Potentiation and depression of the M wave in human biceps brachii

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1. The effects of repeated excitation on the compound action potential, or M wave, of mammalian muscle fibres have been investigated in the human biceps brachii.

2. During continuous indirect stimulation at 10 and 20 Hz the mean voltage–time area of the M wave doubled within the first minute, while the mean peak-to-peak amplitude increased by approximately half. The enlargement of the M wave was sustained during stimulation at 10 Hz but not at 20 Hz. Stimulation at 3 Hz caused a small increase which was significant for M wave amplitude only.

3. When the 20 Hz stimulation was performed under ischaemic conditions, the M wave first enlarged and then gradually declined. After 20 Hz stimulation was discontinued, the M wave increased in size; in the ischaemic experiments the release of the cuff produced a further, rapid augmentation. In both the ischaemic and non-ischaemic experiments, the amplitudes and areas of the M waves during the recovery period became significantly larger than the resting values (range, 15–60% at the endplate zone).

4. The mean muscle fibre impulse conduction velocity decreased to less than half the resting value during 20 Hz stimulation, with or without ischaemia, and then increased above the resting value during recovery.

5. On the basis of previous experiments in animals, the augmentation of the M wave was attributed to enhanced electrogenic Na^+-K^+ pumping, and the biceps brachii appeared to be an excellent preparation for studying the time course of this enhancement.

When muscle fibres contract, the impulse-mediated efflux of K^+ causes the concentration of this ion to rise in the interstitial fluid. The rise is especially marked when a maximal effort is maintained for several seconds (Vyskočil, Hník, Rehfeldt, Vejspada & Ujec, 1983), partly because the perfusion of arterial blood through the muscle belly is prevented by the increase in intramuscular pressure (Barcroft & Millen, 1939). Under the last condition especially, a K^+- induced depolarization of the muscle fibres might be expected, leading to a decline in the size of the compound action potential, or M wave, evoked by a stimulus to the motor nerve. Although such a decline has been observed by some authors (Stephens & Taylor, 1972; Milner-Brown & Miller, 1986; Bellemare & Garzaniti, 1988), others, also studying human muscles *in situ*, have found little or no change (Merton, 1954; Bigland-Ritchie, Kukulka, Lippold & Woods, 1982), or even an increase (Fitch & McComas, 1985). In a microelectrode study of rat soleus muscles, Hicks & McComas (1989) showed that the paradoxical enlargement of the M wave was caused by fibre hyperpolarization, itself resulting from increased electrogenic Na^+-K^+ pumping. Hicks & McComas further suggested that, in intact human muscles, the M wave could be used as an indirect index of Na^+-K^+ pumping and it is this possibility which has been explored in the present study.

In order to standardize the excitation of the muscle fibres, we have employed tetanic stimulation at selected frequencies, rather than voluntary contractions. The results have demonstrated that the efficacy of the Na^+-K^+ pump in maintaining muscle fibre excitability depends on stimulus frequency and on the presence or absence of ischaemia; during the recovery period after fatiguing stimulation, indirect evidence of enhanced pumping has also been found. The study has also included observations on impulse conduction velocities in skeletal muscle fibres during and following fatiguing stimulation.

A preliminary account of this work has appeared elsewhere (Galea & McComas, 1991).

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METHODS

Subjects

Approval of the McMaster University Ethics Committee was obtained for the project and volunteers were provided with an honorarium. The results were obtained from seven men, all in good health, aged 22–29 years. A further seventeen male and female subjects participated in two pilot studies; although the results have not been included in this paper, because of differences in experimental protocol, they were similar in all respects to those reported here and have been described briefly elsewhere (Galea & McComas, 1991). All the experiments were performed on the biceps brachii since, of the various muscles examined in our laboratory, this was the one which exhibited the largest increases in M wave size.

Stimulating and recording system

The subject lay supine on a couch and the right arm was used for the experiment, being fully extended at the elbow and strapped down at the wrist. The stimulating electrodes were two lead plates, approximately 3·5 cm × 2·5 cm, coated with conducting cream. The cathode was attached to the skin overlying the main innervation zone of the biceps brachii, just distal to the confluence of the long and short heads; the anode was placed 3 cm proximally (Fig. 1*A*). Both electrodes lay under the blood pressure cuff in the ischaemic experiments. The stimuli were rectangular pulses, 50 μs in duration, delivered from a high-voltage stimulator (Model 3072; Digitimer Ltd, Welwyn Garden City, Herts, UK); the onset and frequency of the stimuli were controlled by a digital timing device (Digitimer, Model 3290) and a gated pulse generator (Digitimer, Model 2521).

The surface recording electrodes were the disposable AgCl type used for electrocardiography (Sentry Medical Products, Irvine, CA, USA). One of the recordings from the biceps brachii was made close to the innervation zone and the other was derived from the distal extremity of the muscle belly, just before the emergence of the tendon; the common reference electrode was fixed to the skin just beyond the elbow (Fig. 1*A*). The evoked muscle responses were amplified, using a 10 Hz–2 kHz passband, and were displayed on a variable persistence storage oscilloscope (Model 141B; Hewlett Packard Ltd). The signals were simultaneously recorded by a digital acquisition system (CODAS; Dataq Instruments Inc., Akron, OH, USA), from whence they could be retrieved both for display and analysis.

Muscle temperature measurements

Intramuscular and surface probes (hypodermic probe, Model No. 524; surface probe, Model No. 409A; telethermometer, Model No. 43; Yellow Springs Inc., Yellow Springs, OH, USA) were used to monitor temperature during the 20 Hz stimulation experiments. The surface probe was secured to the skin between the two recording electrodes, while the intramuscular probe was inserted deeply into the biceps muscle belly at the same level.

Measurements and data analysis

The sizes of the M waves, recorded at the innervation zones, were assessed in terms of their peak-to-peak amplitudes and voltage–time areas. Since the muscle action potentials could not propagate beyond the distally situated electrode, the corresponding M wave consisted of a negative deflection without an ensuing positivity. The area of this M wave was therefore taken as that of the negativity above the baseline, while the peak-to-peak amplitude was measured from the foot of the negative wave to the summit of the latter. The measurement techniques for the proximal and distal M waves are illustrated in Fig. 1*B*. The muscle fibre impulse conduction velocity was determined by dividing the distance separating the centres of the two active recording electrodes by the time interval between the negative peaks of the M waves, monitored at the two locations.

Experimental protocol

Each subject underwent four experiments, with at least 1 week elapsing between consecutive studies. The subjects were instructed not to exercise with their arms or to engage in heavy physical work for a day beforehand. At each session, the stimulating and recording electrodes were attached (see above) and the maximal stimulus intensity was determined by noting the value beyond which there was no further increase in M wave amplitude; the intensity was then increased by 20–30% to ensure that it remained supramaximal throughout the examination.

Once the appropriate stimulus intensity had been determined (see above), four control responses were evoked over a 5 min period, with the muscle relaxed. These responses, which showed no detectable deviation from each other, were used for comparison with the M waves elicited by single shocks during the recovery periods. In the case of the ischaemic studies the arterial cuff was inflated between the 2nd and 3rd stimuli in order to acquire appropriate control values for the ischaemic and non-ischaemic recovery periods, respectively. For assessing the changes in amplitude and voltage–time area of the M waves evoked by repetitive stimuli, as opposed to single shocks, the control values were the means of the 6th to the 10th responses elicited after the start of the tetanus; earlier M waves were likely to have been subjected to mechanical artefact due to shortening of the muscle belly.

The tetanic stimuli were applied continuously at either 3, 10 or 20 Hz for 3 min. Single supramaximal stimuli were then given at 10 s intervals for 2 min, at 30 s intervals for the next 4 min, at 1 min intervals for the next 4 min, and at 5 min intervals for the next 50 min; the recovery period was investigated for 60 min altogether. Finally, in each subject, an additional, fourth, experiment was performed in which the 20 Hz stimulation was carried out in the presence of ischaemia imposed by application of a blood pressure cuff inflated to 300 mmHg; at the end of the 3 min tetanic train, single stimuli were delivered at 10 s intervals for 1 min before the arterial cuff was released and the usual measurements resumed (see above). The experiments were run in a random sequence so as to minimize any carry-over of effects from one study to another.

Statistical treatment

Repeated measures analysis of variance designs were used in which M wave values at selected time points were averaged and compared. A two-way ANOVA was employed to investigate M wave changes during the same tetanic run, or during runs at different stimulus frequencies. When F ratios were significant at the $P = < 0{\cdot}05$ level, Tukey's *post hoc* A tests were performed to test differences between means.

RESULTS

M wave characteristics

In all subjects a region of low stimulus threshold could be detected across the belly of the biceps brachii and, using electrophysiological criteria, this has been identified previously as the main innervation zone (McComas,

Kereshi & Manzano, 1984). Relatively weak shocks applied through the skin, slightly proximal to this region, evoked M waves, each of which, when recorded with the more proximal electrode, consisted mainly of a negative-going deflection (trace *b*, Fig. 1*B*). The short latency of the latter, typically 1–2 ms, confirmed that the electrode was indeed close to the endplate zone. The M wave recorded with the distal electrode was preceded by a slowly increasing positivity (trace *a*, Fig. 1*B*), the onset of which corresponded to muscle fibre excitation in the endplate region; the foot of the negative-going deflection was taken as signifying the arrival of the impulse volley under this electrode. The absence of a late positive wave in many experiments indicated that the distal electrode was, as intended, close to the ends of the majority of muscle fibres.

M waves during tetanic stimulation

In Fig. 2, the sizes of the M waves during the 3 min of tetanic stimulation have been expressed in terms of their mean peak-to-peak amplitudes and of their mean voltage–time areas (see Methods). It can be seen that the M waves enlarged significantly during stimulation at 10 and 20 Hz, whereas only the amplitude changes were significant at 3 Hz (see Table 1). At the two higher frequencies the mean area more than doubled, while the peak-to-peak amplitude increased by approximately half. Figure 3 shows examples of M waves, recorded at different times from the endplate and distal regions in the same subject, during and after 20 Hz stimulation in the presence of ischaemia.

The increases in area and amplitude could be detected within 10 s of starting the tetanic train (Fig. 2) while the times at which the maximal values were reached depended on the frequency of stimulation (Table 1). Thus, in the endplate zone, the maximum amplitude occurred at approximately 30 s during 20 Hz stimulation, at 90 s during 10 Hz stimulation, and at 140 s during 3 Hz stimulation. While the enlargement of the M wave was maintained throughout the 3 min of stimulation at 3 Hz

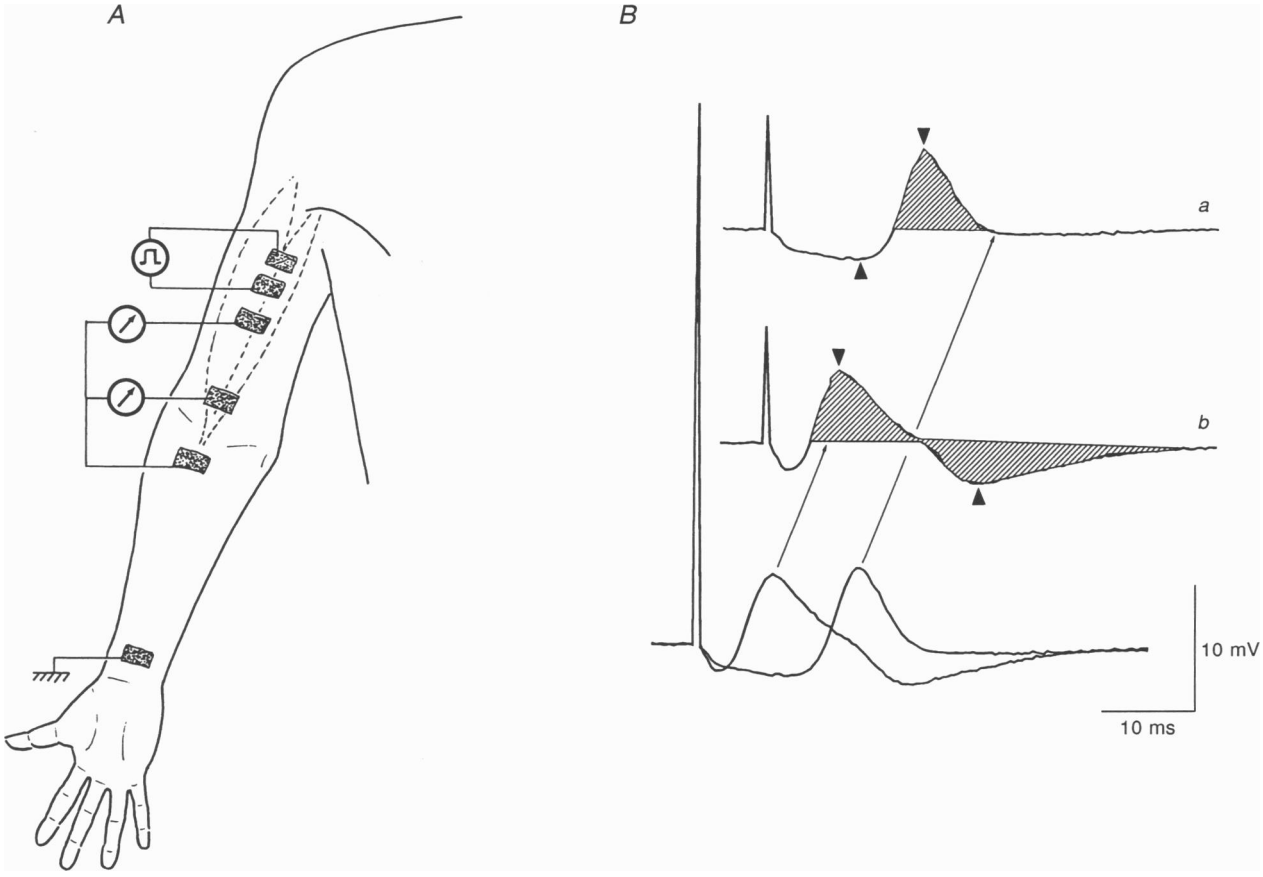

Figure 1. Experimental arrangements and typical M waves

A, experimental arrangements for stimulating, and recording from, the biceps brachii (dashed lines); see text. *B*, typical M waves recorded from the distal region (*a*) and endplate zone (*b*) of a resting muscle; the shock artefacts are shown in their full extents in the superimposed traces at the bottom of the figure, and have been digitally attenuated in the separate traces above (and in Fig. 3). For each type of response the hatched area indicates the determination of area, while the amplitude was measured between the two arrowheads. Negativity is shown as an upward deflection.

Table 1. Maximum M wave sizes and minimum conduction velocities during repetitive stimulation at different frequencies

Stimulus frequency (Hz)	M wave amplitude	Time (s)	M wave area	Time (s)	Conduction velocity
3	116 ± 5	140	115 ± 4	130	97 ± 3
10	147 ± 9	90	223 ± 77	130	87 ± 14
20	158 ± 15	30	216 ± 37	40	45 ± 9
20, ischaemia	149 ± 15	30	202 ± 30	40	49 ± 16*

Values are expressed as mean percentages ± S.D. of controls and are given for the endplate zone recordings only; all values were significantly different from controls ($P = < 0{\cdot}01$), except for the 3 Hz M wave area and conduction velocity results. Mean value from six subjects only indicated by the asterisk.

and at 10 Hz, there was a progressive decline of the response at 20 Hz. The decline was greater in the ischaemic experiments and in six of the seven subjects the M wave disappeared altogether (Fig. 2).

Muscle fibre impulse conduction velocities during tetanic stimulation

It was often difficult to be sure when the negative component of the M wave began, especially in the endplate zone recordings in which the shock artefact was unavoidably large due to the proximity of the stimulating and recording electrodes. For this reason the determinations of impulse conduction velocity were based on the times, following stimulation, at which the maximum negativity of the M wave developed. Whereas measurements made from the onset of the negative wave would have provided information about the fastest-conducting fibres only, the measurements based on peak negativity reflected the state of the majority

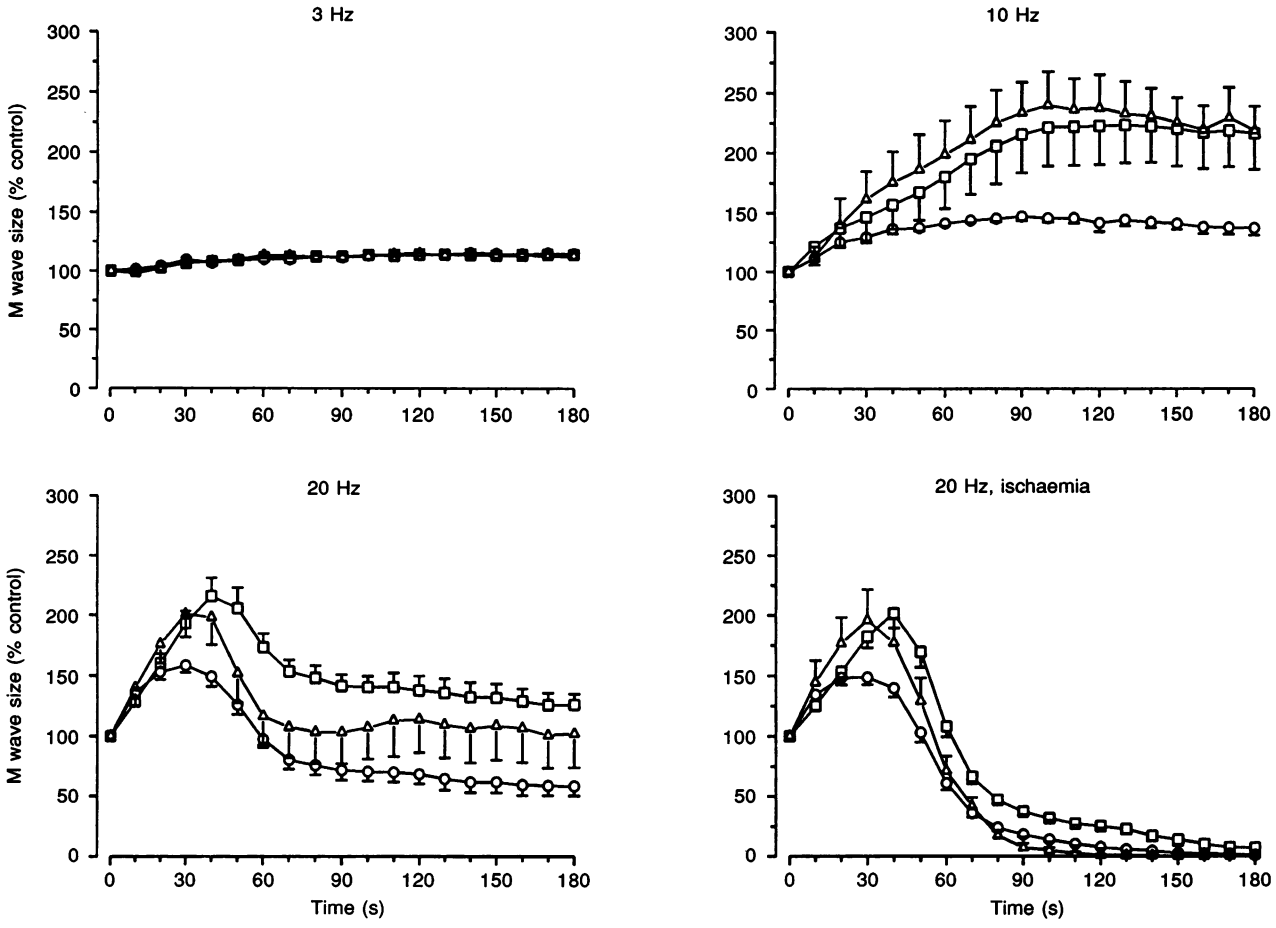

Figure 2. Mean M wave sizes

Mean M wave sizes (± S.E.M.) during 3 min tetanic stimulation at frequencies indicated. □, △, M wave areas at innervation and distal zones, respectively; ○, M wave amplitude at innervation zone; M wave amplitude values for distal zone omitted for clarity.

of muscle fibres. Since the muscle fibres in the biceps brachii run longitudinally between the tendons of origin and insertion, the distance separating the proximal and distal electrodes would have corresponded to the full paths traversed by the impulses. In the resting state, the impulse conduction velocities ranged from 3·7 to 7·2 m s^{-1} in the seven subjects, with a mean value of 4·5 m s^{-1}.

At the onset of tetanic stimulation, there was a marked shortening of the response latencies, in both the proximal and distal recordings (Fig. 3: 0·005 min). Since the distal response remained almost free of a late positivity, this response must still have been derived from the terminal regions of the muscle fibres (see above). Hence the reduction in distal latency signified that the impulses were reaching the fibre ends sooner, presumably due to the shortening and bulging of the contracted fibres. In view of this phenomenon, the mean latency of the 6th–10th tetanic responses was adopted as a reference throughout the period of repetitive stimulation (see Methods). Using this approach, the muscle fibre conduction velocity did not change during tetanic stimulation at 3 Hz, but the 13% mean decline during 10 Hz stimulation was found to be statistically significant (Fig. 4). In the 20 Hz experiments there was little change for the first 20 s, after which the mean conduction velocity fell to less than half by the end of the 3 min period (Table 1).

Temperature measurements during tetanic stimulation

With the subjects resting, the temperatures of the muscle bellies were 1·5–4·3 °C higher than those of the skin (mean difference, 2·6 ± 1·1 °C). Neither temperature changed appreciably throughout tetanic stimulation under ischaemia but there was a slight elevation in intramuscular temperature (mean, 1·6 ± 0·6 °C) in the non-ischaemic experiments.

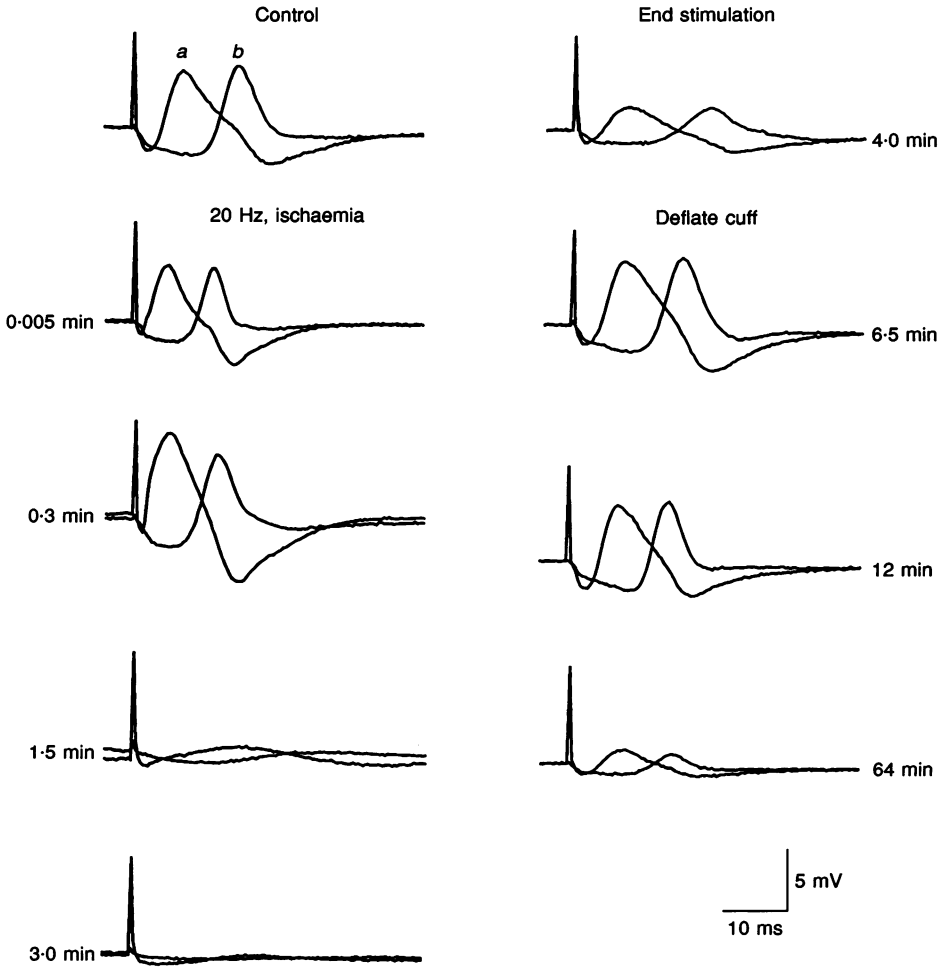

Figure 3. Typical M wave responses

Recordings made at innervation zone (*a*) and distal region (*b*) during and following 20 Hz stimulation under ischaemic conditions in a 29-year-old male subject. Negative-going waves are shown as upward deflections and the times after onset of tetanic stimulation are given beside each pair of traces; the recording at 0·005 min is the 6th response after the onset of the tetanus. The shock artefacts have been digitally truncated. See text.

Table 2. Maximum M wave sizes and impulse conduction velocities during recovery from repetitive stimulation

Stimulus frequency (Hz)	M wave amplitude	Time (s)	M wave area	Time (s)	Conduction velocity
3	112 ± 3*	10	116 ± 4**	20	114 ± 35
10	120 ± 9**	20	119 ± 12**	20	117 ± 10*
20	137 ± 34**	20	136 ± 15**	10	119 ± 51*
20, ischaemia	120 ± 5**	240	125 ± 12**	180	114 ± 30

Values are expressed as mean percentages ± S.D. of controls and are given for the endplate zone recordings only. In all four experimental conditions the corresponding times are referred to the end of the tetanic stimulation. Values significantly different from control at $P = < 0{\cdot}05$ and $P = < 0{\cdot}01$ levels are shown by single and double asterisks, respectively.

M wave sizes during recovery period

Not surprisingly, the most marked changes in the sizes of the M waves during recovery occurred in those experiments in which the tetanic stimulation had produced the greatest depression (Table 2). Following 20 Hz stimulation without ischaemia the mean increase in area amounted to 37% at the endplate zone and was rather larger (75%) in the distal region. When 20 Hz stimulation had been combined with ischaemia, the cessation of the tetanic train caused a prompt reappearance of M waves at both recording locations with a gradual recovery to 42–77% of the control values for amplitude and area during the next 60 s (Fig. 5). Release of the arterial cuff produced a further increase in response amplitude within seconds, and this augmentation continued for 90–120 s; at the end of this time the mean areas of the M waves at the endplate zone and distal region were, respectively, 25 and 47% greater than the control values. As in the other experiments, this enhancement was succeeded by a slow decline, such that by 60 min the mean M wave areas were two-thirds of the initial values, and were associated with rather smaller reductions in mean amplitude (Fig. 5). Although their mechanism remains obscure, the declines could not be attributed to raised axonal thresholds, since the responses were unaffected by momentarily increasing the stimulus intensity.

Muscle fibre impulse conduction velocities during recovery

During the recovery period from the higher rates of stimulation the mean muscle fibre impulse conduction

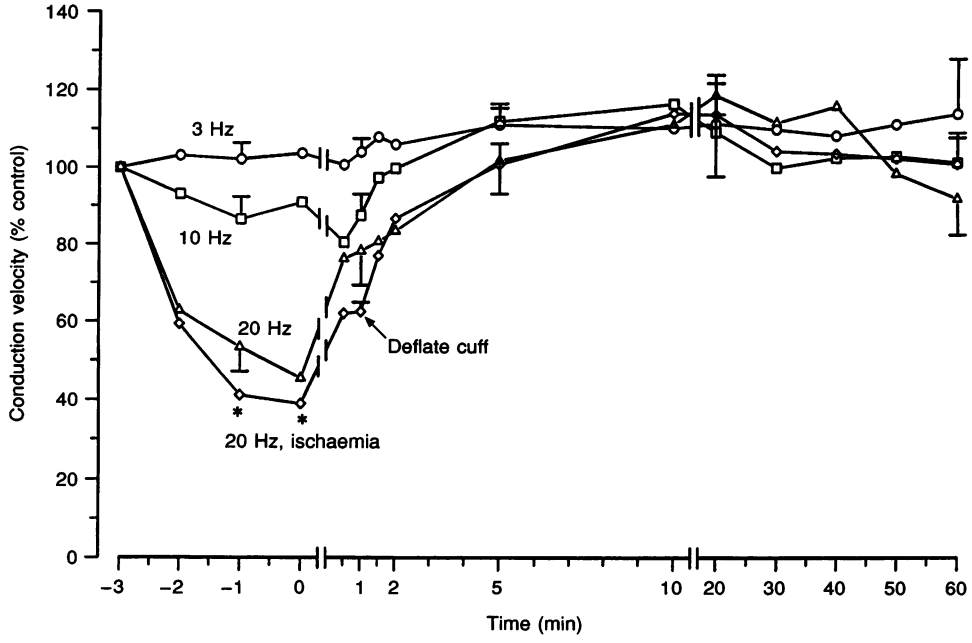

Figure 4. Mean muscle fibre impulse conduction velocities during and following tetanic stimulation at different frequencies

Time 0 = end of stimulation. S.D. values shown where possible. Asterisks show results for single subject only.

velocity slowly increased, reaching a maximum value approximately 20% greater than control by 6–20 min (Fig. 4). By the end of the hour, however, the velocity had fallen to control values.

Temperature measurements during recovery

A rise in temperature began immediately following cessation of tetanic stimulation and continued for approximately 5 min, before slowly falling to control values. This trend was observed at both surface and deep sites and was evident during ischaemic and non-ischaemic experiments. The mean maximum increases in intramuscular and surface temperatures were 2·3 ± 0·7 and 1·6 ± 1·0 °C, respectively. Both surface and intramuscular temperatures were slightly below resting values after 60 min of recovery, but not significantly so.

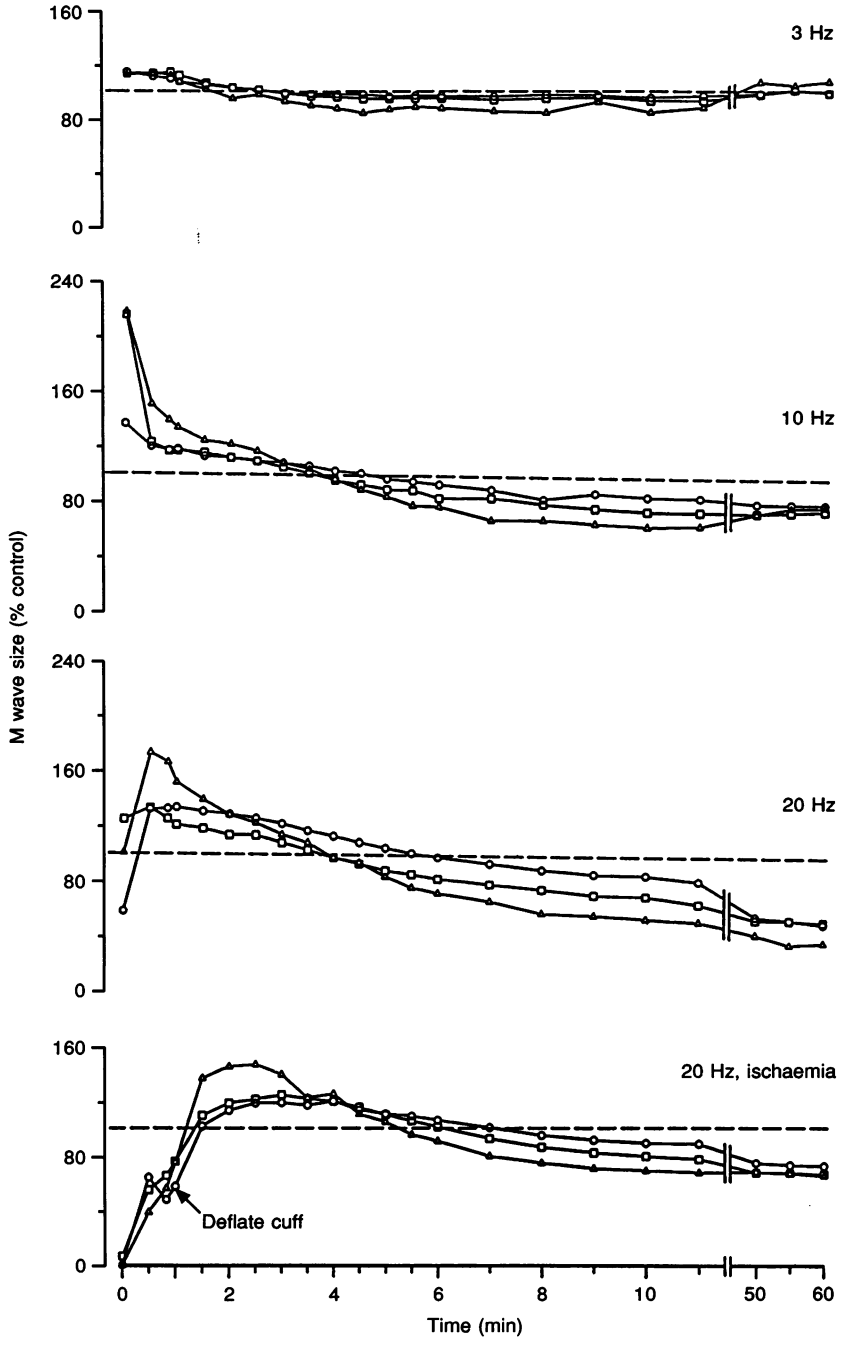

Figure 5. M wave sizes at different times during recovery from tetanic stimulation at different frequencies

Time 0 = end of stimulation. Error values have been omitted for clarity (see, however, Table 2), as have M wave amplitude results for distal zone. □, △, M wave areas at innervation and distal zones, respectively; ○, M wave amplitude at innervation zone.

DISCUSSION

The aim of the present study was to discover the extent to which muscle fibre excitation, as reflected in the M wave of the human biceps brachii muscle, might change in the course of repeated contractions. In order to standardize the number of excitations, stimulated contractions were employed rather than voluntary effort; the highest frequency used, 20 Hz, was rather lower than the mean motor unit firing rate of 31 Hz, found by Bellemare, Woods, Johansson & Bigland-Ritchie (1983) during brief maximum voluntary contractions of the same muscle but was similar to rates observed during more prolonged but less intense effort (Dorfman, Howard & McGill, 1990).

M wave enlargement during tetanic stimulation

On theoretical grounds, it might be expected that the M wave would begin to diminish from the onset of contraction. Thus, the progressive rise in $[K^+]$ in the narrow interstitial spaces of the muscle, due to impulse-mediated K^+ efflux, should depolarize the muscle fibres according to the Goldman–Hodgkin–Katz equation (Hodgkin & Katz, 1949). It was therefore surprising that an immediate decline in the M wave was not observed in any of the twenty-eight experiments, involving different subjects and various rates of stimulation, with and without ischaemia. Instead, there were increases in both the amplitude and the area of the M wave, with maximum values attained at times which depended on the frequency of stimulation, being 30–40 s at 20 Hz and more than 2 min at 3 Hz. Increases in M wave amplitude during repetitive stimulation are sometimes observed in clinical electromyography, and are termed 'pseudofacilitation' (Kimura, 1983), but the rather brief tetani employed have not enabled their full extents to be determined. In the present experiments, however, the mean M wave amplitude could be seen to increase by half, and in some subjects it more than doubled the resting value. It is evident from previous work (Hicks, Fenton, Garner & McComas, 1989) and from the way that the control measurements were made in the present study, that the increased amplitude could not have been a mechanical artefact, due to shortening of the muscle fibres under the surface recording electrodes. Nor could the enlargements be due to muscle fibre discharges becoming more synchronous, the usual explanation given for pseudofacilitation (Duchateau & Hainaut, 1985), in view of the brief synaptic delay and the short period of transmitter release, typically 0·2 and 0·3 ms, repectively, in mammalian neuromuscular preparations (Eccles & Liley, 1959; Hubbard & Schmidt, 1963). Instead, animal experiments in our laboratory have shown that contractile activity is accompanied by an increase in electrogenic Na^+-K^+ pumping by the muscle fibres, and that this results in hyperpolarization and a corresponding increase in the amplitudes of the fibre action potentials (Hicks & McComas, 1989).

From the present study it would appear that Na^+-K^+ pumping is sufficient to maintain the fibre action potentials at, or above, the control amplitude for almost a minute under the most adverse circumstances (20 Hz stimulation with ischaemia). During stimulation at 3 or 10 Hz, the enlarged action potentials can be sustained for at least 3 min; under these conditions not only would less K^+ be released than at 20 Hz, but the lower intramuscular pressures would allow greater diffusion of K^+ into the bloodstream.

The fact that the increase in M wave area was approximately twice that in amplitude also deserves comment. Slowed impulse conduction cannot be the explanation since the enlargement was already present in the endplate region, before the impulses had propagated away. Further, temporal dispersion of the biphasic extracellular action potentials of single fibres would cause phase cancellation, and hence a reduction in M wave area. A more attractive explanation is that individual transmembrane action potentials become longer and have prominent negative after-potentials (Hanson, 1974); being less biphasic, the extracellular potentials would summate more effectively and cause greater enlargement of the M wave.

Variability in M wave potentiation

The question arises as to whether or not the enlargement of the compound action potential is equally prominent in all types of motor unit. The results of Enoka, Trayanova, Laouris, Bevan, Reinking & Stuart (1992) in the cat tibialis posterior muscle suggests that this is not so, and that the potentiation is greatest in the fast-twitch fatiguable (FF) and fast-twitch intermediate (FInt) units. In the human biceps brachii, approximately half of the muscle fibres are fast-twitch, as determined histochemically (Brooke & Engel, 1969), and these fibres were presumably responsible for the M wave enlargement in the present experiments. However, the prominent M wave potentiation in the biceps brachii cannot have been entirely due to the incidence of fast-twitch fibres, since the latter is little different from those in other human muscles, such as the tibialis anterior and the intrinsic muscles of the hand and foot (Johnson, Polgar, Weightman & Appleton, 1973), which show less M wave enlargement (Hicks *et al.* 1989). It is possible that differences in the amount of muscle usage are important; thus a muscle such as the biceps, which is hardly ever used maximally by sedentary individuals, might have low resting Na^+-K^+ pump activity and the relative increase during stimulated or voluntary contractions would be all the greater. In keeping with such a possibility, Everts, Retterstøl & Clausen (1988) showed that pump activity could increase considerably in rat hindlimb muscles stimulated *in vitro*.

M wave decline during tetanic stimulation

It would appear that the electrogenic Na^+-K^+ pump cannot keep pace indefinitely with the K^+ efflux, for during 20 Hz stimulation the M wave eventually declined; impaired neuromuscular transmission may also have been a factor. Studies in animals would suggest that excitation failure occurs first in the fast-twitch fatiguable motor units (Burke, 1967; Clamann & Robinson, 1985; Hamm, Reinking & Stuart, 1989; Enoka *et al.* 1992). In the present experiments employing 20 Hz stimulation, it is probable that the biceps brachii was ischaemic during the early part of the tetanus, even when the arterial cuff had not been applied; thus, the rise in intramuscular pressure would have exceeded the systolic blood pressure, preventing the inflow of arterial blood (cf. Barcroft & Millen, 1939). However, the fact that the M wave was only abolished in the experiments with the cuff suggests that, in the non-cuff experiments, as the muscle became fatigued, the intramuscular pressure fell below the systolic blood pressure and allowed some re-perfusion to occur.

Impulse conduction velocities during tetanic stimulation

In the present investigation the impulse conduction velocities in the muscle fibres fell during stimulation at 10 and 20 Hz, even at a time when the M waves were potentiated. Reduced conduction velocities have been observed in other studies of muscle fatigue, usually involving voluntary contractions rather than tetanic stimulation (e.g. Kereshi, Manzano & McComas, 1983; Eberstein & Beattie, 1985). The present studies of impulse conduction differ from others, however, in that we have been able, in the 20 Hz ischaemic experiments, to stimulate the muscle to the point of unresponsiveness and to determine how slowly impulses may propagate before the fibres are no longer excited. Since the values were similar in the 20 Hz experiments with and without ischaemia, despite the much greater depression of the M wave in the former, it would appear that a 50% reduction in velocity is the most that can be tolerated before conduction is abolished. The fall in conduction velocity did not appear to be an effect of intramuscular temperature since the latter was unchanged in the ischaemic experiments, while the slight rise in temperature during non-ischaemic stimulation should have increased, rather than decreased, velocity.

Muscle excitation during recovery from tetanic stimulation

The responses of the muscle fibres to single stimuli, following the end of the tetanic train, depended on their previous behaviour. In the experiments employing low rates of stimulation, during which the M waves had remained enlarged until the end of the tetanic trains, the evoked potentials slowly diminished, reaching the control values after 1–2 min in the 3 Hz experiments, and after 3–4 min in the 10 Hz experiments. In the absence of any other plausible explanation, these declines can best be attributed to corresponding reductions in the amount of electrogenic Na^+-K^+ pumping to resting levels, and would be consistent with the results of microelectrode recordings in single fibres of the rat soleus (Hicks & McComas, 1989). Similarly, the persistence of enhanced electrogenic Na^+-K^+ pumping is likely to have caused the rapid rises in M wave amplitude and area, during the early part of the recovery period in the 20 Hz non-ischaemic experiments. It is interesting that enlarged M waves were present during the recovery phase of the study by Stephens & Taylor (1972; see their Fig. 7), though this was not commented upon by the authors.

When the 20 Hz stimulation had been combined with ischaemia, the responses to single stimuli also became substantially larger, even though the arterial cuff remained inflated for the first minute of recovery. While the greater response to the first stimulus could have been due to excitation occurring beyond the extended relatively refractory period of the fibres, the progressive improvement over the next 50 s is more difficult to explain. The improvement cannot have been due to a reduction in interstitial $[K^+]$, since the capillary blood flow should still have been occluded. On the other hand, the very rapid increase in M wave size, once the arterial cuff was released, was almost certainly due to flushing out of K^+ from the interstial spaces by the restored capillary circulation; this would have increased E_k, the K^+ equilibrium potential, and hence the amplitudes of the resting and action potentials. The subsequent slow potentiation of the M wave, with maximum values at approximately 3 min, can only have been due to the resumption of electrogenic Na^+-K^+ pumping. The conduction velocities of the muscle fibres also changed during the recovery period. Thus, there was a significant increase in impulse conduction velocity above resting values which was maximal at 10–30 min in the 20 Hz experiments and at approximately 6 min in the 10 Hz experiments. An increase in impulse conduction velocity has also been recently reported by Van der Hoeven, Van Weerden & Zwarts (1993) in human biceps brachii during the recovery period after maximal voluntary contractions and was attributed to swelling of the muscle fibres. In the present experiments swelling may also have been a major factor; in contrast, neither an increase in temperature or in muscle blood flow are likely to have been involved, since the intramuscular temperature had reverted to normal by the time impulse conduction velocity was maximal.

Finally, we return to the dispute as to whether or not muscle fibre excitation is depressed in prolonged maximal voluntary contractions (see the introduction). On the strength of our present studies, it seems that the answer is complex. Thus, the instantaneous size of the M wave will

depend on the sum of a number of factors – the choice of muscle, the amount of antecedent activity, the motor unit firing rates, the time elapsing since the start of the contraction, and whether the contractions are intermittent or sustained.

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