CHANGES IN

ELECTROMYOGRAPHIC ACTIVITY

IN

HUMAN MUSCLE FATIGUE

By

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ABSTRACT

The purpose of this research was to determine the physiological mechanism(s) underlying the reduction in voluntary electromyographic (EMG) activity with maximal contractions during fatigue. The specific hypothesis was that this reduction in EMG activity results from reflex inhibition of homonymous motoneurons by afferents from the fatigued muscles. The experiments were conducted on the human ankle dorsiflexor and plantarflexor muscles, with the use of ischemia to accelerate the fatigue process. In the first part of the study it was shown that, within 3-4 minutes, repetitive indirect stimulation of the dorsiflexor muscles via the peroneal nerve, at either 15 Hz or 30 Hz, could abolish dorsiflexion torque elicited by single shocks and trains of stimuli. During the 15 Hz fatiguing frequency the relative loss of dorsiflexion torque was greater than the decline in the amplitude of the evoked muscle compound action potentials (M-waves). Thus 15 Hz was the rate of fatiguing stimulation used in the remainder of the study. In the second part of the study, fatiguing indirect stimulation at 15 Hz caused significant reductions in both the dorsiflexion torque and EMG activity associated with subsequent maximal voluntary contractions (MVCs) of 66.1 ± 16.4% and 50.3 ± 27.0% respectively. The relative preservation of M-wave amplitudes in the fatigued muscle (reduction of 13.1 ± 22.9%) indicated that most of the loss of EMG activity was not due to inexcitability of the neuromuscular junctions or muscle fibre membranes. Nor could the reduction

in voluntary EMG activity have been due primarily to failure of subjects to exert maximal voluntary effort since supraspinal motor pathways had not been involved in the fatigue process. Furthermore, during the MVC, no additional force was evident with a supramaximal interpolated stimulus. Hence, reflex inhibition appeared to be the most likely mechanism. The third part of the study explored the possibility that the reduction in voluntary EMG activity during fatigue was due to a lower level of alpha motoneuron excitability; for these experiments recordings were made of the electrically-induced homonymous response (H-reflex) of the soleus muscle. It was shown that the H-reflex excitability was depressed by 44.4 ± 25.1% during fatigue of the right soleus muscle using 15 Hz stimulation under ischemic conditions; the H-reflex excitability in the nonfatigued left soleus did not change significantly. The maximum M-wave amplitudes demonstrated a mean decline of only 8.8 ± 11.9%, indicating good peripheral excitability of the muscle fibre membranes. Control experiments performed under ischemic conditions alone (without induced fatigue) or with electrical stimulation alone (without ischemia and hence without fatigue) failed to demonstrate any significant changes in reflex excitability. Thus, in the absence of fatigue, the depression could not be accounted for on the basis of either ischemia or electrical stimulation; instead the findings were consistent with the presence of an inhibitory reflex from the fatigued muscle onto the homonymous alpha motoneurons. In the final part of the study it was demonstrated, by means of pressure-induced impulse blockade of large myelinated afferents in the sciatic nerve prior to fatigue, that the mean plantarflexor torque

produced during MVCs decreased by 38.0 ± 18.6 % from the postblock value compared to a decrease of 5.2 ± 7.0 % in the ischemia control; the mean EMG activity decreased from postblock values by 43.4 ± 15.6 % following fatigue and by only 6.6 ± 5.5 % following ischemia alone. These results were very similar to those demonstrated without any blockade of large diameter afferents. This suggested that the afferents involved in the putative reflex inhibition of the alpha motoneuron pool with fatigue were likely to have been those with small diameters (Group III and IV). This research provided evidence suggestive that the reduction in voluntary EMG during fatigue, in both tibialis anterior and soleus muscles, resulted from reflex inhibition of the motoneuron pool.

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LIST OF ABBREVIATIONS

ACh	acetylcholine
ANOVA	analysis of variance
ATP	adenosine triphosphate
Ca^{2+}	calcium ion
DC	direct current
deg	degree
df	degrees of freedom
EMG	electromyogram
F	Fisher's variance ratio
HP	Hewlett Packard
H^+	hydrogen ion
Hmax	maximum H-reflex
K^+	potassium ion
IT	interpolated twitch
La^-	lactate ion
max	maximum
min	minute
Mmax	maximum M-wave
M-wave	muscle compound action potential
MSE	mean square error
MVC	maximum voluntary contraction
Na^+	sodium ion
Nm	newton meter
ns	nonsignificant
p	theoretical probability of an event
r^2	square of correlation coefficient
RE	reflex excitability
rec	recovery
s	second
SD	standard deviation
SE	standard error
SS	sum of squares
t	Student's t distribution statistic
torr	millimeters of mercury

Chapter 1

GENERAL INTRODUCTION

1.1 Overview of Fatigue

1.1.1 Introduction

Muscle fatigue may be defined as the failure to maintain the required or expected force (Edwards, 1981). This definition has been criticized because it implies that fatigue appears suddenly after a period of activity (Bigland-Ritchie, 1984). Bigland-Ritchie (1984) has suggested instead that any reduction in the force-generating capacity of the total neuromuscular system, regardless of the force required in a given situation, is a more appropriate definition. This statement encompasses the concept that physical changes which culminate in muscle fatigue start at the onset of activity although they are not readily detected until force declines. Edward's definition of fatigue has been chosen for the purposes of this research in view of its simplicity as a practical and measurable definition.

The generation of voluntary muscle activity involves a sequence of events (Table 1), any of which might conceivably fail and result in loss of force ie. muscle fatigue. The factors influencing the rapidity of muscle fatigue are diverse and include the duration and intensity of work, muscle fibre composition, environmental

conditions, and the degree of training (Fitts et al, 1982). This review will show that it is appropriate to characterize muscle fatigue as a sum of several events depending on the circumstances, rather than a single phenomenon. In this review the chain of command for muscle activation will be examined (cf. Edwards, 1981 and also Table 1). Although depletion of energy supply (Hermansen, 1981) and increased energy cost (Dawson et al, 1978; Bergstrom, Hultman, 1988) or accumulation of lactate (Duchateau et al, 1987) and diprotonated inorganic phosphate (Miller et al, 1988; Nosek et al, 1987) may cause the contractile mechanism to fail, this aspect of muscle fatigue will not be reviewed.

1.1.2 Central Fatigue

a) Excitatory Input to the Motor Cortex

The work of Mosso in 1892, as recounted by Asmussen (1979), dramatically illustrated that improved work performance could occur if the mind was in a state of increased "nervous energy" or arousal; this suggested that the amount of muscular force depended on its activation by the central nervous system. An obvious method to measure the completeness of muscle activation by the central nervous system has been to compare the force of an MVC with that elicited by supramaximal tetanic nerve stimulation (Bigland-Ritchie et al, 1978). A second and rather more subtle approach has been to interpolate a maximal stimulus, either of the motor nerve (Belanger, McComas, 1981) or of the motor cortex (Merton et al, 1981), in the course of an MVC. In either case, the superimposition of a twitch on the voluntary

Table 1

Chain of Command for Muscle Contraction

Command Chain	Site of Fatigue	Examples of Fatigue Sites
Impulse in central nervous system	Central	Excitatory input to the motor cortex ie. motivation
↓ Lower Motoneuron		Motoneuron excitability ie. descending excitatory drive and sensory feedback from muscle or movement
↓ Sarcolemma	Peripheral	Transmission of excitation at neuromuscular junction including sarcolemma excitability
↓ T-tubule		Excitation-Contraction Coupling
↓ Ca^{2+} Release		
↓ Actin-Myosin Interaction		Contractile Mechanism
↓ Force Output		Energy Supply Metabolite Accumulation

(adapted from Edwards, 1981; Gibson, Edwards, 1985)

force recording would indicate that muscle activation by the central nervous system was incomplete. A review of these methods can be found in Bigland-Ritchie et al (1986a).

There are reports which argue both for and against the role of declining excitatory input to the motor cortex in fatigue. Direct massive stimulation of adductor pollicis muscle (1000 V) has been used in conjunction with MVCs and no increment in force was evident, a finding which indicated that the loss of contractility of the muscle was independent of the motor drive (Merton et al, 1981). Ikai (1967), however, demonstrated an increase in force by indirect electrical stimulation of the adductor pollicis muscle via the ulnar nerve when compared to the force produced by MVCs, a finding interpreted as evidence for decreased muscle activation by the central nervous system during fatigue (cf. Asmussen, 1979). This work has been questioned on methodological grounds since it is deemed very difficult to achieve a full MVC in the 1 second trials used in that protocol (Bigland-Ritchie, 1984). Nevertheless, the hypothesis of inadequate activation from higher centers, ie. central fatigue, has been partially supported by the finding of faster declines in voluntary force as opposed to force elicited by intermittent supramaximal tetanic nerve stimulation during sustained MVCs (Bigland-Ritchie et al, 1978; Grimby et al, 1981b). Central fatigue was most apparent in the latter half of a 60 s MVC and could account for 10 - 30% of the loss in force (Bigland-Ritchie et al, 1978). The subjects were only able to overcome the drop in voluntary force with "super efforts" but were unable to maintain the force at the higher level for more than a few

seconds. In this work, visual feedback and verbal encouragement were used extensively to counteract central fatigue. In fact, central fatigue appears relatively more important in subjects with little training (Grimby et al, 1981b). This illustrates the importance of motivation and practice in overcoming central fatigue.

The contribution of central factors in fatigue induced by sustained submaximal isometric contractions varied in different muscles. In one study by Bigland-Ritchie et al (1986b), brief MVCs and contractions elicited by 50 Hz stimulation declined in parallel during fatiguing submaximal contractions of the quadriceps and the adductor pollicis muscles, and no superimposed twitch was evoked by an interpolated stimulus. Since the MVC did not decline more rapidly than the electrically stimulated contraction, this indicated peripheral rather than central fatigue mechanisms. On the other hand, using the same protocol in soleus, central fatigue was evident because the brief MVCs declined faster than the tension during 50 Hz stimulation (Bigland-Ritchie et al, 1986e); the ability to remain motivated to sustain a contraction over longer periods of time, in soleus muscle, could be an important factor in soleus muscle. It is probably relevant that, even in rested ankle plantarflexor muscles, approximately half of the subjects have been unable to activate the muscles maximally (Belanger, McComas, 1981). Central fatigue in the ankle plantarflexors might have been due to the differences in synaptic inputs to the ankle plantarflexor muscles versus the small hand muscles and quadriceps. It seems reasonable to suggest that in normal daily activities, sustained force in some muscles was partially limited by a reduction in net excitatory input to the spinal motoneurons.

b) Motoneuron Excitability

Excitatory drive to the motoneuron comes from both higher centers ie. motor cortex, and lower centers ie. muscle receptors; the net drive is reflected by the number of active motoneurons and their firing rates. Conduction along the corticospinal pathway has been considered complete during fatigue since single pulse stimulation of the motor cortex has evoked muscle action potentials of maximal amplitude despite fatigue (Merton et al, 1981). Nevertheless, it has been argued that the motor path may be able to respond to single stimuli yet remain unable to transmit trains of impulses characteristic of normal muscle activity (Wiles, 1981).

Alpha motoneuron firing rates probably vary with the size of the cell soma as well as with the mode of activity of the motoneuron. For example, during voluntary contractions of extensor digitorum brevis, those motoneurons with the highest impulse conduction velocities (and hence with the largest axons and cell bodies) tend to discharge phasically with rates above 40 Hz (Borg et al, 1978). In contrast, putatively smaller motoneurons with lower impulse conduction velocities tend to fire tonically at lower discharge rates (Borg et al, 1978). There is another study which demonstates that during a prolonged contraction of constant strength continuously firing motor units discharge between 10 and 30 Hz; there are also intermittently discharging motor units which fire between 20 and 100 Hz during bursts of activity (Grimby, Hannerz, 1977). These observations are consistent with Henneman's Size Principle which states that the recruitment of motoneurons is orderly and according to their size, such that smaller motoneurons (with tonic

discharges and low firing rates) are recruited before large motoneurons (with phasic discharges and higher firing rates) (Henneman, Mendell, 1981).

There have been few reports which indicated that there was a reduction in number of active motoneurons during fatigue. In one study (Grimby et al, 1981a), some motor units ceased firing within the first 10 seconds of a maximal voluntary contraction. These were usually high threshold units. Other smaller motor units with low thresholds fired tonically for several minutes. It has been suggested that large motor units ceased firing tonically to protect fast twitch muscle fibres from fatigue while allowing them to respond phasically as needed (Grimby et al, 1981a).

There have been many reports of decreased firing rates of motoneurons in fatigue. Firing rates of motor units have been shown to decrease during sustained MVCs (Bigland-Ritchie et al, 1983a; Grimby et al, 1981b). A difficulty in studying individual motor unit firing rates is the inability, because of electrode movement, to follow a single unit during strong contractions. In another study, an ischemic or local anaesthetic block of the ulnar nerve was used to measure firing rates in a few median-innervated motor units in the adductor pollicis muscle (Marsden et al, 1971). The authors found these latter motor units to fire initially at 60-80 Hz, but occasionally up to 100 Hz, and then drop to 20 Hz within a 30 s MVC. Another group used a "self inflicted injury in the cause of science" (editorial, Lancet, 1981) to damage intramuscular nerve fibres until only a few excitable motor units remained in the extensor hallucis brevis (Grimby et al, 1981a). The authors found that the discharge rates of the residual motor units dropped from 60 Hz to 25 Hz within 4

s of MVC. Fast twitch motor units have been found to be particularly susceptible to reduced firing rates with fatigue (Grimby et al, 1981a; Kernell, Monster, 1982).

Motoneuron firing rates have also been studied using population responses rather than continuous recordings from individual motor units. A 45% decrease in firing rates in a population of motor units was found after a 60 s MVC of adductor pollicis (Bigland-Ritchie et al, 1983b). Another group found that during a 45 s MVC of biceps brachii, the MVC declined by 70%, the smooth rectified EMG fell by 50% and the computed firing rates fell by 60% (Kranz et al, 1985).

It has been proposed (Bigland-Ritchie, 1981b) that decreases in the firing rates should not be included as central fatigue since they are not necessarily responsible for loss of force but rather serve to optimize muscle function by matching the motoneuron firing rates with the muscle fusion frequency. The reductions in motor unit firing rates during fatigue have been correlated with increased relaxation and contraction times (Bigland-Ritchie, Woods, 1984; Bellemare et al, 1983). Motor units have been shown to fire at fusion frequency in voluntary contractions of extensor digitorum brevis and the interspike intervals were crudely indicative of the contraction time of the motor unit (Grimby et al, 1979). The reduced motor unit firing during voluntary contractions would be sufficient to maintain fusion with the slower contraction speed during fatigue, and hence improve the economy of muscle contraction.

It would seem that the change in contractile properties during fatigue must be detected by the central nervous system so the motor unit discharge rates could

be regulated to match these changes; such regulation would likely require sensory feedback from the muscle (Bigland-Ritchie, Woods, 1984). It has been suggested that spinal inhibitory reflexes from free nerve endings, Golgi tendon organ discharges, Renshaw cell inhibition or increased spike after-hyperpolarizations could be implicated in matching contraction time to motor unit firing rates (Bigland-Ritchie, 1981b).

Feedback from the muscle is known to influence motor unit behaviour in previously rested muscles. Freychuss and Knutsson (1971) eliminated all activity-induced sensory feedback by curarizing the hand (which prevents any contraction) and measured firing rates in the median and ulnar nerves during attempted maximal handgrip exercise; the maximum motor unit firing rate was limited to 30 Hz and observed to decrease further during sustained effort. With ischemic blockade of large afferent fibres from the muscle receptors in tibialis anterior and the short toe extensor muscles, altered threshold and maximum firing rates were evident (Hannerz, Grimby, 1979); they concluded that proprioceptive afferent activity had both facilitating and inhibitory effects on the motor unit firing ranges in sustained isometric voluntary contraction. Grimby and Hannerz (1976) demonstrated a reversal of motor unit recruitment order in tibialis anterior muscle with ischemic or lidocaine blockades of proprioceptive afferent activity. Therefore, while muscle afferent activity has been shown to modulate aspects of motor unit behaviour in the rested muscle, there is a paucity of research on the influence of afferent inflow during fatigue on motor unit firing and recruitment.

Chemosensitive structures within the exercising muscle may provide the feedback to the motoneuron pool to control motoneuron firing rates. Discharge rates in fatigued muscles have been found to remain reduced as long as an ischemic cuff is inflated even if the muscle is resting (Lippold et al, 1960; Bigland-Ritchie et al, 1985, 1986c). This finding may implicate a chemical stimulus from the muscle that triggers the motoneuron changes. Possible candidates for the chemical mediator include potassium, phosphate, and lactate. Potassium (Hnik et al, 1986), phosphate, and lactate stimulate free nerve endings of group III and IV afferents (Mense, 1977; Kniffki et al, 1978); however, potassium ions apparently do not affect the sensitivity of muscle spindles (Prochazka, Somjen, 1985), or Golgi tendon organs (Mense, 1977). Another study has found that venous blood lactate levels correlated well with the decrease in electromyographic (EMG) mean power frequency during fatigue ($r=.86-.98$) (Moritani et al, 1984). Nevertheless, the rise in lactate concentration in that study is an unlikely cause of the EMG changes since decreases in EMG mean power frequency occurring with fatigue have also been found in patients with myophosphorylase deficiency; in this condition there is no lactate accumulation or acidosis (Mills, Edwards, 1984).

Inhibition of motoneurons by muscle mechanoreceptors is another possible explanation for reduced firing rates in fatigue. Increased muscle spindle responsiveness has been found in fatigue (Christakos, Windhorst, 1986) and proposed by others (Lippold et al, 1960; Hakkinen, Komi, 1983). However, higher spindle gain would serve to reinforce, rather than diminish motoneuron excitation. Golgi

tendon organ firing could be increased with fatigue, due to the longer duration of a twitch contraction, but is more likely decreased due to the loss of tension development (Hasan, Stuart, 1984). Although one study has found decreased Golgi tendon organ responses to stretch in fatigued muscle (Hutton, Nelson, 1986), only a very small proportion of these receptors are responsive to stretch (Hasan, Stuart, 1984). It would appear, therefore, that muscle spindle and Golgi tendon organ input can only play a limited role in reflex inhibition of motoneurons during fatigue. Other possibilities for such an effect are the Group III and IV afferents; these afferents are polymodal responding to such mechanical stimuli as pressure, stretch, and contraction (Kniffki et al, 1978; Houk, Rymer, 1981; Kaufman et al, 1984). There is recent evidence that these small diameter afferents increase their firing rate during fatigue, supporting their suggested role as fatigue-sensitive muscle afferents (Hayward et al, 1988).

In summary, there appears to be a reduction in motoneuron excitation during fatigue since both the number of active motoneurons and their firing rates decrease. So far, however, there have been no direct studies of motoneuron excitability nor is it clear whether afferents from the fatigued muscle depress motoneuron responsiveness to descending motor drive.

1.1.3 Peripheral Fatigue

a) Neuromuscular Transmission Failure

The process of neuromuscular transmission involves the motor nerve impulse arriving at the presynaptic terminal, causing an influx of Ca^{2+}, and evoking release of acetylcholine (ACh). ACh diffuses to the postsynaptic endplate and binds to receptors; the consequent increase in muscle fibre membrane permeability to sodium and potassium ions depolarizes the membrane. When this depolarization, or endplate potential, exceeds a critical value (15 to 20 mV; Katz, 1966), an action potential is initiated which propagates along the muscle fibre. The possible causes for neuromuscular transmission failure can be divided into four categories (Grob et al, 1956):

1) deficient release of ACh from the presynaptic terminal,
2) excessive removal of ACh from the synaptic cleft,
3) inhibition of depolarizing action of ACh on the endplate,
4) abnormally prolonged depolarization of endplates.

Mechanisms for failure of neuromuscular transmission have been investigated in rat muscles, in vitro, using intracellular recordings with microelectrodes. Endplate potentials were normal with stimulation frequencies below 14 Hz, but above 40 Hz, endplate potential failures occurred within 1 s (Krnjevic, Miledi, 1958). This was reaffirmed by Thesleff (1959) who demonstrated a decline in endplate potential amplitude with stimulation frequencies above 20 Hz. The mechanism underlying the decline in endplate potential could be receptor desensitization since applications of

ACh did not increase the membrane depolarization (Thesleff, 1959). There has also been some evidence for presynaptic failure based on the temporal distribution of endplate potential failures (Krnjevic, Miledi, 1958).

The viability of neuromuscular transmission in human muscle fatigue can be indirectly assessed by the shape of the muscle compound action potential (M-wave). Methods of measuring the M-wave vary greatly. Some investigators report amplitude (Merton, 1954; Luttgau, 1965, Marsden et al, 1983; Miller et al, 1987), amplitude and duration (Naess, Storm-Mathisen, 1955; Lippold et al, 1960; Hultman, Sjoholm, 1983, Milner-Brown, Miller, 1986), while others additionally measure area (Stephens, Taylor, 1972; Bigland-Ritchie et al, 1979, 1982; Pagala et al, 1984). Alterations of different aspects of the M-wave shape are thought to represent different locations for action potential failure (Milner-Brown, Miller, 1986). The M-wave amplitude can be considered as a measure of the level of muscle fibre membrane excitation since it is dependent on the resting membrane potential and any presynaptic events. The duration is inversely proportional to the velocity of propagation along the sarcolemma and is affected by the rates of Na^+ and K^+ conductance changes. Area is strictly proportional to the number of muscle fibres excited only if the duration of the waveform is constant (Milner-Brown, Stein, 1975; Milner-Brown, Miller, 1986).

In keeping with the results of microelectrode studies in animal muscles (see above), declining M-waves have been shown to depend on the frequency of motor nerve stimulation in both humans and animals. Low frequency motoneuron discharge results in a limited decline of the M-wave amplitude even though the

force shows substantial decreases (<40 Hz, Naess, Storm-Mathisen, 1955; 25 Hz, Johns et al, 1956; 10 Hz, Sandercock et al, 1985; 15 Hz, Garland et al, 1987). At higher frequencies, there is a substantial decline in the M-wave amplitude of mammalian muscles (40 Hz, Naess, Storm-Mathisen, 1955; 50 Hz, Johns et al, 1956; 80 Hz, Bigland-Ritchie et al, 1979; 80 Hz, Sandercock et al, 1985). In isolated amphibian muscle fibres, however, action potential failure can occur at frequencies as low as 25 Hz (Luttgau, 1965). The results of stimulating at intermediate frequencies (between 25 and 40 Hz) have not been reported.

High frequency stimulation induces an elevation in the excitation threshold of the muscle fibre membrane (Jones, 1979); a 2-6 fold rise in excitation threshold may exceed the safety margin for neuromuscular transmission (Krnjevic, Miledi, 1958; Jones, 1979). However, Jones et al (1979) have reported circumstantial evidence that fatigue induced by high frequency stimulation may not necessarily be the result of neuromuscular transmission failure; they found that direct stimulation of a curarized mouse muscle resulted in a loss of force which mimicked that evoked by nerve stimulation at 100 Hz in intact human muscle (Jones et al, 1979). Since changing from this high frequency to a lower frequency of stimulation increased the force production, failure of the contractile machinery was not the limiting factor; this suggested that the fatigue resulted from failure of action potential propagation along the muscle fibre membrane or impaired excitation-contraction coupling.

Fast twitch muscles ie. rat extensor digitorum longus (97% fast twitch fibres) may be particularly susceptible to neuromuscular transmission failure since the

decline in the muscle compound action potential correlates highly with the reduction in force (Pagala et al, 1984). There is one report that some fast twitch motor units in extensor digitorum brevis with high thresholds for voluntary activation tend to be more sensitive than other motor units to failure of neuromuscular transmission during voluntary contractions (Borg et al, 1983). However, other studies have shown that fatigue of slow twitch muscles may not be associated with reductions in single motor unit potentials in soleus (84% slow twitch fibres; Pagala et al, 1984) and medial gastrocnemius (31% slow twitch fibres; Sandercock et al, 1985).

Action potential failure could be uncommon during MVCs (Merton, 1954; Bigland-Ritchie, Lippold, 1979; Bigland-Ritchie et al, 1982) because of the drop in the firing rates of motor units as the contraction continues (Bigland-Ritchie, 1978, 1981b; Grimby et al, 1981b; Moritani et al, 1986). Marsden and colleagues (1983) tested the aforementioned statement by a technique they coined as "artificial wisdom". By progressively decreasing the frequency of electrical stimulation from 60 to 20 Hz, they were able to mimic the force production of a maintained MVC; this was suggestive of similar reductions in motor unit firing rates in voluntary contractions. However, Miller et al (1987) found decreased M-wave amplitudes during 4 minutes of maximal voluntary contractions of the adductor pollicis muscle. Stephens and Taylor (1972) also documented action potential failure during maximal voluntary contractions of the first dorsal interosseus muscle based on measures of M-wave area over a fixed time. Nevertheless the area in a fixed time might be reduced if the impulse conduction velocities along the muscle fibres were lower; this

measure was therefore not a reliable index of the transfer of excitation to the muscle fibre (Bigland-Ritchie, 1981a). Bigland-Ritchie and coworkers (1979) measured total area of the M-wave during fatigue in adductor pollicis and found it to increase initially and thereafter remain constant; the MVC force declined steadily during this time. In a later study, both M-wave amplitude and area were found to show no decrement with fatigue (Woods et al, 1987). Although these latter studies suggest that action potential failure is unlikely to be a prime cause for fatigue in the majority of voluntary contractions, there has been further support for decreased muscle fibre excitation. Recently, Bellemare and Garzaniti (1988) demonstrated that M-waves evoked in the human adductor pollicis by brief tetani became smaller as the voluntary contractions continued.

Apart from that due to neuromuscular transmission (see above), muscle fibre membrane excitation may be lost if impulse propagation is blocked along the fibre. Failure of propagation of the action potential along the sarcolemma has been attributed to changes in the Na^+/K^+ gradients across the muscle fibre membrane (Krnjevic, Miledi, 1958; Jones et al, 1979; Edwards, 1981; Milner-Brown, Miller, 1986). Increased external K^+ from 5 mM to 10 mM in a nonfatigue situation decreased the muscle action potential evoked by single shocks by 70% (Jones, 1981). There have been several studies of ionic changes in Na^+ and K^+ during fatigue. Gonzalez-Serratos and colleagues (1978) found intracellular increases in Na^+ (from 29 to 140 mM/kg dry weight) and decreases in K^+ (from 488 to 332 mM/kg dry weight) following 40 Hz stimulation in isolated frog muscle. Efflux of K^+

(Heigenhauser et al, 1985) and accumulation of K^+ in the extracellular space (Sjogaard et al, 1985) have been documented with fatigue. Determination of the interstitial K^+ has been made with ion-sensitive electrodes. In one such study, performed on human forearm muscles, Hnik et al (1986) found rises in interstitial K^+ concentration to 10 mM and occasionally to 15 mM (see their Figure 11). A rise in extracellular K^+ plays a critical role in reducing sarcolemmal excitability by decreasing the resting membrane potential and inactivating Na^+ channels. According to the Nernst equation, a 2-fold increase in external K^+, e.g. from 2.5 to 5 mM, would decrease the resting membrane potential by 9 mV (Katz, 1966). Juel (1986) found that after 960 stimuli delivered intermittently at 40 Hz the mean resting membrane potentials decreased by 11.9 mV and 18.2 mV in mouse soleus and extensor digitorum longus muscles respectively; the corresponding internal K^+ concentrations decreased by 32 mM and 48 mM, and the K^+ concentration in the interstitium doubled. A decline in the transmembrane muscle action potentials of 35 mV has been associated with a fall in resting membrane potential of 15 mV, following low frequency stimulation in isolated amphibian muscle (Eberstein, Sandow, 1963); similar results have been obtained by Luttgau (1965) and Grabowski et al (1972).

Further support for sarcolemmal inexcitability due to ionic changes across the membrane is derived from experiments with low extracellular Na+ concentration; the action potential remained normal at the beginning of stimulation but declined with repetitive stimuli at 50-60 Hz (Bezanilla et al, 1972; Jones et al, 1979) and the rate of force decrement was roughly proportional to the degree of sodium depletion

(Jones et al, 1979). In an experiment in which contractility was abolished by metabolic inhibitors, single amphibian muscle fibres were able to transmit several thousands of action potentials at 100 Hz without failure (Luttgau, 1965); however, it should be noted that this single fibre preparation would be free of naturally-occurring ionic changes in the interstitial fluid. These data suggest that changes in membrane permeability consequent to contraction may adversely affect the height of the action potential. On the basis of the above studies, changes in Na^+/K^+ gradients would appear to provide a reasonable explanation for action potential failure either at the neuromuscular junction or elsewhere along the sarcolemma.

There is, however, experimental evidence against such an hypothesis. Krnjevic and Miledi (1958) reported that a decrease of external Na+ by 70 mM did not produce inexcitability in the rat diaphragm muscle fibre soaked in Ringer-Locke solution. This amount of external Na^+ was calculated to have been exchanged after 10000 stimuli, at which time fatigue was evident. Others have concurred with Krnjevic and Miledi and ruled out Na^+/K^+ changes since inexcitability has occurred after many fewer stimuli than the 10000 calculated by Krnjevic and Miledi (Steiman, 1943 - 300 stimuli at 5 Hz; Naess, Storm-Mathisen, 1955 - 2500 stimuli at 50 Hz; Pagala et al, 1984 - 1000 stimuli at 30 Hz). Furthermore, in the study by Pagala et al (1984) the action potential declined more slowly than the tension and the calculated increase in internal Na^+ was only 10 mM; the latter was not deemed sufficient to cause muscle membrane inexcitability. The ionic theory for failure of action potential propagation during fatigue needs to be substantiated with further

recordings of ionic composition in the interfibre milieu during fatigue (cf. Juel, 1986; Hnik et al, 1986).

In summary, muscle action potential failure during fatigue may result from presynaptic changes, endplate receptor desensitization, and sarcolemmal inexcitability; the vulnerability of the action potential is particularly high during high frequency stimulation and in fast twitch muscles. It is still uncertain if neuromuscular transmission failure occurs in normal voluntary contractions.

b) Excitation-Contraction Coupling

Excitation-contraction coupling involves the link between propagation of the action potential along the sarcolemma and the interaction of the contractile proteins to produce mechanical output. The transport of Ca^{2+} is integral to this process. Ca^{2+} must be released from the terminal cisternae of the sarcoplasmic reticulum and diffuse to the contractile proteins. When the myoplasmic Ca^{2+} rises above threshold, the inhibitory action of tropomyosin is overcome by binding of Ca^{2+} to troponin C. The muscle fibre relaxes as Ca^{2+} is removed via the longitudinal reticulum and restored to the terminal cisternae. (Ebashi, Endo, 1968; Winegrad, 1968, 1970; Bianchi, Narayan, 1982)

Donaldson (1986) summarizes the possible links between the action potential and release of Ca^{2+} from the sarcoplasmic reticulum, as follows:

1) electrical - depolarization travelling down the t-tubule induces sarcoplasmic reticulum depolarization,

2) mechanical - molecules bridging the t-tubule and sarcoplasmic reticulum,

3) chemical - Ca^{2+} entry or inositol triphosphate release with depolarization of t-tubule.

There has been much indirect evidence implicating excitation-contraction coupling dysfunction in fatigue. In isolated amphibian muscles, stimulation at 1 Hz produced fatigue of the twitch independent of action potential fatigue or failure of contractile proteins; the tension was restored during caffeine contracture (Eberstein, Sandow, 1963; Grabowski et al, 1972; Nassar-Gentina et al, 1981). The long lasting fatigue (approximately 24 hr recovery) to low frequency stimulation has been hypothesized to result from excitation-contraction coupling failure (Edwards, 1981). During this prolonged recovery, muscle phosphagen content and action potential amplitudes were normal, torque in response to high frequency and voluntary contractions was restored, yet the tension produced to low frequency (<20 Hz) stimulation remained depressed from prefatigue values (Edwards et al, 1977; Hultman, Sjoholm, 1983; Jami et al, 1983; Bigland-Ritchie et al, 1986b). This could be explained by less Ca^{2+} release from the sarcoplasmic reticulum or less affinity of troponin binding sites for Ca^{2+} (Grabowski et al, 1972; Edwards et al, 1977; Jones, 1981; Jami et al, 1983). However, Metzger and Fitts (1987) have shown faster recovery from 5 Hz stimulation than from 75 Hz stimulation. This result would appear to contradict the dogma of low-frequency fatigue; however, 5 Hz stimulation normally caused unfused twitches and the improved recovery of force may have been

due more to the increase in fusion of the twitches, from the slowing of relaxation in fatigue, than to excitation-contraction coupling mechanics.

The acidosis produced by fatigue alters the excitation-contraction coupling. Inhibition of Ca^{2+} release from the sarcoplasmic reticulum has been suggested to result from decreased pH in fatigue (Nassar-Gentina et al, 1978). The optimum pH for Ca^{2+} binding to the sarcoplasmic reticulum is 6.1-6.3; thus as pH decreases to this level during fatigue (Sahlin, 1978), Ca^{2+} release would be expected to be substantially decreased (Nakamuru, Schwartz, 1972). It was also reported that the Ca^{2+} bound to fragmented sarcoplasmic reticulum increased 50% with decreased pH and failure of the tubular action potential was secondary to swelling induced by the pH changes (Gonzalez-Serratos et al, 1978).

Another hypothesis is that, due to the large safety margin in Ca^{2+} release, decreased myoplasmic Ca^{2+} may not be as important in fatigue as decreased Ca^{2+} kinetics or the ability of the fibre to respond to Ca^{2+} (Vergara et al, 1977). The Ca^{2+}-tension relation (tension developed as a function of free Ca^{2+} ion concentration) is sigmoidal in shape and steep near threshold but flattens out quickly with rising Ca^{2+} levels (Ebashi, Endo, 1968). It follows then that any small decreases in muscle action potential with fatigue would be less likely to effect tension changes by reducing myoplasmic Ca^{2+}. The actual amount of Ca^{2+} released from the sarcoplasmic reticulum in relation to the height of the action potential is still not known. However, the rise in free Ca^{2+} in the cytosol has been measured with Ca^{2+}-sensitive dyes injected intracellularly. Blinks and colleagues (1978), using aequorin,

found that while the amplitude of the aequorin response reflected the amount of Ca^{2+} liberated into the cytoplasm; attempts to quantify the aequorin signals in terms of Ca^{2+} concentration have failed. More recently, Miledi et al (1982) demonstrated with Arsenazo III that moderately stretched frog muscle fibres were capable of releasing 8 uM of Ca^{2+} from the sarcoplasmic reticulum with a single action potential. Baylor et al (1983), also using Arsenazo III, found that the Ca^{2+} transient increased markedly with step depolarizations of the muscle fibre membrane near threshold; Ca^{2+} transients in response to action potentials with different-sized overshoots were not investigated. It has been suggested that during tetanic stimulation all of the available Ca^{2+} is released into the myoplasm (Miledi et al, 1982); hence the amount of Ca^{2+} may be above that required to saturate the contractile apparatus.

The interaction of Ca^{2+} with the contractile protein is affected by low pH. Ca^{2+} activation of actomyosin ATPase is inhibited by intracellular acidosis (cf Edwards, 1986). Bolitho-Donaldson and Hermansen (1978) found acidotic depression of maximum tension at pH 6.5 in skinned rabbit soleus fibres. Another group found that when the muscle pH dropped from 7.4 to 6.2, three times as much Ca^{2+} was required for myofibrils to develop 50% of their maximal tension (Fabiato, Fabiato, 1978). In this case, maximal tension was reduced in the presence of ample amounts of myoplasmic Ca^{2+}. This suggests that the Ca^{2+}-troponin binding constant is affected by decreased pH. The ability of Ca^{2+} to bind to troponin, expressed as the apparent binding constant K', decreased from 8×10^{-6} M to 0.5×10^{-6} M when

the pH was dropped from 8.5 to 6.5 (Fuchs et al, 1970). Thus the interaction of Ca^{2+} with the contractile proteins would be reduced at pH levels which correspond to those produced in fatigue (Sahlin, 1978).

Bianchi and Narayan (1982) found that 0.9% of Ca^{2+} released with a twitch is translocated to the transverse tubule. The slowly increasing external Ca^{2+} concentration raises the threshold for coupling the action potential to contraction because hyperpolarization of the muscle fibre resting membrane potential has been demonstrated (cf. Ebashi, Endo, 1968). Since membrane permeability for Ca^{2+} increases with depolarization (Endo, 1977), the depolarization-Ca^{2+} release coupling would be depressed with hyperpolarization.

The effects of high frequency stimulation on excitation-contraction coupling are probably complex. During high frequency stimulation, the free Ca^{2+} ion in the cytosol between stimuli could well be kept consistently higher than at rest (Endo, 1977). However, lactate production is evident in the rat hindlimb muscles following fatigue evoked by 100 Hz stimulation (Heigenhauser et al, 1985) and hence it could be assumed that Ca^{2+}-troponin binding would be adversely affected by the pH even with adequate myoplasmic levels of Ca^{2+}. Furthermore, Ca^{2+} would be expected to build up in the transverse tubule faster at higher frequencies than at lower rates of stimulation and to exert more inhibitory effects. It is likely that action potential failure, occurring quickly in high frequency stimulation (see above), could be more important than loss of excitation-contraction coupling in producing fatigue.

Many research questions remain. Little is known about the role of excitation-contraction coupling failure in fatigue following voluntary contractions in man. Recently, Bigland-Ritchie and colleagues (1986b) have made the intriguing suggestion that fatigue of repeated submaximal contractions may be largely due to impaired excitation-contraction coupling since electrical and metabolic parameters appear normal in that experimental protocol. Clearly, this possibility must be investigated further, ideally with experimental techniques giving a direct approach to the coupling mechanism.

1.1.4 Summary

Aspects of central and peripheral fatigue have been reviewed. It has been shown that decreased motivation and motor drive, particularly during sustained voluntary contractions, could result in fatigue by failing to deliver sufficient activation to the muscle. Muscle action potential failure, either from impaired neuromuscular transmission or from impulse propagation block along the muscle membrane, may be present during fatigue from high frequency stimulation, but is less likely to occur during lower frequency stimulation or voluntary contractions. During prolonged muscle activity the progressive loss of force is considered to result not so much from a loss of muscle fibre membrane excitation but rather from impairment of some subsequent step, probably excitation-contraction coupling. The decline in muscle fibre excitation during fatigue can be considered a form of 'muscular wisdom' (Marsden et al, 1983) which eliminates superfluous impulse activity in muscle fibres.

1.2 Purpose

The problem to be addressed in this thesis stems from the observation by Bigland-Ritchie et al (1981a) that, during fatigue induced by sustained MVCs, the smoothed rectified EMG activity decreases roughly in parallel with the loss of force. This research will investigate the physiological mechanisms responsible for the declining EMG activity.

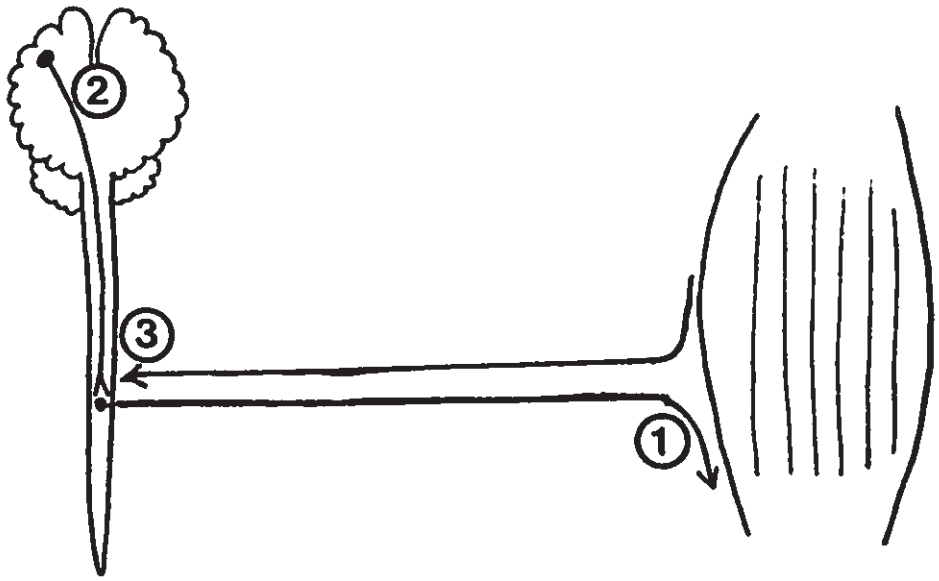
Any reduction in EMG could, in theory, have resulted from one or more of the following mechanisms: (1) a loss of peripheral excitability, from impairment of neuromuscular transmission or of muscle fibre impulse propagation, (2) decreased descending motor drive from 'higher centers' in the nervous system, and (3) reflex inhibition of the motoneurons by afferents from the exercising muscle (Figure 1).

It is the hypothesis of this research that this reduction in EMG activity results from reflex inhibition of homonymous motoneurons by afferents excited by biochemical or mechanical changes in the fatigued muscle. This hypothesis is essentially similar to that propounded by Bigland-Ritchie et al (1985).

The hypothesis is tested in conditions that either excluded or directly assessed the contribution of motor drive factors. The conditions minimize the possibility that large decreases in voluntary motor drive could contribute to any reduction in motoneuron output during fatigue; this is achieved by bypassing higher 'motor centres' through the use of tetanic motor nerve stimulation, rather than voluntary contraction to fatigue the muscle. Further, the failure of descending motor drive during attempted maximal voluntary contractions is assessed with the interpolated

Figure 1: Possible explanations for declining EMG with fatigue.

1) peripheral inexcitability of the neuromuscular junction or muscle fibre membrane
2) failure of descending motor pathways
3) reflex inhibition of motoneurons


2
3
1

twitch technique (Belanger, McComas, 1981). An approach is used to minimize the role of muscle fibre membrane inexcitability as a factor in the decline of EMG activity. This is accomplished by selecting a frequency for the 'fatiguing' stimuli which would minimize action potential failure; being essential to the remainder of the experiments, these results are the first to be reported. Relatively direct estimates of motoneuron excitability during fatigue are made using the Hoffmann reflex; this reflex is an electrically-induced excitatory reflex predominantly between Ia fibres and homonymous motoneurons (Hugon, 1973). Finally, the study explores the possible role of large diameter afferent fibres from the fatigued muscle in causing any reflex inhibition of motoneurons; this part of the investigation employs pressure over the motor nerve to produce selective blockade of impulse conduction.

If there is a reflex inhibitory signal, or loss of excitatory signal, during fatigue from the exercising muscle to the homonymous motoneurons, the nature of the stimulus remains to be determined; chemoreceptors or mechanoreceptors, either separately or in combination, would be possible candidates. The significance of the present research is to provide information relevant to the understanding of the interaction between the fatigued muscle and the central nervous system.

Chapter 2

GENERAL METHODOLOGY

2.1 Introduction

Electromyographic and force recordings were taken from the dorsiflexors and plantarflexors of the ankle. Since soleus and tibialis anterior have quite different patterns of use and contractile properties (Kugelberg, Edstrom, 1968; Belanger et al, 1983), the use of different muscle groups allowed for the detection of any generalized mechanisms underlying the decline in EMG.

2.2 Evaluation Procedure

Schema of the evaluation procedure for experiments on tibialis anterior and soleus muscles are given in Figures 2 and 3 respectively. Subjects sat in a chair of adjustable height; the right leg was securely strapped into a metal frame which maintained the knee joint at a constant angle of 90°. The foot was held down with two wide Velcro straps onto the aluminium footplate of an adjustable foot holder (Figure 4). In experiments on tibialis anterior, the ankle joint was plantarflexed by 30°, the reference being perpendicularity of the sole to the tibia. Ankle plantarflexion in these experiments reduced the contribution of the plantarflexor

Figure 2: **Evaluation procedure for studies on tibialis anterior.**

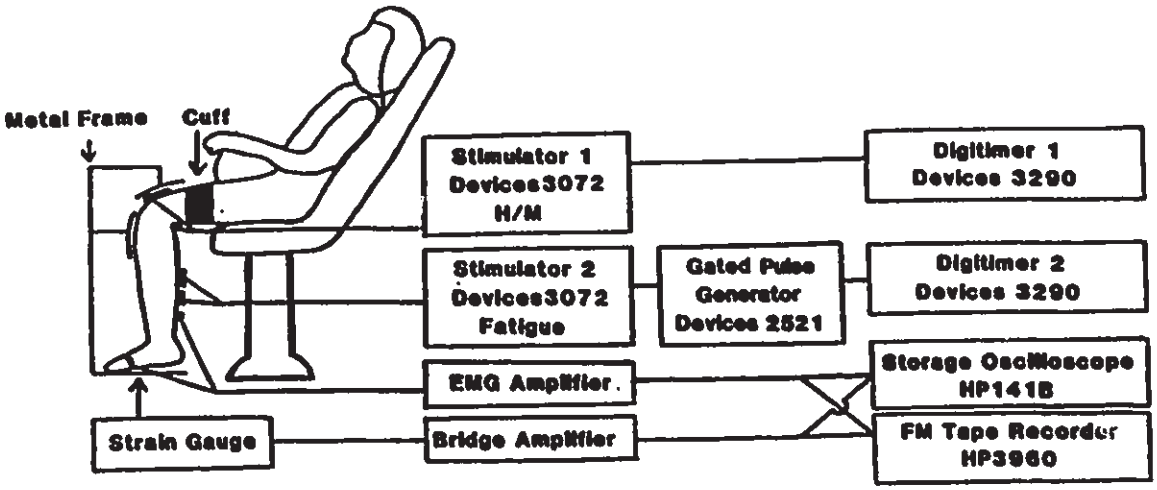

Metal Frame
Cuff
Stimulator 1
Devices3072
H/M
Stimulator 2
Devices3072
Fatigue
Gated Pulse
Generator
Devices 2521
Digitimer 1
Devices 3290
Digitimer 2
Devices 3290
EMG Amplifier
Storage Oscilloscope
HP141B
Strain Gauge
Bridge Amplifier
FM Tape Recorder
HP3960

Figure 3: **Evaluation procedure for studies on soleus.**

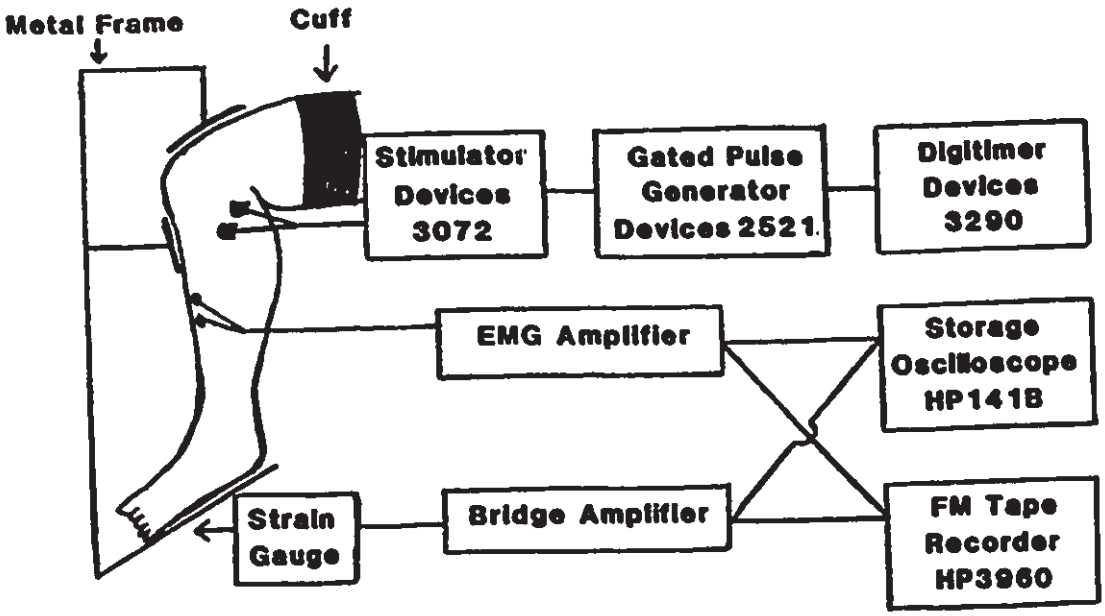
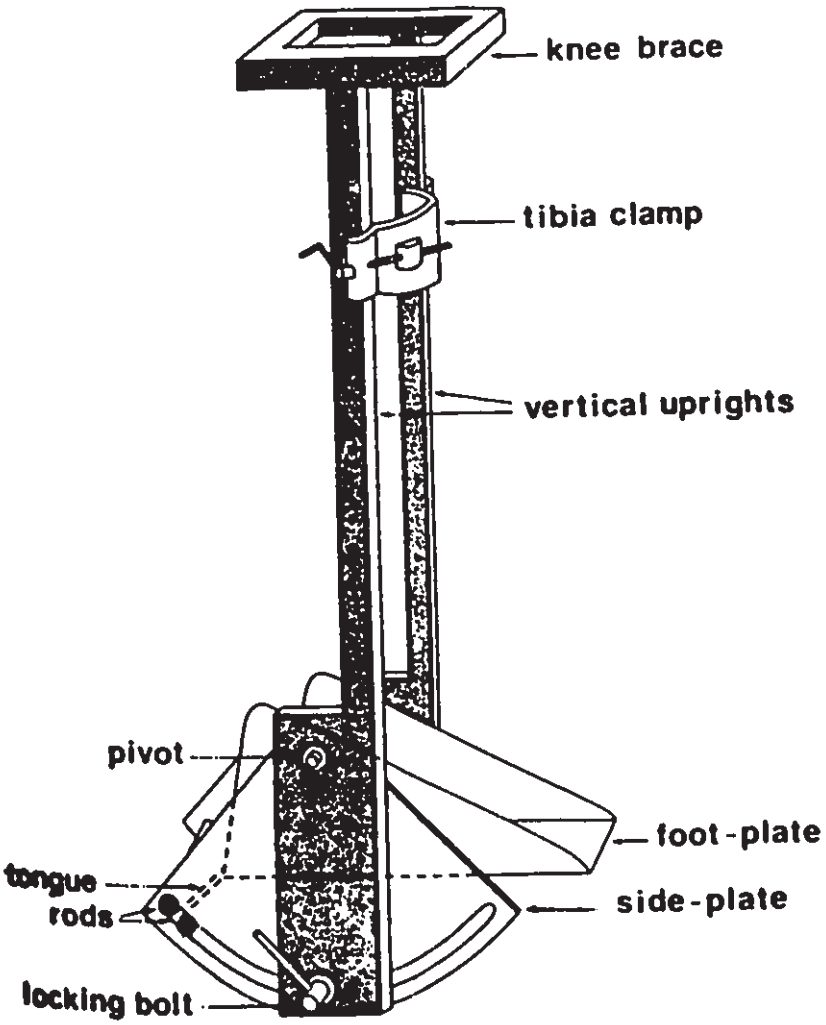

Metal Frame
Cuff
Stimulator Devices 3072
Gated Pulse Generator Devices 2521
Digitimer Devices 3290
EMG Amplifier
Storage Oscilloscope HP141B
Strain Gauge
Bridge Amplifier
FM Tape Recorder HP3960

Figure 4: Diagram of Foot Holder
From Marsh et al, 1981


knee brace
tibia clamp
vertical uprights
pivot
foot-plate
tongue
rods
side-plate
locking bolt

muscles to the measured force since peroneal nerve stimulation would have activated the two peroneal muscles, both of which are plantarflexors of the ankle.

The experiments on soleus muscle involved H-reflex testing, hence subjects were seated in a semi-reclined position with the head and arms supported (cf. Hugon, 1973). Subjects were advised to keep their body position stationary and to relax as much as possible. Both legs were placed in metal holders which kept the knees and ankles at 90°; the right foot rested on the adjustable foot holder.

A blood-pressure cuff was wrapped around the lower thigh and kept inflated to 350 - 400 torr during the experiments, so as to produce ischemia. Ischemic conditions were used to hasten the onset of fatigue; this made the experimental conditions more tolerable for the subjects as they were subjected to fewer tetani. Furthermore, since soleus muscle is very fatigue-resistant, ischemic conditions were necessary to induce fatigue within a reasonable time. Preliminary experiments demonstrated that a subject could endure 35 minutes of tetanic stimulation of soleus muscle with an intact circulation and yet only realize a 15% decline in the force. Hence to keep the experimental conditions constant, ischemia was used in all experiments. During the experiments on soleus muscle, the blood pressure cuff was momentarily released in some subjects to relieve the pressure-pain underneath the cuff. The cuff was never deflated for more than 1 second and the pressure never fell below 200 torr (usually 250 torr). Although muscle ischemia was employed to hasten fatigue, muscle ischemia would also be expected to occur whenever prolonged MVCs were attempted (Barcroft, Millen, 1939). It is quite probable that during

during certain activities (e.g. rock climbing, sailing) these muscles also experience periods of sustained or repeated maximal voluntary contraction. Therefore, in this study, the ischemic conditions would have been similar to those when sustained MVC was employed to induce fatigue; the latter conditions were used when the observation of declining EMG was made (Bigland-Ritchie et al, 1981a).

2.3 Electrical Recordings

Surface electrical recordings included M-waves, evoked by single shocks or tetanic stimulation, H-reflexes, and voluntary EMG activity. Electrodes were placed on the skin after it had been prepared by rubbing with sandpaper and an alcohol swab, followed by a mildly abrasive electrode jelly (Drug Trading Company). All surface-recorded electrical signals were amplified using a 10 Hz to 1 kHz bandpass and were displayed on a variable persistence cathode ray storage oscilloscope (Hewlett-Packard model 141B). The recordings were stored on FM tape (Hewlett-Packard recorder 3960) for detailed analysis after the experiment.

Intramuscular electromyographic recordings of tibialis anterior were made with bifilar and coaxial electrodes (type 13L49 and 9013 L0501, Dantec Inc.). The electrodes were inserted into the muscle belly of tibialis anterior, midway in the rostrocaudal axis, and motor unit recordings were taken with the electrode stationary. The activities of different units were recorded by altering the depth of the electrode tip. The intramuscular EMG recordings were amplified using a 10 Hz to 10 kHz bandpass and were recorded on FM tape before being photographed on

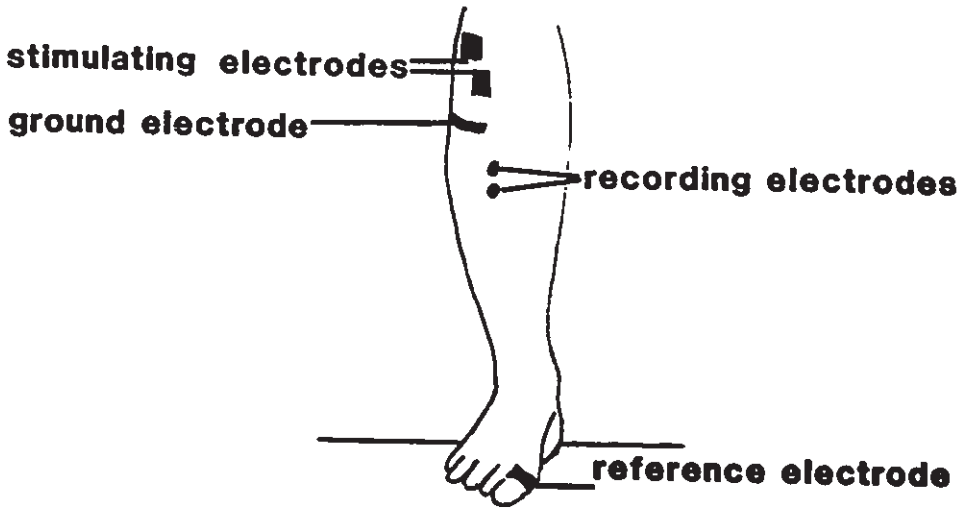
35 mm negative film for projection (enlargement) and analysis. Analysis involved identification of individual motor units according to the shape and amplitude of the potentials, measuring the intervals between potentials, and calculating the firing rates.

2.3.1 Electrode Placement: Tibialis Anterior

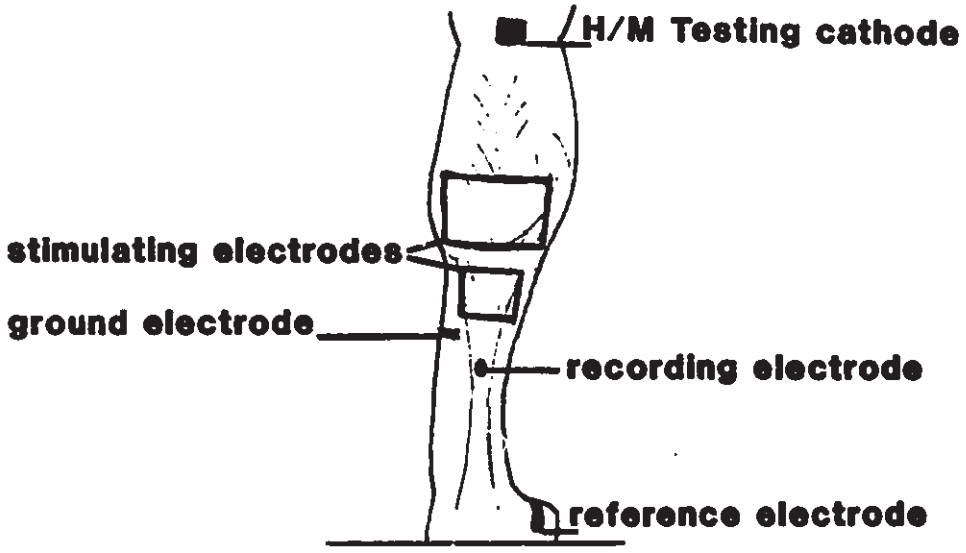
Two stimulating lead electrodes (4.5 cm by 2.5 cm) were wrapped in gauze and soaked in conducting gel (Cardio-Cream, Ingram & Bell Medical). The cathode was placed over the common peroneal nerve behind the neck of the fibula and the anode was placed inferomedially. To record voluntary EMG activity, two silver chloride cup electrodes (9mm in diameter) were placed, approximately 3 cm. apart, over the middle third of the muscle belly of tibialis anterior with a ground silver chloride strip electrode (5 cm by 0.7 cm) placed between the stimulating and recording electrodes (bipolar derivation, Figure 5a). M-waves, evoked by peroneal nerve stimulation, were recorded by the proximal cup electrode and a reference silver chloride strip electrode (5 cm by 0.7 cm) wrapped around the great toe (monopolar derivation). The monopolar derivation was used for the recording of M-waves since it gave a much cleaner signal. The bipolar derivation was preferred for the voluntary EMG since it reduced the contribution to the EMG recordings of other muscles which may brace the ankle during MVCs.

Figure 5: Electrode placement for:
a) tibialis anterior, and
b) soleus.

a) TIBIALIS ANTERIOR



b) SOLEUS



2.3.2 Electrode Placement: Soleus

Two stimulating lead electrodes were placed bilaterally for H-reflex and M-wave testing; the cathode (4.5 cm by 2.5 cm) was placed in the popliteal fossa over the posterior tibial nerve and the anode (8 cm by 8 cm) was placed superior to the patella on the anterior thigh (Figure 5b). Stigmatic recording electrodes were disc electrodes (0.9 mm in diameter) placed bilaterally, approximately 6 cm above the superior aspect of the calcaneus (cf. Hugon, 1973). The ground and reference electrodes were silver chloride strips (5 cm by 0.7 cm) and were placed over the lateral gastrocnemius muscle belly of each leg and on the dorsum of each foot respectively.

2.3.3 M-waves

The maximum M-wave is a measure of the total electrical activity of all the muscle fibres in response to a supramaximal stimulus to the motor nerve. Neuromuscular junction transmission and muscle fibre membrane excitability can be determined in human subjects by measuring the amplitude of the M-wave (Pagala et al, 1984; Milner-Brown, Miller, 1986). M-wave amplitude has been shown to adequately represent the muscle fibre membrane excitability in cat tibialis anterior muscle particularly since M-wave area was not found to be a independent parameter but rather was proportional to the product of amplitude and duration (Enoka et al, in press). In the present studies, muscle fibre membrane excitability was determined from M-waves elicited by single shocks; tetani were also employed since it is

that neuromuscular transmission could be maintained for single stimuli but not for trains of stimuli (cf. Bellemare, Garzaniti, 1988). M-waves were elicited by rectangular voltage pulses of 50 us duration; the latter were delivered from a high-voltage dual stimulator (Devices Ltd., model 3072). Maximal M-waves were determined by increasing the voltage until the evoked response became no larger; the stimulus intensity was then increased by 20%, both prior to the experiment and again in the posttest period, to be certain it remained supramaximal during the experiment. Consecutive recordings of maximal M-waves could be superimposed on the oscilloscope screen. Any measurement error in these recordings would have been less than 1%; this represents the visual discrimination of the divisions on the oscilloscope.

M-waves, evoked by single shocks of 120% of maximal stimulus intensity, were analyzed by measuring the peak-to-peak amplitude and the duration on the oscilloscope screen. If the M-wave duration increased more than 10%, then the areas were calculated. This enabled the shape of the M-wave to be studied in detail. A computer program was devised by Mr. Glen Shine to calculate the amplitude, duration and area of the M-waves. The computer digitized the M-wave potentials every 0.1 ms. Computations began at the beginning of a series of 10 successive data points which showed progressively larger values and ended when the M-waves returned to 10% of the baseline. The M-waves evoked by tetanic stimulation were analyzed by measuring the peak-to-peak amplitudes; durations could not be measured since the M-waves occurred too close in succession. The M-waves within

each train of stimuli were analyzed to determine whether action potential failure occurred during the train.

2.3.4 Voluntary EMG

The EMG associated with a voluntary contraction underwent full-wave rectification and integration over 1 second. The integrator was triggered manually at the onset of the contraction; the manual trigger ensured that any noise in the recordings could not trigger the integrator. Since some subjects had difficulty holding a maximal voluntary contraction for 2 seconds during fatigue, a 1 second interval was chosen for the integration. A programmable desk-top calculator (Hewlett Packard model 9810A) computed the integral. The voluntary EMG activity had a 3.8 ± 3.9% intratest measurement error since the integrator was triggered manually. To overcome this, the EMG associated with each MVC was integrated 3 times over the same period and the mean value was taken. The voluntary EMG activity had an 8.8 ± 7.7% intertest error ie. the difference in integrated EMG activity during contractions of the same force made by the same subject on different trials (Appendix I).

2.3.5 H-Reflex

The Hoffmann or H-reflex is a reflex evoked by electrical stimulation of the Ia afferent fibres coming from the muscle spindles (Hugon, 1973). Electric pulses of long duration (0.5 ms) and low intensity favour preferential activation at threshold

of Ia afferents; the latter appear to have lower accommodation than the motor axons in the nerve bundle. Baseline EMG activity and force measures indicated that the subjects were relaxed at the time of administration of the stimuli. This avoided the pitfall, outlined by Verrier (1985), that the H-reflex amplitude changed according to the level of background EMG activity. Single rectangular pulses of 0.5 ms duration were delivered from the Devices stimulator (model 3072). A digital timing unit (Digitimer model 3290) triggered the stimulator every 5 seconds. The 5 second interval prevented stimulation-induced depression of the H-reflex (Hugon, 1973). The H-reflex peak-to-peak amplitude is variable; accordingly the criterion measure was taken as the average of 5 H-reflexes after the largest and smallest responses had been removed (Hugon, 1973).

In this study, the maximal H-reflex was expressed as a proportion of the maximal M-wave as the criterion measure. The Hmax/Mmax ratio was first described by Angel and Hofmann (1963) and has been used effectively in at least one previous exercise study (Bulbulian, Darabos, 1986). This strategy was necessary to calibrate for any changes in the peripheral excitability of the muscle fibre membrane consequent to fatigue. Other studies (non-fatigue) which employ submaximal H-reflexes use the consistency of a small accompanying M-wave as evidence of a constant stimulation current density and stable electrode placement (Kukulka et al, 1985; Morin et al, 1982). In fatigue the assumption of a constant M-wave does not hold true since both potentiation (Garner et al, 1987) and

depression (Garland et al, 1987; Miller et al, 1987) of the maximum M-wave have been reported.

2.4 Force Recordings

Force produced by the ankle dorsiflexors or ankle plantarflexors was determined from two strain gauges mounted on an aluminium foot plate on the underside of the adjustable foot holder (see Figure 4). The foot holder mass was 1.51 kg with a resonance frequency of 80 Hz when the foot was in position (Marsh et al, 1981). The apparatus was calibrated by measuring the DC voltage developed when known weights were suspended from the foot-plate; over the range of torque values encountered, the deviation of the system from linearity was less than 5% (Marsh et al, 1981). Compliance of the foot holder and Velcro straps was determined using a gauge placed on the head of the first metatarsal bone. The displacement measured by the gauge for an exerted ankle dorsiflexion torque was .0469 deg/Nm, ie. 40 Nm of dorsiflexion torque produced an angular displacement of 1.88°. Force recordings were amplified and displayed on the storage oscilloscope. The peak amplitude of the oscilloscope trace served as the force measure. All data were stored on FM tape (Hewlett-Packard recorder 3960) for detailed analysis after the experiment.

2.4.1 Force Produced by Voluntary Contraction

It was important to determine whether during an attempted MVC the motor drive to the muscle was complete (ie. no central fatigue). Subjects would attempt MVCs twice since the maximal force production was found to vary by 3.4 ± 3.7% (Appendix I). The mean of the two attempts was used as the outcome measure. A strong interpolated stimulus was delivered, at intensities above that required for a maximal twitch, during a voluntary contraction; the force recording was analyzed for the presence of any interpolated twitch. This interpolated twitch technique was based on the theory that motor units that were either not activated or submaximally activated during a voluntary contraction should give an observable twitch response to supramaximal stimulation of the motor nerve during the contraction (Denny-Brown, 1929; Belanger, McComas, 1981). If the MVC was truly maximal, a decrement in the force should be evident after the interpolated stimulus. This decrement was due to a transient silent period after a synchronous volley along the motor nerve (Merton, 1951) and to collision of antidromic impulses from the volley with impulses travelling down the motor nerve from the spinal cord. The silent period could have been the result of inhibition from synchronous activation of Renshaw cells and Golgi tendon organ afferents, or to unloading of the muscle spindle by the twitch contraction (Angel et al, 1965). During higher force contractions (90% of maximum or greater), it was sometimes difficult to ascertain if an interpolated twitch was present (Appendix IV).

2.4.2 Force Produced by Electrical Stimulation

A gated pulse generator (Devices Ltd, model 2521) was set to deliver stimuli at the desired frequency. Manual adjustments of the frequencies could have caused the actual frequencies delivered to have varied up to 10% from those indicated on the dial. Rectangular voltage pulses of 50 us duration were delivered from a stimulator (Devices Ltd, model 3072) which received triggering pulses from a digital timing device (Devices Ltd., Digitimer model 3290).

a) Tibialis Anterior:

When electrical stimulation was used to produce ankle dorsiflexion torque, the stimuli were delivered to the common peroneal nerve behind the neck of the fibula. The intensity was above that required for the maximum twitch.

b) Soleus:

When electrical stimulation was used to produce force from the soleus muscle only, the stimuli were delivered through large lead plate electrodes placed end-to-end over the soleus muscle; this stimulated intramuscular nerve fibres. The cathode (7.5 cm by 5.5 cm) was 2 cm above the stigmatic recording EMG electrode (section 2.3.2) and the anode (12 cm by 7 cm) was placed just below the bulk of the gastrocnemius muscle. Because of the discomfort experienced with this stimulation, submaximal voltages (50 to 80% of that for the maximal M-wave) were used; this prolonged the time required to induce

fatigue but should not have influenced the data from the voluntary test contractions since the force production at the end of the fatiguing stimulation was equivalent in all subjects.

Chapter 3

DETERMINATION OF FATIGUE PROTOCOL

3.1 Introduction

The first problem to be addressed in this thesis was the choice of a stimulating protocol for inducing muscle fatigue. To ascertain the contribution of reflex inhibition to the decline in voluntary EMG during fatigue it was necessary to ensure that peripheral inexcitability, from failure of neuromuscular transmission or of muscle fibre impulse propagation, was minimized. As discussed in the General Introduction (section 1.3.3a), muscle action potential failure has been shown to be dependent on the frequency of stimulation; it was prominent during high frequency stimulation (>40 Hz) but limited during lower frequency stimulation (Naess, Storm-Mathisen, 1955; Johns et al, 1956, Sandercock et al, 1985).

It was hypothesized that 15 Hz frequency of stimulation would minimize muscle action potential failure. Two different low frequencies of stimulation (15 Hz and 30 Hz) were tested to determine the respective amounts of muscle action potential failure. The frequency which resulted in the least decline in the M-wave, for a given reduction in force, was to be used in the subsequent experiments. These two frequencies of stimulation were appropriate since they were within the

physiologic firing ranges for the tibialis anterior and soleus muscles. The tibialis anterior has motoneuron firing rates during MVCs of 30 to 65 Hz (Hannerz, 1974) and, under the conditions of fatigue, this rate has been shown to drop to between 15 and 20 Hz (Grimby et al, 1981a). The soleus has mean motoneuron firing rates of 11 Hz prior to fatigue (Bellemare et al, 1983). It was also important that the frequency of stimulation chosen should be sufficient to evoke a fused tetanus, thereby maximizing force production and speeding up the fatigue process. Preliminary experiments indicated that frequencies below 15 Hz failed to evoke a fused tetanus in the tibialis anterior. Tibialis anterior was used to test the hypothesis since in mammalian muscle, soleus was found to have good peripheral excitability of the muscle fibre membrane and neuromuscular junction with 30 Hz stimulation however, muscles with more phasic activity (similar in muscle fibre composition to tibialis anterior) demonstrated action potential failure (Pagala et al, 1984). Hence it was deemed that if the reduction in the muscle compound action potential could be minimized in tibialis anterior, that frequency of stimulation would also be appropriate for soleus muscle.

3.2 Methods

3.2.1 Subjects

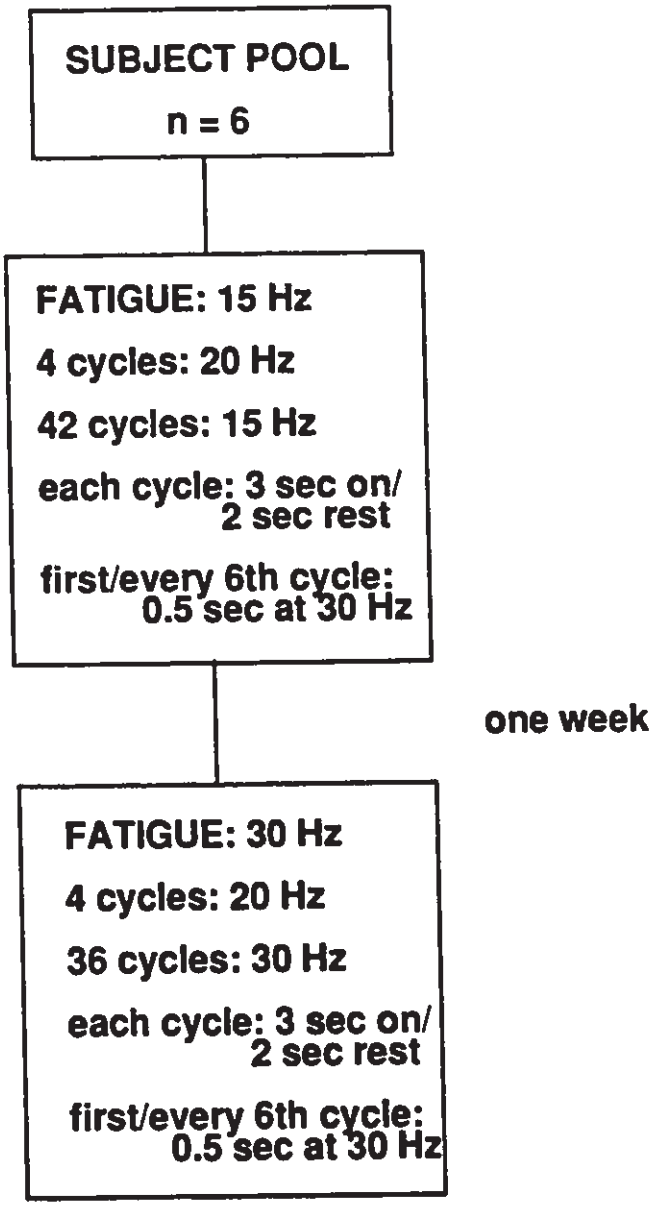
Six human volunteers (3 male, 3 female), ranging in age from 27 to 52 years (mean of 33.8 years), participated in the study. All were in good health and had no previous history of neuromuscular disorders.

3.2.2 Experimental Protocol

Each subject was tested on two different occasions with at least one week between tests (Figure 6). The two tests were performed on the same leg, the only difference being the frequency of peroneal nerve stimulation (15 Hz or 30 Hz) employed to induce fatigue. Recordings of M-waves from tibialis anterior and ankle dorsiflexion torque elicited by single shocks and trains of stimuli were monitored throughout each experiment. The single shocks enabled any changes in the amplitude, duration, and area of the M-waves to be studied in detail. Such measurements could not be made from the first responses in each of the fatiguing trains since, at 30 Hz, the next M-wave began before the previous one was completely over. In addition, comparison of the electrical and mechanical responses to single stimuli was relevant to a consideration of the factors responsible for "peripheral" fatigue and, as such, are discussed in Chapter 7 and Appendix II. The trains of stimuli enabled any changes in the amplitude of the M-wave during prolonged stimulation to be detected.

The fatigue procedure consisted of cycles, 5 s in duration, each containing a single shock and followed, 0.5 s later, by 3 s of tetanic stimulation at 20 Hz; this sequence was repeated for 4 cycles. By this time any enlargement of the twitch resulting from the stimulation (post-tetanic twitch potentiation; cf. Vandervoort et al, 1983) was usually maximal and the fatiguing stimulus frequency was then changed from 20 Hz to either 15 Hz or 30 Hz. This produced a standardized starting point, in terms of force production, before assessing the difference between 30 Hz and

Figure 6: Experimental Protocol



* M-waves and torque monitored throughout

15 Hz stimulation frequencies. The fatiguing stimulation was continued until the dorsiflexion torque had been completely abolished. The fatigue procedure took a maximum of 36 cycles using 30 Hz and 42 cycles using 15 Hz stimulation.

Based on preliminary results from the McComas laboratory, this study was used to answer another question unrelated to the theme of this thesis (see Appendix II). Accordingly, a brief (0.5 s) testing burst of stimuli at 30 Hz was delivered at the end of the first, sixth, and then every sixth cycle, even when 15 Hz was employed as the fatiguing frequency. If 30 Hz was being used as the fatiguing frequency, the effect of the additional burst was to prolong the 30 Hz fatiguing tetanus by 0.5 s. The results of these data are presented in Appendix II.

3.2.3 Statistical Analysis

The peak-to-peak amplitudes of the M-waves, together with the peak dorsiflexion torques, were measured from the oscilloscope screen. In addition, durations and areas were calculated for the M-waves responses to single stimuli; these could not be reliably measured during the tetanic stimulation due to slight overlap of the responses at 30 Hz. All values were expressed as percentages of the corresponding values in the first cycle.

Of prime concern was whether the amplitude of the M-wave at the end of the fatiguing stimulation was better maintained, in relation to force, with either of

the fatiguing frequencies. To determine whether the M-wave responses obtained after fatiguing stimulation at 15 Hz and at 30 Hz differed significantly from each other, a paired t-test was performed. The final M-wave in the last fatiguing train (prior to the 30 Hz testing burst) was used in the analysis (Figure 7). The significance level was set at $p=0.05$.

3.3 Results

3.3.1 Responses to Tetanic Trains

Typical recordings of the dorsiflexion torque elicited by a single stimulus and tetanic stimulation, together with the corresponding M-wave responses, are displayed in Figure 7; the photographs were taken at different times (indicated by the numbers to the left of the figure) during the fatiguing stimulation in one of the subjects, a 29 year old woman (Appendix III). In Figure 8, the peak-to-peak amplitudes of the final M-wave in each fatiguing train (prior to the 30 Hz testing burst) have been plotted as a function of the time after the start of the experiment. Both 15 Hz and 30 Hz stimulation caused potentiation of the M-wave (cf. Garner et al, 1987) followed by declines; the decline began earlier and appeared to proceed more rapidly during the higher of the two rates of fatiguing stimulation. The maintenance of the M-wave relative to the decline in the dorsiflexion torque is clearly evident using 15 Hz fatiguing stimulation. At both frequencies of stimulation, the loss of dorsiflexion torque produced by tetanic stimulation began before the reduction in M-wave amplitude; the fall in dorsiflexion torque became noticeably steeper after

Figure 7: Dorsiflexion torque produced by single stimuli and tetanic trains (lower traces), together with M-waves (upper traces), recorded in same subject at different stages during the fatigue at either 15 Hz (left column) or 30 Hz (right column). At the end of each tetanus, there was a burst of testing stimuli at 30 Hz. Numbers at the left indicate the duration of intermittent fatiguing stimulation. The arrows mark the points where the M-wave and dorsiflexion torque were measured.

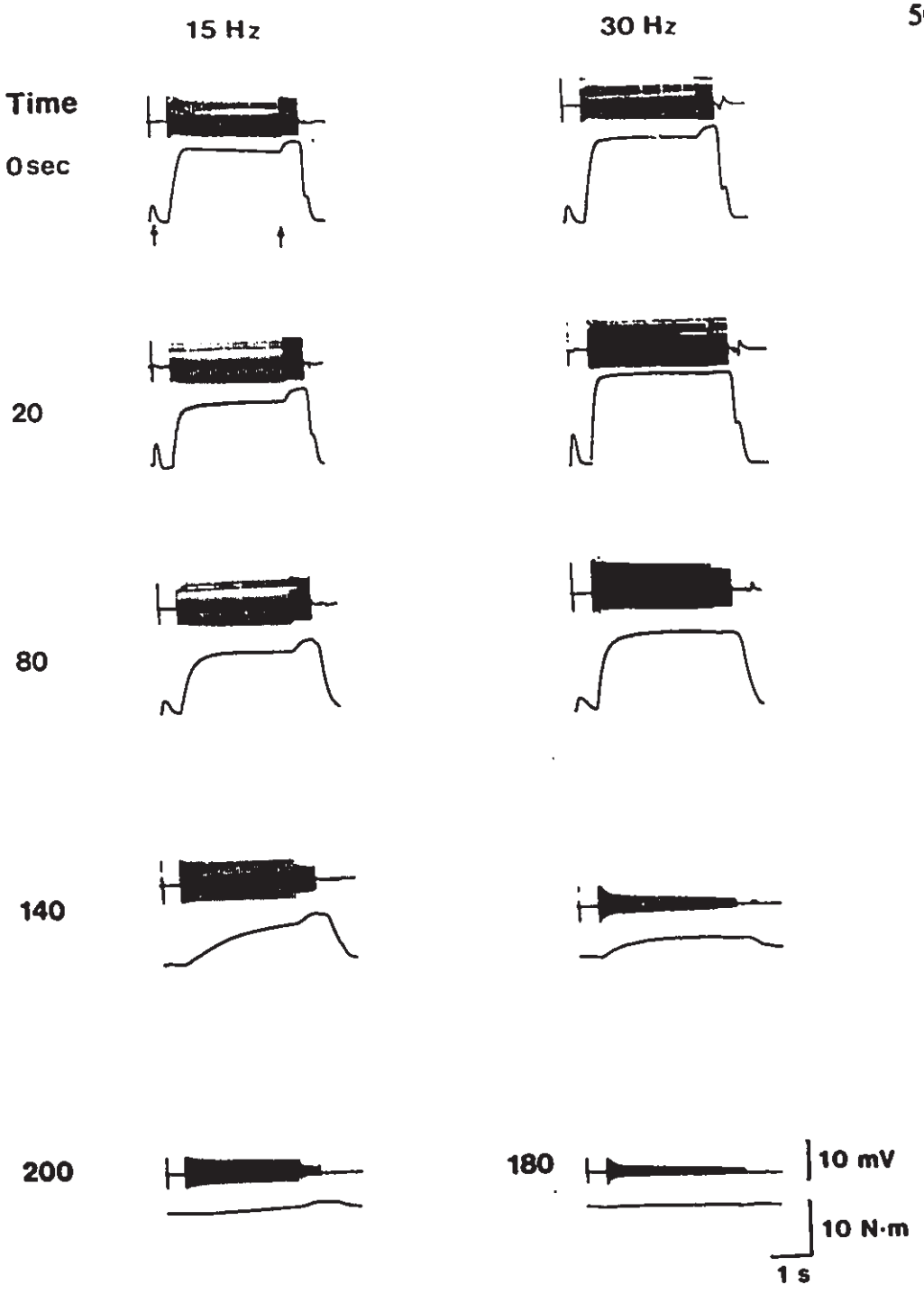
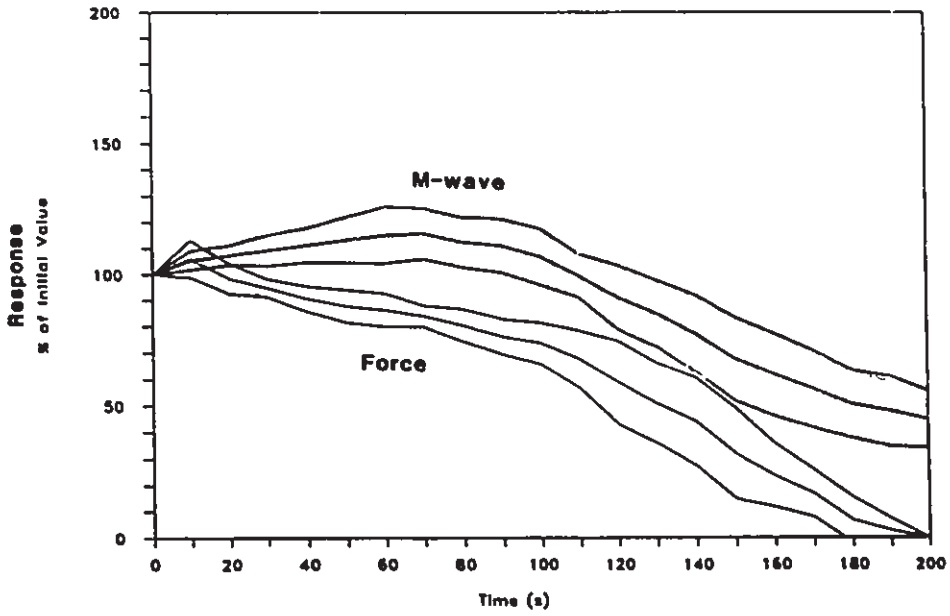
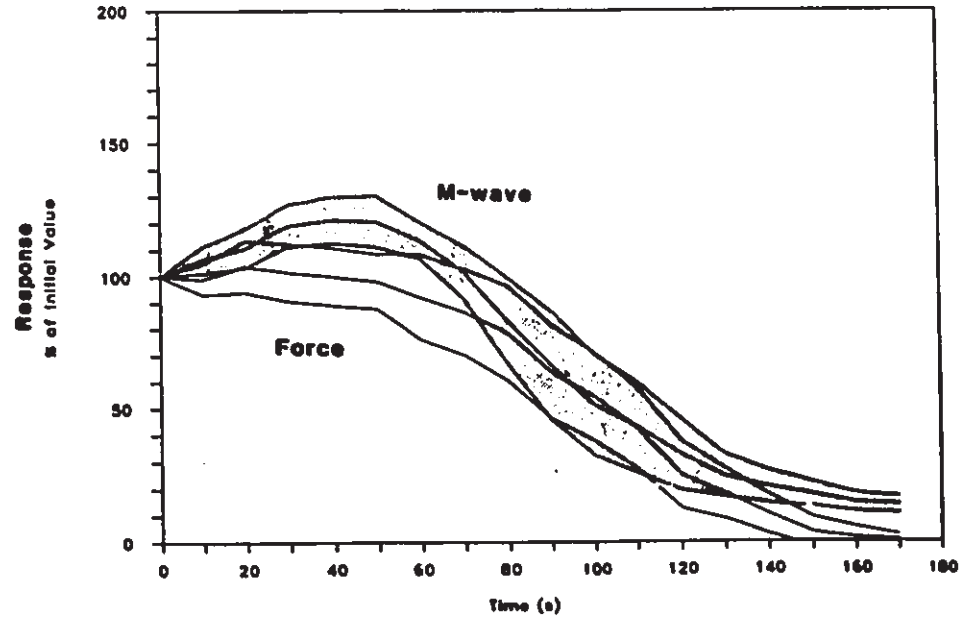

15 Hz
30 Hz
Time
0 sec
20
80
140
200
180
10 mV
10 N·m
1 s

Figure 8: Dorsiflexion torque and final M-wave (stippled curve) responses of ankle dorsiflexor muscles during successive fatiguing tetani at 15 Hz (upper figure) or 30 Hz (lower figure). Values are means ± SDs.

15 Hz


200
150
100
50
0
Response
% of Initial Value
M-wave
Force
0
20
40
60
80
100
120
140
160
180
200
Time (s)

30 Hz


200
150
100
50
0
Response
% of Initial Value
M-wave
Force
0
20
40
60
80
100
120
140
160
180
Time (s)

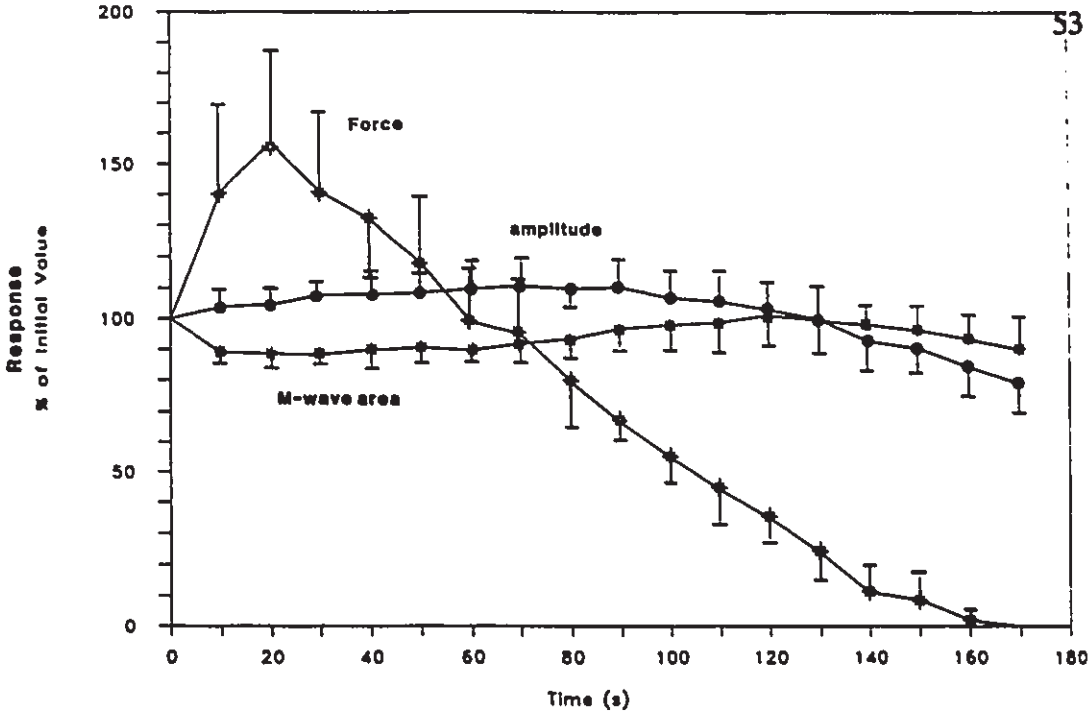
80 s when 30 Hz was used as the fatiguing frequency. This higher rate of stimulation abolished tetanic dorsiflexion torque in all subjects by 175 s; when 15 Hz was used, however, there was a mean decline in dorsiflexion torque of 83.2% at this time. At the time of total fatigue, the M-wave amplitude decreased by 56.8 ± 8.9% of its original amplitude following 15 Hz stimulation, but fell by 85.9 ± 3.3% following 30 Hz stimulation; the amplitudes of the M-waves were significantly different between the two frequencies ($t=8.122$, 5 df, $p<0.001$).

3.3.2 Responses to Single Stimuli

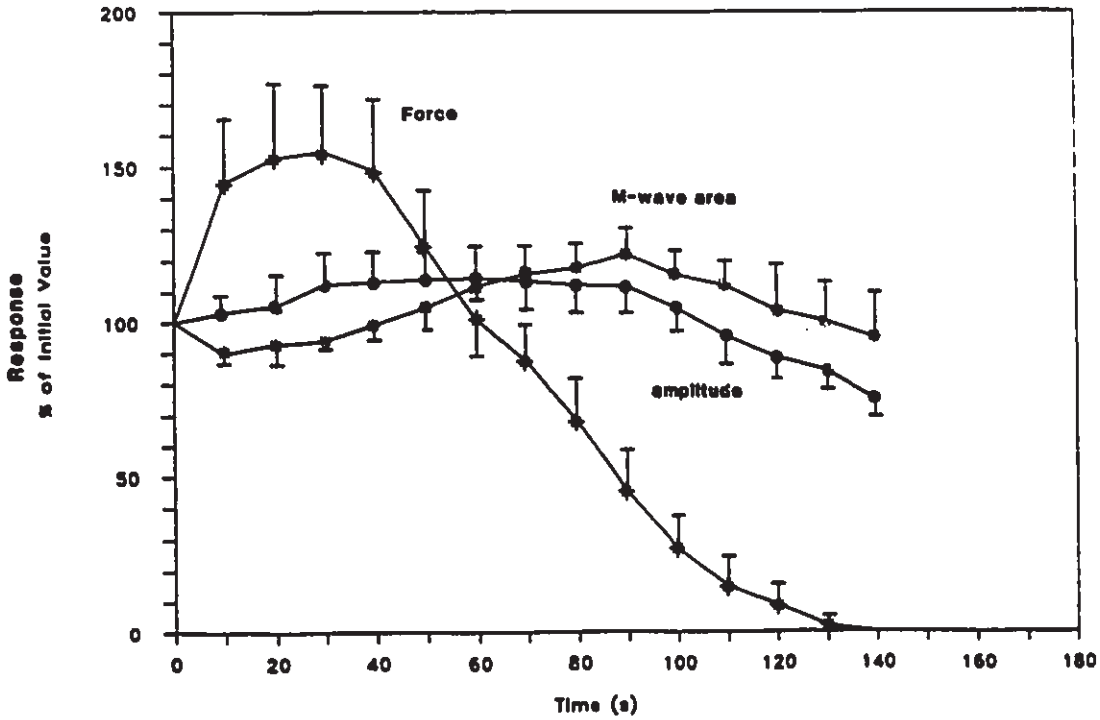
In Figure 9, the mean M-waves and mean dorsiflexion torques elicited by single stimuli are shown at different times during the fatiguing stimulation at 15 Hz (upper figure) and 30 Hz (lower figure). In both sets of results, there was appreciable potentiation of the dorsiflexion torque such that by 20 - 30 s, the mean potentiated values exceeded the initial values by 57% and 55% respectively. The dorsiflexion torques then declined in an approximately linear manner with time. In all subjects, the dorsiflexion torque elicited by single stimuli had been completely abolished by 170 s, following 15 Hz stimulation, and by 140 s, following 30 Hz stimulation. The M-wave curves were quite different to those for dorsiflexion torque, showing slight enlargement of the amplitude (by 11%, 15 Hz and by 14%, 30 Hz) followed by modest declines of 21.1 ± 9.7% and 24.9 ± 5.3% of original with 15 Hz and 30 Hz fatiguing stimulation respectively; a paired t-test indicated that these end values were not significantly different. Regardless of the stimulation

Figure 9: Mean responses (±SD) of ankle dorsiflexor muscles to single stimuli during fatiguing stimulation at 15 Hz (upper figure) or 30 Hz (lower figure). Symbols are ⌖ for force, • for M-wave amplitude, and • for M-wave area.

15 Hz


Force
amplitude
M-wave area
Response
% of Initial Value
Time (s)
0
50
100
150
200
0
20
40
60
80
100
120
140
160
180

30 Hz


Force
M-wave area
amplitude
Response
% of Initial Value
Time (s)
0
50
100
150
200
0
20
40
60
80
100
120
140
160
180

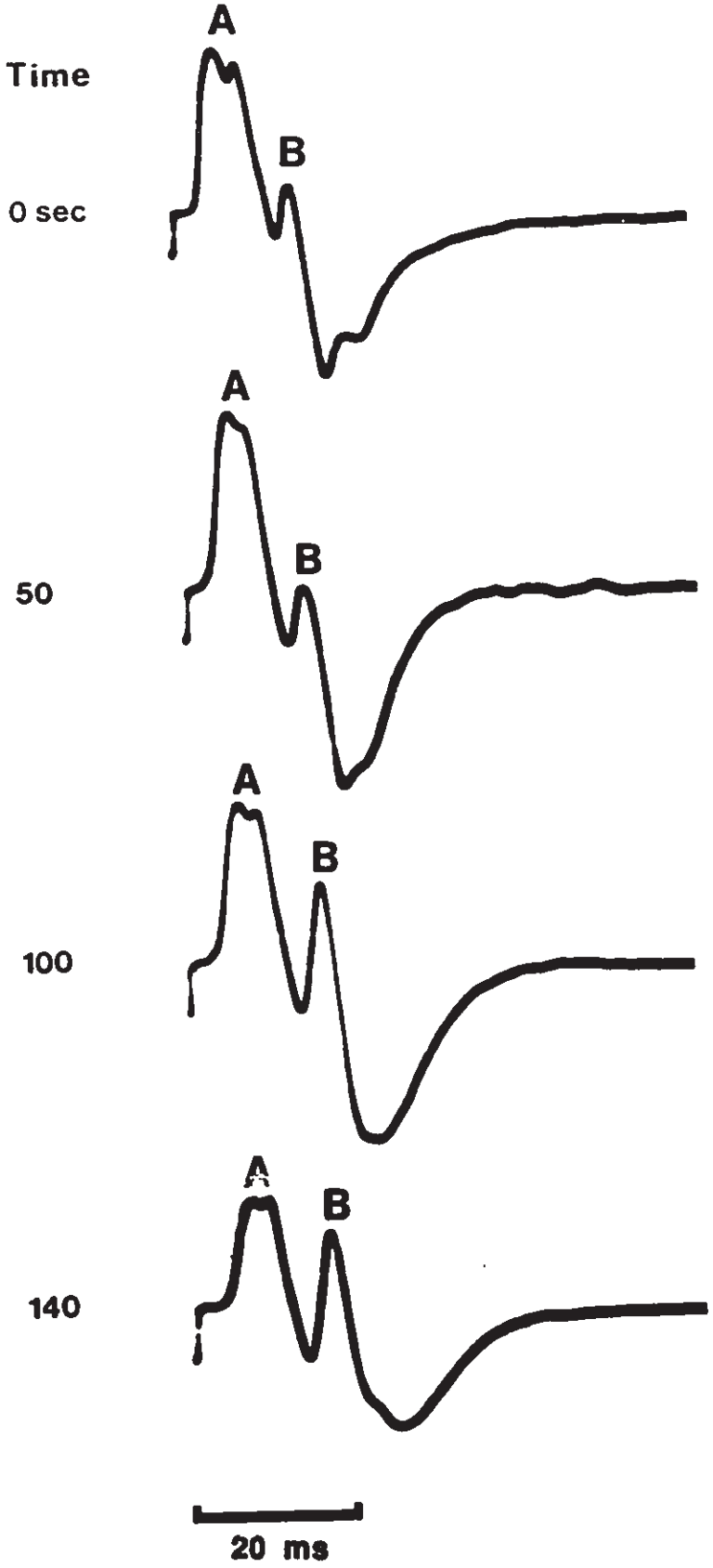
frequency employed, the amplitude of the M-wave response to single stimuli did not fall below the initial value until the dorsiflexion torque had become markedly attenuated.

Associated with the reduction in M-wave amplitude were increases in duration of 25.7 ± 13.2% and of 25.2 ± 14.1% with 15 Hz and 30 Hz fatiguing frequencies respectively, as evident in the typical recordings depicted in Figure 10. Also, some of the reduction in amplitude would have been due to temporal dispersion of the muscle impulse volley as a result of slowed impulse conduction (Bigland-Ritchie, Thomas, 1986). The effect of such slowing was evident in the recordings of individual M-waves in the present experiments (Figure 10) and was consistent with the results of other studies in which the slow conduction velocities were observed during maximal sustained contraction (Kereshi et al, 1983; Mortimer et al, 1970). The increase in duration of the M-wave was associated with better maintenance of the area than the amplitude; the mean area fell only 10.0 ± 13.8% and 5.2 ± 11.1% of original with 15 Hz and 30 Hz respectively (Figure 9).

3.4 Discussion

The objective of this part of the study was to determine the frequency of fatiguing stimulation which would result in loss of force production with the least amount of muscle action potential failure. The data also allowed speculation as to the site of fatigue in this experiment (Appendix II).

Figure 10: M-waves, evoked by a single stimulus, from one subject taken at different stages during the 30 Hz fatiguing stimulation. Numbers at the left indicate the duration of the intermittent fatiguing stimulation. The M-wave decomposed over time with two clearly separable negative waves marked A and B. It is likely that with fatiguing stimulation the second negative wave became progressively delayed in relation to the first.


Time
A
B
0 sec
A
B
50
A
B
100
A
B
140
20 ms

3.4.1 Responses to Tetanic Trains

The results of tetanic stimulation were of particular relevance to the remainder of the thesis. These showed, in keeping with the results of single stimuli, that it was possible to dissociate electrical and mechanical events in the muscle fibres during fatigue with 15 Hz stimulation. The present results demonstrated a greater loss of dorsiflexion torque than of M-wave amplitude during 3 s trains of 15 Hz stimulation. During these trains at 30 Hz stimulation, however, the dissociation was not apparent. Stimulation rates of 15 Hz are closer than 30 Hz to the motoneuron firing frequencies observed during fatigue (Grimby et al, 1981; see also this study, Chapter 4).

From a practical standpoint, the experiments reported in this part of the thesis demonstrated that 15 Hz, rather than 30 Hz, should be used as the frequency of fatiguing stimulation in the remainder of this research. By minimizing peripheral inexcitability, the application of stimuli at this frequency made it possible to examine the contribution of central nervous system factors to the decline in EMG observed during MVCs.

3.4.2 Responses to Single Stimuli

The experiments described above showed that, under ischemic conditions, both 15 Hz and 30 Hz stimulation were effective in abolishing dorsiflexion torque produced by single stimuli while maintaining the M-wave at approximately 75% of its initial amplitude. These findings demonstrated that the muscle fibre membrane

could remain excitable to single stimuli yet be less able to transmit trains of stimuli. In the next study, M-waves in the middle and at the end of a train of stimuli were used as indicators of peripheral excitability of the neuromuscular junction and muscle fibre membrane.

Chapter 4

MECHANISM FOR DECLINING EMG

4.1 Introduction

The main objective of this research was to determine the mechanism(s) for the declining EMG activity during maximal voluntary contractions with fatigue. As discussed in the General Introduction (section 1.1.5), the decrease in EMG activity could have been due to a loss of excitability at the neuromuscular junction or muscle fibre membrane. Alternatively, it might have resulted from failure of the descending motor pathways in the central nervous system or from reflex inhibition of the motoneurons by afferents from the exercising muscle, or from a combination of factors (see Figure 1).

In this thesis, it is hypothesized that reflex inhibition of the alpha motoneuron pool is responsible, at least in part, for the declining EMG during MVCs with fatigue.

Bigland-Ritchie and coworkers (1986c), in a recent attempt to distinguish between the possible causes of declining EMG activity during fatigue, studied the effects of ischemia on biceps brachii motoneuron firing rates. They observed that, following a period of sustained MVC, firing rates (based on population responses)

declined and remained low during a three-minute rest period of ischemia; when the arterial cuff was released, the firing rates returned to control values within three minutes. Hence, the authors demonstrated that without ischemia, the 3 minute rest period was sufficient time for the recovery of motoneuron firing rates; since the motoneuron firing rates remained depressed in the postfatigue ischemic period, substances trapped within the muscle probably inhibited the motoneurons. Lippold and colleagues (1960) also demonstrated a lack of recovery of submaximal EMG while the muscle remained ischemic. The findings of these two studies were suggestive of reflex inhibition from afferents within the exercising muscle.

In the study by Bigland-Ritchie et al (1986c), the authors did not eliminate the first possibility, that of a peripheral loss of excitability at the neuromuscular junction or muscle fibre membrane although results from experiments on the adductor pollicis (Bigland- Ritchie et al, 1979, 1982; Woods et al, 1987) made such an explanation unlikely. The amplitude of the maximal M-wave, evoked by stimulation of the ulnar nerve, remained unchanged at a time when voluntary EMG activity was depressed during fatigue. It could be possible nonetheless that significant decreases in M-wave amplitude might have been observed if muscle fibre excitability had been tested with low frequency (15 or 30 Hz) tetani rather than single shocks (see Chapter 3).

In the present experiment, the mechanism for the declining EMG was determined in a different way, by fatiguing the muscle through indirect electrical stimulation (via the peroneal nerve) at 15 Hz under ischemic conditions and then

determining whether voluntary EMG activity was reduced. This frequency of fatiguing stimulation has been shown to minimize the loss of excitability of the muscle fibre membrane (see Chapter 3). The use of electrical stimulation to induce fatigue has also minimized any contribution from failure of descending motor pathways since these were not used during the fatigue; this eliminated the motivational difficulties ("central fatigue") in attempting to sustain voluntary contraction to induce fatigue. Further, to assess the completeness of the alpha motoneuronal activation during MVCs, a strong interpolated stimulus was employed. Although supraspinal drive could still be reflexively inhibited after fatigue, this technique enabled the detection of submaximal efforts during the attempted MVC contractions ie. detect if the subject had "given up". Although muscle ischemia was employed to hasten fatigue, muscle ischemia would also be expected to occur whenever prolonged MVCs were attempted (Barcroft, Millen, 1939). If, under these conditions, voluntary EMG activity was still reduced, then reflex inhibition of motoneurons would appear to be the most likely mechanism.

An extension of this study was to investigate how any inhibition of motoneurons was manifested ie. decreased motoneuron firing rates or decreased number of active motoneurons. It was hypothesized that both of the aforementioned would be present in the fatigued muscle. While many reports existed of decreasing motoneuron firing rates in fatigue (Bigland-Ritchie et al, 1983b; Bigland-Ritchie, Woods, 1984; Grimby et al, 1981b), there were few reports of decreasing numbers of active motoneurons (Grimby et al, 1981a).

Intramuscular EMG recordings were taken in an attempt to distinguish between these possibilities. Bigland-Ritchie and colleagues (1985, 1986) studied motoneuron discharges using population responses rather than continuous recordings from individual motor units; this strategy was required because the electrode movement during strong contractions made it impossible to follow the activity of a single unit. This approach, however, did not enable the investigators to determine whether some motoneurons ceased firing. In the present experiment, it was therefore considered important to record from individual motor units. It was hoped that during fatigue, the force of contraction would be reduced and hence single motor units would be more easily identified and tracked.

4.2 Methods

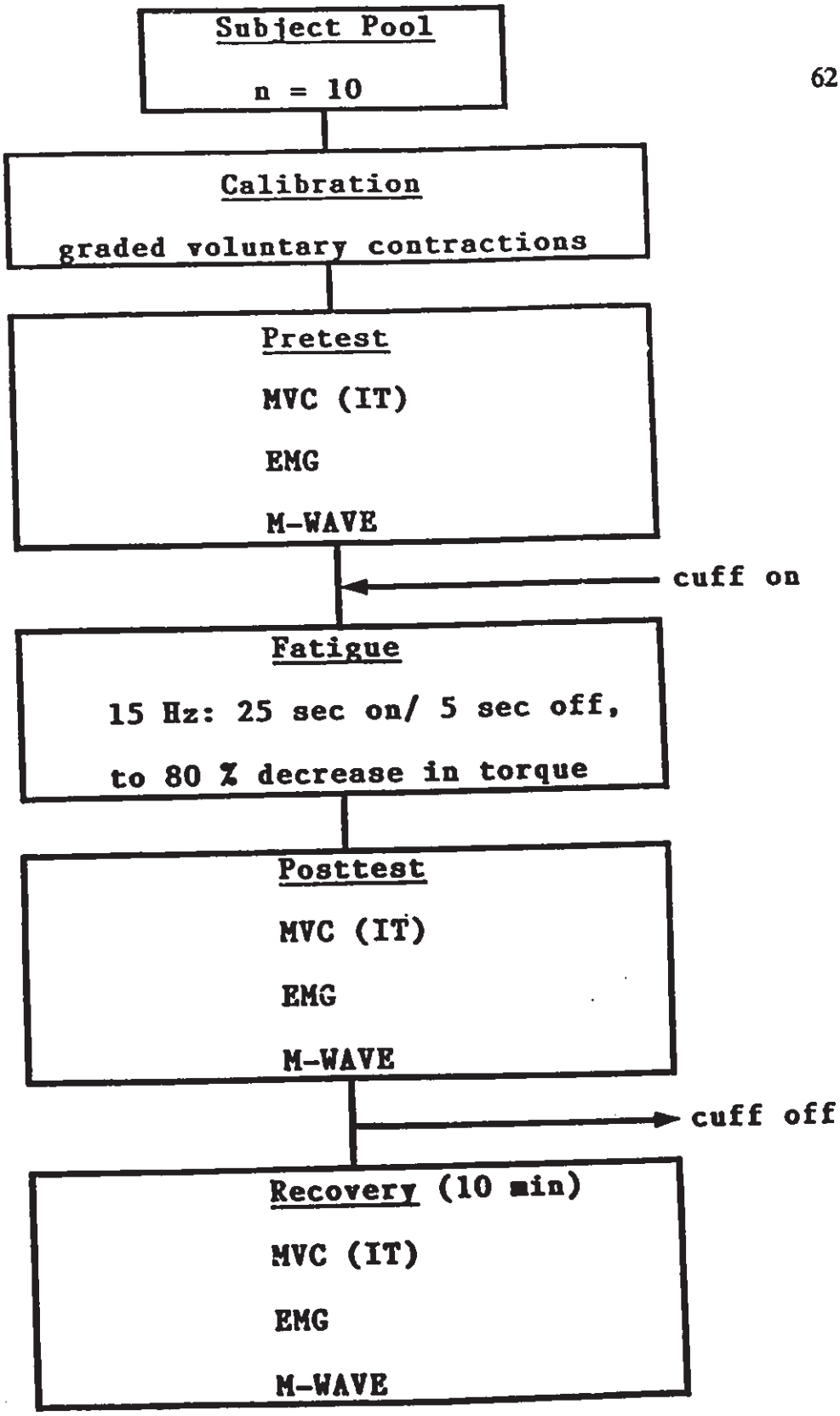
4.2.1 Subjects

Ten adult subjects, five male and five female, aged 24 to 52 years (mean of 31 yrs) were recruited on a volunteer basis (Appendix IV). No subject reported any history of neuromuscular disorders.

4.2.2 Experimental Protocol

To establish the relationship between tibialis anterior EMG and dorsiflexion torque, each subject was requested to perform two series of brief graded isometric contractions of the ankle dorsiflexor muscles with each contraction lasting 2 s (Figure 11). The subject performed an MVC and then in each series of 2 s contractions,

Figure 11: Experimental Protocol. The calibration recordings, which were used to determine the EMG/force relationship, and the pretest recordings were taken in the prefatigue period. The interpolated twitch (IT) was measured to determine the amount of voluntary effort. The M-waves were elicited by trains of 15 Hz stimulation. The posttest (fatigue) recordings were taken while the muscle remained ischemic.


Subject Pool
n = 10
Calibration
graded voluntary contractions
Pretest
MVC (IT)
EMG
M-WAVE
cuff on
Fatigue
15 Hz: 25 sec on/ 5 sec off,
to 80 % decrease in torque
Posttest
MVC (IT)
EMG
M-WAVE
cuff off
Recovery (10 min)
MVC (IT)
EMG
M-WAVE

contractions, attempted to generate 20, 40, 60, 80 and 100% of maximal force. In the second series of prefatigue graded contractions, a train of 5 pulses were delivered to the peroneal nerve at 100 Hz to serve as the interpolated stimulus; each pulse was supramaximal to that required for maximal dorsiflexion twitch torque. This train of stimuli was employed to compensate for the profound decline of the force produced by a single stimulus during fatigue, thereby aiding the detection of any interpolated twitch. The interpolated stimulus was administered after 2 s of each contraction; the perception of the stimulus was used as a signal for the subject to relax.

Prefatigue measures of dorsiflexion torque, EMG, and M-wave amplitude were made on the ankle dorsiflexor muscles in response to the following three conditions: 1) a train of 5 stimuli at 100 Hz (interpolated stimulus) with the subject relaxed, 2) a 2 s MVC with the interpolated stimulus and 3) a 2 s tetanic train at 15 Hz with the interpolated stimulus to assess the degree of M-wave decline over 2 seconds of muscle contraction. There were 2 s of rest after each of the three tests. These tests were repeated twice within 10 s of the end of the fatiguing stimulation (posttest). The arterial cuff was then released and further measures were taken at 1, 3, 5, and 10 min into recovery to chart the resumption of function.

As an extension of this study, intramuscular electrodes were inserted into tibialis anterior muscles of two subjects in order to record motor unit potentials during MVCs held for 10 seconds before and after fatigue (induced by 15 Hz electrical stimulation).

The fatigue procedure consisted of trains of stimuli at 15 Hz; each train lasted 25 s and was preceded by a single shock of sufficient voltage to evoke a maximal twitch response and followed by a 5 s rest period. The length of each train was such as to hasten the onset of fatigue and to make the subjects more comfortable, in comparison with the previous protocol in which trains of 3 s duration with only 2 s rest were employed (see Chapter 3). The purpose of the single shock was to monitor the development of fatigue in the twitch. In one subject, 20 Hz frequency was used because fusion of the tetanus did not result from 15 Hz stimulation; this did not result in any systematic difference in the results from this subject. The fatiguing stimulation continued until approximately 20% of the original dorsiflexion tetanic tension remained. The number of cycles required ranged from 4 to 12 with a median of 6. In some subjects, the development of fatigue was associated with a poorly localized but tolerable pain in the anterior tibial region.

4.2.3 Statistical Analysis

Linear and polynomial regression analyses were used to determine the relationship between voluntary dorsiflexion torque and the associated EMG during graded contractions in the prefatigue period. The posttest (fatigue) values for the EMG activity and torque from the ankle dorsiflexor muscles were calculated as the mean of two posttests. In the few cases where the voluntary dorsiflexion torque declined over the 2 s contraction, the average amplitude [max - (max - min /2)]

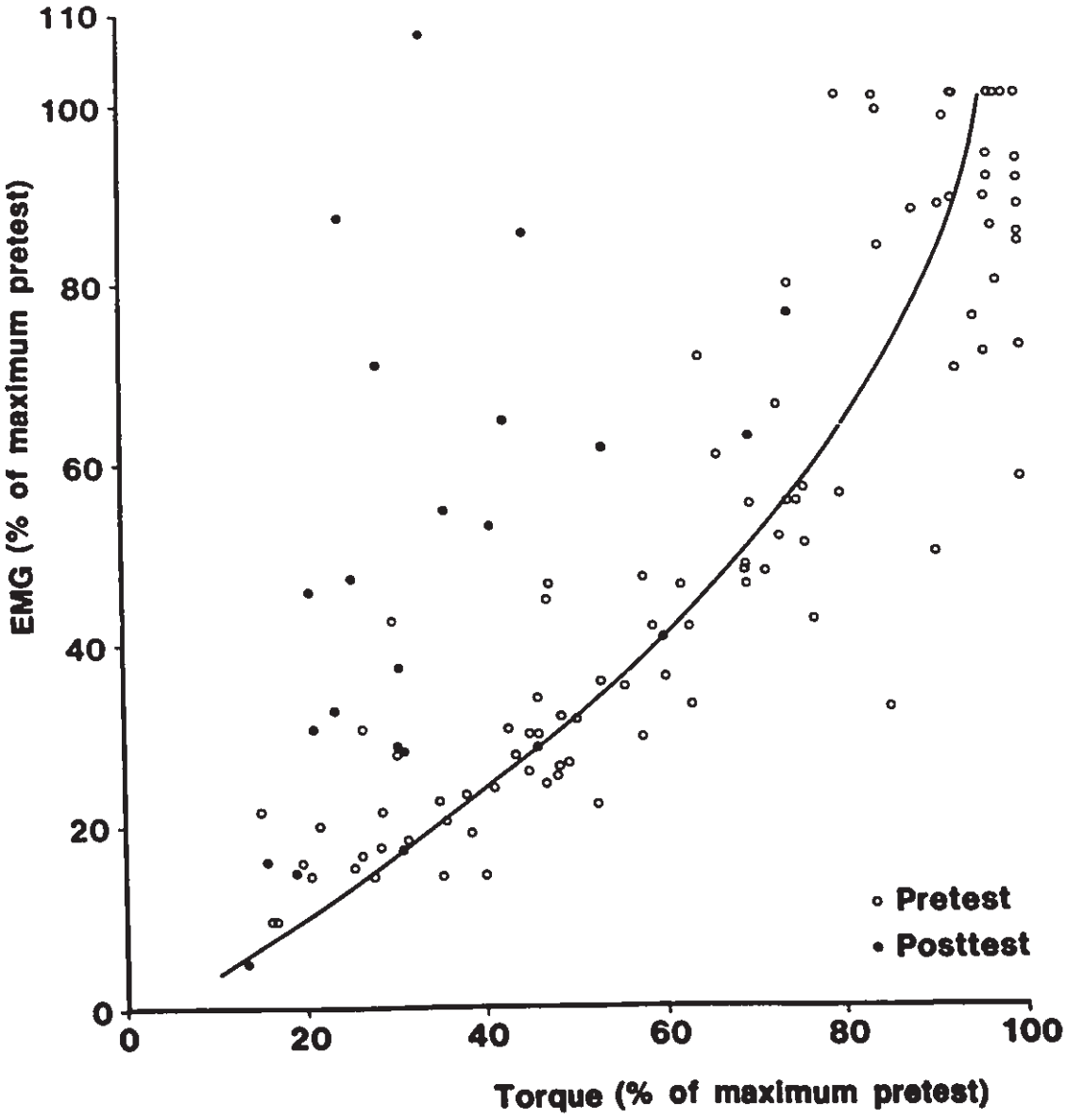
was chosen as the posttest (fatigue) measure. The peak-to-peak amplitude of the M-wave in middle of the test tetanic train was used in the analysis; this M-wave was chosen since it corresponded with the end of the integration period for the voluntary EMG activity. All posttest (fatigue) and recovery measures of voluntary EMG, M-waves, and dorsiflexion torque were expressed as percentages of the prefatigue value. Paired t-tests were used to 1) test the declines in the M-wave amplitude against those of the voluntary EMG, 2) test for any differences in the M-wave within the posttest train of stimuli, and 3) test for any differences in the MVC dorsiflexion torque between the two posttest trials. The fatigue and recovery measures were subjected to repeated measures two-way ANOVA, with subject and time as the factors, and Tukey's multiple comparisons. The alpha level of significance was set at $p=0.05$.

4.3 Results

4.3.1 Prefatigue Period

Before subjecting the ankle dorsiflexor muscles to fatiguing stimulation, the relationship between tibialis anterior EMG activity and voluntary dorsiflexion torque was determined for each of the ten volunteers. A curvilinear relationship was evident from Figure 12, which showed the pooled results from all ten subjects. There was a large increase in EMG as voluntary torque approached its maximal value. The curve providing the best fit for the data points ($r^2=.85$) was described by the equation:

Figure 12: EMG/Force Relationship: Integrated EMG activity as a function of dorsiflexion torque, during voluntary contractions of different intensities in previously rested ankle dorsiflexor muscles (open circles); the regression curve was calculated for these prefatigue observations. Filled circles (posttest) indicate values for maximal voluntary contractions taken immediately after muscles had been fatigued by electrical stimulation and while the cuff remained inflated.


110
100
80
60
40
20
0
EMG (% of maximum pretest)
0
20
40
60
80
100
Torque (% of maximum pretest)
Pretest
Posttest

$$T = 4.63 + 1.68\ E - 0.0076\ E^2$$

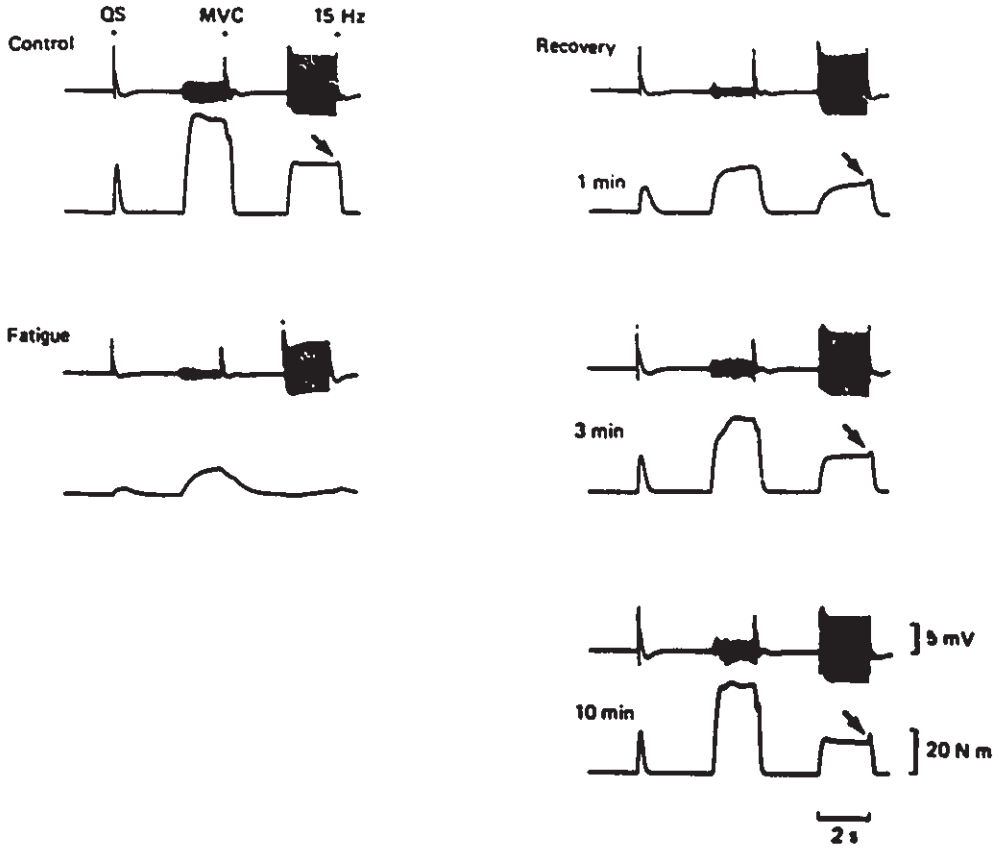
where T=dorsifiexion torque (Nm) and E=integrated EMG activity (mv.s).

Since there were reports of both linear and curvilinear relationships in the literature (Fuglsang-Frederikson, 1981), a linear regression analysis was also performed which yielded an equation, T=22.63 + .81 E, and a rather smaller value for r^2 (.81); however, the lower part of the linear plot diverged quite markedly from the experimental observations. Further, the curvilinear relationship yielded a significantly better fit than the linear plot (p=.001).

That the greatest observed dorsiflexion torque corresponded to maximal voluntary effort was confirmed by the eventual disappearance of the interpolated twitches in all subjects as the contractions became stronger. It was noted, however, that any increments in force evoked by the interpolated stimulus would have been difficult to recognize with contractions greater than 90% of maximum.

After the graded voluntary contractions were performed and while the subjects remained relaxed, the ankle dorsiflexors were tested for the prefatigue values of dorsiflexion torque and EMG activity; Figure 13 shows typical responses in one of the subjects. This figure illustrates that the interpolated stimulus evoked a compound twitch which was approximately the same size as the dorsiflexion torque developed during the 15 Hz tetanus. In all subjects the 15 Hz dorsiflexion torque was approximately one-third to one-half of the MVC dorsiflexion torque. This discrepancy was due to the stimulus frequency being suboptimal for force development, as shown by the presence of a small interpolated twitch. Another

Figure 13: Data from one subject illustrating the prefatigue (control), fatigue and recovery responses. Electrical responses are on the top trace and mechanical activity are on the lower trace. Responses to the interpolated stimulus (IT) given with the muscle resting are in the left column; the voluntary EMG and MVC dorsiflexion torque are in the middle column, and the M-waves and the dorsiflexion torque elicited by 15 Hz stimulation are in the right column. In the first trace, the dots indicate the timing of the interpolated stimulus; the arrows in the torque recordings identify interpolated twitches.


QS
MVC
15 Hz
Control
Recovery
1 min
Fatigue
3 min
5 mV
10 min
20 N m
2 s

factor was the unavoidable activation of antagonist muscles; thus peroneal nerve stimulation would have activated the long and short peroneal muscles, both of which are plantarflexors of the ankle.

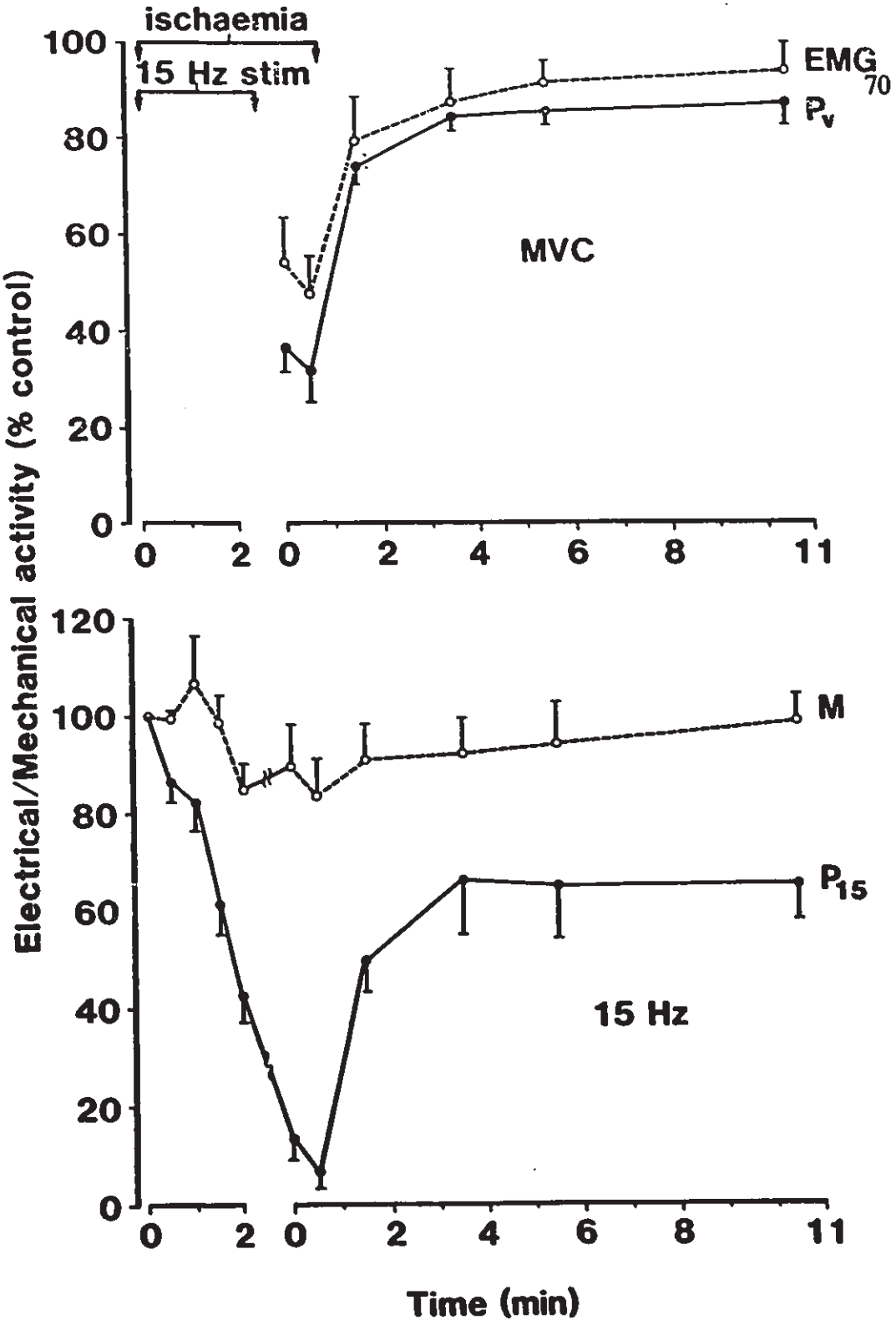
4.3.2 Responses During Fatiguing Stimulation

As described in Methods, fatiguing stimuli were delivered at 15 Hz (or 20 Hz in one subject) in tetani lasting 25 seconds. The mean dorsiflexion torque elicited by the last tetanic train of the fatiguing stimulation decreased by 84.3 ± 6.6% from the prefatigue value. By this stage the dorsiflexion torque elicited by single stimuli was either very small (less than 1 Nm) or had disappeared; in contrast to the mechanical responses, the M-waves responses to single stimuli were well preserved (20% decrease from initial). Mean amplitudes of M-waves at the beginning, mid-point (12 seconds into tetanus) and conclusion of the last fatiguing tetanic train decreased by 20.0 ± 16.1%, 32.3 ± 18.3%, and 40.0 ± 20.4% of original respectively.

4.3.3 Fatigue Period

After the fatiguing stimulation was stopped, and while the ischemic cuff remained inflated, some subjects were aware of a deep-seated discomfort in the anterior tibial area which persisted while the postfatigue measures were taken. After the fatiguing stimulation, MVC dorsiflexion torque was greatly reduced (Figure 14), the mean reduction for the first 2 s trial being 62.9 ± 17.1% of control and that of the second trial being significantly less (69.3 ± 16.3%; $p<.05$). The overall mean

Figure 14: Mean ± SD of electrical and mechanical activity after fatiguing stimulation at 15 Hz. Top, maximal voluntary torque (P_v) and EMG activity of the ankle dorsiflexor muscles. Bottom, amplitudes of the M-waves after 1 second of contraction (M) and dorsiflexion torques evoked by 15 Hz stimulation (P_{15}) at different stages of the experiment.


ischaemia
15 Hz stim
EMG70
Pv
MVC
M
P15
15 Hz
Electrical/Mechanical activity (% control)
Time (min)
100
80
60
40
20
0
120
0
2
4
6
8
11

overall mean reduction in dorsiflexion torque following fatigue of 66.1 ± 16.4% was significantly lower than the prefatigue value (Table 2a). In all subjects it was not possible to detect an interpolated twitch during the maximal voluntary effort, even though such responses to the interpolated stimulus were easily discerned with the subject relaxed (Figure 13). Control experiments on four separate subjects confirmed that 3-5 minute periods of ischemia, without fatiguing electrical stimulation, produced only minor changes in tibialis anterior EMG activity and dorsiflexion torque during MVCs (mean decreases of 4% and 11.3% respectively; Appendix V).

The mean EMG reduction of 50.3 ± 27.1% (Figure 14) was significantly lower than the pretest (Table 2b). In nine of the ten subjects the EMG activity was reduced during each of two maximum voluntary contractions. In the remaining subject, the activity during the first posttest (fatigue) trial was rather larger (by 7%) than the prefatigue value and that in the second trial was slightly reduced (by 13%). This behavior did not appear to be an artifact, for example due to electrode shift, since the M-waves evoked by 15 Hz posttest stimulation were decreased by 13% of the prefatigue value. In 14 of the 19 remaining MVCs the declines in EMG were less than the reductions in maximum voluntary dorsiflexion torque; the mean dorsiflexion torque reduction, however, was not statistically lower than the mean reductions in EMG activity during MVCs. The discrepancy between EMG and dorsiflexion torque became more obvious when the results were plotted on the graph relating the two variables (Figure 12). As in the subject depicted in Figure

Table 2

ANOVA Tables and Tukey's Multiple Comparisons

a) ANOVA TABLE: MVC

Source	df	SS	MS	F	p
model	14	28504.64	2036.05	31.14	.0001
residual	42	2746.22	65.38		
total	56	31250.86			
Partial F					
subject	9	2978.02	330.89	5.06	.0001
time	5	25526.61	5105.32	78.08	.0001

Tukey's Multiple Comparisons

alpha=.05 df=42 MSE=65.38 critical value=4.222

Test Comparison	Difference (%) Between Means	Confidence Limit	Statistical Significance
(Pre=100%)			
Pre-Post	66.07	±10.79	***
Pre-1min rec	26.43	±10.79	***
Pre-3min rec	15.94	±10.79	***
Pre-5min rec	15.29	±10.79	***
Pre-10min rec	14.21	±10.79	***

b) ANOVA TABLE: EMG

Source	df	SS	MS	F	p
model	14	26744.07	1910.29	9.35	.0001
residual	42	8581.82	204.33		
total	56	35325.89			
Partial F					
subject	9	11383.83	1264.87	6.19	.0001
time	5	15360.24	3072.05	15.03	.0001

Tukey's Multiple Comparisons

alpha=.05 df=7 MSE=399.7 critical value=4.16

Test Comparison	Difference (%) Between Means	Confidence Limit	Statistical Significance
(Pre=100%)			
Pre-Post	50.31	±19.08	***
Pre-1min rec	20.75	±19.08	***
Pre-3min rec	12.69	±19.08	ns
Pre-5min rec	8.61	±19.08	ns
Pre-10min rec	6.72	±19.08	ns

13, the mean decline in voluntary EMG activity (50.3 ± 27.1%) was significantly greater than the mean reduction in amplitude of the middle M-wave evoked by 15 Hz stimulation (13.1 ± 22.9%; $t=4.27$, 9 df, $p<.01$; see Figure 14). During the 2 sec 15 Hz train, the M-waves remained the same amplitude in 7 out of the 10 subjects. In the other 3 subjects, the M-wave responses declined in amplitude (6, 16, 33%); these reductions were not accompanied by any decrease in dorsiflexion torque. The final M-wave of the train showed a mean decline of 19.9 ± 27.2%; this was not significantly different from the first M-wave in the train. Therefore, the excitability of the neuromuscular junction and muscle fibre membrane remained stable during trains of stimuli.

Intramuscular EMG recordings were made in two subjects, one subject on two occasions. It was very difficult to follow an individual motor unit during an MVC particularly in the prefatigue period because of the density of the EMG signal. Although it was hoped that during fatigue, the force of contraction would be reduced enough to enable the investigator to identify and follow a single motor unit over time, the EMG recordings remained too dense. As such, 5 prefatigue trials and 3 postfatigue trials could not be analyzed. In the best experiment, only 3 motor units could be identified in one subject during the prefatigue trials; these motor units demonstrated firing rates of 29 Hz, 34 Hz, and 38 Hz. During the first MVC performed under fatigued conditions, 6 motor units were identified in the same subject; the mean discharge frequency of 11.8 Hz (range: 7.2 - 22 Hz) was lower than the prefatigue values. During the second fatigued MVC, 8 motor units were

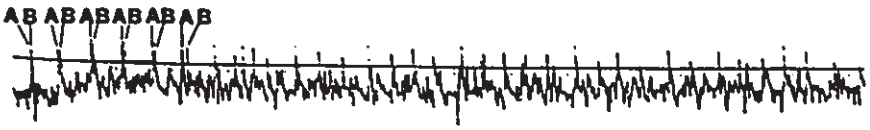
identified that had a mean frequency of 10.8 Hz (range: 4.2 - 14.3 Hz; Figure 15). In the second subject, prefatigue motoneuron firing rates could not be determined but during fatigue, 3 motor units were identified which had a mean firing rate of 7 Hz (range: 5.6 - 8.7 Hz). It was often difficult to ascertain whether individual motor units had ceased firing consequent to fatigue; some units were found to cease firing and later become reactivated after a period of quiescence.

4.3.4 Responses During Recovery Period

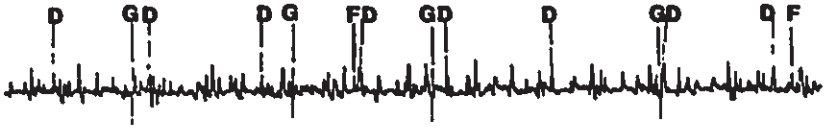
Following release of the arterial cuff, there was usually an immediate sensation of warmth through the previously ischemic leg and this was associated with a reduction in the deeply felt pain. The subjects remained relaxed until tested at 1, 3, 5, and 10 min into recovery; on each occasion, a control interpolated stimulus was followed by a 2 s MVC with an interpolated stimulus, and by a 2 s tetanus at 15 Hz with an interpolated stimulus. From Figure 14, it can be seen that most of the recovery in voluntary dorsiflexion torque and EMG activity took place within the first minute with a smaller amount in the following 2 min and little change thereafter. Tukey's multiple comparisons indicated that the EMG had returned to prefatigue values by 3 min of recovery, whereas the voluntary dorsiflexion torque never returned to prefatigue levels within the 10 min recovery period (Table 3a). None of the 10 subjects demonstrated an interpolated twitch during MVCs at any stage during recovery.

Figure 15: Intramuscular EMG recordings from tibialis anterior before and during fatigue. The fatigue recordings (all four strips) are continuous. Note motor units labelled A and B in the prefatigue strip which fire simultaneously and then separate; B has a slightly lower firing rate than A. Also note in the fatigue recordings that the firing rates of motor units C,D,F,G fall during the contraction and motor units (D,E) drop out and reappear at later times; motor unit D demonstrated both lower firing rates and temporary derecruitment.

PRE



FATIGUE



100 ms

The dorsiflexion torque generated by 15 Hz stimulation also rapidly increased during the first min of recovery but failed to reach the prefatigue value even at the end of 10 min (Table 3a). The M-waves generated during tetanic stimulation, measured after 1 second of stimulation, were significantly different from prefatigue values during the posttest (fatigue) recordings (Table 3b) but returned to prefatigue levels by the first min of recovery. The mean dorsiflexion torque produced by 15 Hz stimulation at 10 min of recovery remained depressed by 38.6% of prefatigue values whereas the reduction in mean MVC dorsiflexion torque was 16.5% of the prefatigue level. This provides an illustration of fatigue with low frequency stimulation ("low frequency fatigue", Section 1.1.3b).

4.4 Discussion

It was the objective of this study to determine if EMG activity still declined during MVCs if fatigue had been induced by electrical stimulation. The relationship between EMG and isometric force in the rested muscle was determined first, followed by the response of EMG and dorsiflexion torque to fatigue.

4.4.1 EMG/Force Relationship

In this experiment, voluntary contractions of graded intensities demonstrated a curvilinear relationship between the EMG activity of the ankle dorsiflexor muscles and the dorsiflexion torque, such that larger dorsiflexion torques were associated with disproportionately greater EMG activity. In other studies, EMG activity has

Table 3

ANOVA Table and Tukey's Multiple Comparisons

a) ANOVA TABLE: 15 Hz torque

Source	df	SS	MS	F	p
model	14	44.03	3.14	20.11	.0001
residual	42	6.57	.16		
total	56	50.67			
Partial F					
subject	9	25.73	2.86	18.28	.0001
time	5	18.30	3.66	23.40	.0001

Tukey's Multiple Comparisons
alpha=.05 df=42 MSE=.16 critical value=4.222

Test Comparison	Difference (div) Between Means	Confidence Limit	Statistical Significance
Pre-Post	1.88	±.53	***
Pre-1min rec	.96	±.53	***
Pre-3min rec	.77	±.53	***
Pre-5min rec	.77	±.53	***
Pre-10min rec	.72	±.53	***

b) ANOVA TABLE: M-waves

Source	df	SS	MS	F	p
model	14	39.79	2.84	39.59	.0001
residual	42	3.02	.07		
total	56	42.81			
Partial F					
subject	9	37.02	4.11	57.29	.0001
time	5	2.78	.56	7.74	.0001

Tukey's Multiple Comparisons
alpha=.05 df=7 MSE=.07 critical value=4.222

Test Comparison	Difference (div) Between Means	Confidence Limit	Statistical Significance
Pre-Post	.66	±.36	***
Pre-1min rec	.19	±.36	ns
Pre-3min rec	.19	±.36	ns
Pre-5min rec	.16	±.36	ns
Pre-10min rec	.13	±.36	ns

been found to increase either linearly (Lippold et al, 1960; Lind, Petrofsky, 1979; Hakkinen, Komi, 1986; Moritani et al,1986) or non-linearly (Bigland-Ritchie, 1981b; Woods, Bigland-Ritchie, 1983) during sustained submaximal contractions (for review, see Fuglsang-Frederiksen, 1981). Bigland-Ritchie and colleagues (1986e) found the EMG of muscles of mixed fibre type (eg. quadriceps) to increase nonlinearly while those of predominantly one fibre type (eg. soleus) to increase linearly during submaximal contractions. Tibialis anterior has a mixed composition with 34% type II (thought to be correlated with fast twitch) fibres (Henriksson-Larsen et al, 1985). Another muscle of mixed fibre type, triceps brachii (67% fast twitch), has a curvilinear EMG/force relationship similar to that found in this study (Clamann, Broecker, 1979).

The nonlinearity of the EMG/force relationship may be due to the EMG activity being recorded from different types of motor units at different stages of the contraction. The amount of EMG activity recorded will depend on the number, size, and distribution of active motor units as well as the size of individual muscle fibre action potentials, their firing frequency and synchronization (Bigland-Ritchie, 1978). Since smaller motor units are recruited first (Henneman, Mendell, 1981), recruitment of larger motor units would contribute larger potentials at higher forces (Bigland-Ritchie, 1981a). Furthermore, if deeper motor units (predominantly slow twitch) are recruited first (Clamann, 1970; Gonyea, Ericson, 1977), the EMG activity when recorded with surface electrodes, would be attenuated at low forces; greater increases in EMG would be evidenced when more superficial motor units (predominantly fast twitch) were recruited at higher forces (Woods, Bigland-Ritchie,

1983). Nonlinearity in the EMG/force relationship could also result from motor units being driven at rates above those required for fused tetani (Bigland-Ritchie, 1981a). While it is probable that extensor hallucis longus and extensor digitorum longus also act as dorsiflexors of the ankle (Marsh et al, 1981), the contributions of these muscles to the surface-recorded EMG would be expected to be smaller than that of the more superficially situated tibialis anterior.

4.4.2 Mechanisms for Declining EMG

In this study, the EMG activity during maximal voluntary contractions fell to approximately half its former value after fatigue had been induced by electrical stimulation. This decline was as great as those reported by other workers when sustained voluntary contractions, rather than electrical stimulation, were used to induce fatigue (Stephens, Taylor, 1972; Bigland-Ritchie et al, 1982). For example, Bigland-Ritchie and colleagues (1979) reported a case study in which there were declines of 55% in smoothed rectified EMG and 60% in force following fatigue induced by a sustained MVC. Hence, although the use of electrical stimulation to induce fatigue could be considered unphysiological, the effect on the EMG was as if voluntary contractions had been used.

There is argument as to the mechanism(s) responsible for the declining EMG activity in MVC during fatigue, both peripheral and central factors being implicated (Stephens, Taylor, 1972; Bigland-Ritchie et al, 1982). In the present experiments, the fall in EMG activity can not be explained by peripheral inexcitability of the

neuromuscular junction or muscle fibre membrane since the maximum evoked M-waves showed only modest reductions. Although these findings and conclusion are similar to those of Woods et al (1987), it should be noted that, in addition to single shocks, 15 Hz tetanic trains were used as testing stimuli and revealed good maintenance of neuromuscular transmission during tetani. Further, tetani at 15 Hz are within the range of motor unit discharge rates for fatigued tibialis anterior (4 to 22 Hz), as measured in the present study with the intramuscular EMG electrodes.

The present experiments not only point to a central mechanism as being mainly responsible for the reduced EMG, but they have enabled a distinction to be made between the two main possibilities, that is failure of descending motor pathways or reflex inhibition of motoneurons. The loss of motoneuron activation was unlikely to have been due to failure of subject motivation to perform MVCs since the descending motor pathways had not been employed to produce fatigue. Furthermore, during the MVC, no additional dorsiflexion torque was evident with the interpolated stimulus; this indicated that the subjects remained highly motivated throughout the experiment.

However, it is conceivable that the descending motor pathways could have been subjected to reflex inhibition from afferents arising in fatigued muscle. The results of the interpolated stimulus experiments were relevant in that no additional force could be evoked in the subjects as the muscle became fatigued. This negative finding meant that either (i) there was still full descending motor drive, or (ii) there was a slight reduction in descending motor drive that the interpolated twitch

technique was unable to detect (<10%, Appendix IV), or (iii) the descending motor drive, although reduced, was still able to cause the motoneurons to fire at rates appropriate for maximal force development, or (iv) there was no descending drive onto those motor units which had developed mechanical fatigue. If some supraspinal inhibition was present, it was either very small or insufficient to interfere with the force output of the muscle, due to reasons (iii) and (iv) above. The results do not exclude the possibility that supraspinal inhibition could have reduced the voluntary EMG output of the muscle, by decreasing motoneuron firing rates, but to frequencies which were still optimal for force production or by affecting those motoneurons no longer able to generate force within their muscle fibre colonies.

The latter relationship would be advantageous, in that muscle fibre membrane function would not be compromised by ineffectual activity, but would be better preserved for the time when force production was restored within the fibres. Those fibres incapable of producing force would have to preferentially instruct their own motoneurons to stop firing. There is some precedence for this concept in that Ia afferents within a compartment (sub-regions of the muscle innervated predominantly by a primary branch of the motor nerve) of the cat lateral gastrocnemius muscle have been shown to synapse most strongly with their motoneurons innervating that compartment (Vanden Noven et al, 1986). The presence of neuromuscular compartments has been demonstrated in tibialis anterior (Iliya, Dum, 1984). The notion that the nervous system may be able to exert differential control within a single muscle (partitioning hypothesis) date back to the

early 1900s but the detailed study of the generality of partitioned connections is in its infancy (Stuart et al, 1988). It remains unknown 1) if the partitioned Ia afferent connections induce functional consequences rather than reveal a feature of neuromuscular design and 2) if afferents with more widespread terminations on the spinal interneurons would demonstrate partitioned connections (cf. Stuart et al, 1988). Nevertheless, the idea of linking the afferent signal with the motoneuron impulse is not heretic; Loeb (1984, 1985) suggests that the motor system employs small groups of motor units within a muscle which are independent of compartmentalization. Those motor units involved in a task must tailor their output to afferent information flow in order to have such fine control of sub-sections of a muscle.

The findings of the present study were compatible with those of Bigland-Ritchie et al (1986b) who observed that fatigue after voluntary contraction persisted for as long as an ischemic cuff was maintained. They postulated that central pathways would have recovered within the same time period with the cuff released. In the present study, subjects were generally aware of a deep-seated pain in the fatigued muscle which diminished within seconds of the arterial cuff being deflated and was followed by a rapid return in EMG activity. Taken together, the observations during ischemia would be consistent with a mechanism whereby the motoneurons were reflexively inhibited by nociceptive afferents in the exercising muscle.

It is theoretically possible, however, that the motoneuron could be rendered inexcitable by prolonged activation from antidromic stimuli during the fatigue procedure. In gastrocnemius muscles, Kernell and Monster (1982) have shown that the "late adaptation" of motoneurons, evidenced by the drop in firing rate, is strongly correlated to the initial firing rate and to the type of motor unit ie. it is more prominent in fast twitch units (Kernell, 1986). Extrapolating from Kernell (1986), the frequency of stimulation (15 Hz) used in the present study would be sufficiently low that very little late adaptation would have occurred. Furthermore, the experimental conditions in which late adaptation of motoneurons has been demonstrated (constant intracellular injection of current for up to 4 min) are very different to those in this study. Late adaptation was unlikely to have occurred because of the long intervals between successive stimuli in the tetanic train (67 ms). It was probable that any effects due to late adaptation or Renshaw inhibition would have largely worn off in the even longer interval elapsing between termination of the fatiguing stimulation and the posttest recordings (typically 3-5 s). The rapid improvement in voluntary EMG and dorsiflexion torque following the release of the ischemic cuff also suggested that EMG declines had emanated from the muscle rather than the spinal cord.

The present experiments have also shown that, at a time when the EMG activity was reduced, it was not possible to record an interpolated twitch superimposed on the recording of voluntary torque. This observation implied, in part, that in the fatigued state, motoneurons could have been discharging at optimal

frequencies for force development. It remains in question as to whether there was drop out of motor units or a decline in motor unit firing rates with all motor units remaining active. While Grimby et al (1981a) found evidence of drop out of phasic motor units within 2 s of maximal activity in previously rested muscle, Bigland-Ritchie et al (1983) has maintained that all motor units remain active although discharging at lower rates during fatigue. A loss of excitable motoneurons in fatigue was also suggested by the restoration of EMG activity during the first minute of the recovery period, a process which occurred more rapidly than the increase in motoneuron discharge rates observed in Bigland- Ritchie et al (1986c).

The limited number of intramuscular electrode recordings were able to identify motor units which were firing throughout the contraction but with lower firing rates by the end of the contraction. Motor units were also identified which fired phasically, stopped, and returned a few seconds later for short bursts of activity. The drop out of motor units was not an artifact of electrode movement because during the same period other units could be followed which did not demonstrate any change in their shape or amplitude. It was difficult to interpret the recruitment and derecruitment of motor units since it is a normal phenomenon in maximal voluntary contractions and therefore not unique to the fatigue process (Hannerz, 1974). Hannerz, using a bipolar electrode, demonstrated high-threshold motor units which showed discontinuous or phasic discharges in the rested tibialis anterior; the minimum firing rates ranged from 7 Hz to 35 Hz and maximum firing rates ranged from 25 Hz to 65 Hz in sustained voluntary contractions. What could

not be determined from the recordings in the present study was whether certain motor units failed to be recruited at all during fatigue.

In conclusion, after ankle dorsiflexor muscles had been fatigued by repetitive stimulation of the peroneal nerve at 15 Hz under ischemic conditions, there was a reduction in voluntary EMG activity which persisted as long as the arterial cuff remained inflated (30 s). The reduction in voluntary EMG activity could not have been primarily due to failure of subject to remain motivated to perform maximal efforts or loss of excitability at the neuromuscular junction or muscle fibre membrane. The preceding observations were consistent with the view that the reduction in EMG activity was due to reflex inhibition of motoneurons by afferents from the fatigued muscles.

Chapter 5

MOTONEURON EXCITABILITY WITH FATIGUE

5.1 Introduction

The experiments reported in the previous chapter demonstrated that EMG activity decreased during MVCs of fatigued ankle dorsiflexor muscles. Because of the design of the experiment, failure of subject motivation and peripheral inexcitability of the neuromuscular junction or muscle fibre membrane could only have been minor factors in the decline in EMG..

Instead, it is hypothesized that depressed alpha motoneuron excitability is responsible for the declining EMG during fatigue and that this, in turn, was the result of reflex inhibition of the motoneuron pool (see Chapter 4; also Bigland-Ritchie et al, 1986c; Woods et al, 1987). There do not appear to have been any previous attempts to measure the excitability of the motoneuron pool in fatigue. In the present study the approach has been to use excitatory inputs from group Ia fibres to evoke an electrical response in the muscle (H-reflex). The Hoffmann or H-reflex can be used to measure the excitability of the alpha motoneuron pool since, under the conditions of constant input, variations in the size of the H-reflex can provide a reliable criterion of the net excitatory and inhibitory influences on the

alpha motoneurons from other sources (Taborikova, 1973). The soleus muscle was used for this experiment since the H-reflex cannot be elicited in tibialis anterior under normal circumstances.

Few studies to date have used the H-reflex technique to investigate changes following fatigue. During prolonged heel-raising exercise, Ete-Okoro (1982) found elevated H-reflexes in soleus which would indicate increased alpha motoneuron excitability. The experimental protocol, however, failed to control for the facilitation of H-reflexes by contractions of other simultaneously active muscles (Delwaide, Toulouse, 1980; Tarkka, 1986). Voluntary contraction of many muscles has been shown to enhance or reveal H-reflex activity (Upton et al, 1971) and this is true of soleus (Morin et al, 1982; Tarkka, 1986); however, isometric contractions of 50% and 100% of maximum, too brief to induce fatigue, inhibit the H-reflex both in the immediate release phase ie. relaxation from contraction (Schieppati, Crenna, 1984) and over the next minute (Enoka et al, 1980).

In the present study it was considered that any depression of the H-reflex excitability would provide additional evidence for reflex inhibition of the alpha motoneuron pool consequent to fatigue.

5.2 Methods

5.2.1 Subjects

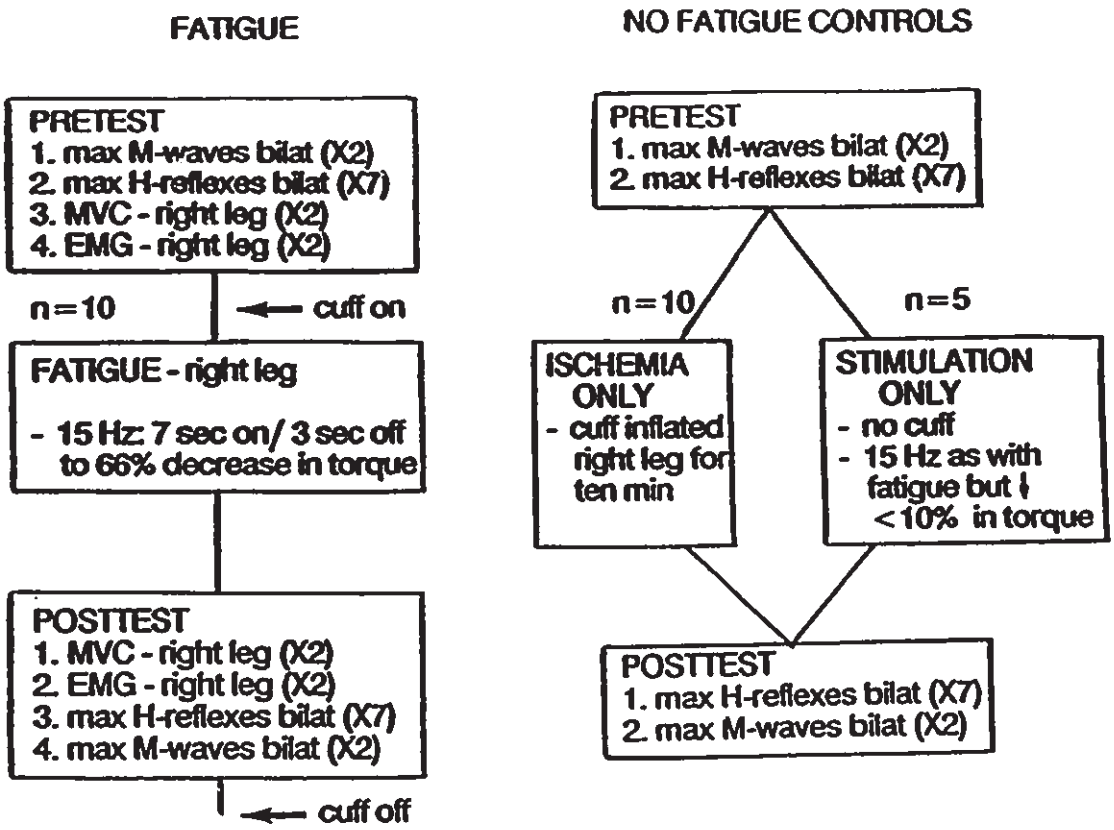
Ten healthy volunteers, six male and four female, aged 22 to 53 years (mean of 30.0 years) participated in the study; none reported any history of neuromuscular disease (Appendix VI).

5.2.2 Experimental Protocol

Ten subjects were tested on two occasions with at least one week between tests. The tests included: Test 1) fatigue under ischemic conditions and Test 2) ischemia alone without fatigue. Five of the subjects also volunteered for a further test: Test 3) electrical stimulation without ischemia and hence without fatigue (Figure 16). Test 1 was repeated on five of the subjects to determine the reproducibility of the results.

On the right side, pretest measures were taken which included peak-to-peak amplitudes of maximum M-waves and maximum H-reflexes to single shocks, together with MVC plantarflexion torque and voluntary EMG activity in soleus muscle. Since, in Chapter 4, M-waves were found to be stable during trains of stimuli at 15 Hz, single M-waves were used as the indicator of muscle fibre membrane excitability. Contralateral maximum H-reflexes and M-waves were also recorded to control for any generalized environmental effects which influence the amplitude of the H-reflex; these would be evident in the nonischemic and nonfatigued leg. Maximum H-reflexes were found by increasing the stimulus voltage

Figure 16: Experimental Protocol


FATIGUE
PRETEST
1. max M-waves bilat (X2)
2. max H-reflexes bilat (X7)
3. MVC - right leg (X2)
4. EMG - right leg (X2)
n=10
cuff on
FATIGUE - right leg
- 15 Hz: 7 sec on/ 3 sec off
to 66% decrease in torque
POSTTEST
1. MVC - right leg (X2)
2. EMG - right leg (X2)
3. max H-reflexes bilat (X7)
4. max M-waves bilat (X2)
cuff off
NO FATIGUE CONTROLS
PRETEST
1. max M-waves bilat (X2)
2. max H-reflexes bilat (X7)
n=10
n=5
ISCHEMIA ONLY
- cuff inflated right leg for ten min
STIMULATION ONLY
- no cuff
- 15 Hz as with fatigue but ↓ <10% in torque
POSTTEST
1. max H-reflexes bilat (X7)
2. max M-waves bilat (X2)

between 15V and 40V until the H-reflex just started to decline (cf. Magladery et al, 1950). Maximal M-waves were elicited using stimulus intensities (200V - 320V) which failed to evoke any further increase in response amplitude. The right leg was strapped securely into the metal frame and the blood pressure cuff was placed around the thigh, at the junction of the middle and distal thirds; it was sufficiently far from the stimulating electrodes to limit electrode shift during inflation and any consequent artifactual changes in the M-wave or H-reflex (cf. Gandevia, McKenzie, 1986). An interpolated stimulus (2 pulses at 100 Hz) was administered during MVCs of the right soleus to assess the level of voluntary effort (Belanger, McComas, 1981). The disposition of the stimulating and recording electrodes for these various measurements was as described in Chapter 2 (section 2.3.2).

In Test 1, the right soleus was fatigued with submaximal electrical stimulation (120-160 V) at 15 Hz under ischemic conditions until the evoked plantarflexion torque decreased at least 50%. The electrical stimulation was repeated every 10 s (7 s on, 3 s off). This intermittent protocol was chosen since subjects in the previous study found 25 s trains too uncomfortable. Submaximal stimulation intensities were also used to increase the subjects' comfort; these intensities prolonged the time required to induce fatigue so that the entire procedure took approximately 10 min.

All posttest measures were taken while the limb remained ischemic. Posttest measures of the maximum voluntary plantarflexion torque and EMG were taken within a few seconds after the fatiguing stimulation had stopped; maximal voluntary

effort was determined using the interpolated twitch technique. Posttest maximum H-reflexes and maximum M-waves were then recorded; the voltages were increased between evoked M-waves in the postfatigue period to be certain of maximality. The comparison of pretest and posttest values for the right leg served to identify the experimental effects. At least 2 min elapsed between MVCs and H-reflex recordings to avoid post-contraction depression of the H-reflex (which lasts for approximately 1 min, cf. Enoka et al, 1980). The maximum M-wave served to identify a decrease of excitability of the neuromuscular junction or muscle fibre membrane as a result of the fatigue.

In Test 2, the effects of ischemia alone on the reflex excitability were determined. Pretest maximum M-waves and maximum H-reflexes were compared to those taken in the posttest period after 10 min of ischemia. The MVC plantarflexion torque and EMG activity were not tested in the control experiments since force and EMG measures were found to be unchanged with ischemia in a separate group of subjects (Appendix V; see also Chapter 6).

In Test 3, the effects of the electrical stimulation on reflex excitability, in the absence of ischemia and hence fatigue, were measured. Pretest maximum M-waves and maximum H-reflexes were compared to those taken in the posttest period after electrical stimulation. This protocol limited the loss of force production to approximately 10% of original.

5.2.3 Statistical Analysis

Maximum H-reflex amplitudes were expressed as a proportion of the maximum M-wave amplitude. This ratio calibrated for any changes in the peripheral excitability of the muscle fibre (as assessed by the amplitude of the M-wave) consequent to fatigue. This Hmax/Mmax ratio was calculated for the pretest and posttest; the posttest measure was expressed as a percent change from the pretest value. The percent change in Hmax/Mmax ratio from the left (control) leg was subtracted from the percent change in Hmax/Mmax ratio obtained on the right (experimental) side so as to give the "reflex excitability" (RE). In this way the left leg served as a calibration for any generalized environmental factors affecting reflex excitability.

The statistical significance for the Hmax/Mmax ratio between tests and between legs was determined using analysis of variance (ANOVA) with repeated measures with test (1,2,3) and leg (right, left) as the factors. Post hoc Tukey's multiple comparisons tested for specific differences between the three tests (fatigue, ischemia alone, electrical stimulation alone). In Test 1 changes between prefatigue and postfatigue in MVC plantarflexion torque, voluntary EMG activity, and maximum M-waves following fatigue were analyzed with paired t-tests. Polynomial regression analysis and the correlation coefficient were calculated to determine the relationship between posttest values of RE and EMG. The alpha level of significance was set at $p=0.05$.

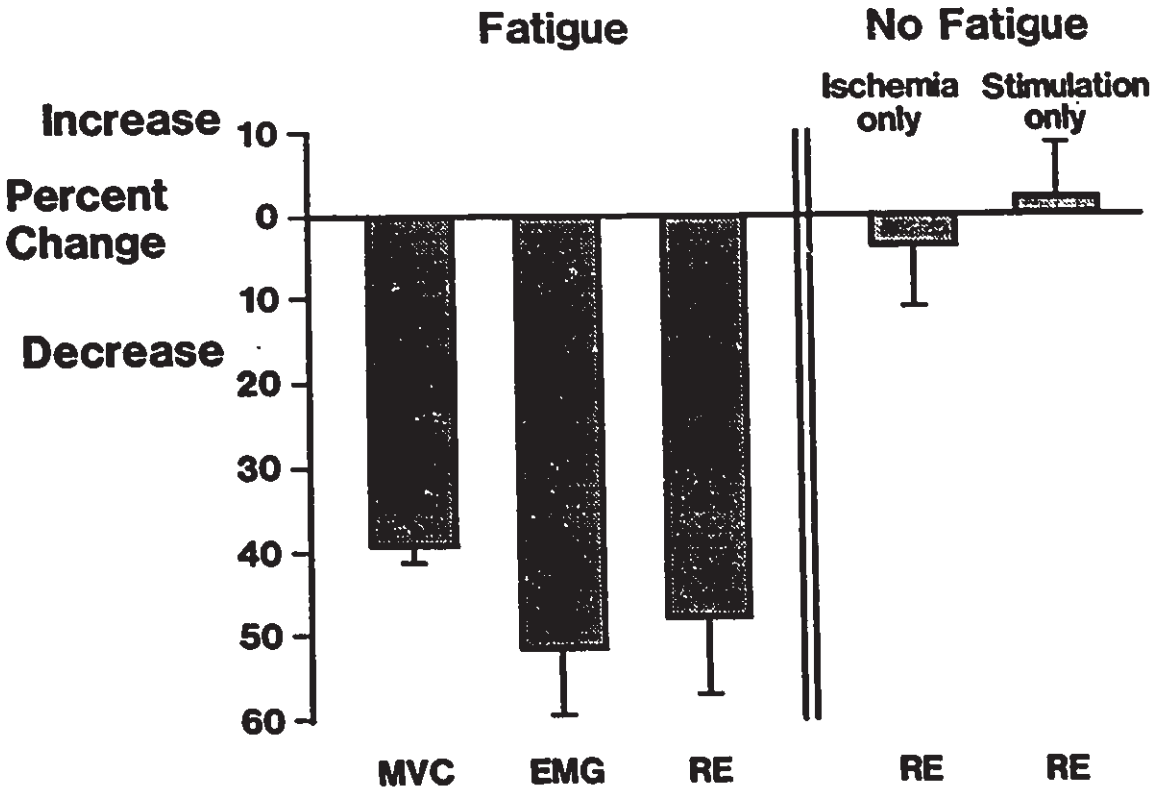
5.3 Results

5.3.1 Effects of Fatigue

The fatigue condition in Test 1 demonstrated mean decreases in MVC plantarflexion torque of 38.5 ± 8.6% (SD), and in EMG activity of 51.9 ± 17.9% (Figure 17). The results of the paired t-tests for MVC and EMG showed that these differences from respective control values were highly significant (MVC: $t=11.958$, 9df, $p<.001$; EMG: $t=12.935$, 9 df, $p<.001$).

The Hmax/Mmax ratio decreased 44.4 ± 25.6% on the right (fatigued) leg but was not significantly altered on the left (control) leg (mean increase of 2.9 ± 20.7%); the RE (right Hmax/Mmax ratio minus left Hmax/Mmax ratio) declined 47.3 ± 27.1%. The mean Hmax on the fatigued right leg decreased 47.9 ± 7.6% in comparison to 0.8 ± 18.5% on the left side. The fatigue protocol induced a small degree of peripheral inexcitability of the muscle fibre membrane reflected in the decline of the M-wave by 8.8% ± 11.9% ($t=2.343$, 9 df, $p<0.05$); any decrease of excitability of the muscle fibre membrane should have affected the H-reflex to a similar extent and hence should have left the Hmax/Mmax ratio unchanged. The duration of the M-wave, measured on the oscilloscope screen, usually stayed the same or increased up to 1 ms (7% of original) and never increased more than 2 ms. Repeated measures ANOVA for the Hmax/Mmax ratios (Table 4) indicated a significant interaction between the three tests and the two legs ($p=.001$). Hence the effect of the test intervention depended on the leg being tested; this was to be expected since the left leg served as a control.

Figure 17: Mean changes (± SE) in MVC plantarflexion torque and EMG activity, and in reflex excitability (RE) following fatigue, ischemia control and electrical stimulation control. Reflex excitability was calculated as the percent change in Hmax/Mmax ratio from the left (control) leg subtracted from the percent change in Hmax/Mmax ratio from the right (experimental) leg.



RE = reflex excitability

⊥ = SE

Table 4

ANOVA Tables and Tukey's Multiple Comparisons

ANOVA TABLE: Hmax/Mmax ratio

Source	df	SS	MS	F	p
model	5	14597.18	2919.436	8.62	.001
residual	44	14905.40	338.759		
total	49	29502.58			
Partial F					
test	2	3041.38	1520.69	4.49	.016
leg	1	6028.02	6028.02	17.79	.0001
interaction	2	5527.78	2763.89	8.16	.001

Tukey's Multiple Comparisons

alpha=.05 df=44 MSE=338.759 critical value=4.73

Test Comparison	Difference Between Means	Confidence Limit	Statistical Significance
Fat vs Isch	52.640	± 29.495	***
Fat vs Stim	59.397	± 29.495	***

Tukey's multiple comparisons demonstrated that the significant difference between tests lay between the fatigue test and the other two non-fatigue control tests.

When the posttest EMG value was plotted against the posttest RE value for each subject, there was a curvilinear relationship with the values for reflex excitability usually remaining larger than those for EMG (Figure 18). The subjects denoted by open triangles experienced less than 30% reduction in the MVC and were unwilling to continue fatiguing stimulation; hence these subjects were excluded from the study (Appendix VI). These data points were included in the calculation of the relationship between EMG and RE. The nonlinear curve (r^2=.92) which provided a significantly better fit over the linear curve (r^2=.85) for the data points was:

$$E = 1.75\ RE - 0.11\ RE^2$$

where E = integrated EMG (mv.s).

The correlation coefficient for the two variables was .67; this was significant at $p<.025$. From Figure 19, it can be seen that three subjects (2, 4, 10) showed little reduction in the RE; these subjects also showed the smallest reductions in voluntary EMG activity during fatigue. Hence, not only did the mean values change in the same proportion but within subjects the postfatigue values were in proportion.

The mean plantarflexion torque evoked by the fatiguing stimulation declined 66.6 ± 10.7% by the end of stimulation. The difference between this decline and the smaller one in MVC probably illustrated the contribution that the nonfatigued gastrocnemius muscle was able to make to voluntary plantarflexion torque.

Figure 18: Posttest EMG plotted against posttest reflex excitability (RE) for each of the ten subjects. The subjects denoted with open triangles experienced less than 30% reduction in the MVC and hence were excluded from the study; these data points were included in the calculation of the relationship between EMG and RE.

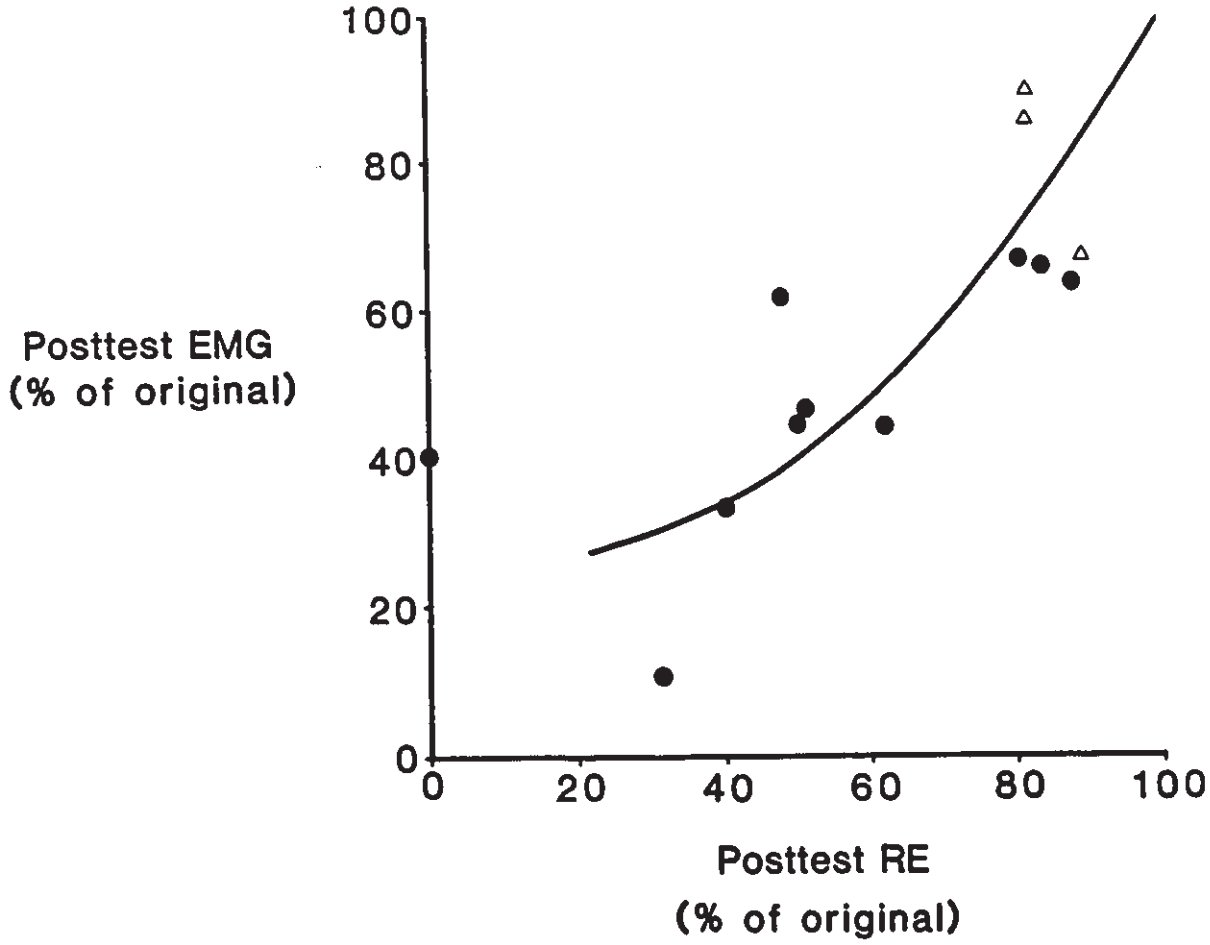
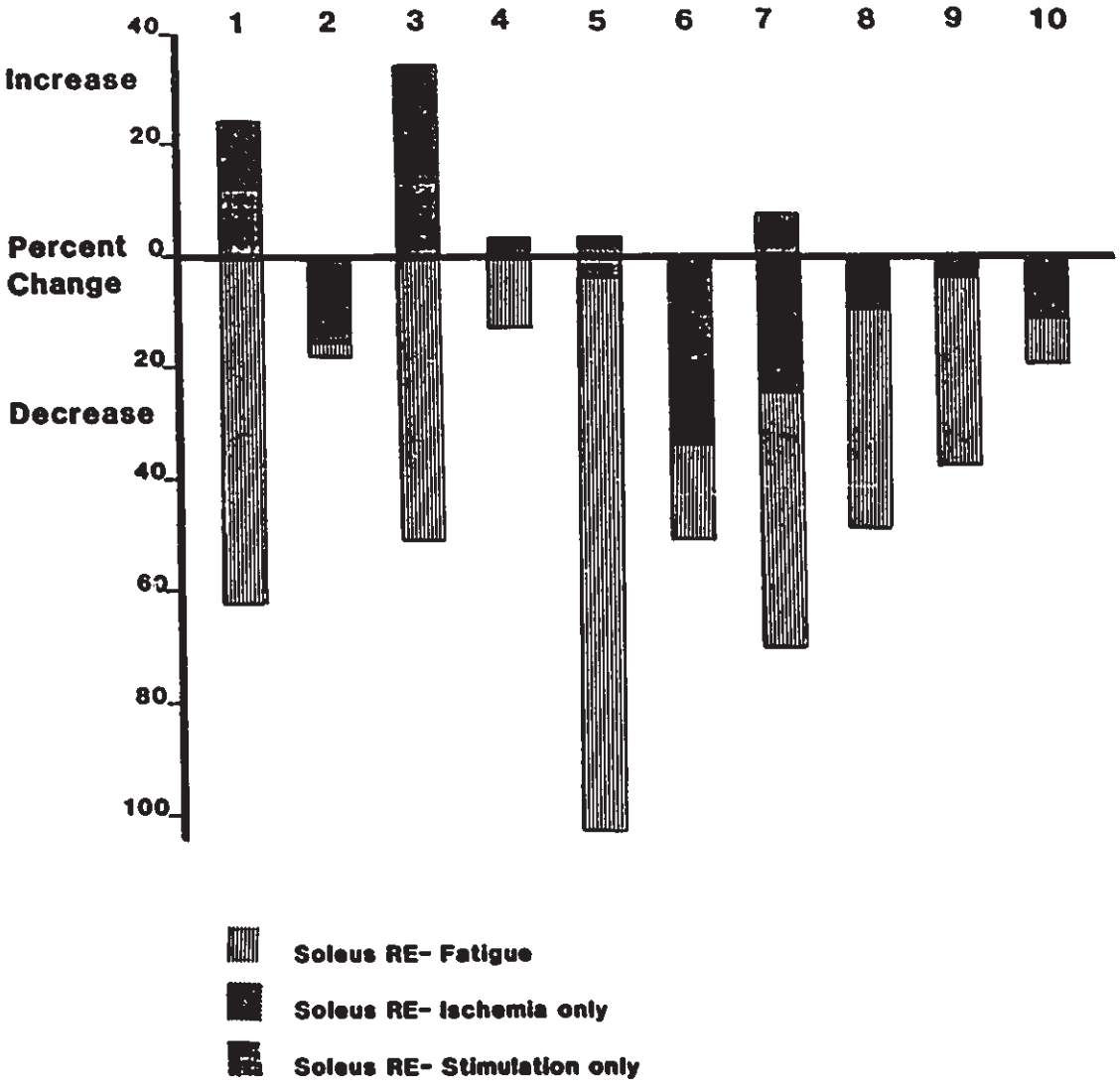

Posttest EMG
(% of original)
100
80
60
40
20
0
0
20
40
60
80
100
Posttest RE
(% of original)

Figure 19: Individual subject data for reflex excitability for the three tests. In none of the subjects were the decreases in control values (Tests 2 and 3) greater than those under the fatigue condition (Test 1); however, there was marked variability between subjects.


1
2
3
4
5
6
7
8
9
10
40
Increase
20
Percent
Change
0
20
Decrease
40
60
80
100
Soleus RE- Fatigue
Soleus RE- Ischemia only
Soleus RE- Stimulation only

Results from administration of strong interpolated stimuli on the MVC after fatigue indicated that 9/10 subjects remained highly motivated to activate the motoneurons. In one subject a small interpolated twitch (8% of the twitch at rest) was evident during the pretest trial and could not be overcome despite encouragement and instruction. A slightly larger interpolated twitch (17% of the rested value) was seen in the posttest trial in this subject (Appendix VI).

For the 5 subjects who repeated the fatigue test (on two separate occasions), the mean MVC plantarflexion torque, EMG activity, and Hmax/Mmax (H/M) ratios declined to similar extents during fatigue on the two occasions; paired t-tests demonstrated that the results of the two trials were not significantly different. The mean declines ± SD were:

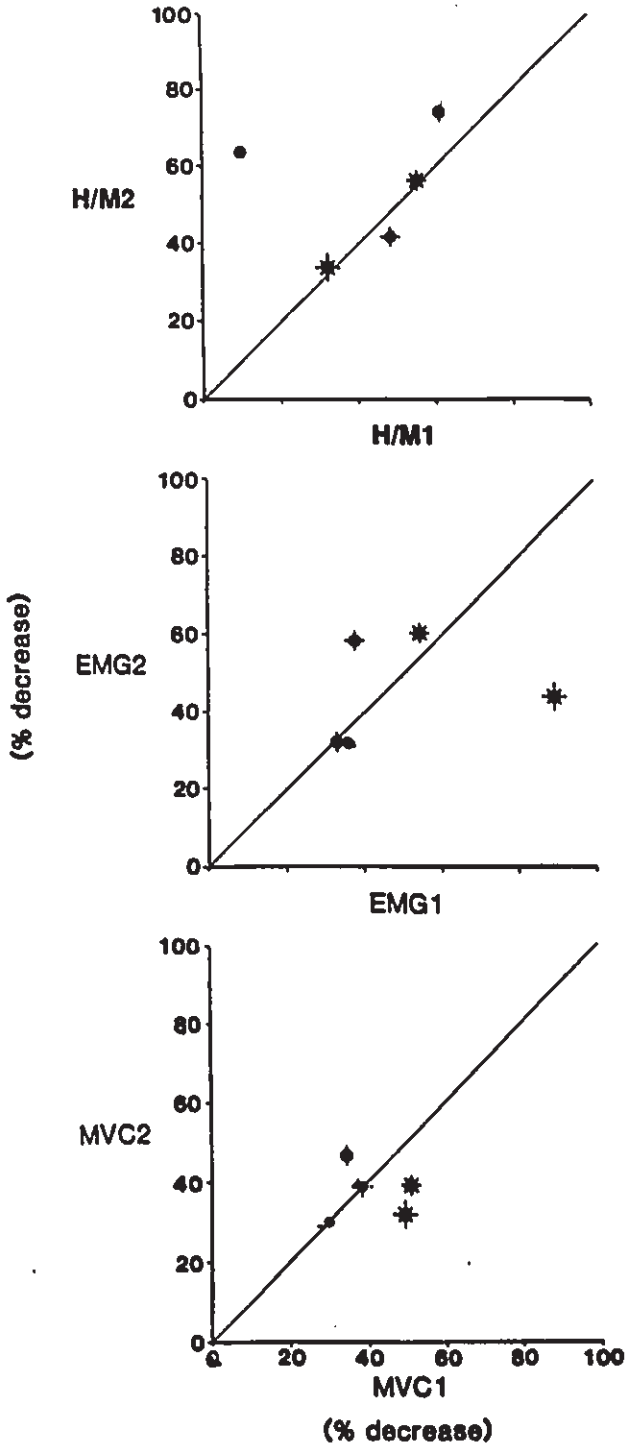
MVC 1	= 40.6 ± 8.8%,	MVC 2	= 37.6 ± 6.8%;
EMG 1	= 50.0 ± 23.3%,	EMG 2	= 44.6 ± 14.3%;
H/M 1	= 43.0 ± 24.5%,	H/M 2	= 54.0 ± 16.4%.

When the results for individual subjects were examined, as opposed to the mean values, there was good agreement between MVC plantarflexion torques in all subjects and between EMG activities and Hmax/Mmax ratios in 4 of the 5 individuals (Figure 20).

5.3.2 Effects of Non-Fatigue Tests

The ischemic condition alone in Test 2 showed a nonsignificant mean decline in RE of 3.7 ± 20.8% from the pretest value (Figure 20). Thus the reduced reflex

Figure 20: The decreases in Hmax/Mmax ratio (H/M), EMG activity, and MVC plantarflexion torque in trial 1 (abscissa) and trial 2 (ordinant). The line of unity represents perfect reproducibility.


H/M2
H/M1
EMG2
EMG1
MVC2
MVC1
(% decrease)
(% decrease)
0
20
40
60
80
100

excitability during fatigue could not be accounted for by ischemic factors alone. Since the effects of ischemia varied considerably from one person to another (Figure 19), the change in RE following Test 2 (ischemia alone) was subtracted from that following Test 1 (fatigue with ischemia) for each subject. However, even when the contributions of ischemia within each subject were removed, the mean change in RE due to fatigue was still significant (43.6 ± 36.5%; $p<.01$).

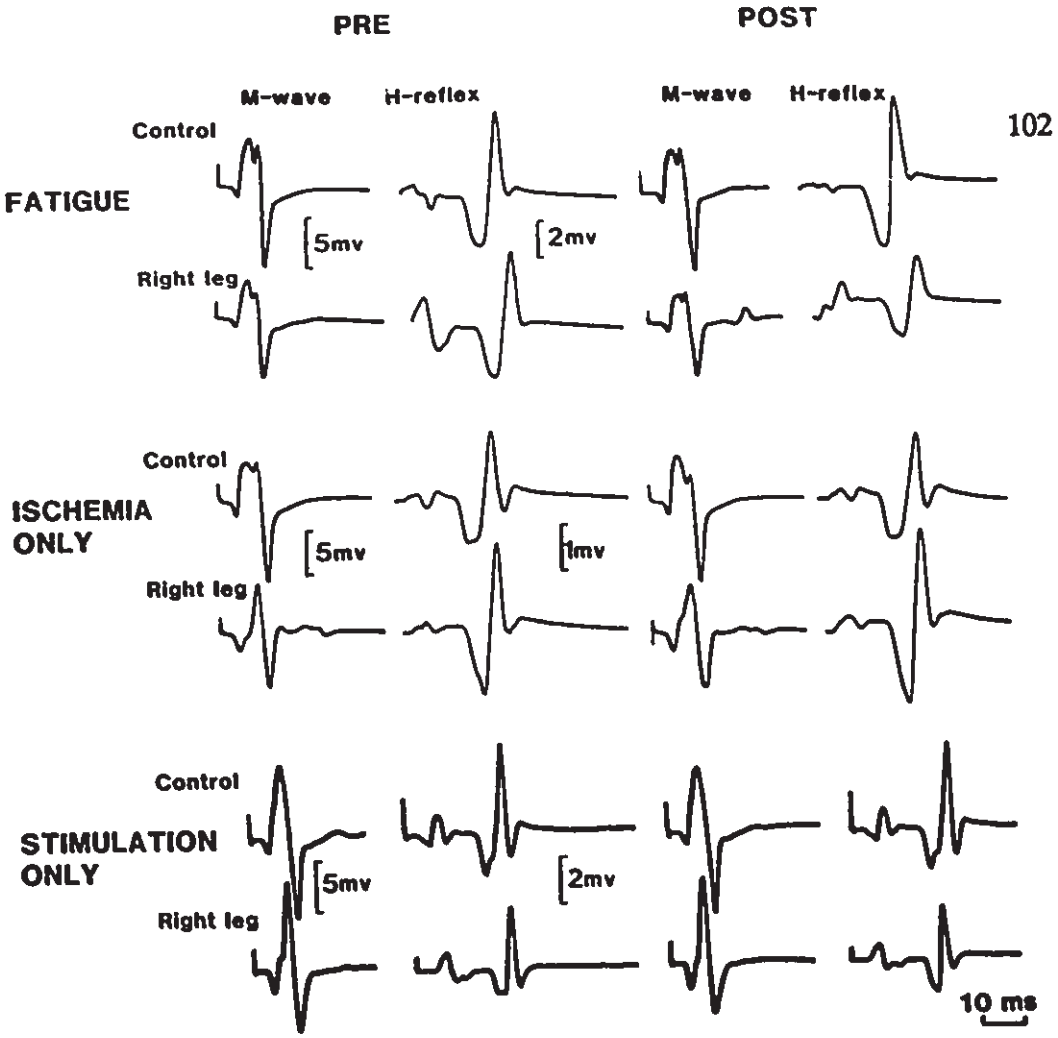
Electrical stimulation, when delivered without the ischemic cuff, could be continued for the same length of time as in Test 1 yet without the occurrence of significant changes in plantarflexion torque or in reflex excitability (mean changes of 8.2 ± 9.6% and 1.8 ± 13.4% respectively; Figure 20). The data from one subject for all three experimental conditions are shown in Figure 21. It can be seen that the only critical change in H-reflex amplitude occurred with the fatigue intervention.

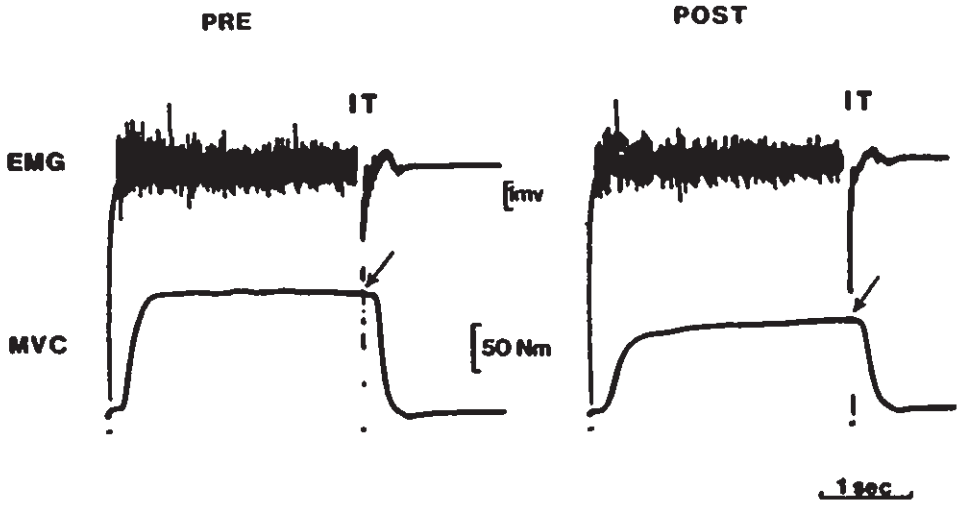
5.4 Discussion

The present study was undertaken to determine the electrophysiological mechanism(s) responsible for the decline in voluntary EMG activity observed following electrically-induced fatigue of the soleus muscle. Depression of the reflex excitability of the motoneuron pool would be consistent with the presence of reflex inhibition during fatigue.

Figure 21: Top, data from one subject illustrating H-reflex and maximum M-wave changes during the three tests. The first two columns from the left are pretest values of M-wave and of H-reflex amplitude respectively; the next two columns are posttest values of M-wave (third column) and H-reflex amplitude (far right column). Within each test (fatigue, ischemia, and stimulation), the electrical responses from the left (control) leg are above those from the right (experimental) leg. The only major change occurs in the H-reflex following the fatigue intervention. It should be noted that the dissimilarity in the shapes of the H-reflex and M-wave probably reflects the contributions of muscles, additional to soleus but also supplied by the tibial nerve, to the M-wave.

Bottom, the EMG activity and MVC plantarflexion torque for the same subject before and after the fatiguing stimulation.


PRE
POST
M-wave
H-reflex
M-wave
H-reflex
Control
FATIGUE
5mv
2mv
Right leg
Control
ISCHEMIA ONLY
5mv
1mv
Right leg
Control
STIMULATION ONLY
5mv
2mv
Right leg
10 ms


PRE
POST
IT
IT
EMG
1mv
MVC
50 Nm
1 sec

5.4.1 Mechanism for Declining EMG

The results of this study indicated that the decline in mean voluntary EMG activity of 51.9% during fatigue of soleus was associated with a mean decrease of the reflex excitability of the alpha motoneuron pool of 43.7%. Further, the decline in EMG activity was significantly correlated with the decline in reflex excitability ($r=.67$). The decline in EMG activity was unlikely to be explained by inadequate motivation resulting in decreased motor drive since these descending motor pathways had not been used during the fatiguing procedure and the interpolated twitch data demonstrated adequate activation of the motoneurons during the brief tests of MVCs.

Similarly, the decline in reflex excitability could not be explained by loss of excitability of neuromuscular junctions or muscle fibre membranes. Although the small decline in the maximum M-wave indicated the presence of some muscle fibre membrane inexcitability, this was much less than the fall in voluntary EMG activity. To this extent, the results for the soleus muscle were consistent with those obtained by a similar experimental strategy for the human ankle dorsiflexors; the M-wave from tibialis anterior declined to a slightly greater extent, most likely caused by greater fatigue in the ankle dorsiflexion experiments than the ankle plantarflexion experiments (see Chapter 4). Further, any changes in muscle fibre membrane excitability could not have influenced the change in reflex excitability since the RE was determined using a Hmax/Mmax ratio. Thus the Hmax/Mmax ratio declined substantially despite the small decrease in Mmax; this decline in the

Hmax/Mmax ratio could have occurred only by depressed H-reflex excitability of the alpha motoneuron pool.

It should be noted that in those 3 additional subjects, who were not included in the study because the MVC plantarflexion torque fell less than 30% after fatigue, the EMG and RE values decreased to levels of less than one half of those reported above (Appendix VI). Hence, if reflex inhibition is occurring in the early stages of fatigue i.e. less than 30% MVC fatigue, its effects on EMG are indeed very small (cf. Hakkinen, Komi, 1986).

It would appear from Figures 18 and 19 that the contribution of reflex inhibition to the declining EMG can vary. In one subject, the right Hmax/Mmax ratio decreased by 5% in the first trial and by 63% in the second trial; this corresponded to similar voluntary EMG declines of 36% and 32% respectively. In another subject, the EMG decreased by 89% in the first trial and by 44% in the second trial with equivalent decreases in the right Hmax/Mmax ratio (32% and 34% respectively). In both of these subjects the maximum M-wave showed a rather large decline from the prefatigue value in the first trial (15% and 24%) possibly indicating that peripheral inexcitability of the muscle fibre was contributing to the decline in EMG. Another possibility to explain the larger declines in EMG than RE is that of reflexively-induced supraspinal inhibition of descending motor drive. Even though interpolated twitches were absent during fatigue, it is still possible that there were some motor units which were no longer capable of either descending activation or force generation in response to direct stimulation of motor axons (see

discussion, Chapter 4). The two aforementioned possibilities could explain why the decrease in reflex excitability may not have been sufficient to account for all the reduction in voluntary EMG.

Equally difficult to explain were those instances in which the RE decreases were much larger than the reduction in EMG. For example, in one subject the H-reflex was completely abolished while the EMG was reduced by 60% of original. It may be possible that some subjects are able to overcome the reflex inhibition with large volleys of descending excitatory drive to the motoneuron pool.

In reviewing these discrepancies, it should be acknowledged that there is no a priori reason why the declines in EMG and RE should be identical. Indeed, there might well be important differences in the locations of the Ia afferent terminations and the synapses for voluntary pathways, and in the sizes and shapes of the respective excitatory postsynaptic potentials. These differences would cause a reduction in motoneuronal excitability to affect the voluntary EMG and the H-reflex excitability to different degrees.

5.4.2 Possible Confounding Factors

The use of an ischemic cuff presented a possible confounding factor. The ischemic cuff was necessary to induce fatigue in soleus within a reasonable period of time. The pressure exerted by a blood pressure cuff on the motor nerve might compromise impulse conduction in afferent fibres and the H-reflex would consequently decrease in the absence of any fatigue effect (Mayer, Mawdsley, 1965).

Three additional subjects were found in whom this appeared to be the case since their H-reflexes were depressed with the ischemic cuff inflated in the absence of fatiguing stimulation; these subjects were excluded from the study. In the remaining 10 subjects, the decreases in reflex excitability were greater during the fatigue protocol (Test 1) than in the presence of ischemia alone (Test 2). Metabolic changes in the leg (eg. reduced oxygen tension) consequent to 10 minutes of ischemia alone might have been sufficient to induce inputs from muscle chemoreceptors to the alpha motoneuron pool; if so, the present results suggest that their effect on motoneuron excitability was minimal (see also Harris et al, 1975; Dery et al, 1965). Therefore, in this study the presence of ischemia can be discounted as an explanation for the loss of reflex excitability.

It was thought that indirect electrical stimulation of soleus motor axons could have depressed motoneuron excitability through antidromic impulse activity (see Kernell, Monster, 1982). Recurrent inhibition from antidromic alpha motoneuron activation, via Renshaw cells and possibly other interneurons, is unlikely to explain the changes since the duration of recurrent inhibition of alpha motoneurons from antidromic stimulation in the cat is only 20 - 50 ms (Ellaway, Murphy, 1979) and, in the present study, the H-reflexes were elicited after much longer intervals. The cutaneous afferent input from the fatiguing stimulation might also have affected motoneuron excitability but these effects would more likely be excitatory than inhibitory (Hagbarth et al, 1960). To control for these effects, electrical stimuli were delivered without ischemia or fatigue; in this condition it was found that the

reflex excitability remained unaffected. Recently, the effect of antidromic activation of the soleus motoneuron pool was investigated in a cat preparation (Hayward et al, 1988); these authors found no evidence of soleus inhibition when the proximal end of a cut nerve was stimulated at intensities and rates equal to those used to fatigue the muscle.

It was felt that the presence of any pain during the experiment was not a confounding factor. As mentioned earlier, pain associated with the electrical stimulation over the soleus muscle belly (cutaneous in origin) would tend to be facilitatory to soleus motoneurons (Hagbarth et al, 1960). Furthermore, pain associated with the ischemic cuff and electrical stimulation was present in the control tests and yet depression of the reflex excitability was not evident. Despite any pain experienced, subjects were able to remain well-motivated as evidenced by the absence of interpolated twitches during MVCs. Muscle pain, mediated by nociceptive endings within skeletal muscle, was an inevitable and quite natural part of the muscle fatigue process and hence did not introduce any foreign variable; rather it has been speculated that nociceptive afferents could mediate the reflex inhibition.

The depression of H-reflexes was not a postcontraction phenomenon of the kind demonstrated by Enoka et al (1980); this phenomenon was thought to result from postcontraction discharge and increased sensitivity of the muscle spindle receptors. The H-reflex depression in this experiment was at least twice as great as that found by Enoka and colleagues (1980). More importantly, Test 2 and Test

3 (control conditions) also had H-reflexes taken after the MVC and did not demonstrate the decline seen following fatigue.

It could be argued that use of Hmax/Mmax ratio biased the study toward finding inhibition rather than facilitation since the prefatigue responses were maximal. However, 7/10 subjects in this study demonstrated an increase in the Hmax/Mmax ratio after testing under control (nonfatigue) conditions. Hence, the ratio was sensitive to change in either direction and provided a meaningful measure of reflex excitability.

5.4.3 Motoneuron Excitability with Fatigue

In summary, both the voluntary EMG and the H-reflex excitability of the alpha motoneuron pool were significantly depressed during fatigue of soleus induced by electrical stimulation. The depression of the H-reflex was not the result of ischemia or electrical stimulation alone and hence represented a true response to the fatigue procedure. The reduction in EMG was greater than could be accounted for by peripheral failure of muscle fibre membrane excitability. The interpolated twitch findings suggested that subjects remained motivated to perform maximal efforts ie. little "central" fatigue. These findings are consistent with the existence of a reflex (spinal or supraspinal) whereby the alpha motoneurons are inhibited by afferents from the exercising muscle.

Chapter 6

TYPE OF AFFERENT IMPLICATED IN REFLEX INHIBITION

6.1 Introduction

The final problem to be addressed in this research concerns the type of afferent fibre mediating the putative reflex inhibition during fatigue (see Chapter 5). This information would help to elucidate the nature of the stimulus which mediates the depression of voluntary EMG during fatigue. There has been much speculation on the type of afferent that could exert reflex inhibition of motoneuron firing. The sensory discharge could arise from receptors sensitive either to mechanical changes in the contractile properties of the exercising muscle or to metabolic and ionic changes (Bigland-Ritchie et al, 1986c). Large myelinated afferents responding to mechanical events include Ia and II afferents from muscle spindles and Ib afferents from Golgi tendon organs (Guyton, 1980). Smaller diameter afferents, among the group III (Mense, 1977) and group IV (Kniffki et al, 1978) fibre populations, can be activated by chemical stimulation as well as by muscle contraction (Kniffki et al, 1978); some of the mechanoreceptors exhibit a low sensitivity to muscle stretch (Houk, Rymer, 1981).

In this thesis, it is hypothesized that the afferents mediating the reflex inhibition of motoneurons during fatigue do not have large diameters. Smaller diameter fibres are the likely candidates for two reasons. First, both in this study and that of Bigland-Ritchie and colleagues (1986c) EMG during fatigue remained depressed as long as the limb was rendered ischemic; this suggested a chemical stimulus and it is known that only small diameter afferents are chemosensitive. Second, in Chapter 4, it was shown that release of the ischemic cuff was associated with a reduction in muscle pain, and only group III and IV fibres are nociceptive (Mense, 1983).

In this study a partial compression block of the impulse conduction in the sciatic nerve was employed to elucidate the contribution of the different-sized afferents to the reflex inhibition. Compression blocks are known to be more effective for afferent than for efferent fibres (Gottlieb et al, 1983; Magladery et al, 1950; Moddel et al, 1977). The impulse conduction block progresses according to fibre size, with large myelinated afferents being affected first, followed by small myelinated afferents and lastly by unmyelinated afferents (Gasser, Erlanger, 1929; MacKenzie et al, 1975; Torebjork, Hallin, 1973). In the present study, if the same decrease in voluntary EMG during maximal voluntary contractions was seen following fatigue, with or without impulse blocking in the large afferent fibres, then smaller diameter afferents would be indirectly implicated in the reflex inhibition.

There are two other means to selectively block different-sized afferents. Local anaesthesia can block small diameter afferents preferentially but the selectivity

of the block is difficult to maintain in human subjects (personal communication, Bigland-Ritchie, 1987) and the degree of blocking changes rapidly with time (Torebjork, Hallin, 1973). Prolonged ischemia can also be used to block large diameter afferents but the prolonged ischemia increases the risk of arterial or venous thrombosis and the pain experienced by the subjects is unacceptable. Further, inducing fatigue after prolonged ischemia could evoke different metabolic processes within the limb; this could make the results difficult to interpret in the context of the previous studies. The compression block was chosen since it was the least invasive and had fewer confounding factors.

6.2 Methods

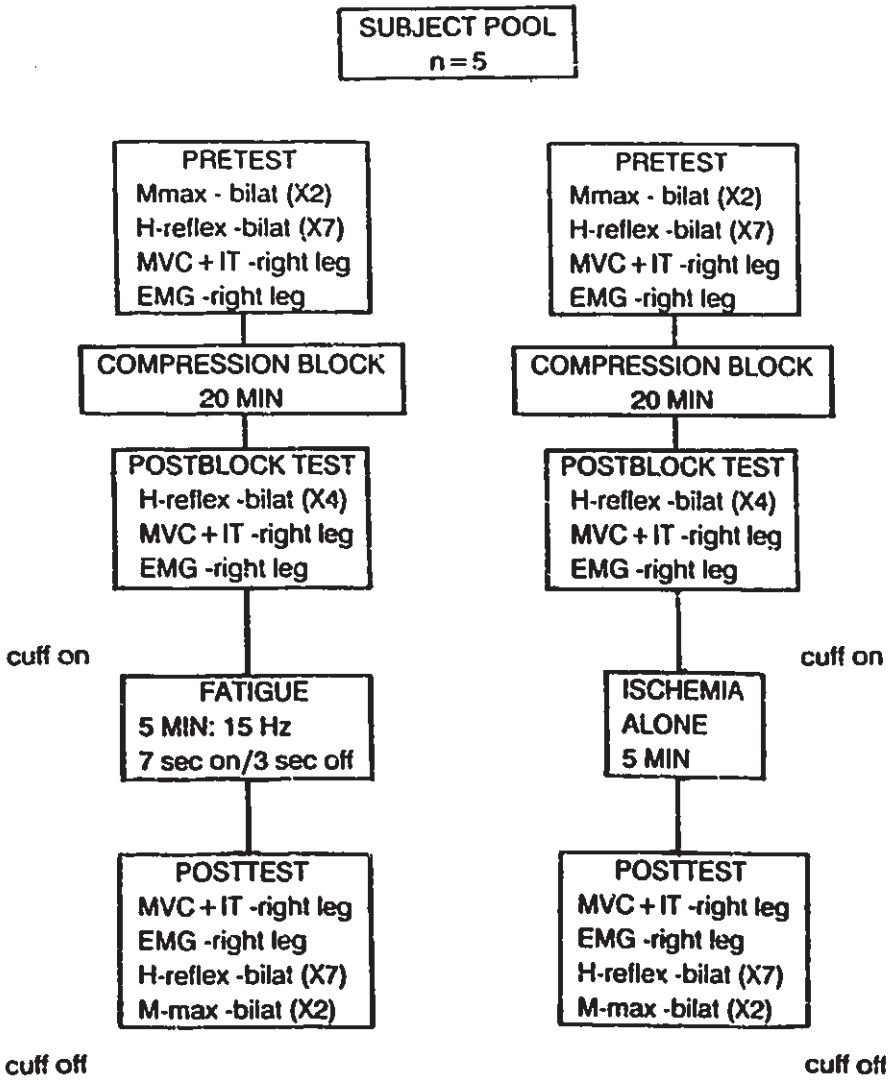
6.2.1 Subjects

Five healthy female subjects aged 25 - 35 years (mean of 28 years) participated in the study (Appendix VII). None had any history of neuromuscular or vascular disease.

6.2.2 Experimental Protocol

The five subjects were tested on 2 occasions with at least 3 days between experiments. The tests consisted of: Test 1) compression block of the sciatic nerve followed by fatigue of the soleus muscle under ischemic conditions, and Test 2) compression block of the sciatic nerve followed by ischemia but without fatigue (Figure 22).

Figure 22: Experimental Protocol


SUBJECT POOL
n = 5
PRETEST
Mmax - bilat (X2)
H-reflex -bilat (X7)
MVC + IT -right leg
EMG -right leg
COMPRESSION BLOCK
20 MIN
POSTBLOCK TEST
H-reflex -bilat (X4)
MVC + IT -right leg
EMG -right leg
cuff on
FATIGUE
5 MIN: 15 Hz
7 sec on/3 sec off
POSTTEST
MVC + IT -right leg
EMG -right leg
H-reflex -bilat (X7)
M-max -bilat (X2)
cuff off
PRETEST
Mmax - bilat (X2)
H-reflex -bilat (X7)
MVC + IT -right leg
EMG -right leg
COMPRESSION BLOCK
20 MIN
POSTBLOCK TEST
H-reflex -bilat (X4)
MVC + IT -right leg
EMG -right leg
cuff on
ISCHEMIA
ALONE
5 MIN
POSTTEST
MVC + IT -right leg
EMG -right leg
H-reflex -bilat (X7)
M-max -bilat (X2)
cuff off

Pretest measures consisted of maximum H-reflex and M-wave amplitudes to single shocks in the soleus muscles of both sides; on the right side recordings were also made of MVC plantarflexion torque (with an interpolated stimulus, 2 pulses at 100 Hz, applied to the tibial nerve), and the associated voluntary EMG. The disposition of the stimulating and recording electrodes for these various measurements was as described in Chapter 2 (section 2.3.2).

The differential compression block of the sciatic nerve was achieved by placing a wooden bar, 6 cm high and 2 cm wide, under the thigh just distal to the ischial tuberosity. The subject sat with as much body weight as possible over the bar. The maximum H-reflex was monitored over the next 20 minutes until it was completely abolished; at this point it was assured that the group Ia fibres had been blocked. The MVC plantarflexion torque and EMG activity were then retested (Postblock test). Ruskin and coworkers (1967) and Magladery et al (1950) found the ischemia time for the onset of efferent block to be 17-33 min and 36 min respectively. If, during an attempted MVC in the postblock period, a sizeable interpolated twitch was present then the possibility of conduction block in the efferent fibres was substantial and the experiment was discontinued. As the study progressed the importance of fatiguing the soleus muscle quickly, before the compression block progressed to the efferent fibres, became evident. Hence, in order to save time, the maximum M-waves were taken only in 3 subjects in the postblock period; the M-waves were still taken in all subjects in the pretest and posttest periods. Following the compression block, subjects reported a loss of

position sense around the ankle joint and an inability to perceive the development of plantarflexion torque; they were instructed therefore to view the force tracing on the oscilloscope while developing MVCs. The subjects were aware of crude touch, although there was noticeable hypaesthesia over the lower leg and foot. Others have found similar sensory losses after 20 min of compression (Clark et al, 1935; MacKenzie et al, 1975). It was imperative that the subjects maintain their body weight over the wooden bar otherwise the compression block would be lost within seconds.

In Test 1 the fatigue was induced under ischemic conditions after the compression block. The fatiguing electrical stimulation was repeated every 10 seconds (7 seconds on, 3 seconds off) for 4 - 5 minutes; brief voluntary contractions of toe flexors were periodically monitored so as to detect the onset of any block of alpha-motor axons.

In the control experiment, Test 2, the ischemic cuff was inflated in the absence of stimulation for the same period of time as in the fatigue condition. Posttest measures of MVC and EMG were followed by those of maximal H-reflexes and M-waves.

6.2.3 Statistical Analysis

Changes in voluntary EMG activity and MVC plantarflexion torque resulting from application of the compression block were determined by subtracting the postblock value from the pretest value and then expressing it as a percent of the

pretest value. Further changes in EMG activity and MVC plantarflexion torque resulting from Tests 1 and 2 were determined by subtracting the posttest value from the postblock value and expressing it as a percent of the postblock value.

The statistical significance of the effects of the compression block and the fatigue on the EMG and MVC plantarflexion torque was determined with repeated measures ANOVA, with subject and treatment (pretest, block, fatigue, ischemia) as the factors, and Tukey's multiple comparisons. Because of the small sample size, there was some concern that the data would not approximate to the normal distribution. The assumption of normal distribution was tested with the Shapiro-Wilk statistic as part of the ANOVA testing; this test confirmed that the data approximated the normal distribution and served to validate the use of ANOVA.

The effects of fatigue on the maximum M-wave (pretest - posttest) were determined with a two-way paired t-test. The alpha level of significance was set at $p=.05$.

6.3 Results

6.3.1 Effects of Compression Block Prior to Fatigue

Following sciatic nerve compression for approximately 20 minutes, the H-reflex was completely abolished in 3 subjects and reduced by at least 90% in the other two subjects. The mean decreases in the H-reflex were 97.8 ± 3.2% in the fatigue test and 97.2 ± 4.4% in the ischemia control test (pooled mean of 97.5 ± 3.6%). The percent reductions in individual subjects are presented in Table 5.

Table 5

Part D: Individual Subject Data

Subject	% reductions from pretest														
	H-reflex			MVC			EMG			Mmax Amp			Mmax Area		
	B	C	F	B	C	F	B	C	F	B	C	F	B	C	F
NI Test1	100		100	37		53	41		63	–		19	–		+2
Test2	100	100		24	32		37	39		–	+4		–	+15	
LM Test1	100		64	9		47*	+11		43	21		+6	–		7
Test2	90	70		6	15		27	33		5	19		28	4	
TE Test1	100		100	9		72*	+1		61	4		50^	0		40
Test2	96	100		29	29*		15	31		0	+3		15	5	
KH Test1	96		85	0		48	28		71	+4		+14	+8		+26
Test2	100	60		5	1		+1	5		2	+15		+5	+10	
WH Test1	93		100	26		51	+2		34	–		26	–		20
Test2	100	100		20	33		+24	+21		–	+11		–	+7	
Mean	97.5	86.0	89.8	16.5	22.0	54.2	10.9	17.4	54.4	5.5	+2.8	6.5	5.8	+4.4	8.0

B : postblock period
C : post ischemia control
F : post fatigue test
(*) : indicates an interpolated twitch was present
^ : M-wave configuration altered, amplitude not included in mean

In 3 subjects M-waves were taken in the postblock period during both Tests. The mean reduction of the 6 maximum M-wave amplitudes and areas was 5.5 ± 7.9% and 5.8 ± 15.1% respectively. The duration of the M-waves did not usually change (decreases of 32%, 1%, and increases of 3%, 3%, 1%), as such the reduction in M-wave area was usually a reflection of the decrease in amplitude.

Table 5 shows that there was considerable variation between subjects and even for the same subject in the declines of EMG activity and voluntary plantarflexion torque resulting from the compression block alone. For this sample of subjects, the mean MVC torque fell 16.2 ± 15% in Test 1 and 16.8 ± 10.8% in Test 2 (pooled mean of 16.5 ± 12.3%) after administration of the compression block. These declines in MVC following compression block in both the fatigue (Test 1) and ischemia control (Test2) were statistically significant (Table 6). No additional torque could be elicited by interpolated stimuli.

The voluntary EMG associated with the MVC fell 11 ± 22.3% in the fatigue test and 10.8 ± 24.1% in the ischemia control test (pooled mean of 10.9 ± 21.8%). The decrease in EMG was not statistically significant with Tukey's multiple comparisons (Table 7). The mean changes with the compression block are depicted in Figure 23.

Although maximum M-waves were not taken after the compression block in all subjects, it was deemed unlikely that the MVC or EMG declined because of inexcitability of the muscle fibre membrane for the following reasons. First, those subjects in whom M-waves were measured showed limited mean reductions. Second,

Table 6

ANOVA Table and Tukey's Multiple Comparisons

ANOVA TABLE: MVC

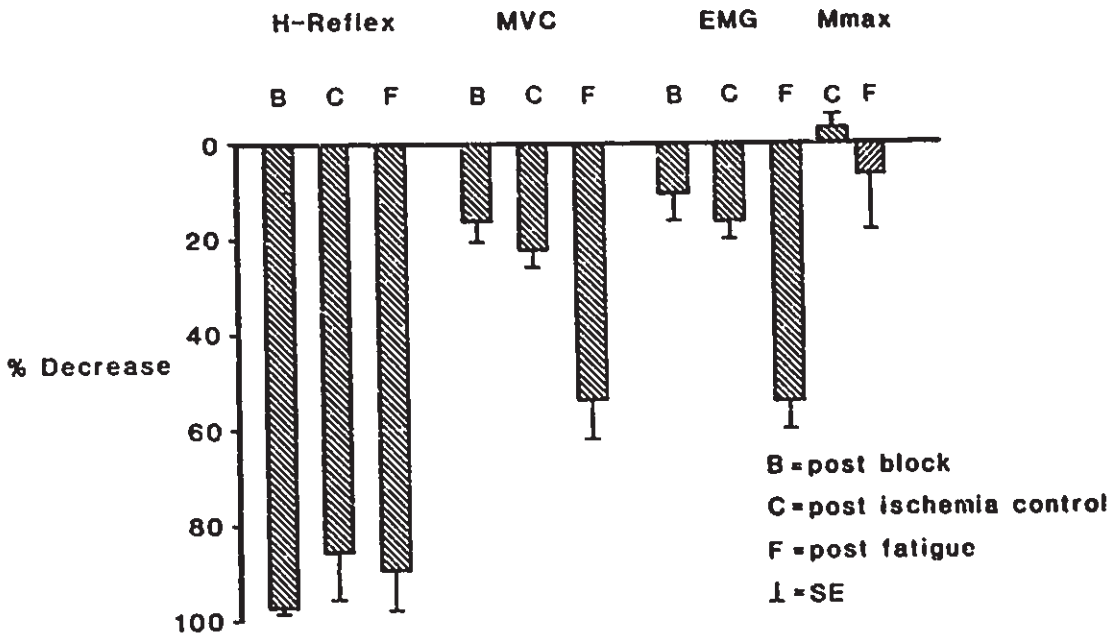
Source	df	SS	MS	F	p
subject	4	1351.76	337.94	4.55	.05
treatment	4	7906.96	1976.74	26.61	.001
residual	16	1188.64	74.29		
total	24	10447.36			

Tukey's Multiple Comparisons

alpha = .05 df = 16 MSE = 62.89 critical value = 4.33

Test Comparison (Pre = 100%)	Difference (%) Between Means	Confidence Limit	Statistical Significance
Pre-Fat Block	16.20	±15.36	***
Pre-Isch Block	16.80	±15.36	***
Fat Block - Fat	38.00	±15.36	***
Isch Block - Isch	5.20	±15.36	ns
Isch - Fat	32.20	±15.36	***
Isch Block-Fat Block	0.60	±15.36	ns

Figure 23: Mean changes (±SE) from the pretest value in maximum H-reflex amplitude, MVC plantarflexion torque, EMG activity, and maximum M-wave amplitude following compression block (B), ischemia only control (C), and fatigue (F).


H-Reflex
MVC
EMG
Mmax
B C F B C F B C F C F
0
20
40
60
80
100
% Decrease
B = post block
C = post ischemia control
F = post fatigue
⊥ = SE

the muscle was resting and adequately perfused so there is little reason to suspect change in excitability. Third, any M-wave decline would be equal or less than that during the posttest in which the M-waves showed limited mean declines. Fourth, Magladery et al (1950) found normal M-waves for up to 20 min of nerve compression induced by an ischemic cuff. Hence the decline in MVC after the afferent blockade was most likely due to the lack of proprioceptive sensory input to the spinal cord.

6.3.2 Effects of Fatigue

The compression block was maintained at 100% in 3 subjects but in the other two there were varying degrees of recovery of the H-reflex following the fatigue procedure. This could be explained by the subjects inadvertently shifting their body weight off the wooden bar because of local discomfort or through excessive effort during the MVC trials. However, the mean decline of the H-reflex was 89.8 ± 15.8% and this suggested that most of the large myelinated afferents remained blocked.

By the end of 5 - 10 minutes of fatiguing stimulation, the block had been in place for 25 - 30 minutes. In 2 subjects there was evidence of slight increments of torque with the interpolated twitch. Despite encouragement and obvious effort on the part of the subject, this could not be overcome.

The MVC plantarflexion torque showed a statistically significant decrease of another 38.0 ± 18.6% from the postblock value (Table 6); further, this decrease

Table 7

ANOVA Table and Tukey's Multiple Comparisons

ANOVA TABLE: EMG

Source	df	SS	MS	F	p
subjects	4	3814.64	953.66	3.86	.025
treatment	4	8737.84	2184.46	8.85	.001
residuai	16	3950.56	246.91		
total	24	16503.04			

Tukey's Multiple Comparisons

alpha = .05 df = 16 MSE = 246.91 critical value = 4.33

Test Comparison (Pre = 100%)	Difference (%) Between Means	Confidence Limit	Statistical Significance
Pre-Fat Block	11.00	±30.43	ns
Pre-Isch Block	10.80	±30.43	ns
Fat Block - Fat	43.40	±30.43	***
Isch Block - Isch	6.60	±30.43	ns
Isch - Fat	37.00	±30.43	***
Isch Block-Fat Block	0.20	±30.43	ns

was significantly greater than that in the control Test 2. This decline in MVC was similar to the value of 38.5 ± 8.6% found in Chapter 5 in which fatigue was induced without the block. The MVC plantarflexion torque decline in the 3 subjects without interpolated twitches was still 29.7 ± 16.5%.

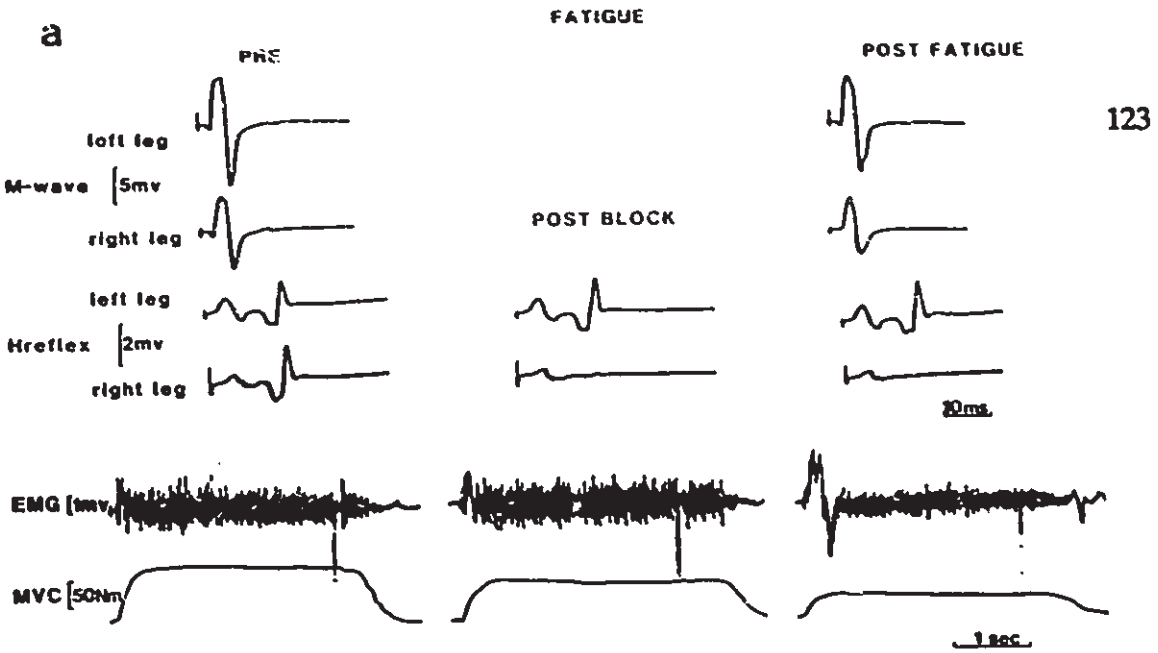
The associated voluntary EMG activity decreased by 43.4 ± 15.6% from postblock levels and this reduction may be compared with the value of 51.9 ± 17.9% found in Chapter 5 (without the block). Hence, fatigue produced very similar changes in MVC plantarflexion torque and EMG activity both with and without the majority of sensory input from large diameter afferents. The most convincing results were those for subjects TE, KH, and WH (Table 5), in whom the reduction in EMG activity following fatigue greatly exceeded that due to compression alone or compression with ischemia.

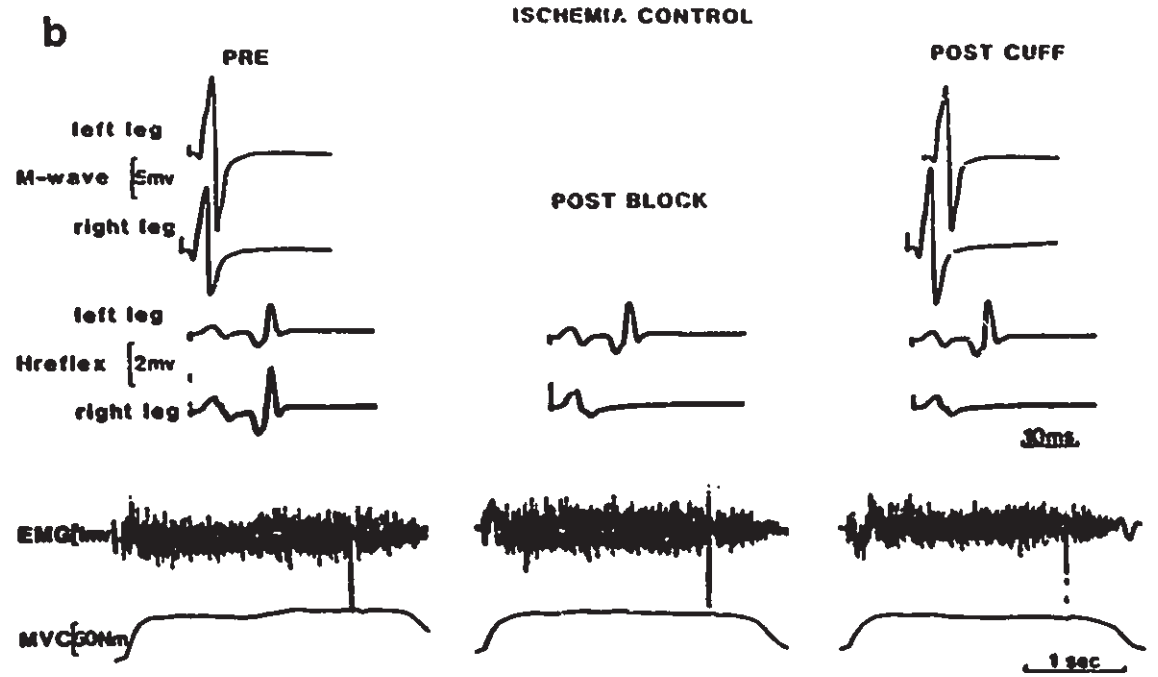
This decline in EMG activity was statistically significant (Table 7); further there was a significant difference between tests. Even when the results for the 2 subjects with small interpolated twitches were excluded, the mean EMG decrease was 33.7 ± 19.9%. The mean amplitude of the maximum M-wave fell by 6.5 ± 19.1% from the pretest value; the corresponding area of the M-wave fell by 8.0 ± 24.4%. The M-wave duration increased from 2% to 15% (.4 ms to 2.6 ms).

The mean changes following fatigue are illustrated in Figure 23. In Figure 24, the data from one subject show the effectiveness of the block and the substantial decline in EMG activity and MVC plantarflexion torque during fatigue. In this subject the M-wave also showed a moderate decline.

Figure 24 a): Data from one subject illustrating the effectiveness of the compression block and the substantial decline in EMG and MVC following fatigue. Top 2 rows: maximum M-waves from control (left) leg above and right (experimental) leg below. Middle 2 rows: Maximum H-reflexes from left leg above and right leg below. Bottom 2 rows: voluntary EMG activity above and MVC plantarflexion torque recordings below. Left column are pretest values, middle column are postblock values and right column are postfatigue values. Note the abolition of the right H-reflex postblock which was maintained postfatigue.

b): Same subject's data in the ischemia control test. These data illustrate little change in MVC and EMG despite total block of the H-reflex and 5 minutes of ischemia.


a
FATIGUE
PRE
POST FATIGUE
left leg
M-wave 5mv
right leg
POST BLOCK
left leg
Hreflex 2mv
right leg
10ms
EMG 1mv
MVC 50Nm
1 sec


b
ISCHEMIA CONTROL
PRE
POST CUFF
left leg
M-wave 5mv
right leg
POST BLOCK
left leg
Hreflex 2mv
right leg
10ms
EMG 1mv
MVC 50Nm
1 sec

6.3.3 Effects of Ischemia Control

The H-reflex was completely abolished after the period of ischemia in 3 subjects. The other 2 subjects had small H-reflexes after the ischemia as in the fatigue test. The mean decline of the H-reflex in the 5 subjects was 86.0 ± 19.5%.

The mean MVC plantarflexion torque only decreased 5.2 ± 7.0% from the postblock value; the change in the fatigue experiments was significantly greater (Table 7a). Similarly, the associated voluntary EMG activity only decreased 6.6 ± 5.5% from the postblock value; while the change in the fatigue experiment was significantly greater (Table 7b). This decrease in EMG activity in the presence of ischemia alone was similar to that previously reported for tibialis anterior (4.0 ± 11.7%) in Appendix V.

The mean changes in the ischemia control experiments are presented in Figure 23 and the data from one subject are illustrated in Figure 24. There is little change in EMG activity or MVC plantarflexion torque despite the total block of the H-reflex and the 5 minutes of ischemia.

The 2 subjects with interpolated twitches evident following fatigue also had small interpolated twitches following the ischemia test. Since these were not accompanied by substantial decreases in EMG activity or MVC plantarflexion torque, it is unlikely that the changes in EMG and MVC seen following fatigue in these subjects were due solely to conduction block of the motor axons.

the prime candidates for matching motoneuron output to the functional status of the muscle fibres. Approximately half of the small diameter afferents have been shown to respond to noxious chemical, mechanical and thermal stimuli (Kniffki et al, 1981). Other small afferents were activated by moderately innocuous stimuli such as stretch, contractions, and touch (Kniffki et al, 1981; Kaufman et al, 1984; Hayward et al, 1988).

Afferents from group III and IV can be activated by fatigue (Hayward et al, 1988b) and by chemical agents which are known to increase during fatigue (Sreter, 1963; Karlsson, Saltin, 1970; Edwards et al, 1975a; Fitts, Holloszy, 1976; Dawson et al, 1980a,b; Sjogaard et al, 1985; Juel, 1986; Lindinger, Heigenhauser, 1987) and are associated with muscle pain; these agents include bradykinin and potassium (Mense, 1977), lactate and phosphate (Kniffki et al, 1978). The rapid return of EMG activity following release of the ischemic cuff in Chapter 4 and the concurrent reduction in muscle pain are suggestive of such a chemical mediation.

The central actions of noxious stimuli could induce inhibition at the spinal and/or supraspinal level. Nociceptive afferents (group A delta, III, IV) are known to synapse in the dorsal horn of the spinal cord, the pathway to the somatosensory cortex and frontal brain continuing via the brain stem reticular formation and ventral basal and posterior nuclear group of the thalamus (Mense, 1983). It is possible that these terminations could influence alpha motoneuron excitability via reticulospinal tracts or via frontal and premotor areas feeding the motor cortex (Schell, Strick, 1984). Inputs from the reticulospinal system on spinal motoneurons are

predominantly excitatory to limb muscles (Wilson, Peterson, 1981). Repetitive stimulation, however, (as in this protocol) causes a decrement in activity in reticulospinal neurons (Wilson, Peterson, 1981) which could remove excitatory influences on the alpha motoneuron pool. Hence, it is possible that stimulation of nociceptive afferents during fatigue produces inhibition of motoneurons via spinal or supraspinal reflexes. If there is supraspinal reflex inhibition, the descending motor drive to the motoneurons during MVC is still able to fully utilize the available force-generating capacity of the muscle (as evidenced by the absence of interpolated twitches in 3 subjects).

Group III and IV afferents have been implicated in cardiovascular and cardiorespiratory reflexes evoked by contraction (Kalia et al, 1972; McCloskey, Mitchell, 1972; Rowell et al, 1986); the reflex stimulus may have been metabolite accumulation as reflected in the increased venous concentration of lactate (Sheriff et al, 1986). However, it is also possible that the cardiovascular reflexes may have arisen from mechanosensitive afferents signalling the number of working motor units or the force of contraction (Kaufman et al, 1984).

A mechanosensitive model is also attractive for explaining the reflex inhibition of motoneurons during fatigue. Group III afferents may signal the event of contraction rather than the degree of contraction; the activity of Group III afferents may be related to enhanced spindle activity (via gamma motoneurons) after the onset of contraction (Ellaway et al, 1982). These authors suggest that if group III afferents signal the time course of twitch contractions, then they might be able to

sense changes in the contractile speed which occurs in fatigue and cause an adjustment in motoneuron output. Pressure-pain receptors connected to group III afferents in triceps surae have been shown to facilitate the flexor reflex in the leg and inhibit their own (Paintal, 1961). Both group III and IV afferents are included in the flexor reflex afferent category which preferentially inhibit their own extensor motoneurons (Houk, Rymer, 1981).

It is doubtful that the flexor reflex afferent system could be exclusively involved since the same declines in EMG with fatigue were seen in soleus, an extensor of the ankle joint, as in tibialis anterior, a flexor (Chapter 4). Tibialis anterior is normally facilitated to some extent from autogenetic flexor reflex afferents (Paintal, 1961). This does not preclude the possibility that other group III and IV mechanosensitive afferents could signal the declining force of contraction (and possibly the declining number of working motor units) and remove excitation to the tibialis anterior motoneuron pool. Force sensitive interneurons can be excited by tendon squeezing and stroking of the muscle surface and also by muscle contractions too weak to stimulate Golgi tendon organs; their excitation produces abrupt and prolonged inhibition of motoneuron output (Cleland et al, 1982). Kukulka et al (1985) and Robinson et al (1984) also found pressure on the human triceps surae tendon to decrease alpha motoneuron excitability.

There have not been any studies to date which demonstrate whether the nature of the signal is mechanosensitive or chemosensitive. Chemical changes in fatigue were more likely than mechanical changes to mediate reflex inhibition for

several reasons. First, both in this study and that of Bigland-Ritchie and colleagues (1986c) EMG during fatigue remained depressed as long as the limb was rendered ischemic; this suggested a chemical stimulus. Second, in Chapter 4, it was shown that release of the ischemic cuff was associated with a reduction in muscle pain, and chemosensitive afferents are known to be nociceptive (Mense, 1983). Third, Marsden and colleagues (1983) were unable to change EMG activity by slowing muscle contraction through cooling rather than fatigue; hence in the absence of chemical changes associated with fatigue, the EMG activity was unaffected. Lastly, the magnitude of chemical changes observed in fatigue eg. extracellular K+ concentrations rising as high as 15 mM (Hnik et al, 1986) and the strong correlation between the decline in force and the concentration of diprotonated inorganic phosphate (Miller et al, 1988; Nosek et al, 1987) have lent support to the contention that the decline in EMG activity which occurs in fatigue could also be mediated by such chemical changes.

To conclude, there appears to be a reflex inhibitory system active during fatigue; it serves to decrease motoneuron activity in parallel with the reduction in force. This reflex is probably not mediated by larger diameter afferents, rather it is speculated that the reflex is mediated from small diameter afferent from within the exercising muscle. Whether this reflex is chemical in nature (consequent to metabolite accumulation or deprivation of energy substrate) or mechanical (consequent to low sensitivity stretch or pressure) requires further investigation.

Chapter 7

SUMMARY AND CONCLUSIONS

7.1 Summary

This research has investigated the neurophysiological factors contributing to the decline in voluntary electromyographic activity which occurs in a muscle as it becomes fatigued. It has been the hypothesis of this research that the declining EMG activity is a result of inhibition of alpha motoneurons by afferents sensitive to mechanical or chemical changes in the muscle. Several research questions have been raised and answered.

1) **What is the optimal frequency of electrical stimulation for inducing fatigue while minimizing muscle action potential failure?**

Two different frequencies of stimulation (15 Hz and 30 Hz) were employed to induce fatigue. While normal muscle action potentials were present in response to single stimuli, there was evidence of some peripheral inexcitability with repetitive stimulation. The muscle fibre membrane excitability was better maintained, however, during the lower frequency (15 Hz) of fatiguing stimulation.

2) What is the contribution of the declining M-wave to the fatigue process?

Observations made throughout this research are suggestive that declining excitation of the muscle fibre membrane was not the primary cause of the fatigue induced in these experiments. Some observations from this research that make it unlikely that declining peripheral excitation was causing the fatigue are: 1) mechanical failure of the twitch occurred without loss of M-wave amplitude to single shocks. Thus fatigue of the twitch contraction was independent of its activation and further even when the M-wave evoked by trains of stimuli started to decline there was already fatigue of the twitch and tetanic contractions, 2) loss of M-wave amplitude by as much as 55% within trains of stimuli was not accompanied by any loss of force production (see Figure 7, Chapter 3), 3) the EMG and dorsiflexion torque lacked parallelism during recovery in Chapter 4; there was fast improvement in voluntary EMG and slow recovery of MVC dorsiflexion torque, and there was fast return of the M-wave amplitude to pretest values while the dorsiflexion torque produced by the 15 Hz stimulation remained depressed. This demonstrated that peripheral excitation was adequate at a time when force generation was depressed.

Two other experiments are suggestive that the reductions in M-wave amplitude did not cause the muscle fatigue in this research. It has been shown that individual amphibian muscle fibres still twitched when transmembrane action potentials had been reduced by 40 mV by reduction in extracellular sodium concentration (Grabowski et al, 1972). More recently, the slowing and reduction in amplitude of the M-wave evident in fatigue, was reproduced in nonfatigued single

amphibian fibres by increasing extracellular potassium to 14 mM; in this case the muscle action potential was decreased but the peak twitch tension was actually increased (Lannergren, Westerblad, 1986). In summary, it was unlikely that muscle action potential failure was the primary cause of the muscle fatigue.

3) What is the mechanism for the declining EMG which occurs with fatigue?

The decrease in electrical activity could, in theory, be due to a loss of excitability at the neuromuscular junction or muscle fibre membrane. Alternatively, it might result from failure of the descending motor pathways (consequent to lack of subject motivation) or from reflex inhibition of the motoneurons by afferents from the exercising muscle. Special conditions have been utilized to solve this problem. Fatiguing the muscle by indirect electrical stimulation minimized lack of subject motivation as a factor in declining EMG. Similarly, the use of a low frequency of stimulation minimized any loss of excitability in the periphery (verified by only modest reduction in maximum M-wave amplitude). It was also noted that at a time when the EMG was reduced, it was not possible to record an interpolated twitch superimposed on the MVC. This indicated that the subjects remained motivated to perform MVCs. The reduction in voluntary EMG activity could not have been primarily due to lack of voluntary effort or loss of peripheral excitability of the neuromuscular junction or muscle fibre membrane. Hence, reflex inhibition of motoneurons was indirectly implicated as a plausible mechanism.

The results of the interpolated stimulus experiments were interesting in that no additional force could be evoked in the MVCs as the muscle became fatigued. If reflex inhibition was decreasing the voluntary EMG output of the muscle, it must have done so by either affecting those motoneurons no longer able to generate force within their muscle fibre colonies and/or by decreasing motoneuron firing rates, but to frequencies which were still optimal for force production. Such a relationship would be advantageous, in that muscle fibre membrane function would not be compromised by ineffectual activity, but would be better preserved for the time when force production was restored within the fibres.

4) What is the level of alpha motoneuron excitability during fatigue?

To examine further the role of reflex inhibition in the declining electrical activity in fatigue. the Hoffmann reflex was implemented. The amplitude of this reflex reflects the net excitatory and inhibitory influences on the alpha motoneuron pool. Reflex inhibition of the motoneuron pool was suggested by the data showing declining H-reflexes concurrent with reductions in voluntary EMG and MVC torque. Possible confounding factors (such as the presence of ischemia, antidromic and reflex inputs from the electrical stimulation, and pain) were minimized.

The parallel reduction in voluntary EMG activity and reflex excitability of the alpha motoneuron pool provided further evidence compatible with a process whereby the motoneuron pool is reflexively inhibited by afferents arising from within the exercising muscle.

5) What type of afferent is involved modulating motoneuron excitability during fatigue?

To elucidate the contribution of large and small diameter afferents in the reflex inhibition during fatigue, a compression block of the sciatic nerve was used to block large myelinated afferents selectively prior to fatigue. Since the same decrease in voluntary EMG during MVC was seen following fatigue irrespective of any blockade of larger afferents, then smaller diameter afferents must be implicated in the reflex inhibitory process.

There is much speculation as to whether the signal activating the afferents is chemical or mechanical in nature. Theoretical discussion is presented which illustrates the feasibility of either a chemosensitive or mechanosensitive model. However, the rapid return of EMG activity following release of the ischemic cuff and the concurrent reduction in muscle pain are suggestive of chemical mediation.

6)How "physiologically-relevant" are the results of these experiments?

It is worthwhile noting that although the use of ischemia and electrical stimulation to induce fatigue may be considered "unphysiological", the results of this study are very similar to those of Bigland-Ritchie et al (1983, 1986) in which voluntary contractions were used as the fatiguing procedure. Further, the motoneuron firing rates of the fatigued ankle dorsiflexor and plantarflexor muscles approximate to the 15 Hz stimulation frequency used in this study. Finally, although ischemia was employed in the fatiguing procedure, maximal voluntary contractions

would also render the muscle ischemic; natural activities such as rock climbing or sailing could employ sustained contractions strong enough to induce ischemic conditions.

7.2 Future Research

The nature of the signal mediating reflex inhibition warrants further study. An animal model would be ideal for performing the following experiments:

1) selective afferent blockade (including the use of local anesthetics) while recording reflex changes during stimulated fatigue,
2) recordings from alpha motoneurons during perfusion of muscle with levels of lactate or potassium expected in a fatigue protocol so as to observe the end result of any group III and IV activation,
3) monitor mechanosensitive Group III and IV afferents to determine any changes in their activity with fatigue,
4) record Group III and IV afferent activity and alpha motoneuron excitability in both tibialis anterior and soleus to determine any differences given the disparity in FRA input to the motoneuron pools,
5) induce fatigue or simulate fatigue in single motor units and determine whether the homonymous motoneuron ceases firing.

7.3 Conclusions

1) Muscle compound action potential failure was minimized during low frequency stimulation at 15 Hz.
2) The reduction in torque during fatigue, when induced by electrical stimulation and tested with either MVCs or tetanic stimulation, was not primarily due to muscle action potential failure. Rather, fatigue appeared to be the result of either failure of excitation-contraction coupling and/or the contractile machinery.
3) After fatiguing ischemic ankle dorsiflexors by repetitive stimulation at 15 Hz, there was a reduction in voluntary EMG activity associated with declining MVC torque. The reduction in EMG was not due to primarily peripheral inexcitability of the muscle fibres or lack of subject motivation to do MVCs.
4) After fatigue of soleus muscle under ischemic conditions using 15 Hz stimulation, there was a reduction in both voluntary EMG activity and reflex excitability of the alpha motoneuron pool. The reduction in reflex excitability gave further evidence suggestive of reflex inhibition (spinal or supraspinal) consequent to fatigue.
5) Compression block of the larger myelinated afferents in the sciatic nerve prior to fatigue did not change the reductions in MVC torque and EMG following fatigue. This finding implicated smaller diameter muscle afferents in mediating the reflex inhibition; these afferents could signal either chemical or mechanical events during fatigue.

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Appendix I: Calculation of Measurement Errors

Table I-A

Intertest measurement error

Voluntary contraction (Nm) (Pretest MVC repeated twice - difference between two attempts)		EMG activity (mv.s) (Difference in EMG for two MVCs with the same force production)	
Difference Between Trials	Mean Force of Contraction	Difference Between Trials	Mean EMG Activity
2.82	31.3	23.83	256.52
0	54.1	13.07	134.05
0	51.3	0.87	112.60
2.85	96.12	3.59	435.18
1.42	45.60	28.37	408.23
0	42.70	1.70	91.59
0	54.11	59.87	335.97
2.85	34.18	39.43	364.71
0	99.68	15.67	159.27
0	48.42	5.20	92.63
2.85	56.96	69.33	287.67
0	74.05	22.53	289.58
5.7	48.42	18.58	215.77
5.7	54.11	321.32	3648.45
6.1	19.94	x=22.95	x=260.60
5.7	54.11	SD=20.05	
2.85	41.30		
0	105.38	(22.951/260.604)*100=8.81%	
0	99.68	(20.050/260.604)*100=7.69%	
0	76.90		
5.7	41.30		
0	18.51		
2.85	65.50		
1.43	72.62		
0	51.26		
2.85	48.42		
2.85	66.81		
2.85	62.66		
0	52.69		
57.37	1668.13		
X=1.98	X=57.52		
SD=2.12			
(1.978/57.522)*100=3.44%			
(2.123/57.522)*100=3.69%			

Table I-B

Intratest Measurement Error

Integrated EMG (mv.s)
(Difference between 3 integrations of the same EMG pattern)

Largest difference Between integrations	Mean EMG Activity	Largest difference Between integration	Mean EMG Activity
2.8	122.1	9.5	363.43
2.9	355.00	2.0	179.23
0.4	159.27	8.8	365.13
10.1	239.10	0.2	179.85
0.4	105.50	2.6	146.10
19.1	248.67	1.7	326.67
0.1	269.87	0.4	309.30
30.6	257.70	1.8	286.90
11.3	124.93	17.8	200.17
0.0	116.90	0.3	171.35
11.7	172.42	4.7	253.73
2.7	206.91	2.2	215.77
15.6	149.40	1.8	199.23
22.0	173.52	21.8	224.57
14.1	146.0	12.3	106.1
11.6	119.1	21.2	283.57
10.7	229.47	39.7	255.63
16.9	285.27	32.0	382.50
1.7	380.63	15.5	344.60
8.7	327.33	29.3	408.67
35.87	407.80	5.7	397.63
2.3	472.73	8.2	375.27
0.1	464.68	2.5	432.18
1.1	429.47	5.8	452.55

Sum of Difference = 480.50 Sum of EMG = 12823.9
Mean of Difference = 10.01 Mean of EMG = 267.16
Standard Deviation = 10.36

(10.010/267.165)*100 = 3.75%
(10.365/267.165)*100 = 3.88%

Appendix II: Electrical versus Mechanical Fatigue

It was hypothesized that the loss of force production was not dependent solely on the number of stimuli delivered to the muscle, as had been suggested by Merton (1981). This proposition, enunciated by Merton, is important to the understanding of fatigue because, if true, it would imply that loss of force depended on 'electrical' rather than 'mechanical' events in the contracting muscle. This argument depends on the fact that, above an optimal frequency of stimulation, no further force can be generated by the muscle. Expressed differently, at higher than optimal frequencies of stimulation a certain proportion of stimuli would be redundant in terms of force output and the biochemical events associated with contraction, but would nevertheless continue to cause the ionic perturbations associated with impulse activity. If the latter changes were ultimately responsible for fatigue, then fatigue would be related to the number of shocks delivered rather than to their frequency. The present study reinvestigated the relationship between stimulus frequency and fatigue; the hypothesis was that the amount of fatigue would depend on the frequency of stimulation in addition to the number of stimuli delivered.

This study employed the methods outlined in Chapter 3. Dorsiflexion torque and M-wave responses to single shocks and tetani were analyzed during fatigue induced by two different frequencies (15 Hz and 30 Hz); a common stimulus frequency (30 Hz) was used to test tetanic responses. To determine whether the responses obtained after fatiguing stimulation differed significantly from each other, the following procedure was adopted. First, the values for 15 Hz stimulation were

subtracted from those for 30 Hz stimulation. The resulting differences were then plotted as functions of the numbers of fatiguing stimuli delivered. These plots were parabolic; the plots were obviously different from a straight line of zero slope (indicating no effect of the number of stimuli on the difference). A paired t-test was performed on the differences observed for a given number of stimuli; the latter number corresponded to the peak difference in most of the subjects.

Figure II-1 shows the mean dorsiflexion torque elicited by single shocks as a function of the numbers of stimuli delivered at the two fatiguing frequencies (15 Hz or 30 Hz). It can be seen that the curves became clearly separated after the initial potentiation was over, such that fewer stimuli were required at 15 Hz than at 30 Hz to produce the same amount of fatigue. The difference between the two curves was statistically significant ($p<.001$), with the greatest discrepancy occurring after the delivery of 1200 stimuli. At this point the mean dorsiflexion torque had dropped by 62% from its original value, with 15 Hz used as the fatiguing stimulation, compared with a reduction of 13% of its original value when 30 Hz stimulation was employed. In all subjects the dorsiflexion torque elicited by single shocks was completely fatigued by 1720 stimuli, with 15 Hz used as the fatiguing frequency and by 2500 stimuli, with 30 Hz stimulation.

The mean dorsiflexion torque developed by the 30 Hz testing bursts with the two fatiguing frequencies is shown in Figure II-2. As in Figure II-1, the results have been expressed in terms of the numbers of stimuli delivered; for both curves the initial dorsiflexion torque generated by the first 30 Hz testing burst has been used

Figure II-1: Mean dorsiflexion torque elicited by single stimuli expressed as percentages of control values, after different numbers of fatiguing stimuli delivered at either 15 Hz or 30 Hz.

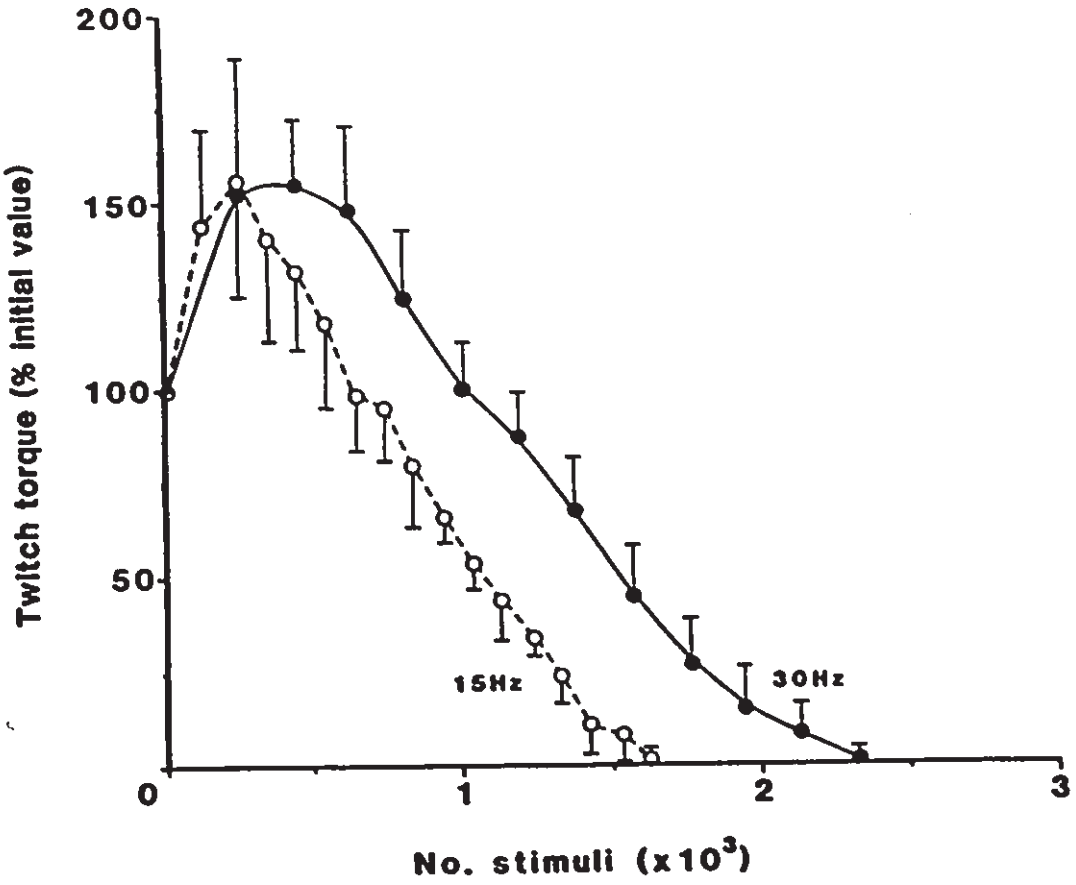
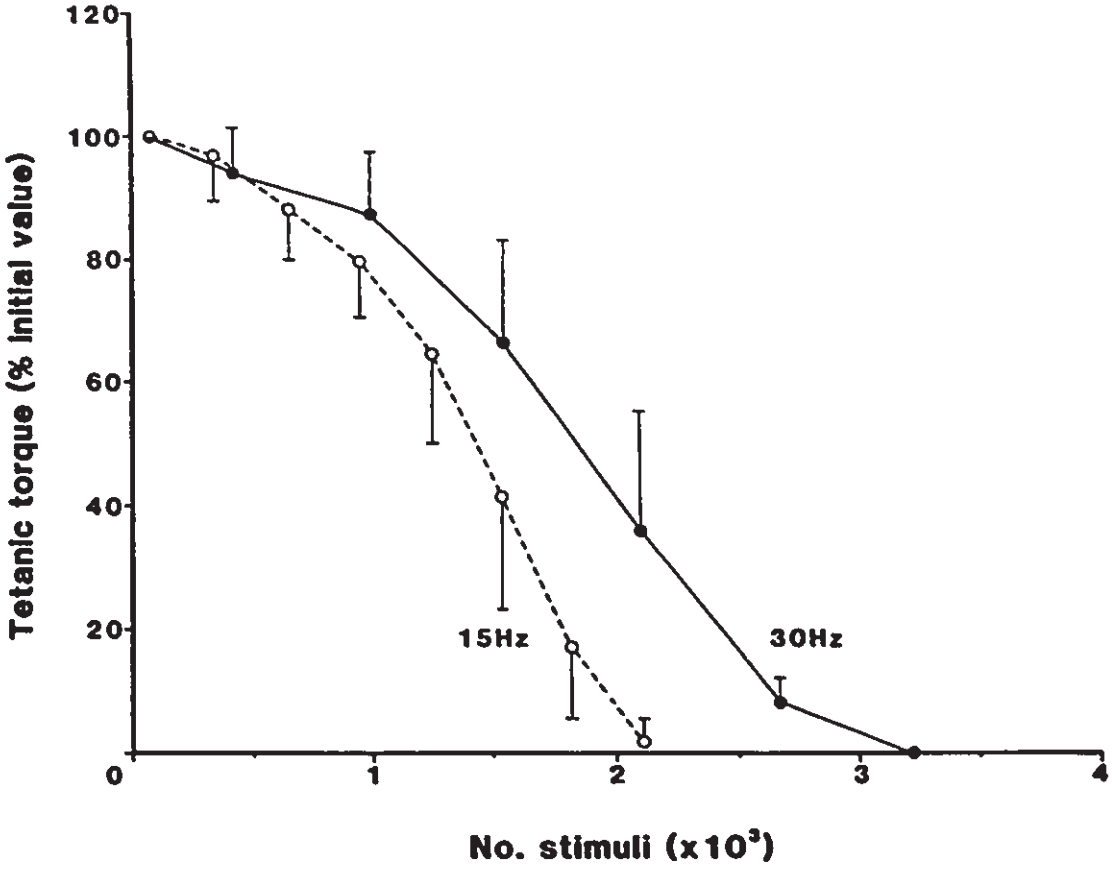

200
150
100
50
0
1
2
3
Twitch torque (% initial value)
No. stimuli (x10³)
15Hz
30Hz

Figure II-2: Mean dorsiflexion torque elicited by testing 30 Hz bursts of stimuli developed during fatiguing stimulation at 15 Hz and 30 Hz.


120
100
80
60
40
20
0
1
2
3
4
Tetanic torque (% initial value)
No. stimuli (x10³)
15Hz
30Hz

as a reference for each of the six subjects. It can be seen that the curves for the two stimulation frequencies diverged, such that fatigue occurred after fewer stimuli at 15 Hz than at 30 Hz. The difference between the two curves was most significant after 1800 stimuli, when the torque at 30 Hz decreased by 46% from the initial value, compared with a decrease of 82% at 15 Hz ($p<.01$). The contrast between the results for the two stimulus frequencies was also reflected in the numbers of stimuli required for total mechanical fatigue. After the results for one subject with a high resistance to fatigue are omitted, it can be seen that total fatigue was achieved after a mean of 2010 stimuli at 15 Hz and after 2880 stimuli at 30 Hz.

In Figure II-3 and II-4, the data have been plotted differently, with the dorsiflexion torque and M-wave amplitude shown against each other rather than against the number of stimuli. In Figure II-3, it was clearly evident that the responses to single stimuli, for 15 Hz and 30 Hz fatiguing stimulation, were very similar. In each instance, there was a relatively flat portion of the curve as substantial twitch enlargement (157%, 15 Hz and 155%, 30 Hz) was accompanied by modest enlargement of M-wave amplitude (111%, 15 Hz and 114%, 30 Hz). The flat region continued as mechanical fatigue caused the twitch to decline to less than half of its initial value. Beyond this point the M-wave amplitude became progressively attenuated but, by the time total twitch fatigue had been achieved, the M-wave amplitude fell by only 21.1 ± 9.7% from its original value following 15 Hz stimulation, and decreased by 24.9 ± 5.3% from control, following 30 Hz stimulation; a paired t-test indicated that these values were not significantly different. In Figure

Figure II-3: Mean (± SD) amplitudes of M-wave elicited by single shocks, as a function of corresponding twitch torques.

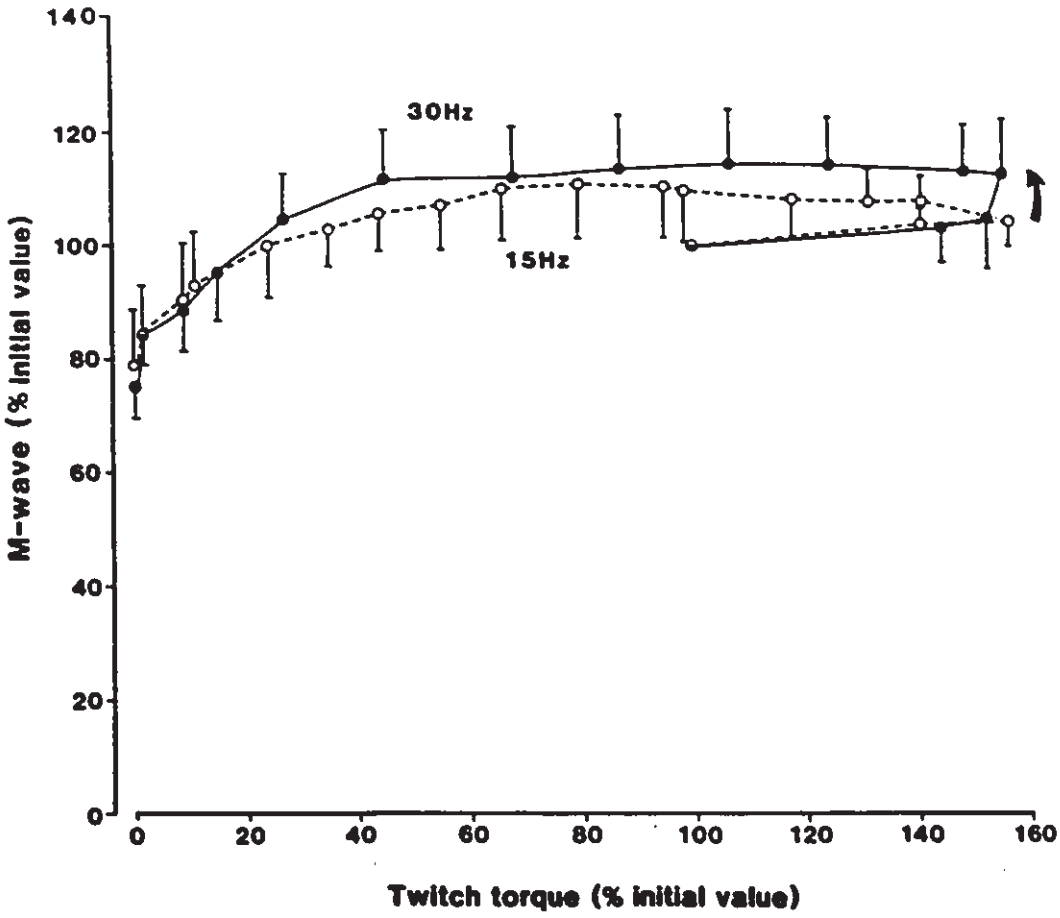
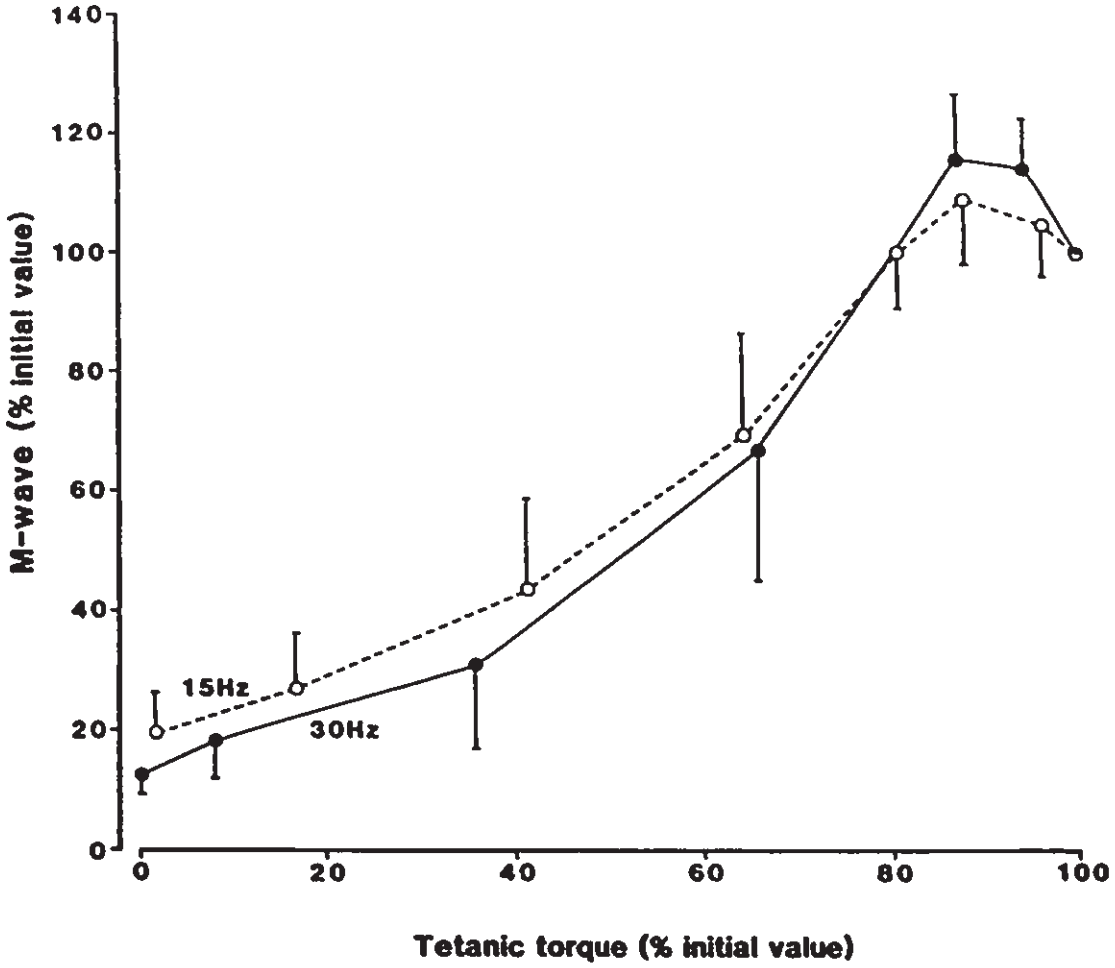

140
120
100
80
60
40
20
0
30Hz
15Hz
M-wave (% initial value)
0
20
40
60
80
100
120
140
160
Twitch torque (% initial value)

Figure II-4: Mean (±SD) M-wave amplitudes, measured at the end of each 30 Hz testing tetanus, as a function of corresponding tetanic torques.


140
120
100
80
60
40
20
0
M-wave (% initial value)
15Hz
30Hz
0
20
40
60
80
100
Tetanic torque (% initial value)

II-4, the tetanic responses to both 15 Hz and 30 Hz fatiguing stimulation showed initial potentiation of the M-wave (109% at 15 Hz and 112% at 30 Hz) which was statistically significant from the pretest value using a paired t-test with the 30 Hz stimulation ($t=3.44$, 5 df, $p<.05$); it caused the relative M-wave values to exceed those of the tetanic dorsiflexion torques for the regions of the curves between 80 and 100% of initial torque. Between 40 and 70% of initial torque, the electrical and mechanical responses did not depart from each other. Below this level of torque, mechanical fatigue proceeded more rapidly than action potential failure, as judged by the amplitudes of the M-waves; thus small M-waves (20% of the initial value with 15 Hz and 13% of initial with 30 Hz) could still be evoked without any accompanying dorsiflexion torque. The similarity between the two curves is suggestive that while action potential failure could have contributed to the tetanic fatigue, it could not account for the differences in fatigue between the two stimulation frequencies. The testing bursts of 30 Hz stimulation were revealing in showing that, regardless of which fatiguing frequency had been employed, the relationships between M-waves and dorsiflexion torques were similar when the muscles were examined at the same excitation frequency. Although the corollary experiment was not performed, it is likely that, had the fatiguing trains of 30 Hz stimulation been followed by short bursts of 15 Hz stimulation, the M-wave amplitudes would have increased during the latter. Indeed, the M-waves at the end of the 15 Hz fatiguing stimulation were better maintained (decreases of 57% from original amplitude; see Chapter 3). These results clearly imply that there is less

opportunity for recovery of impaired excitatory mechanisms, pre- or post-synaptic, when the interval between successive stimuli is shortened from 67 ms (during 15 Hz stimulation) to 33 ms (during 30 Hz stimulation).

The main finding of this study was that muscle fatigue depended not only on the number of muscle fibre excitations but also on impulse frequency. This observation is true for the dorsiflexion torque elicited both by single stimuli and by trains of stimuli; in both instances fatigue occurred after fewer stimuli when the lower frequency was employed. It was noted that, at the stage when the terminal M-waves in the testing tetani began to decline, the twitch was already smaller (by 40% with 15 Hz stimulation and 15% with 30 Hz stimulation). Since it is reasonable to assume that all the muscle fibres had responded to every stimulus until this point, these observations would indicate that not only did muscle fatigue occur after fewer stimuli at 15 Hz than at 30 Hz, but the discrepancy could not be ascribed to failure of muscle fibre membrane excitation.

The loss of dorsiflexion torque, in the presence of adequate muscle fibre membrane excitation, clearly indicated that the fatigue of the twitch must have been due to a breakdown of excitation-contraction coupling or to failure of the contractile machinery. The balance of evidence presently available favours the first explanation. Thus Eberstein and Sandow (1963) were able to induce a contracture through the application of potassium or caffeine, in muscles which had become totally fatigued by low frequency electrical stimulation. This result suggested that the actin filaments and myosin cross-bridges were still in a state conducive to interaction and that local

energy reserves were still adequate. Further, in the present experiment, it was also apparent from Figure 7 (Chapter 3) that at later stages of fatigue, the motor response outlasted the electrical activity for approximately 1 second. This was suggestive of contracture; if so, this indicated that the contractile machinery was capable of producing force although there was no force produced by a single stimulus. The phenomenon of persisting fatigue at low frequencies of stimulation, but not at high frequencies, would also be consistent with impaired excitation-contraction coupling (Hultman, Sjoholm, 1983).

Another possibility consistent with the present findings is that fatigue may be related to the amount of "internal work" performed by the muscle, that is, the number of actin and myosin cross-bridge interactions responsible for the development and maintenance of dorsiflexion torque. Such a correlation would be attractive if there was proportionality between the amount of internal work and the production of fatigue-producing metabolites. The results of Dawson et al (1980) who used nuclear magnetic resonance spectroscopy in isolated frog muscle, are strongly suggestive of such an interpretation; these authors were able to demonstrate that the amount of fatigue was correlated with the metabolic state of the muscle. The results of Loiselle and Walmsby (1982) are also pertinent; myothermal recordings from rat soleus muscles showed that more energy was expended per unit force during intermittent contractions, compared with steady contractions. This effect may have contributed to the present findings in two ways, since more tetani were delivered at 15 than at 30 Hz to achieve fatigue, and the tetani were less well fused at 15 Hz that

at 30 Hz. Bergstrom and Hultman (1988) delivered the same number of stimulation pulses to human quadriceps with a train duration of 0.8 s or 3.2 s and found that fatigue was more pronounced in the intermittent protocol with only 0.8 s train duration; this protocol was associated with more contractions and higher ATP utilization. In addition, the present results are in agreement with those of Fitch and McComas (1985) who, by making use of the length-tension relationship of human ankle dorsiflexor muscles, found that fatigue appeared to depend on the amount of dorsiflexion torque generated and not merely on the number of stimuli delivered.

Appendix III: Individual subject data for Chapter 3

Determination of Fatigue Protocol.

TABLE III-A

Twitch Data with 15Hz Stimulation

Variables are time (s) and dorsiflexion torque (Nm) for each subject.

	Subject					
Time	BK	BH	TH	LT	KH	WH
0.0	6.4	5.7	3.6	2.8	2.1	3.6
10.0	7.8	10.0	6.4	3.6	2.8	3.9
20.0	8.5	10.7	7.1	4.3	3.2	4.3
30.0	8.5	9.3	6.4	3.9	2.8	3.6
40.0	7.8	8.5	5.7	3.6	2.8	3.6
50.0	7.1	7.1	5.7	3.2	2.1	3.6
60.0	5.7	7.1	3.6	2.8	2.1	2.8
70.0	5.0	6.4	3.6	2.8	2.1	2.8
80.0	4.3	5.7	3.6	2.1	1.4	2.5
90.0	4.3	3.6	2.8	1.8	1.4	2.1
100.0	2.8	3.6	2.1	1.8	1.1	1.8
110.0	2.1	2.1	2.1	1.4	0.7	1.8
120.0	2.1	1.4	1.4	1.1	0.7	1.4
130.0	1.4	0.7	0.7	0.7	0.7	1.1
140.0	0.7	0.4	0.0	0.4	0.4	0.7
150.0	0.7	0.0	0.0	0.4	0.2	0.7
160.0	0.4	0.0	0.0	0.2	0.0	0.0
170.0	0.0	0.0	0.0	0.0	0.0	0.0

Twitch Data with 15Hz Stimulation

Variables are time (s) and M-wave amplitude (amp, mv) and area (uv.s) for each subject.

	Subject											
	BK		BH		TH		LT		KH		WH	
Time	Amp	Area	Amp	Area	Amp	Area	Amp	Area	Amp	Area	Amp	Area
0	19	104	28	132	27	111	22	116	32	125	23	95
10	20	88	28	129	28	100	22	98	34	109	25	84
20	20	85	29	134	29	101	21	96	34	111	25	83
30	20	86	31	129	30	101	22	96	34	112	26	82
40	21	88	31	132	30	105	21	93	34	115	26	83
50	21	87	32	131	30	107	21	99	34	117	26	83
60	21	88	33	121	32	108	21	98	33	116	26	84
70	22	89	33	124	32	113	21	102	33	119	26	85
80	23	91	33	126	31	114	21	108	33	121	26	87
90	23	97	32	129	31	116	21	109	33	125	26	88
100	22	97	30	129	31	121	21	110	32	128	25	87
110	22	95	28	129	30	124	21	117	33	132	25	88
120	20	95	26	128	30	126	22	114	32	138	25	90
130	20	94	24	119	30	127	22	117	30	139	24	88
140	18	95	23	114	27	125	21	120	26	135	24	87
150	18	88	21	107	26	121	22	115	26	137	22	85
160	16	85	20	102	25	120	20	112	24	133	21	84
170	15	82	19	97	24	117	20	112	22	130	18	77

Table III-B

Twitch Data with 30 Hz Stimulation

Variables are time (s) and dorsiflexion torque (Nm) for each subject.

	Subject					
Time	BK	BH	TH	LT	KH	WH
0	5.7	5.7	5.0	3.6	2.1	2.8
10	7.1	7.8	8.5	4.6	3.6	3.9
20	7.1	8.5	9.3	5.0	3.6	4.3
30	7.8	8.5	9.3	5.0	3.6	4.3
40	7.1	7.8	9.3	5.0	3.6	3.9
50	6.4	5.7	6.4	4.3	3.2	3.9
60	5.0	5.0	5.0	3.6	2.5	3.2
70	5.0	4.3	3.6	3.2	2.1	2.8
80	4.3	3.6	2.1	2.5	1.8	2.1
90	2.8	2.1	1.4	1.8	1.4	1.1
100	2.1	0.7	0.7	1.4	0.7	0.7
110	1.4	0.4	0.0	1.1	0.4	0.4
120	0.7	0.0	0.0	0.7	0.2	0.4
130	0.4	0.0	0.0	0.2	0.0	0.0
140	0.0	0.0	0.0	0.0	0.0	0.0

Twitch Data with 30 Hz Stimulation

Variables are time (s), M-wave amplitude (amp, mv) and area (uv.s) for each subject.

	Subject											
	BK		BH		TH		LT		KH		WH	
Time	Amp	Area	Amp	Area	Amp	Area	Amp	Area	Amp	Area	Amp	Area
0	18	89	25	114	23	98	24	100	31	135	22	95
10	18	83	25	105	26	88	23	90	33	116	23	85
20	18	82	26	121	27	93	22	92	34	110	24	85
30	18	83	29	111	29	98	24	94	35	115	26	89
40	20	89	29	121	29	103	24	96	35	123	25	92
50	20	91	29	130	29	111	24	100	35	133	26	95
60	21	94	27	134	29	122	24	109	35	144	27	102
70	20	98	27	140	29	125	24	108	35	159	27	103
80	12	101	26	135	28	127	24	110	35	167	27	106
90	11	102	26	133	28	129	25	124	35	179	27	109
100	17	98	24	124	26	118	25	115	33	175	25	104
110	15	95	23	119	21	109	24	115	30	176	24	98
120	15	88	20	109	20	94	24	113	28	172	20	88
130	14	85	20	107	19	95	22	103	27	164	19	85
140	12	80	19	101	19	94	18	95	24	156	16	80

TABLE III-C

Tetanic Data for 15 Hz Stimulation

Variables are time (s), dorsiflexion torque (Tor, Nm) and M-wave amplitude (Mw, mv) for each subject.

	Subject											
	BK		BH		TH		LT		KH		WH	
Time	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw
0	23.6	18	22.1	27	22.1	31	12.5	21	9.7	33	11.1	28
10	23.6	18	23.6	30	26.5	33	12.5	22	10.0	34	11.1	30
20	21.4	19	23.6	30	22.9	35	11.8	22	9.3	34	11.1	30
30	21.4	19	22.2	32	20.0	35	11.4	22	9.3	34	10.4	31
40	20.7	20	21.5	32	20.0	37	10.4	22	8.9	34	10.4	31
50	20.0	21	20.7	34	18.6	37	10.0	22	8.6	34	10.7	31
60	19.3	21	20.7	35	17.9	39	10.4	22	8.6	34	10.4	31
70	19.3	22	19.3	34	17.9	39	10.0	22	8.2	35	10.0	31
80	18.6	22	18.6	32	16.4	38	10.0	22	7.9	34	10.0	29
90	17.9	23	15.7	29	15.7	37	10.0	23	7.9	33	9.7	29
100	17.9	22	14.3	27	15.0	36	9.3	23	7.9	31	9.3	28
110	17.2	20	12.2	24	12.9	32	8.9	22	7.2	30	9.3	28
120	15.0	19	7.9	20	11.4	28	8.6	22	7.2	27	8.6	26
130	12.2	18	7.2	18	9.3	25	7.9	21	6.4	26	7.9	24
140	10.7	17	6.4	16	5.7	22	6.4	20	6.1	22	7.2	22
150	5.7	15	2.1	14	5.0	18	5.4	19	5.4	20	5.4	19
160	5.0	14	2.1	13	2.9	18	4.7	18	3.6	18	4.3	15
170	3.6	12	1.4	13	2.1	17	3.9	17	2.9	17	2.9	14
180	0.0	11	0.0	11	0.0	16	2.9	15	2.1	16	1.1	12
190	0.0	11	0.0	11	0.0	15	1.8	14	0.7	13	1.1	9
200	0.0	10	0.0	11	0.0	15	1.1	12	0.0	13	0.0	8
210	0.0	9	0.0	11	0.0	15	0.0	11	0.0	13	0.0	8

TABLE III-D

Tetanic Data for 30 Hz Stimulation

Variables are time (s), dorsiflexion torque (Tor, Nm) and M-wave amplitude (Mw, mv) for each subject.

	Subject											
	BK		BH		TH		LT		KH		WH	
Time	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw
0	25.0	17	25.0	25	28.6	27	15.0	22	8.9	32	10.4	26
10	23.6	17	22.9	27	28.6	31	14.7	23	9.3	22	11.1	28
20	24.3	19	22.2	28	32.2	33	15.4	33	10.0	34	11.4	29
30	22.9	20	21.5	35	30.7	35	15.4	24	10.0	37	11.4	30
40	22.9	21	20.8	32	30.7	36	15.0	24	9.7	38	11.4	30
50	23.6	22	20.0	30	29.3	35	14.7	23	9.7	40	11.1	30
60	22.9	21	15.7	26	25.8	31	24.7	24	9.7	38	10.7	29
70	22.2	19	15.0	22	21.5	25	14.3	23	9.3	36	10.0	26
80	20.0	16	12.9	17	17.9	18	13.2	22	8.6	32	9.7	18
90	17.2	14	9.3	12	13.6	12	11.4	20	7.5	26	7.2	13
100	15.0	11	8.6	9	10.0	8	10.8	17	6.4	20	5.7	10
110	12.9	10	7.2	7	6.4	6	8.9	14	5.0	16	3.9	9
120	7.2	8	3.6	6	3.6	5	6.4	11	3.2	11	1.8	6
130	5.7	6	3.6	5	2.1	4	5.0	7	1.8	9	1.1	5
140	3.6	5	2.1	4	1.4	4	3.6	6	0.7	7	0.4	4
150	1.4	4	0.4	4	0.0	3	2.1	5	0.0	6	0.0	4
160	0.0	3	0.0	3	0.0	3	1.4	4	0.0	6	0.0	3
170	0.0	3	0.0	3	0.0	3	0.7	3	0.0	6	0.0	3
180	0.0	3	0.0	3	0.0	3	0.0	3	0.0	6	0.0	3

TABLE III-E

Tetanic Testing with 30 Hz burst

Variables are time (s), dorsiflexion torque (Nm) and M-wave amplitude (mv) for each subject.

15 Hz Fatiguing Stimulation

	Subject											
	BK		BH		TH		LT		KH		WH	
Time	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw
0	24.2	19	22.8	26	30.6	30	14.6	20	12.5	30	12.1	28
25	23.5	19	24.2	30	27.0	34	13.5	20	11.4	28	12.8	30
55	22.1	20	21.4	32	23.5	36	12.1	22	10.7	28	12.1	29
85	19.9	21	17.1	24	19.9	31	12.1	22	10.0	26	11.1	28
115	16.3	16	12.1	12	12.8	16	10.7	17	8.5	20	10.0	23
145	9.3	11	5.0	6	5.7	8	7.8	11	7.1	15	7.1	14
175	3.2	8	0.7	4	1.4	6	4.3	6	3.6	8	2.9	8
205	0.0	6	0.0	4	0.0	6	1.4	4	0.0	6	0.0	3

30 Hz Fatiguing Stimulation

	Subject											
	BK		BH		TH		LT		KH		WH	
Time	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw	Tor	Mw
0	24.9	17	25.6	25	34.2	26	17.1	22	11.0	30	11.4	28
25	23.5	19	21.4	30	32.0	33	15.3	23	10.7	35	12.1	30
55	23.5	22	18.5	28	27.8	31	14.2	22	10.3	38	11.4	30
85	18.5	16	11.4	9	15.7	12	12.1	18	8.5	22	9.6	20
115	17.1	9	5.8	5	3.6	4	7.5	9	4.3	10	3.2	7
145	2.9	5	1.4	3	1.4	4	2.5	4	0.7	6	0.7	4
175	0.0	3	0.0	3	0.0	3	0.0	2	0.0	5	0.0	3

Appendix IV: Individual subject data for Chapter 4

Mechanism for Declining EMG

TABLE IV-A

EMG/Force Relationship

Variables are voluntary dorsiflexion torque (Nm), integrated electromyographic activity (IEMG, mv.s), and interpolated twitch torque (IT, Nm).

Subject ID	Sex	Age	Trial 1 Torque	Trial 1 IEMG	Trial 2 Torque	Trial 2 IEMG	Trial 2 IT
BK	M	51	11.39	72.58	8.54	74.93	14.95
			17.09	109.83	14.24	86.15	10.68
			24.92	164.28	20.64	140.20	4.98
			31.33	258.53	26.34	195.63	2.14
					32.75	309.73	0.71
					41.30	457.60	0.00
WH	F	32	9.97	98.05	10.68	83.83	3.56
			14.24	52.88	12.82	150.45	2.85
			19.94	469.95	18.51	285.18	0.71
			24.92	592.63	18.51	271.85	0.71
					22.78	193.50	0.36
					24.21	292.65	0.00
					25.63	422.93	0.00
TH	M	31	25.63	170.65	11.39	86.30	18.51
			38.45	287.18	24.21	166.28	8.54
			52.69	450.78	39.87	287.70	2.85
			54.11	599.50	54.11	474.63	0.00
HH	M	28	11.39	115.98	5.70	90.35	15.66
			17.80	186.00	9.97	128.35	12.10
			24.92	252.20	17.80	193.08	7.12
			32.04	348.48	24.21	297.35	2.85
					32.04	410.23	0.00
KH	F	27	6.76	77.90	3.56	40.95	9.61
			10.68	118.58	10.32	109.47	4.63
			13.88	188.29	16.38	249.52	1.42
			21.36	421.05	21.36	451.62	0.00

TABLE IV-A - cont

Subject ID	Sex	Age	Trial 1 Torque	Trial 1 IEMG	Trial 2 Torque	Trial 2 IEMG	IT
OX	F	26	11.39	176.75	6.41	81.09	0.71
			13.53	219.57	9.61	133.62	0.71
			17.09	298.69	12.46	200.17	0.36
			23.14	556.50	17.80	282.47	0.36
					22.78	474.84	0.00
HK	M	28	12.10	92.65	7.12	48.15	14.24
			19.22	134.60	12.82	221.50	11.39
			25.63	211.18	19.22	156.60	5.70
			32.04	290.70	26.34	240.15	1.42
					32.75	296.53	0.71
					39.87	521.33	0.00
BO	F	24	8.90	118.98	6.05	104.88	4.98
			10.68	175.88	8.19	90.08	2.85
			13.88	226.63	11.39	168.48	1.78
			18.51	351.68	14.60	208.95	1.07
					17.80	265.43	0.36
					22.43	569.85	0.00
SV	M	25	18.51	92.72	14.24	127.35	9.97
			23.50	267.23	21.36	123.30	7.12
			34.18	504.74	37.02	498.40	0.00
			39.16	568.40	37.74	396.19	0.00
					40.58	327.70	0.00
DD	F	37	8.90	58.23	5.34	57.33	2.85
			12.46	90.20	7.12	61.45	2.14
			19.94	286.70	10.68	87.83	1.07
					22.07	250.68	0.00

TABLE IV-B

Fatigue and Recovery

Variables are maximum voluntary dorsiflexion torque (MVC, Nm), integrated electromyographic activity (IEMG, mv.s), dorsiflexion torque evoked by 15 Hz stimulation (15 Hz, Nm), maximum M-wave amplitude (Mmax, mv) for prefatigue (Pre), fatigue (Fat) and recovery (Rec).

Subject	Time	MVC	IEMG	15 Hz	Mmax
BK	Pre	44.86	469.48	22.78	19
	Fat	10.32	218.79	1.42	17
Rec -	1 min	21.36	221.80	13.53	21
	3 min	34.18	384.53	21.36	22
	5 min	37.74	443.40	16.38	22
	10 min	41.30	439.20	15.66	22
WH	Pre	26.70	426.55	4.63	30
	Fat	12.82	269.15	0.00	25
Rec -	1 min	20.65	306.50	1.42	28
	3 min	22.78	352.28	2.14	26
	5 min	23.14	315.93	2.85	27
TH	Pre	55.54	502.15	24.21	31
	Fat	21.36	270.32	0.00	25
Rec -	1 min	41.30	467.58	12.82	31
	3 min	45.57	473.78	14.24	31
	5 min	45.57	463.38	12.82	29
	10 min	44.14	437.83	12.82	31
HH	Pre	37.74	416.40	16.38	16
	Fat	11.04	404.84	0.36	14
Rec -	1 min	25.63	548.43	6.41	13
	3 min	28.46	544.78	8.54	15
	5 min	26.34	476.88	8.54	15
	10 min	25.63	490.38	9.26	16
KH	Pre	22.07	408.40	9.26	20
	Fat	5.70	139.19	0.00	12
Rec -	1 min	14.24	222.25	3.56	15
	3 min	16.73	314.97	5.70	16
	5 min	16.55	289.64	6.76	16
	10 min	18.51	382.83	5.70	19

TABLE IV-B cont

Subject	Time	MVC	IEMG	15 Hz	Mmax
OX	Pre	23.50	487.67	2.14	16
	Fat	16.91	338.43	0.36	13
Rec -	1 min	22.43	454.27	1.42	14
	3 min	23.85	368.24	1.78	14
	5 min	22.07	412.65	1.78	15
	10 min	23.50	455.85	2.14	14
HK	Pre	42.72	441.08	20.65	17
	Fat	15.66	344.21	4.98	16
Rec -	1 min	35.60	447.20	17.09	17
	3 min	38.45	482.08	17.09	16
	5 min	35.60	450.53	16.38	19
	10 min	37.74	453.55	14.24	20
BO	Pre	23.14	626.90	3.56	10
	Fat	7.12	175.86	1.42	4
Rec -	1 min	17.44	265.58	1.07	4
	3 min	21.00	458.08	0.71	4
	5 min	22.07	580.58	0.71	3
	10 min	21.72	403.63	1.42	8
SV	Pre	37.74	503.34	21.36	16
	Fat	6.05	49.97	2.14	9
Rec -	1 min	28.48	373.92	17.09	17
	3 min	32.04	439.93	17.80	16
	5 min	32.75	451.39	17.09	16
DD	Pre	24.92	265.83	7.12	19
	Fat	4.81	65.58	0.00	13
Rec -	1 min	18.51	220.68	1.42	22
	3 min	19.58	162.23	1.42	22
	5 min	22.43	262.25	1.42	25

Appendix V: Individual Subject Data for Ischemia Control

Effects of 3-4 Minutes of Ischemia

Variables are maximal voluntary dorsiflexion torque (MVC, Nm) and integrated electromyographic activity (IEMG, mv.s) for before ischemia (Pre) and after ischemia (Post).

	MVC			IEMG		
Subject	Pre	Post	% Change	Pre	Post	% Change
BI	54.1	51.3	-5	268.10	225.20	-16
BJ	31.3	25.6	-23	256.52	270.45	+5
WH	54.1	48.4	-11	381.57	335.97	-12
GT	45.6	42.7	-6	408.23	435.18	+7

Appendix VI: Individual Subject Data for Chapter 5
Motoneuron Excitability with Fatigue

TABLE VI-A

Motoneuron Excitability

Variables are Hmax/Mmax ratio for Test 1 (Fatigue condition), Test 2 (Ischemia only), and Test 3 (Stimulation only) from the right (R) leg and control left (L) leg.

Subject			Test 1				Test 2				Test 3			
			R		L		R		L		R		L	
ID	Sex	Age	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
KG	M	23	.33	.17	.26	.29	.39	.44	.27	.24	.29	.32	.44	.43
UU	M	24	.37	.14	.48	.26	.38	.31	.44	.43				
HK	M	29	.33	.13	.35	.32	.31	.40	.33	.32	.38	.42	.45	.44
IK	F	23	.37	.35	.45	.48	.40	.35	.44	.37				
MD	F	35	.42	.03	.34	.38	.32	.29	.40	.34	.43	.34	.57	.47
LF	F	29	.47	.27	.42	.45	.58	.35	.47	.44	.56	.48	.38	.40
TH	M	32	.59	.40	.36	.49	.57	.56	.39	.48	.39	.39	.30	.28
HH	M	30	.53	.24	.51	.48	.50	.45	.40	.40				
SM	F	27	.37	.26	.25	.27	.46	.41	.35	.32				
BN	M	52	.44	.38	.34	.36	.52	.47	.47	.48				

TABLE VI-B

Fatigue Data

Variables are integrated electromyographic activity (IEMG, mv.s), maximum voluntary plantarflexion torque (MVC, Nm), plantarflexion torque evoked by 15 Hz stimulation (Tet, Nm), and maximum M-wave (Mmax, mv) for Test 1 (Fatigue condition - right leg).

	IEMG		MVC		Tet		Mmax		IT	
Subject	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
KG	119.27	39.62	142.40	71.20	64.08	21.36	21	20	0	0
UU	316.90	211.07	119.62	62.66	45.57	17.09	23	19	0	0
HK	164.72	101.93	124.60	76.54	35.60	13.24	24	21	0	0
IK	261.87	166.77	66.22	46.28	17.09	2.85	20	17	0	0
MD	177.37	72.27	68.35	44.86	15.66	4.27	18	16	0	0
LF	270.47	121.75	71.20	48.42	14.24	5.70	18	19	0	0
TH	160.43	17.62	147.74	96.12	21.36	10.68	17	13	0	0
HH	205.45	94.87	133.50	65.86	42.72	10.68	20	18	1.91	3.82
SM	195.22	86.27	59.81	43.43	12.82	5.70	23	19	0	0
BN	317.37	214.83	128.16	81.88	24.92	5.34	23	21	0	0

TABLE VI-C

Reproducibility

Variables are Hmax/Mmax ratio (H/M), integrated electromyographic activity (IEMG, mv.s), and maximum voluntary plantarflexion torque (MVC, Nm) for Test 1 (Fatigue condition - right leg) on two occasions (T1, T2).

	H/M				IEMG				MVC			
	T1		T2		T1		T2		T1		T2	
ID	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
HK	.33	.17	.38	.22	164.72	101.93	205.58	85.82	124.60	76.54	156.64	94.34
IK	.37	.35	.48	.18	261.87	166.77	216.70	148.14	66.22	46.28	59.81	41.72
UU	.37	.14	.32	.08	316.90	211.07	371.39	263.79	119.62	62.66	112.78	76.90
HH	.53	.24	.34	.15	205.45	94.87	198.99	78.84	133.50	65.86	122.82	74.76
TH	.59	.40	.59	.39	160.43	17.62	154.74	86.80	147.74	96.12	174.44	92.56

TABLE VI-D

Subjects not achieving 30% decline in MVC

Variables are Hmax/Mmax ratio (H/M) for right (R) leg and control left (L) leg, integrated electromyographic activity (IEMG, mv.s) and maximum voluntary plantarflexion torque (MVC, Nm) for right leg.

Subject			H/M				IEMG		MVC	
			R		L					
ID	Sex	Age	Pre	Post	Pre	Post	Pre	Post	Pre	Post
MX	F	22	.30	.25	.34	.32	217.74	145.52	56.96	42.72
SH	M	22	.63	.55	.63	.66	347.73	300.30	99.68	74.05
BI	F	35	.23	.16	.24	.21	220.50	198.07	62.66	51.26

Appendix VII: Individual Subject Data for Chapter 6

Type of Afferent Implicated in Reflex Inhibition

TABLE VII
Compression Block Data

Variables are maximum H-reflex/maximum M-wave (H/M) ratios, maximum voluntary torque (MVC, Nm), integrated electromyographic activity (IEMG, mv.s), maximum M-wave amplitudes (Mmax, mv) and areas (uv.s), and interpolated twitch torque (IT, Nm). Pretest (Pre) values, postblock (PB) values and postfatigue (PF) values are given for Test 1 (Fatigue condition) and Pre, PB, and postcuff (PC, values are given for Test 2 (Ischemia control condition).

Test 1

Subject		H/M			MVC			IEMG			Mmax amp			Mmax area			IT		
		Pre	PB	PF	Pre	PB	PF	Pre	PB	PF	Pre	PB	PF	Pre	PB	PF	Pre	PB	PF
NG	(R leg)	.24	0.0	0.0	108.2	68.4	57.3	179.9	105.5	66.8	27	–	22	55.7	–	56.5	0.0	0.0	0.0
	(L leg)	.34	.34	.33							23	–	22						
LM	(R leg)	.34	0.0	.12	128.2	116.8	68.4	224.6	250.2	127.0	18	18	19	56.8	–	60.9	2.8	2.8	5.7
	(L leg)	.29	.28	.29							19	19	20						
TE	(R leg)	.37	0.0	0.0	131.0	119.6	37.0	171.4	172.4	66.5	23	22	12	45.0	44.9	26.5	0.0	0.0	11.4
	(L leg)	.31	.31	.35							32	32	29						
KH	(R leg)	.46	.02	.07	79.7	79.7	41.3	162.6	116.9	46.4	17	18	20	79.5	86.2	99.9	0.0	0.0	0.0
	(L leg)	.24	.30	.20							25	25	25						
WH	(R leg)	.35	.03	0.0	99.7	74.1	48.4	182.4	180.5	118.6	21	–	17	56.4	–	43.7	0.0	0.0	0.0
	(L leg)	.21	.26	.18							30	–	28						

Test 2

Subject		H/M			MVC			IEMG			Mmax amp			Mmax area			IT		
		Pre	PB	PC	Pre	PB	PC	Pre	PB	PC	Pre	PB	PC	Pre	PB	PC	Pre	PB	PC
NG	(R leg)	.28	.04	0.0	108.2	82.6	74.1	146.1	92.6	89.0	27	–	28	48.8	–	55.9	0.0	0.0	0.0
	(L leg)	.34	.34	.39							25	–	22						
LM	(R leg)	.25	.03	.08	133.9	125.3	113.9	173.5	126.7	116.1	21	20	17	50.5	36.5	48.4	0.0	2.8	2.8
	(L leg)	.20	.25	.26							22	21	18						
TE	(R leg)	.27	.02	0.0	145.3	102.5	102.5	253.7	215.8	174.3	33	33	34	62.4	53.3	59.5	0.0	0.0	5.7
	(L leg)	.31	.32	.36							33	30	25						
KH	(R leg)	.37	0.0	.15	105.4	99.7	104.0	287.7	289.6	272.3	24	26	28	103.4	108.9	113.4	0.0	0.0	0.0
	(L leg)	.33	.32	.32							26	27	27						
WH	(R leg)	.22	.05	0.0	85.4	68.4	57.0	187.6	232.3	226.3	18	–	20	75.0	–	80.2	0.0	0.0	0.0
	(L leg)	.20	.32	.30							23	–	22						

Appendix VIII: Consent Form

Date:

The study entitled "Electromyographic Changes during Human Muscle Fatigue" is designed to measure the electrical activity and the force produced by my ankle joint muscles during fatigue induced by electrical stimulation.

I understand that a moderate amount of pain will be experienced during the experiment.

I understand that there is a remote possibility of arterial or vascular thrombosis with the use of the blood pressure cuff wrapped around my thigh.

I understand that I am free to withdraw from the study at anytime.

I understand fully the extent and nature of my participation in the study.

I hereby agree to participate in the research study.

Signed:

Printed Name: