicative of a rapid decrease in excitability of surface fibers. The rapidity and extent to which this was observed is rather surprising and difficult to explain because the aforementioned inhibition of 40% of Na\(^+\)-K\(^+\) pump capacity by ouabain, which was noted in cut tissue samples, would not occur within 60 s in intact isolated muscles nor should inhibition of Na\(^+\)-K\(^+\) pumps cause such a rapid effect on excitability. Indeed, addition of ouabain (10\(^{-3}\) M) to muscles causes an immediate depolarization of only 8.5 mV, followed by a much slower phase of depolarization (6.9 ± 0.7 mV/h), which remains constant for up to 4 h (9). These findings, which have been substantiated by others (23), were interpreted as reflecting an initial halt in the rheogenic, electrogenic component of the 3 Na\(^+\)-to-2 K\(^+\) coupled pump that is followed by a slower redistribution of ions and associated depolarization (9). Recently, however, it was reported that ouabain causes a change in Na\(^+\) channels of cardiac myocytes, referred to as “slip-mode conductance” (33), such that the current through Na\(^+\) channels is not only increased, but is more permeant to Ca than Na ions (33). If slip-mode conductance occurs in Na\(^+\) channels of skeletal muscles incu-
bated with ouabain, one might expect 1) a slight increase in Na\(^+\) influx, resulting in a reduction in the transmembrane gradient for Na\(^+\), slight depolarization, and a comparable decrease in excitability, and 2) spontaneous contractions or fibrillations, as indeed have been reported on addition of ouabain (9) as a result of an increase in Ca\(^{2+}\) influx. While it may be debated that part of the rapid decrease in excitability of muscle fibers, on addition of ouabain, may be attributable to slip-mode conductance of Na\(^+\)-channels, it should be noted that this phenomenon alone cannot explain the present findings, which should be the topic of further investigation.

In Fig. 3, it was noted that there was a potentiation of deep M wave area on addition of ouabain to the incubation buffer that increased with time, a finding that was also observed in other muscles. This is perhaps an unexpected result, because it has been demonstrated that, in experiments involving the effects of contractile activity on rat soleus muscles, tetanic stimulation is accompanied by an increase in the resting membrane potential and action potential of individual fibers, which can be suppressed by the addition of ouabain (23), a finding that gives support to the theory that potentiation of M waves in muscles is the result of hyperpolarization, which, in turn, is a consequence of electrogenic Na\(^+\)-K\(^+\) pumping by muscle fibers. An explanation for the present finding, however, may lie with hyperpolarization of deep muscle fibers resulting from increased Na\(^+\)-K\(^+\) pumping as an indirect effect of addition of ouabain, because it is known that neural tissue has a high binding affinity for ouabain, triggering a release of catecholamines and/or calcitonin gene-related peptide, which are known to stimulate the Na\(^+\)-K\(^+\) pump (1, 9, see also review in Ref. 19). However, an alternative explanation for potentiation of M waves is given in the following section of this discussion.

**Effects of Low- and High-Frequency Stimulation**

The initial increase in M wave amplitude, measured using a deep recording electrode, during direct stimulation of isolated soleus muscles at 30 Hz compares favorably with the results obtained by Fitch and McComas (15), who used a surface recording electrode to measure M wave amplitude during evoked contractions of tibialis anterior muscles of human subjects. The present study also serves to extend these results by revealing an initial increase in M wave area of isolated muscles during direct stimulation. The increase in M wave amplitude could simply be the result of increased synchronization of fibers; however, potentiation of M waves has been reported previously in rat soleus muscles (14) and is often seen in clinical electromyography, in which it is termed “pseudofacilitation”. Some reports attribute this initial potentiation to enhanced calcium kinetics, increased phosphorylation, and optimized mechanical properties (12, 14), but these factors would also be likely to improve the contractile force of muscle, an effect that we have not noted in the present study, at least not during the initial period of contraction. Slowed impulse conduction with duration of contractile activity cannot be the explanation, because the resulting temporal dispersion of the action potentials of single fibers would cause phase cancellation and hence a reduction rather than an increase in M wave area. Rather, it seems likely that the phenomenon is due to transmembrane action potentials of longer duration with more prominent negative after-potentials as proposed by Hanson (20), because such “less biphasic” action potentials would summate more effectively, resulting in an increase in the M wave area.

At the higher frequency of 90 Hz, which was used to ensure activation of all functional Na\(^+\)-K\(^+\) pumps (22, 31), deep M wave amplitude decreased in a linear fashion with the commencement of stimulation and in conjunction with the decline in tetanic force. These results suggest, therefore, that the phenomenon of initial potentiation of M wave amplitude and area during periods of continuous stimulation is restricted to lower and more physiological frequencies. This conclusion is in agreement with recent results that showed that, in human subjects, M wave amplitude, recorded from the surface of quadriceps muscles, increased with short- and decreased with long-duration fatigue (2). An explanation for this difference between low- and high-frequency stimulation in terms of M wave potentiation as a result of electrogenic Na\(^+\)-K\(^+\) pumping may lie with the possibility that, during low-frequency stimulation, when the intracellular K\(^+\) concentration ([K\(^+\)]) of fibers would be expected to fall and the intracellular Na\(^+\) concentration ([Na\(^+\)]) to rise (35), the electrogenic effect of Na\(^+\)-K\(^+\) pumping to raise the membrane potential and render Na\(^+\) channels available for excitation may temporarily overcome a fall in membrane potential that would otherwise be sufficient to cause depolarization block (23). Conversely, at higher stimulation frequencies, the rate of fall in [K\(^+\)] and rise in [Na\(^+\)] may be so great as to outweigh any restorative effects on membrane potential of Na\(^+\)-K\(^+\) pumping, ultimately resulting in loss of excitability and a decline in contractility.

Alternatively, because the present study has focused solely on isolated muscles, results may be due, in part, to either buildup of byproducts of contraction or to lack of substrate deep within the center of isolated muscles (16, 38). In support of which, stimulation of muscles leads to an increase in [Na\(^+\)] (34, 35) and a substantial decline in [K\(^+\)] (11, 25), and a recent study involving isolated rat muscles bathed in a low-Na\(^+\) and high-K\(^+\) KR buffer showed an 80% decrease in M wave amplitude and a 56% decrease in M wave conduction velocity (32).

**Correlations of Force With M Wave Parameters**

We have previously shown that a close correlation exists between twitches and M wave area in isolated rat soleus muscle (17). In the present study, we show that the best indicator of a decrease in “excitability” in isolated muscles is of the M wave area, especially when recorded using an electrode positioned deep within the muscle, supporting the work of Enoka et al. (14).
Whereas the results of Fig. 3 show a close correlation between the recovery of twitch area, on washout of ouabain, and deep M wave area for one muscle, the results presented in Fig. 4B, which are the mean of a number of muscles, are less well correlated. Perhaps an explanation for this dissociation of M wave and contractility during the recovery phase lies with 1) insufficient cover of muscle area in terms of recording electrodes, such that regions of the muscle that could not be detected by our two electrodes were excitable and therefore capable of contracting, or 2) that some change in the excitation-contraction coupling process occurred during the period of stimulation or immediately afterwards, such that contractility was enhanced despite little recovery of the M wave. In support of the latter, it is well known that, during periods of sustained contraction, there is an increase in intracellular inorganic phosphate concentration (16) and that conditioning stimuli given to isolated muscles from mice induce phosphorylation of regulatory light chains (RLC), causing an increase in the extent of twitch-force development, which has been explained as an RLC phosphorylation-induced alteration in the Ca$^{2+}$ sensitivity of cross-bridge transitions (37).

Perspectives

This study indicates the importance of simultaneous recordings of both surface and deep M waves in experiments designed to investigate the excitability of muscles and highlights the risk associated with predicting excitability based on surface M wave parameters alone. Data show that incubation of isolated muscles with ouabain results in a rapid and considerable decrease in excitability of surface fibers as indicated by a decrease in surface M wave area. Moreover, results indicate that hyperpolarization of muscle fibers, as measured by potentiation of M waves, perhaps as a result of the electrogenic effect of Na$^{+}$-K$^{+}$ pumping, is limited to low- and not high-frequency stimulation. Finally, this study concludes that changes in excitability can be closely correlated to changes in M wave parameters.

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REFERENCES


